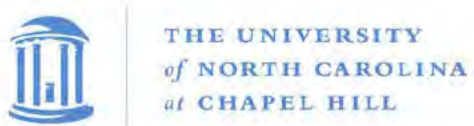

INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
January 11, 2016, 3:30 PM
Burnett-Womack Room 9001

Members present: Doug Cyr, Sandra Bradshaw, Matthew Lawrence, Fred Sparling, Amy Sims, Aravinda de Silva, Craig Fletcher, Mary Beth Koza, Daniel Eisenman,

Members Absent: Peggy Cotter, Dwight Bellinger, Kara Milton

Open Meeting

1. Welcome
2. Review minutes from the December 2, 2015 meeting.
3. Applications under review

Protocol ID #	Investigator	Title
24003	GRIEGER JOSHUA	Production and testing of AAV-Syn-HA-PRR7-IRES2-eGFP
24123	GRIEGER JOSHUA	Production and testing of AAV-pRVPG22_AM
24124	GRIEGER JOSHUA	Production and testing of AAV-p_AAV_CMV_dio_Sun1_flag
24125	GRIEGER JOSHUA	Production and testing of AAV-p_AAV_dio_ER_tags_eGFP_GPI
24126	GRIEGER JOSHUA	Production and testing of AAV-p_AAV_Syn_ER_tags_eGFP_GPI
24127	GRIEGER JOSHUA	Production and testing of AAV-p_AAV_Syn_Sun1_flag
24128	GRIEGER JOSHUA	Production and testing AAV-pUF1-human-OSM
24129	GRIEGER JOSHUA	Production and testing of AAV-AB1025 pAC1-V16-BDNF
24130	GRIEGER JOSHUA	Production and testing AAV-pAAV-DIO-Orexin
24131	GRIEGER JOSHUA	Production and testing of AAV-pAM-cFos-htTA
24132	GRIEGER JOSHUA	Production and testing AAV-pTT-034
24133	GRIEGER JOSHUA	Production and testing AAV-pssHBV-Triple-V1
24134	GRIEGER JOSHUA	Production and testing AAV-AM/CBA-DIO-D1R-HA-WPRE-bGH
24135	GRIEGER JOSHUA	Production and testing AAV-EG6_72 and EG6_73
24136	GRIEGER JOSHUA	Production and testing AAV-CaMKIIa-GCaMP6f-P2A-nls-dTomato
24137	GRIEGER JOSHUA	Production and testing AAV-CaMKIIa-GCaMP6s-P2A-nls-dTomato
24138	GRIEGER JOSHUA	Production and testing AAV-GephyrinK170C-GFP
24140	GRIEGER JOSHUA	Production and testing AAV-GephyrinS305D-GFP
24141	GRIEGER JOSHUA	Production and testing AAV-AB1019 pAC1-V16-eGFP
24142	GRIEGER JOSHUA	Production and testing of Reporter with hKLKB1 containing 3
24143	GRIEGER JOSHUA	Production and testing of AAV-pAAV-hSyn-doubleflexed-hM4D-mCitrine-2ACT
24144	GRIEGER JOSHUA	Production and testing of AAV-pTR-shScram, pTR-shCox6a2A and pTR-Cox6a2O/E
24146	GRIEGER JOSHUA	Production and testing AAV-pAAV-TBG-Ms Sirt6
24183	GRIEGER JOSHUA	Production and testing of AAV-pAAV-TBG-Ms Sirt7
24184	GRIEGER JOSHUA	Production and testing of AAV-pZac2.1-hDPP4
24185	GRIEGER JOSHUA	Production and testing of AAV-pAAV-PACAPshRNA-eGFP
24186	GRIEGER JOSHUA	Production and testing of AAV-pAAV FRT-mG

24187	GRIEGER JOSHUA	Production and testing of AAV-CD40L cocDNA AAV
24188	GRIEGER JOSHUA	Production and testing of AAV-PHPA-TRS-KS
24189	GRIEGER JOSHUA	Production and testing of AAV-pAAV-UbC-eGFP
24190	GRIEGER JOSHUA	Production and testing of AAV-KV3.1-WT, KV3.1b-Gly, KV3.1-Glu
24193	GRIEGER JOSHUA	Production and testing of AAV-IkBa
24194	GRIEGER JOSHUA	Production and testing of AAV-pmiR-210-TD
24195	GRIEGER JOSHUA	Production and testing of AAV-pAAV-hSyn-PRG1-MGCBD-MCBD-HA-IRES-mCitrine
24196	GRIEGER JOSHUA	Production and testing of AAV-scAAV CMV Cre
24197	GRIEGER JOSHUA	Production and testing of AAV-pAAV-tetO-M3-cherry
24198	GRIEGER JOSHUA	Production and testing of AAV-CMV-DIO-AT1R-ferritin-pA
24199	GRIEGER JOSHUA	Production and testing of AAV-CMV-DIO-TREK-1-ferritin-pA
24200	GRIEGER JOSHUA	Production and testing of AAV-pAAV-CMV-DIO-TRPV4-p2A-ferritin-pA
24201	GRIEGER JOSHUA	Production and testing of AAV-rAAV-syn::FLEX-rev::PSAML141F:GlyR-IRES-GFP
24202	GRIEGER JOSHUA	Production and testing of AAV-HTR5a-Flex
24203	GRIEGER JOSHUA	Production and testing of AAV-pAAV/D377Y-mPCSK9
24204	GRIEGER JOSHUA	Production and testing of AAV-pBL-flag Rhes S33N
24205	GRIEGER JOSHUA	Production and testing of AAV-pBL-flag Rhes N77L
24206	GRIEGER JOSHUA	Production and testing of AAV-pAAV-FLEX-NBL10
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24208	GRIEGER JOSHUA	Production and testing of AAV-hSyn-TRPV1
24209	GRIEGER JOSHUA	Production and testing of AAV-TRE-ChR2-EYFP
24210	GRIEGER JOSHUA	Production and testing of AAV-pAAV-Ef1a-ArrTEV
24211	GRIEGER JOSHUA	Production and testing of AAV-pAAV-Ef1a-DIO-GIN
24212	GRIEGER JOSHUA	Production and testing of AAV-pAAV-Ef1a-Receptor (S)
24213	GRIEGER JOSHUA	Production and testing of AAV-pAAV-hSyn-Receptor (L)
24214	GRIEGER JOSHUA	Production and testing of AAV-pAAV-hSyn-Receptor (S)
24243	GRIEGER JOSHUA	Production and testing of AAV-CaV3.2shRNA_368797
24244	GRIEGER JOSHUA	Production and testing of AAV-pAAV-TAS-GFA-gtACR2-YFP
24245	GRIEGER JOSHUA	Production and testing of AAV-pAAV2.6THmye-dsred
24246	GRIEGER JOSHUA	Production and testing of AAV-pAAV-PGK-TTC-CreERT2/WGA-CreERT2
24247	GRIEGER JOSHUA	Production and testing of AAV-pAAV/HCR/apoE-hAAT-promoter-BGHpA
24248	GRIEGER JOSHUA	Production and testing of AAV-NTS siRNA, scramble siRNA
24249	GRIEGER JOSHUA	Production and testing of AAV-pAAV CAG-FLEx-V2T
24250	GRIEGER JOSHUA	Production and testing of AAV-pAAV-Ef1a-fDIO-GCaMP6f
24251	GRIEGER JOSHUA	Production and testing of AAV-pAAV-Ef1a-fDIO-RCaMP2
24252	GRIEGER JOSHUA	Production and testing of AAV-Oxy(2,6)ChR2-mcherry(-WPRE), OX(2.6)HR_EYFP, Oxy(2.6)Venus
24253	GRIEGER JOSHUA	Production and testing of AAV-pAAV-EF1a-DIO-TbRII-WT, TbRII-DN and TbRI-CA(T202D)
24254	GRIEGER JOSHUA	Production and testing of AAV-SiGUCY
24255	GRIEGER JOSHUA	Production and testing of AAV-sgRNA-GFAP-Cre
24256	GRIEGER JOSHUA	Production and testing AAV-pAAV-CRABP2shRNA-eGFP
24257	GRIEGER JOSHUA	Production and testing AAV-pAAV-FABP5shRNA-eGFP
24258	GRIEGER JOSHUA	Production and testing of AAV-pMA-RQ_AAV_H2H
24259	GRIEGER JOSHUA	Production and testing of AAV-pTR-GOI, where GOI represents one of 6 genes

<table>
<tr><td></td><td></td><td>listed on "other commends"</td></tr>
<tr><td>24260</td><td>GRIEGER JOSHUA</td><td>Production and testing of AAV-phIL10</td></tr>
<tr><td>24261</td><td>GRIEGER JOSHUA</td><td>Production and testing AAV-ASPA</td></tr>
<tr><td>24262</td><td>GRIEGER JOSHUA</td><td>Production and testing AAV-pAAV-hSyn-Chrimson-Y261F/S267M-YFP</td></tr>
<tr><td>Approved</td><td colspan="2">AAV mediated transduction of cell lines.
Committee comments: The proposed containment and safety practices are adequate for the experimental design.
Community member comments: None

III-E, BSL-2, E. coli, AAV</td></tr>
<tr><td>23743</td><td>MARGOLIS DAVID</td><td>Studying the role of Histone Deacetylase (HDAC) proteins regulating HIV-1 transcription</td></tr>
<tr><td>Approved w/ Stipulations</td><td colspan="2">Transfection of HDAC into J89 and 2D10 cell lines bearing the HIV genome.
Committee comments: Revise the rDNA category from III-F to III-D. The proposed containment and safety practices are adequate for the experimental design.
Community member comments: None

III-D, BSL-2, E. coli, HIV</td></tr>
<tr><td>23744</td><td>MARGOLIS DAVID</td><td>Creation of a novel HIV-1 latency model</td></tr>
<tr><td>Approved w/ Stipulations</td><td colspan="2">Transfection of cells with the HIV genome. HIV will be produced to infect donor PBMC in vitro.
Committee comments: Revise the rDNA category from III-F to III-D. Revise the application to state the containment level utilized is BSL2. The containment level was originally listed as BSL-3, but the PI lacks a BSL-3 facility. The actual containment level is BSL2+, but the IBC form lacks that as an option. The proposed containment and safety practices are adequate for the experimental design.
Community member comments: None

III-D, BSL-2+, E. coli, HIV</td></tr>
<tr><td>23745</td><td>MARGOLIS DAVID</td><td>Use of shRNA to knock down specific HDAC cellular genes</td></tr>
<tr><td>Approved w/ Stipulations</td><td colspan="2">Lentivirus mediated transduction of cells with shRNA specific to HDAC.
Committee comments: Revise the rDNA category from III-F to III-D. Specify the HDAC genes. The proposed containment and safety practices are adequate for the experimental design.
Community member comments: None

III-D, BSL-2, E. coli, HIV</td></tr>
<tr><td>23746</td><td>MARGOLIS DAVID</td><td>Disrupting HIV latency in cell lines</td></tr>
<tr><td>Approved w/ Stipulations</td><td colspan="2">Transfection of HDAC into J89 and 2D10 cell lines bearing the HIV genome in order to reverse HIV latency.
Committee comments: The containment level listed on the form is BSL2, but the lab is actually operating at BSL2+. BSL2+ is not an available option on the current IBC form. The proposed containment and safety practices are adequate for the experimental design.
Community member comments: None

III-D, BSL-2+, E. coli, HIV</td></tr>
<tr><td>23764</td><td>KELADA SAMIR</td><td>Use of Lentiviral shRNA or cDNA clones</td></tr>
<tr><td>Approved</td><td colspan="2">Lentivirus mediated transduction of cells with shRNA.
Committee comments: The PI must list the genes that will be silenced. The proposed containment and safety practices are adequate for the experimental design.
Community member comments: None

III-E, BSL-2, E. coli, HIV</td></tr>
</table>

23863	KAFRI TAL	Improving biosafety of simple retroviral and lentiviral systems for research applications
Approved	Creation of an EnvA pseudotyped retrovirus with tropism limited to cells expressing the cognate TVA receptor. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, retrovirus	
24023		VEGF-B expression in mice via AAV
Approved	In vivo use of AAV, delivered via IP, IV and during cardiac surgery. **Committee comments:** The application state the mice will be restrained manually or anaesthetized for surgical procedures. A manual restraint is not adequate for tail vein injections. Specify a mouse tube restraint will be utilized for tail vein injections. A biosafety cabinet will not be utilized, but PPE will include gloves, lab coats as well as eye and face protection. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-1, *E. coli*, AAV	
24063		Regulation of smooth muscle differentiation
Approved	Adenovirus mediated transduction of cell lines as well as transfection with plasmids and creation of transgenic mice as well as genome edited mice with the Crisper/Cas system. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, Adenovirus	
24139		Creation of bat coronavirus (Bat-SCoV) reporter viruses (HKU3 and HKU5) expressing reporter proteins
Approved	Aim: HKU3 and HKU5 are Group 2 coronaviruses (CoVs). Our lab created a Bat-CoV infectious cDNA clones based on consensus sequences of HKU3 and HKU5 viruses (Figure 1 and Becker et al 2008). The resultant recombinant viruses were not able to replicate in human and primate cells due to viral attachment protein (Spike) and cellular receptor (ACE2) incompatibilities. We overcame this issue by swapping out the Bat-SCoV receptor-binding domain (RBD) for the RBD to generate HKU3-SRBD (referred to as HKU3 below) and by swapping out the HKU5 Spike ectodomain for the Spike ectodomain to create HKU5-SEcto (referred to as HKU5 below). The aim of this experiment is to create a recombinant bat coronaviruses expressing reporter proteins (i.e., red fluorescent protein (RFP) and nanoluciferase (nLUC)). We will do this by replacing accessory ORF 7 (for HKU3) and ORF3 (for HKU5) with reporter protein sequence. A similar approach was taken for (Sims et. al 2005). Ultimately, we would like to visualize replication in live cells via fluorescence microscopy and be able to measure virus replication by luciferase expression. The recombinant reporter virus would facilitate this. We will create versions with (Bat-SRBD) and without the Spike Iterations described above. While the native Bat-SCoV virus does not infect human or primate cells, having reporter virus with a native spike glycoprotein would allow us to assess replicative fitness in bat cell lines in the lab. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli*, Bat-CoV infectious cDNA clones based on consensus sequences of HKU3 and HKU5	

24145		Expressing MERS-CoV, HKU3, HKU4, HKU5, SHC014, WIV1, other Bat-CoV, Norovirus, and Denguevirus open reading frames in a Venezuelan Encephalitis Virus vector with a Kozak sequence and puromycin cassette
Approved	Aim: We have recently generated a Venezuelan Encephalitis Virus vector with a Kozak translation sequence and a puromycin resistance vector (VEE-pVR21-Kozak-puro). This vector is intended to enhance the expression of the gene of interest in the pVR21 expression cassette and to enable stable transfection in cell lines. This vector will be used in all subsequent cloning of open reading frames (ORFs) or other genetic elements of interest (i.e., nontranslated regions, hypothetical ORFs) from various coronaviruses (MERS-CoV, HKU3, HKU4, HKU5, other Bat- CoVs) Norovirus, and Denguevirus. These expression constructs will then be used in vitro for short- or long-term protein/RNA element expression studies and polyclonal antibody generation in mice. The vectors would be designed so only one ORF would be expressed at a given time. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, VEE 3526 based replicons	
24163		Introduction of mutations affecting nsp14 interactions with other replicase proteins in Betacoronaviruses
Approved	Aim: Previous studies have shown that the CoV replicase proteins, nsp10 and nsp12, can interact with the RNA-editing enzyme, nsp14. It is hypothesized that these interactions are critical regulators of CoV replication fidelity (Smith et al., JVI 89:6418). The purpose of these experiments is to mutate putative and known nsp14 interaction residues in nsp10 and nsp12 proteins in the CoVs MERS-CoV, HKU3, HKU5, and other Betacoronaviruses. The effects of these mutations will be evaluated in vitro in cell cultures and in vivo in mice. Please note: is a select agent; none of the other Betacoronaviruses listed are currently Select Agents. No experiments proposed will alter the Select Agent status of any organism. Additionally, none of the proposed mutations are predicted to enhance the virulence of any construct, particularly any or MERS-CoV construct. Replication and virulence of all viruses generated will be monitored through: 1) viral passage in cell culture and titering and B) weight loss and titering in animals. If any or MERS-CoV viruses show signs of enhanced replication or virulence over WT, we will cease working with the virus and notify the IBC. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli*, MERS-CoV, HKU3, HKU5, and other Betacoronaviruses	
24223	SONDEK JOHN	Intracellular signaling
Approved	Retrovirus mediated transduction of cell lines. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, retrovirus	
24289	SHARPLESS NORMAN	The role of p16Ink4a in in osteoarthritis.
Approved	Lentivirus mediated transduction of cell lines with a Cas9 fusion protein with defective exonuclease function which will be utilized to target the fusion protein to specific genomic sequences. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, lentivirus	

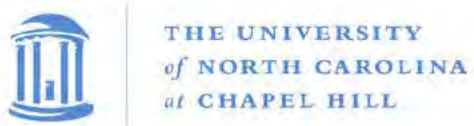
Approved w/ Stipulations	Human gene transfer **Committee comments:** The design of the vector was discussed as well as the method of administration and potential for complications. While the documents submitted provided a great deal of information, the committee felt the requisite safety practices and the agent's biodistribution data must be presented more clearly in a single document prior to releasing approval. The committee discussed requiring future applications for clinical trials to include SOPs clearly stating the containment and safety practices for IBC review as well as for training of study personnel. **Community member comments:** The community members participated in the discussion and expressed comments in line with those stated in Committee Comments. III-C, BSL-2,	
Tabled	Human gene transfer involving lentivirus transduced T cells. **Committee comments:** As NIH RAC review is ongoing, the committee decided to table the application until the RAC response letter is submitted. **Community member comments:** None III-C, BSL-2,	

4. Subcommittee approval of exempt recombinant DNA

Protocol ID #	Investigator	Title
24263	SLEP KEVIN	Functional Analyses of Microtubule Regulators
Involves use of plasmids to express microtubule binding proteins in *E. coli* and cell lines.		

5. Schedule H report: 1
6. Community member IBC training held on 12/29/15
7. Amendment to BSL-3 SOP: The committee requested
8. Incident date 11/30/15, NIH OBA concluded the incident was handled appropriately and no further action is required.
9. DURC policy revision: The revised DURC policy was approved.
10. Dr. Cyr attended and relayed highlights from the January 6th -7th meeting of the National Science Advisory Board for Biosecurity in Bethesda, MD. The topic of the meeting was, "Results of the Risk & Benefit Assessments of Gain-of-Function Studies".

Adjourn. Next IBC meeting on February 3rd at 3:30 PM in Burnett-Womack room 9001



Meeting Minutes
February 3, 2016, 3:30 PM
Burnett-Womack Room 9001

Members Present: Doug Cyr, Sandra Bradshaw, Fred Sparling, Amy Sims, Aravinda de Silva, Barbara Savoldo, Judy Nielsen, Daniel Eisenman,

Members Absent: Matthew Lawrence, Peggy Cotter, Craig Fletcher, Dwight Bellinger, Mary Beth Koza, Kara Milton

Guests Present: [redacted]

Open Meeting

1. Welcome Barbara Savoldo (human gene transfer) and Judy Nielsen (animal containment)
2. Review minutes from the January 11, 2016 meeting.
3. Applications under review

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24183	GRIEGER JOSHUA	Production and testing of AAV-pAAV-TBG-Ms Sirt7
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24261	GRIEGER JOSHUA	Production and testing AAV-ASPA
24262	GRIEGER JOSHUA	Production and testing AAV-pAAV-hSyn-Chrimson-Y261F/S267M-YFP
Approved	AAV mediated transduction of cell lines. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-2, *E. coli*, AAV	
22146	JOHNSON GARY	Analysis of Kinome Dynamics In Cancer
Approved	Plasmids and lentivirus utilized to express fluorescent reporter molecules in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, lentivirus	
24347	KAFRI TAL	Pseudotyping simple retroviral and lentiviral vectors
Approved	Pseudotyping of lentivirus with the following envelope proteins: VSV-G, Syncytin and HIV. Retrovirus is utilized to deliver shRNA against the Syncytin A receptor. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, retrovirus / lentivirus	
24387		AgBiome Agricultural Pest Control in Zea mays and Glycine max

Approved	Genetic modification of plants. Recombinant DNA manipulations will be performed at the company's private lab and the plants would then be transported to the UNC greenhouse. **Committee comments:** The Investigator responded to the various questions asked by the committee's plant containment expert. Topics discussed included containment during transport and in the greenhouse as well as precautions to prevent cross-pollination, heat treatment of waste and waste disposal. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1P, Agrobacterium and Genetically modified plants	
24487	BARIC RALPH	Generation of a cDNA infectious clone system for Zika virus
Approved	Generation of a Zika virus infectious clone. **Committee comments:** The lab's Zika virus SOP details the risks, ensures proper lab signage is in place and provides the necessary information for personnel who are or may become pregnant including the process for requesting a risk assessment and medical monitoring. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, Zika virus	
24527	DUMITRU RALUCA	Genetic Influences on Human Cortical Development, Grant number: 4R00MH102357-03, PI: Jason Stein
Approved	Transduction of cell lines with AAV and lentiviral vectors. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, AAV	
24547	[redacted]	The Role of Coronin 1B in Cell Motility
Approved	Lentivirus mediated transduction of cell lines. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, lentivirus	
24687	[redacted]	Synthesis of a Chinese variant of MERS-CoV (MERS-GD01)
Approved	Creation of a MERS-like virus with variants identified in MERS like coronavirus GD01 for in vitro and in vivo use. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli*, MERS GD01	
24688	[redacted]	Introduction of mutations conferring resistance to nucleoside analogues in Betacoronaviruses
Approved	Mutate residues in nsp12 of [redacted], MERS, HKU3, HKU5 and other beta coronaviruses to confer resistance to nucleoside analogues. Viruses will be utilized in vitro and in vivo. The Investigator would cease all research if the mutants exhibited virulence exceeding that of wild type strains. **Committee comments:** The Investigator provided a letter indicating this study does not fall under the Gain Of Function restrictions. The committee decided DURC classification does not apply as the Investigator is not conferring resistance to known first line therapeutics. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli*, [redacted], MERS, HKU3, HKU5 and other beta coronaviruses	

24708	[redacted]	Generation of a canonical 3ClPro cleavage site at the nsp14-15 junction and combinations of the cleavage site and ExoN inactivation in group 2c Betacoronaviruses
Approved	Restoration of the cleavage site is expected to alter RNA editing and create an attenuated virus with potential as a vaccine candidate. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli*, MERS, HKU4 and HKU5	
24709	ANDERS CAREY	Introduction of p53 knockout using CRISPR/Cas9 technology in human cancer cell lines.
Approved	Use of the CRISPR/Cas system to perform genome editing in cell lines prior to xenografting. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*	
24727	[redacted]	Molecular mechanisms of Zika virus pathogenesis
Approved	Generation of Zika, Dengue clones as well as a Zika / Dengue virus hybrid clone to define epitopes. **Committee comments:** The lab's Zika virus SOP details the risks, ensures proper lab signage is in place and provides the necessary information for personnel who are or may become pregnant including the process for requesting a risk assessment and medical monitoring. Pregnant individuals will make arrangements for others to perform animal inoculations involving sharps. Animal bites are not believed to be a significant route of transmission. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, Zika and Dengue viruses	
24747	[redacted]	Luminescence as a reporter for [redacted] growth.
Approved	Create [redacted] mutant strains that luminesce so that strain of interest are easily studied using a plate reader. The use of the plate reader requires less manipulation (such as in scrape and plate studies) thereby reducing personnel time in the BSL3 and potentially reducing the risk of exposure to [redacted] Plasmids without a [redacted] cloned gene will be transformed into mutants to assess their growth by measuring luminescence. Plasmids containing a gene of interest will be use to complement mutants to confirm the mutant gene was responsible for the growth defect. Genes that may be cloned into the vectors include (but are not limited to) [redacted] as well as other genes involved in nutrient anabolism/catabolism. Hygromycin resistance will be utilized for selection. Hygromycin is toxic to humans and therefore not used clinically. **Committee comments:** The PI has been granted approval by DHHS to perform these experiments with the stipulation that the antibiotics resistance genes utilized for selection do not affect therapeutically useful antibiotics. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli*, [redacted]	
24769	MILEY MICHAEL	Generic use of recombinant nucleic acid plasmids for the purpose of recombinant protein expression
Approved	Use of plasmids and baculovirus to express genes in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-2, *E. coli*, baculovirus	

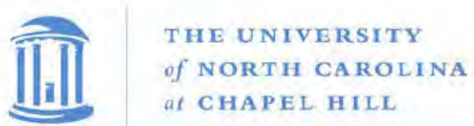
24927		ARF-MDM2-P53 Tumor Suppression Pathway
Approved	Use of plasmids, adenovirus, retrovirus and lentivirus to express genes in cell lines prior to transferring into mice. **Committee comments:** As requested, the PI elaborated on animal procedures and changed the containment to BSL-2. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, adenovirus, retrovirus and lentivirus	
Approved	Use of The clinical trial was reviewed the previous month and tabled pending NIH RAC review. The RAC review documentation has since been provided. No overt toxicities are anticipated. III-C, BSL-2	
Approved	Use of . discussed the experimental design. No overt toxicities are anticipated. III-C, BSL-2,	

4. Subcommittee approval of exempt recombinant DNA

Protocol ID #	Investigator	Title
24567	DOWEN JILL	Genomics of gene regulation in development and disease
Expression of mouse and human genes in cell lines utilizing plasmids.		
24607	TORCHIO JANELLE C	Identifying functional capabilities of the microbiota linked to inflammation and cancer
Expression of genes from RG 1 organisms in non-pathogenic *E. coli* K-12 derivatives.		

5. Schedule H report: 31
6. Amendment to BSL-3 SOP
7. Follow up on the 8/27/15 incident. NIH OBA responded to UNC's report by stating appropriate actions are being taken. NIH OBA requests an update when the new engineering control being developed is completed and implemented.

Adjourn. Next IBC meeting on March 2nd at 3:30 PM in Burnett-Womack room 9001.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
March 2, 2016, 3:30 PM
Burnett-Womack Room 9001

Members Present: Doug Cyr, Sandra Bradshaw, Peggy Cotter, Barbara Savoldo, Judy Nielsen, Mary Beth Koza, Daniel Eisenman

Members Absent: Fred Sparling, Matthew Lawrence, Amy Sims, Aravinda de Silva, Craig Fletcher, Kara Milton

Guests Present: [redacted]

Open Meeting
1. Review minutes from the February 3, 2016 meeting.
2. Applications under review

Protocol ID #	Investigator	Title
24947	COOK JEANETTE	Cell cycle control of DNA replication in mammalian cells
Approved	Transduction of cells with adenoviral, retro/lentiviral vectors. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, adenovirus, retrovirus, lentivirus	
25027	[redacted]	Genetic Dissection of the MEK/ERK and GSK3 Signaling Pathways
Table	Transduction of mouse brains in vivo with AAV. **Committee comments:** Elaborate on purpose of experiment. Revise the recombinant DNA category to III-D. Elaborate on animal restraint / anesthesia, use of BSC and PPE. **Community member comments:** None III-E, BSL-2, *E. coli*, AAV	
25029	[redacted]	Genetic Dissection of the MEK/ERK and GSK3 Signaling Pathways
Table	Transduction of mouse brains in vivo with AAV. **Committee comments:** From the committee's review this application appears redundant to #25027. The PI should either withdraw the application or specify how it is distinct. Elaborate on purpose of experiment. Revise the recombinant DNA category to III-D. Elaborate on animal restraint / anesthesia, use of BSC and PPE. **Community member comments:** None III-E, BSL-2, *E. coli*, AAV	
25087	[redacted]	Human AKR1B10 cDNA for transgenic animal strain
Approved	Creation of vectors expressing human AKRIB10 under control of the bacterial Tet response element to create transgenic mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*	

<table>
<tr><td>25247</td><td></td><td>Peanut DNA Vaccine Therapy</td></tr>
<tr><td>Approved</td><td colspan="2">Creation of a recombinant peanut allergy vaccine. The plasmid will contain peanut allergen and IL-12 and will be injected into allergic mice.
Committee comments: The committee discussed potential risks to personnel with peanut allergies as well as the risk to allergic personnel challenging vaccinated mice with peanut allergen. The proposed containment and safety practices are adequate for the experimental design.
Community member comments: None

III-D, BSL-2, E. coli</td></tr>
<tr><td>25307</td><td></td><td>mouse models of tumor microenvironment</td></tr>
<tr><td>Approved with stipulations</td><td colspan="2">Creation of fluorescently tagged membrane proteins for exosome trafficking.
Committee comments: Include section III for animal experiments.
Community member comments: None

III-D, BSL-2, E. coli, lentivirus</td></tr>
<tr><td>25353</td><td></td><td>Use of a cDNA infectious clone system for Zika virus to examine antibody: virus interactions</td></tr>
<tr><td>Approved</td><td colspan="2">The lab is interested in evaluating the antigenic variability and functional variability of ZIKV structural and non-structural proteins, respectively. In particular, ZIKV recombinant viruses carrying transplanted antibody epitopes and epitope patches within the prM and E glycoprotein genes from variant flaviviruses (primarily DENV and other ZIKV strains) will be generated via site-directed mutagenesis and commercial in vitro gene synthesis (BioBasic). The mutant DNA will be introduced into the relevant clone cDNA fragments, propagated in E. coli, assembled as full-length cDNA, transcribed to RNA and electroporated into Vero, BHK, or C636 cells. Viruses harboring the mutations will be recovered and characterized using growth in cell culture, ELISA, and neutralization assays. Selected mutants will be evaluated for antigenicity, pathogenicity and attenuation in vivo.

Three different infectious clones will be generated representing 3 distinct wild-type ZIKV strains. The prototype MR766 strain, a more current H/PF/2013 (isolated in French Polynesia in 2013), and a chimeric Brazilian 2015 isolate (SPH2015) where we have grafted the 3' untranslated sequence from H/PF/2013 in order to generate a viable virus because this sequence region has not been determined for SPH2015 at this time.

In addition to in vitro characterization including but not limited to virus propagation, titration, ELISA binding, neutralization, antigen depletions, and blockade of binding assays (all under appropriate BSL2 conditions), we plan to use these recombinant ZIKV in murine models of lethal and non-lethal disease to assess the role that particular structural antigenic patches on the virus play in the immune response to viral infection.

Committee comments: The committee questioned the need for intra-cranial inoculations and asked for clarification regarding the nature of the animal restraint / anesthesia that would be utilized for such inoculations.

Investigator response: Intracranial inoculations could become very important for characterizing Zika virus pathogenesis, as there is evidence that the virus is neurotropic. To ensure operator safety, animals that will be intracranially inoculated will first be anesthetized using ketamine/xylasine or isoflurane if the mouse line is ketamine-sensitive. A mechanical restraint device will be used if DLAM requires it for intracranial inoculations.

The proposed containment and safety practices are adequate for the experimental design.
Community member comments: None

III-D, BSL-2, E. coli, Zika virus</td></tr>
</table>

25394		AAVshNPY and AAVshCTL in Ethanol Consumption and Role of Central Peptides
Approved with stipulations	AAV mediated transduction of mouse brains in vivo. **Committee comments:** Elaborate on the use of DREADDs as insert genes. Elaborate on the use of restraints or anesthesia for animal inoculations. **Community member comments:** None III-D, BSL-2, *E. coli*, AAV	
25413	BROWNE EDWARD	Analysis of cells latently infected with lentiviruses
Approved	The study intends to determine the cellular pathways that lead to latent HIV infection and reactivation. In addition to wild type plasmids encoding HIV, the Investigator intends to use modified variants with certain viral genes deleted and replaced with reporter genes such as GFP, Luciferase, and CD24. **Committee comments:** Containment is actually at BSL2+, but the online form lacks that checkbox. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2+, *E. coli*, HIV	
25453		Introduction of p53 knockout using CRISPR/Cas9 technology with lentiviral vectors in human cancer cell lines.
Approved with stipulations	Lentivirus mediated transduction of Cas9 into cell lines to delete p53. The cell lines will be injected into mice intrachranially. **Committee comments:** The form lacked a description of animal procedures. The Investigator must either include the description or delete the animal experiments. **Community member comments:** None III-D, BSL-2, *E. coli*, lentivirus	
25513		Transfection of Cultured Cells with DNA Constructs and Retroviral Vectors
Approved	Retrovirus mediated transduction of GFP, luciferase or siRNA in to cell lines in vitro and neural progenitor cells in utero in mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, retrovirus	
25573		Use of dual guide RNA expressing vectors for CRISPR-mediated genome modification
Approved	AAV mediated transduction of cell lines in vitro or mice in vivo. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, AAV	
25574		Use of Dengue Virus 2 subgenomic flavivirus RNAs to enhance AAV
Approved	We will insert the sequence encoding for Dengue Virus type 2 (DV2) subgenomic flavivirus RNAs (sfRNAs) into the 3' UTR of AAV expression cassettes in order to enhance expression. SfRNAs are generated after XRN1 (an exonuclease) degradation of mRNAs containing sfRNA sequences. These sfRNAs inhibit XRN1 activity which increases mRNA stability/expression. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, AAV	
25593		Gene transfer of mouse cDNA encoding Aquaporin 1 and Aquaporin 4 proteins with AAV in mice
Approved	AAV mediated transduction of aquaporin genes into mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None	

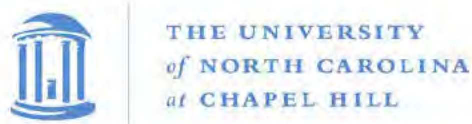
	III-D, BSL-2, *E. coli*, AAV
25594	Study of circRNA expression patterns via delivery into mice using AAV vectors
Approved	Design AAV vector cassettes expressing circular RNAs for in vivo use in mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, AAV
Approved	Revised Investigator's Brochure for a previously approved clinical trial involving human gene transfer. The revisions have no bearing on procedures involving the use of recombinant DNA or modified cells. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-C, BSL-2,
Approved	The study is a clinical trial involving in order to **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-C, BSL-2,

3. Subcommittee approval of exempt recombinant DNA

Protocol ID #	Investigator	Title
25433	RUIZ JOSEPH	Site-specific manipulation of mitochondrial RNA expression
We will express a novel protein which is engineered to bind to specific mitochondrial RNA molecules and degrade them (ASRE) - this is a method that can be used to create in vitro models of mitochondrial disease. In vitro.		

4. Schedule H report: 7
5. Amendment to BSL-3 SOP: The amendment involved
6. 2-4-16 incident reported to CDC and NIH. 2-24-16 incident reported to NIH OBA.
7. The 2-24-16 tornado warning reported to the CDC. BSL-3 labs were closed. No damage or power loss.

Adjourn. Next IBC meeting on April 6th at 3:30 PM in Burnett-Womack room 9001.



Meeting Minutes
April 6, 2016, 3:30 PM
Burnett-Womack Room 9001

Members Present: Doug Cyr, Sandra Bradshaw, Fred Sparling, Amy Sims, Barbara Savoldo, Mary Beth Koza, Daniel Eisenman, Kara Milton

Members Absent: Matthew Lawrence, Peggy Cotter, Aravinda de Silva, Craig Fletcher, Judy Nielsen,

Guests Present:

Open Meeting

1. Welcome
2. Review minutes from the March 2, 2016 meeting.
3. Applications under review

Protocol ID #	Investigator	Title
25693		ELUCIDATING A NOVEL AKT ACTIVATION MECHANISM FOR TARGETED PROSTATE CANCER THERAPY
Approved with Stipulation	Retroviral transduction of cells in vitro prior to use in xenograft studies. **Committee comments:** The Investigator failed to respond to questions pertaining to animal procedures. **Community member comments:** None III-D, BSL-2, *E. coli*, retrovirus	
25733		TetR-based gene regulation system for
Approved with Stipulation	Use of a tetracycline repressor based gene regulation for induction / repression in a deletion mutant model. Growth will be assessed by Tecan plate reader or by counting colonies on agar. Tetracycline repressor does not confer resistance to tetracycline. **Committee comments:** The Investigator possesses a letter from HHS granting permission to perform such experiments. The proposed research does not constitute DURC. The antibiotic resistance utilized for selection would not confer resistance to therapeutics utilized in humans. As tetracycline is the inducer in this system, lab personnel being treated with tetracycline for a community acquired infection should be aware of the potential risk if an exposure takes place. In such an event lab personnel must notify the Biological Safety Officer and the University Employee Occupational Health Clinic to inform them of the exposure, the nature of the experiment and that would likely exhibit altered growth characteristics in the presence of tetracycline. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None **Investigator's response:** Anhydrotetrocyline is not tetracycline and does not impact the anhydrotetrocycline inducible promoter. III-D, BSL-3, *E. coli*,	
25734		Investigating the role of in virulence
Approved with Stipulation	Investigating the role of in by: 1) Repairing the mutation in and compairing to . 2) Delete and create revert ants utilizing from various species. **Committee comments:** The Investigator possesses a letter from HHS granting permission to perform such experiments. The proposed research does not constitute DURC. The antibiotic resistance utilized for selection would not confer resistance to therapeutics utilized in humans. The proposed containment and	

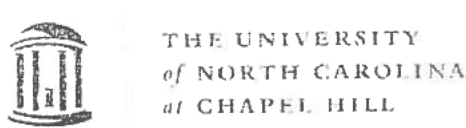
	safety practices are adequate for the experimental design. The submission must be revised to indicate a select agent is utilized. Indicate how virulence will be assessed and provide the threshold for reporting to the IBC if virulence exceeds the WT organism. **Community member comments:** None III-D, BSL-3, *E. coli*,	
25753		AKR1B10 adenovirus for in vitro and in vivo use
Approved	Infection of mice via tail vein injection with adenoviral vectors. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, adenovirus	
25833	ASOKAN ARAVIND	Canine Parvovirus packaging
Tabled	**Committee comments:** The application lacks sufficient detail. **Community member comments:** None III-D, BSL-2, *E. coli*, canine parvovirus	
25834	ASOKAN ARAVIND	Canine Parvovirus packaging
Tabled	**Committee comments:** The application lacks sufficient detail. **Community member comments:** None III-D, BSL-2, *E. coli*, canine parvovirus	
25893		Expressing Zika virus open reading frames in a Venezuelan Encephalitis Virus vector with a Kozak sequence and puromycin cassette
Approved	Insertion of a single Zika virus open reading frame into an attenuated VEE 3526 based replicon in order to vaccinate mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, VEE 3526 based replicon	
25913		A Novel Carcinogen-Induced Cell Cycle Checkpoint
Approved	Transduction of cells with plasmids as well as adenoviral, retro/lentiviral vectors. Adenovirus-Cre will be utilized to infect transgenic mice to induce gene expression. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, adenovirus, retrovirus, lentivirus	
25973		AKR1B10 adenovirus for in vivo use
Approved	Infection of mice via tail vein injection with adenoviral vectors. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, adenovirus	
Approved	The amendment to the previously approved clinical trial was approved. The revision did not alter the risk assessment or any details pertinent to the recombinant DNA manipulations. III-C, BSL-2,	

4. Subcommittee approval of exempt recombinant DNA

Protocol ID #	Investigator	Title
25813	RUSCONI CHRISTOPHER	DNA cloning in small scale cell cultures
Involves cloning into *E. coli* and plasmid mediated expression in cell culture.		

5. Schedule H report: 16
6. NIH OBA stated the 2-24-16 incident was properly handled, no further action required.
7. Revisions to the NIH RAC Review Process for Gene Transfer Protocols
8. USA Today's ongoing coverage of high containment research has won a Scripps Howard Award for public service reporting.

Adjourn. Next IBC meeting on May 4th at 3:30 PM in Burnett-Womack room 9001.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
May 4, 2016, 3:30 PM
Burnett-Womack Room 9001

Members Present: Doug Cyr, Sandra Bradshaw, Fred Sparling, Aravinda de Silva, Judy Nielsen, Mary Beth Koza, Daniel Eisenman

Members Absent: Matthew Lawrence, Amy Sims, Barbara Savoldo, Peggy Cotter, Craig Fletcher, Kara Milton

Guests Present: [redacted]

Open Meeting
1. Review minutes from the April 6, 2016 meeting.
2. Applications under review

Protocol ID #	Investigator	Title
26255	HAHN KLAUS	Design of RhoGEF biosensors
Approved	Retroviral transduction of cells in vitro. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli*, retrovirus	
26273	[redacted]	Redirection of endogenous alternative splicing using antisense oligonucleotides (morpholinos).
Approved	Transfection of anti-sense morphilonos in vitro and in vivo utilizing footpad injections with anaesthetized mice. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, synthetic nucleic acids / morpholinos	
26275	[redacted]	Functional consequences of alternative splicing of clathrin light chain a (Clta), synaptosome associated protein 23kDa (Snap23), and thyroid hormone receptor interactor 10 (Trip10) trafficking genes using plasmids.
Approved	Transfection of plasmids in vitro and in vivo utilizing footpad injections with anaesthetized mice. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli*	
26276	GIUDICE JIMENA	Inhibition of gene expression using small interfering RNAs.
Approved	Transfection of siRNA in cell lines vitro. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli*	

26293	[redacted]	Pathogenesis and therapy of liver human-specific and associated infections in novel humanized mouse model
Approved with Stipulation	Delivery of AAV bearing the complete hepatitis B virus genome to mice via tail vein injection. The HBV polymerase is mutated resulting in replication deficiency. The construct is designed to result in HBV antigen expression without viremia. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. Verify all lab personnel have been offered HBV vaccination. **Community member comments:** None III-D, BSL-2+, *E. coli*, AAV/HBV	
26313	[redacted]	Oncogenic Potential of EBV Latency and Transforming genes
Approved	Expression of EBV genes LMP1 and LMP2 in vitro and in vivo utilizing plasmids and retroviral vectors. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli*, retrovirus	
26314	[redacted]	Molecular Characterization of Tcf4 expression
Approved	Creation of a TCF4 luciferase reporter mouse. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli*	
26315	[redacted]	Spatiotemporal rescue of TCF4 in mice.
Approved	Creation of a TCF4 GFP conditional mutant mouse. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli*	
26353	HAGAN ROBERT	TBK1 and mTOR function in innate and adaptive immunity
Approved	Use of plasmids or retroviral vector to express genes in vitro. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli*, retrovirus	
26433	[redacted]	Introduction of p53 knockout using CRISPR/Cas9 technology with lentiviral vectors in human cancer cell lines.
Approved	Use of CRISPR/Cas9 to delete p53 in cell lines and engraf them into mice. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli*, lentivirus	
26453	SU LISHAN	Zika virus infection and host interaction
Approved	Creation of a luciferase expressing Zika virus infectious clone for in vitro use. **Committee comments:** The lab's BSL2+ SOP has been revised to include Zika virus specific risks and procedures. The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2+, *E. coli*, Zika virus	

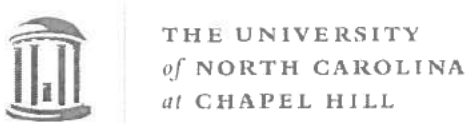
26573	[redacted]	Creation of a Lox-Stop-Lox Cre-inducible Stub1 mouse using CRISPR technology.
Approved	Creation of an inducible stub1 mutant mouse. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-E, BSL-1, *E. coli*	
26593	STEIN JASON	Genetic Influences on Human Cortical Development to study psychiatric disease
Approved	Transduction of cells in vitro with AAV and lentivirus. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2, *E. coli,* AAV and lentivirus	
26735	[redacted]	Coronaviruses that allow for antiviral activity evaluations of inhibitors to MERS nsp12
Approved with Stipulation	The goal of the studies described here is to produce a virulent mouse model for MERS-CoV pathogenesis that allows for antiviral activity evaluations in the context of the MERS-CoV nsp12 RdRp expression in the appropriate host cell targets in vivo. To this end, the recombinant virus HKU5-S MAv-nsp12 (Bat-CoV HKU5 expressing the [redacted] Spike ectodomain and the MERS nsp12) will be produced. Generating this virus will involve constructing infectious cDNAs with substitutions of the nsp12 sequence and the Spike in the HKU5 background (MERS nsp12 will be substituted into the HKU5 cDNA; the [redacted] Spike ectodomain has already been expressed in HKU5 [Agnihothram et al., MBio 2014 5:e00047-14]). We have submitted a request to the NIH to perform this experiment and will provide the IBC with the approval document once it is received. No construct will be generated until approval is given by both the NIH and the UNC IBC. Replication and virulence will be monitored through infections and titering in vitro and in vivo. If the viruses show signs of enhancement over WT, we will cease working with them and will notify the IBC. These enhancements will be defined by a 10-fold increase in the LD50 or a 10-fold increase in virus replication over wild-type. **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. The Investigator must specify how virulence will be assessed and provide the threshold that must be crossed to merit ceasing research and reporting to the IBC. The Investigator must provide the IBC with the NIH letter of exemption from the GOF pause before approval is released. **Community member comments:** None III-D, BSL-3, *E. coli,* HKU-5, MERS	
26753	[redacted]	Exploring Various Humanized Animal Models for the Establishment of Zika Virus Infection
Approved	Creation of a humanized mouse model of Zika virus infection. **Committee comments:** The lab's BSL2+ SOP has been revised to include Zika virus specific risks and procedures. The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-D, BSL-2+, *E. coli,* Zika virus strain MR766	
	[redacted]	[redacted]
Approved	Clinical trial involving [redacted] **Committee comments:** The containment and safety practices described were deemed adequate for the proposed experiments. **Community member comments:** None III-C, BSL-2, [redacted]	

3. Subcommittee approval of exempt recombinant DNA

Protocol ID #	Investigator	Title
25885	JONATHAN BERG	Functional assessment of genetic variants using high-throughput assays
Cloning into *E. coli* and expression in yeast or mammalian cell culture.		
26173	DANIEL J CRONA	Functional gemomics of the androgen receptor signaling pathway
Transfection of plasmids in mammalian cell culture.		
26333	BENJAMIN D PHILPOT	Suplate Cre: enhancer sequences
Transfection of plasmids in mammalian cell culture.		
26493	JONATHAN S SERODY	The role of spliceosome mutations in carcinogenesis and neoantigen production
Transfection of plasmids in mammalian cell culture.		

4. Schedule H report: 10
5. Revisions to the NIH RAC Review Process for Gene Transfer Protocols

Adjourn. Next IBC meeting on June 1, 2016 at 3:30 PM in Burnett-Womack room 9001.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
June 1, 2016, 3:30 PM
Burnett-Womack Room 9001

Members Present: Doug Cyr, Sandra Bradshaw, Peggy Cotter, Aravinda de Silva, Barbara Savoldo, Craig Fletcher, Judy Nielsen, Daniel Eisenman, Kara Milton

Members Absent: Fred Sparling, Matthew Lawrence, Amy Sims, Mary Beth Koza,

Open Meeting

1. Welcome Dr. Rita Tamayo, new IBC committee member.
2. Review minutes from the May 4, 2016 meeting. The minutes were approved.
3. Applications under review

Protocol ID #	Investigator	Title
26834	[redacted]	Etiology of Affective Disorders and Addiction
Approved	Intra-chranial delivery of AAV or lentivirus via stereotaxic surgery. **Committee comments:** Upgrade containment level to BSL-2 for lentivirus. Add eye protection. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, AAV, lentivirus	
26835	[redacted]	Molecular alterations triggered during metastatic TNBC treatment in preclinical murine models.
Approved	Transduction of tumor cells for xenograft studies. **Committee comments:** The PI must include section III detailing animal studies. The investigator complied. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, lentivirus	
26836	PEROU CHARLES	Identification of molecular alterations triggered during metastatic luminal breast cancer progression
Approved	Lentiviral transduction of cells in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, lentivirus	
26917	[redacted]	Engineering Temperate Bacteriophage for Sustained Secretion of Protein Therapeutics or Immunogens by Mucosal Commensals
Tabled	Development of genetically modified phages to transduce commensal bacteria in vitro and in vivo in gnotobiotic mice. **Committee comments:** The committee requires further details about the phages that will be utilized, methods for performing inoculations and the associated IACUC protocol information. The following will be required: All waste must be autoclaved out of the animal facility. ABSL-2 containment. Selection utilizing antibiotics that are not clinically useful. **Community member comments:** None III-D, BSL-2, bacteriophage, *Streptococcus mitis, S. oralis, S. mutans*	

26933	[redacted]	Creation of a Lox-Stop-Lox Cre-inducible Stub1 mouse using CRISPR technology.
Approved	Creation of inducible stub1 mutant mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
26953	COOK JEANETTE	Cell cycle control of DNA replication in mammalian cells
Approved	Use of plasmids, adenovirus or retrovirus to express genes in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, adenovirus, lentivirus	
26979	[redacted]	Oncogenic Potential of EBV Latency and Transforming genes
Approved	Expression of Epstein-Barr virus latent membrane proteins LMP1 and LMP2 in gastric carcinoma cell lines in vivo. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, lentivirus	
26980	[redacted]	IMPG2 Y250C Knock-in Mouse
Approved	Use of CRISPR/Cas to create IMPG2 mutant mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
26981	[redacted]	IMPG2 T807Ter Knock-in Mouse
Approved	Use of CRISPR/Cas to create IMPG2 mutant mice **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
26993	[redacted]	Generation and utilization of a reverse genetics-based viral replicon particle (VRP) system for Zika virus
Approved	Creation of replication deficient Zika virus with structural genes removed and replaced with reporter molecules for use in vitro and in vivo. Inoculations will be performed via the following routes: tail vein, IP, subcutaneous, footpad and intracranial. **Committee comments:** The committee requested further details about the rationale and methodology for intracranial inoculations. The Investigator complied. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, Zika virus	
27033	[redacted]	Immunomodulatory mechanisms in Kras-driven pancreatic cancer and metastasis
Approved	Creation of transgenic mice expressing GFP concurrent to IL-12a expression. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	

27093	HEISE MARK	Influenza C Reverse Genetics System
Approved	Use of reverse genetics to create an influenza C infectious clone. **Investigator comments**: "Using the reverse genetics system allows us to "fix" the virus genome and avoid acquiring cell culture associated mutations through repeated virus passage. These mutations can change cell tropism/virus biology, leading to spurious experimental results. Having the virus derived from the clone minimizes drift in the population and ensures that we are working with a defined stock between experiments and across time. No chimeric viruses will be generated. Our plan is to just study the virus derived from this clone system. If at any point we decide to introduce additional mutations, we will file a new schedule G. We do not intend to introduce any mutations that might alter tropism, virulence, or transmissibility. In fact, at this time, we have no plans to introduce mutations into the influenza C virus infectious clone. If that change, we will of course file a new schedule G." **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* influenza C	
Approved	Creation and use **Committee comments:** The ultimate result is anticipated to be an increased safety profile. is associated with the study and recused herself from voting. The Investigator submitted a cover letter comprehensively addressing the criteria for determining whether NIH RAC review would be required. The committee concurred with the PI's assessment that this study does not meet the criteria requiring RAC review. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-C, BSL-2,	
Tabled	**Committee comments:** Study materials were incomplete and submitted with insufficient time for review. **Community member comments:** None III-C, BSL-2,	

4. Subcommittee approval of exempt recombinant DNA: None
5. Schedule H report: 25
6. 5/20/16 incident reported to NIH OBA.
7. Revisions to NIH Guidelines regarding review of human gene transfer protocols, WIRB Webinar
8. *Scientists Talk Privately About Creating Synthetic Human Genome*, New York Times, May 13, 2016
9. Cyr: NSABB meeting on 5/24/16 to discuss "Evaluation and Oversight of Proposed Gain of Function Research".

Adjourn. Next IBC meeting July 14, 2016 at 9 AM in Burnett-Womack room 9001.

Meeting Minutes
July 14, 2016, 3:30 PM
Burnett-Womack Room 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Aravinda de Silva, Barbara Savoldo, Judy Nielsen, Mary Beth Koza, Daniel Eisenman

Members Absent: Peggy Cotter, Rita Tamayo, Fred Sparling, Matthew Lawrence, Craig Fletcher, Kara Milton

Guest:

Open Meeting

1. Review minutes from the June 1, 2016 meeting. The minutes were approved.
2. Applications under review

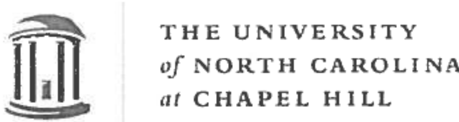
Protocol ID #	Investigator	Title
26153		Dietary Agents that Affect Development
Approved	Use of CRISPR/Cas to create genome edited zebrafish. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
27413		Targeted mutagenesis and transgenesis in zebrafish to study specific gene functions
Approved	Use of CRISPR/Cas and tol2 transposons to modify zebrafish. **Committee comments:** Change the recombinant DNA category from III-F to III-E. Submit section III for animal procedures. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
27453	TARRANT TERESA	GRK3 as an essential regulator of CXCL12-CXCR4 chemokine-receptor interactions in breast cancer metastases
Approved	Lentivirus mediated transduction of cells in vitro to express or silence of G protein coupled receptors in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
27495	HAHN KLAUS	Design of RhoGEF biosensors
Approved	Expression of genes in vitro utilizing plasmids, retrovirus or transposons. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	

27513		Generation of an allelic series within the BAI-1 gene of Collaborative Cross mice
Approved	Creation of genome edited mice. **Committee comments:** Change the recombinant DNA category from III-F to III-E. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
27533		mapping of functioal neural circuits in the mouse brain
Approved	Intrachranial delivery of E1 deleted replication deficient canine adenovirus in mice. **Committee comments:** The injections will take place outside of a biosafety cabinet but with eye and face protection. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, canine adenovirus 2	
27653	HAHN KLAUS	Regulation of the cytoskeleton in hematopoietic cells
Approved	Adenovirus based transduction of cells in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, adenovirus	
27673	PHILPOT BENJAMIN	Delivery of sgRNA to cells using 3rd generation lentivirus delivery
Approved	Lentivirus mediated transduction of CRISPR/Cas in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
27693		Use of a cDNA infectious clone system for Zika virus to examine 5' untranslated region RNA structure mutants
Approved	Creation of Zika virus infectious clones with disrupted 5' UTR for use in vitro and in vivo. **Committee comments:** It was noted that similar experiments conducted with other flaviviruses have resulted in attenuated phenotypes, which is also expected in this case. The committee discussed the methods that will be utilized for restraining and anaesthetizing mice. All procedures will be performed within biosafety cabinets. The lab has an approved SOP for experiments involving Zika virus. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Zika virus	
27745	HEISE MARK	Analysis of RNA structural determinants for their impact on chikungunya virus replication
Approved	Experiments entail mutating the secondary structure of non-coding regions within the Chikungunya virus genome. No determinants of virulence will be manipulated. Experiments will be conducted exclusively in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Chikungunya virus strain 181/25.	
27747	HAHN KLAUS	Biosensors for auto-inhibitory proteins.
Approved	Retrovirus mediated transduction of cells in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* retrovirus	

27773	[redacted]	Pseudotyping retroviral vectors
Approved	In vitro and in vivo comparison of lentivirus pseudotyped with either mouse syncytin A or B vs. VSV-G. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, lentivirus	
27793	[redacted]	Correction of Intron 22 Inversion in Hemonphilia A dogs
Approved	Delivery of chemically synthesized RNA in liposomes in vivo **Committee comments:** The low level of risk allows this study to be conducted at ABSL-1. Change the recombinant DNA category to III-E for animal experiments. The research cannot be performed in the Bingham facility. PPE must be revised to include gowns or lab coats. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, Synthetic RNA	
	[redacted]	[redacted]
Approved	Clinical trial involving [redacted] in [redacted] **Committee comments:** The committee discussed the potential risks to the participant, the clinical trial personnel, the community and the environment [redacted] was discussed as well as the associated safety practices and potential for spills, excretion and spread of contamination. [redacted] The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-C, BSL-2, [redacted]	
	[redacted]	[redacted]
Approved	The study is seeking approval to recruit patients for [redacted]. [redacted] **Committee comments:** The risk to UNC personnel is minimal as [redacted] The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-C, BSL-2, [redacted]	

3. Subcommittee approval of exempt recombinant DNA: None
4. Schedule H report: 25
5. BSL-3 SOP review: [redacted] The SOPs were reviewed and approved.
6. NIH response to 5/20/16 incident report. The incident was properly handled and no further action is required.
7. Update on containment for CC mice.

Adjourn. Next IBC meeting August 3, 2016 at 3:30 PM in Burnett-Womack room 9001.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
August 3, 2016, 3:30 PM
Burnett-Womack Room 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Aravinda de Silva, Barbara Savoldo, Rita Tamayo, Judy Nielsen, Daniel Eisenman

Members Absent: Peggy Cotter, Fred Sparling, Matthew Lawrence, Craig Fletcher, Mary Beth Koza

Guest: [redacted]

Open Meeting

1. Clinical Trial
2. Welcome Dr. Rita Tamayo. Farewell to Kara Milton.
3. Review minutes from the July 14, 2016 meeting. Minutes were approved.
4. Applications under review

Protocol ID #	Investigator	Title
27893	PEROU CHARLES	C-Jun Gene Knockdown in Breast Cancer Cells
Approved	Lentivirus mediated transduction of shRNA against c-Jun in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
27894	PEROU CHARLES	Constitutive Expression of Myc in Breast Cancer Cells
Approved	Lentivirus mediated transduction of mutated c-Myc in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
27913	[redacted]	Cre expression in mice via AAV
Approved	AAV Cre mediated excision of floxed genes in mice. AAV delivered via IV, IP and into myocardium. **Committee comments:** Add face protection as injections are not performed within a biosafety cabinet. The various types of restraints and anesthetics utilized were discussed. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, AAV	
27973	[redacted]	Insertion of human transgene into ES cells
Approved with stipulation	Creation of transgenic mice. **Committee comments:** Provide the list of genes. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	

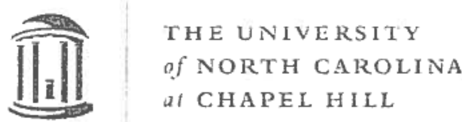
27975	[redacted]	Insertion of bacterial neomycin for selection of targeted ES cell lines
Approved with stipulation	Creation of transgenic mice. **Committee comments:** Provide the list of genes. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
27977	[redacted]	Insertion of bacterial neomycin for selection of targeted ES cell lines
Approved with stipulation	Creation of transgenic mice. **Committee comments:** Provide the list of genes. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
28014	[redacted]	PRE MRNA METABOLISM
Approved with stipulation	Creation of transgenic Drosophila. **Committee comments:** Change rDNA category to III-E. Include section III. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
28033	PURVIS JEREMY	Live-cell reporters for DNA damage, cellular senescence and differentiation
Approved	Expression of genes in vitro via plasmid vector, lentivirus or retrovirus. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* retrovirus, lentivirus	
28185	ASOKAN ARAVIND	Canine Parvovirus packaging
Approved	Use of a canine parvovirus infectious clone in vitro. Replication is limited or permissive cell lines expressing the canine or feline transferrin receptor. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* canine parvovirus infectious clone CPV2	
28186	ASOKAN ARAVIND	Canine Parvovirus packaging study
Approved	Use of a canine parvovirus infectious clone in vitro. Replication is limited or permissive cell lines expressing the canine or feline transferrin receptor. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* canine parvovirus infectious clone CPV2	
28187	[redacted]	Novel Nanoparticle Platform for the Delivery of Vaccines and Adjuvants-VRP vaccination
Tabled	Use of a VEE replicon to vaccinate mice. **Committee comments:** The investigator must provide the list of genes to be expressed as well as the strain of VEE and the source. The committee must be able to determine the virulence, necessary containment level and the select agent status of the virus. **Community member comments:** None III-D, BSL-2, *E. coli,* Venezuelan equine encephalitis virus based replicon	

28193	[redacted]	Mouse adaption of WIV1-CoV
Approved	Creation of a mouse adapted WIV-1 CoV bearing a mutated spike protein. Mice will be anesthetized and undergo intranasal inoculation. The investigator provided a letter of exception from the NIH Gain of Function pause permitting this research to take place. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli,* WIV-1 Coronavirus	
28213	[redacted]	Manipulation of Zika virus envelope to impact stability and neurotropism
Approved	The Zika virus envelope loops will be altered in order to assess stability and neurotropism. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Zika virus	
28214	[redacted]	Incorporating attenuating mutations in the MERS-CoV mouse-adapted infectious clone to determine pathogenicity
Approved	Introduction of established and previously published MERS mutations into mouse adapted MERS. The mutations in parental MERS CoV are found to cause attenuating phenotypes and are not considered to pose a Gain of Function concern. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli,* MERS Coronavirus	
28233	[redacted]	Nanoparticle-based Immune Modulators in Cancer Therapy and Vaccines-CRISPR/Cas9 targeting
Approved	Lentivirus mediated transduction of CRISPR/Cas9 cells in vitro prior to engrafting in mice. **Committee comments:** Increase containment to BSL-2. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
28439	[redacted]	Generation of an allelic series within the Nox4 gene of Collaborative Cross mice
Approved	Use of CRISPR/Cas9 to create nox4 gene edited mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-2, *E. coli*	
28440	HEISE MARK	Analysis of RNA structural determinants for their impact on Zika virus replication
Approved	Mutating Zika virus RNA stem loops while not altering coding sequences in order to characterize effects on replication in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Zika virus H/PF/2013 ZIKV	
28443	[redacted]	Generation of an allelic series within the cKIT and KIT ligand genes of Collaborative Cross mice
Approved	Use of CRISPR/Cas9 to create c-Kit and c-Kit ligand gene edited mice. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-2, *E. coli*	
27993	[redacted]	[redacted]

Approved	Clinical trial involving the inoculation of male study participants with provided a detailed presentation of the study's history and safety profile. The study has been ongoing since 1990, although the creation of a new mutant required precipitated this IBC application **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. The committee discussed the three criteria for RAC review of phase I clinical trials and felt the study did not meet the requirements for such review given the extensive history of this study. **Community member comments:** None III-C, BSL-2,	

5. Subcommittee approval of exempt recombinant DNA: None
6. Schedule H report: 30
7. Revised Schedule G
8. Revised Appendix 10A
9. IBC / IRB review of clinical trials involving recombinant / synthetic nucleic acids

Adjourn. Next IBC meeting September 16, 2016 at 3:00 PM in Burnett-Womack room 9001.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
September 16, 2016, 3:30 PM
Burnett-Womack Room 9001

Members Present: Doug Cyr, Sandra Bradshaw, Peggy Cotter, Aravinda de Silva, Barbara Savoldo, Rita Tamayo, Judy Nielsen, Daniel Eisenman, Mary Beth Koza

Members Absent: Amy Sims, Fred Sparling, Matthew Lawrence, Craig Fletcher,

Open Meeting

1. Review minutes from the August 3, 2016 meeting.
2. Applications under review

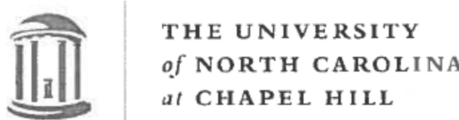
Protocol ID #	Investigator	Title
28284	PEROU CHARLES	Identification of Genetic Drivers in HER2-Enriched/HER2 negative Breast Cancer
Approved	Lentivirus mediated transduction of Cas9 to delete the FGFR4 gene in cell lines in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
28496	DOWEN JILL	Genome editing of cis-regulatory elements in development and disease
Approved	Gene editing of regulatory elements utilizing a plasmid vector in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
28516		Generation of an allelic series within the TEK gene of Collaborative Cross mice
Approved	Gene editing of two missense mutations in B6 mice. **Committee comments:** Revise the recombinant DNA category from III-F to III-E. Provide a current IACUC approval number. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*	
28536		Introduction of Signaling Pathway based Reporter Constructs into TNBC tumors- Wnt Reporter
Approved	Lentivirus mediated transduction of TCF/LET transcription factor in cell lines prior to performing xenograft transplantation. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	

28556		Introduction of Signaling Pathway based Reporter Constructs into TNBC tumors- STAT3 Reporter
Approved	Lentivirus mediated transduction of STAT3 M67 sequence repeats in cell lines prior to performing xenograft transplantation. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
28557		Introduction of fluorescent and bioluminescent markers into Triple Negative Breast Cancer (TNBC) primary tumors to facilitate imaging of metastases or re-isolation of transduced cells.
Approved	Lentivirus mediated transduction of luciferase/GFP in cell lines prior to performing xenograft transplantation. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
28558		Introduction of Signaling Pathway based Reporter Constructs into TNBC tumors- Notch Reporter
Approved	Lentivirus mediated transduction of a 195 bp region of Hes1 eGFP in cell lines prior to performing xenograft transplantation. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
28559		Introduction of Signaling Pathway based Reporter Constructs into TNBC tumors- Notch Reporter #2
Approved	Lentivirus mediated transduction of a 195 bp region of Hes1 eGFP in cell lines prior to performing xenograft transplantation. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
28577		The Role of Apobec3 in Mammary Tumor progression in Transgenic Mouse Models
Approved	Creation of transgenic mice. Apobec3 mice crossed with the following mice: C3 large T antigen, MMTV-Neu and MMTV-PYMT. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
28597		Introduction of targeted mutations of the Polymerase Epsilon (Pole) gene in Oncogenic mouse models of breast cancer
Approved	Editing mouse genome point mutation in Pole locus. Mice will be crossed to existing oncogenic mouse models. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli,*	

28617	MADDOX AMY	Cell biology of cell shape change in cytokinesis
Approved	Creation of GFP tagged fusion proteins (myosin, anillin and other cytoskeletal genes) in *C. elegans*. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1, *E. coli*	
28937	HEISE MARK	Analysis of RNA structural determinants for their impact on Sindbis virus replication
Approved	Disruption of Sindbis virus RNA secondary structure stem loops while preserving coding sequences to study the impact in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Sindbis virus	
29179	CONLON BRIAN	Determining the mechanism of antibiotic tolerance in Staphylococcus aureus and Pseudomonas aeruginosa
Approved	Deletion mutants will be screened for antibiotic resistance. Identified genes of interest will be deleted, mutated or overexpressed to characterize their role in antibiotic resistance. **Committee comments:** The committee expressed concern over the possibility of conferring resistance to clinically relevant antibiotics. Investigator response: "During the generation of S. aureus mutant strains by allelic exchange and for the generation of over-expression strains, antibiotic resistance cassettes will be routinely be introduced on plasmids and/or integrated into the chromosome of S. aureus in order to select for appropriate clones. We will use erythromycin resistant cassettes, chloramphenicol resistance cassettes and tetracycline resistance cassettes for this purpose. Erythromycin, tetracycline and chloramphenicol are not clinically relevant antibiotics for the treatment of S. aureus infection." The proposed containment and safety practices are deemed adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli, S. aureus* JE2 and *P. aeruginosa* PA01	
Approved	Clinical trial involving **Committee comments:** The committee discussed potential toxicities and found it comparable to prolonged low dose immune response to The study drug is considered safe to the environment and workers given the proposed containment and safety practices. **Community member comments:** None III-C, BSL-2	

3. Subcommittee approval of exempt recombinant DNA: 1
   PI: Baric, Title: Overexpression of Protease TMPRSS2 in LLCMK2 Cells, Category III-F
4. Schedule H report: 8
5. BSL-3 SOP Revisions [redacted]
6. 9/9/16 Incident reported to NIH
7. NIH response to CC containment update.
8. Revised Procedures: For review of exempt recombinant DNA. BSO can approve and report to committee at the following meeting. Use of AAV in vitro can be reviewed as III-F.

Adjourn. Next IBC meeting October 25, 2016 at 3:30 PM in Michael Hooker 2005.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
October 25, 2016, 3:30 PM
Michael Hooker, Room 2005

Members Present: Doug Cyr, Sandra Bradshaw, Barbara Savoldo, Judy Nielsen, Daniel Eisenman, Mary Beth Koza

Members Absent: Peggy Cotter, Aravinda de Silva, Amy Sims, Rita Tamayo, Fred Sparling, Matthew Lawrence, Craig Fletcher

Guests: [redacted]

Open Meeting

1. Welcome [redacted]
2. Review minutes from the September 16, 2016 meeting. Minutes were approved.
3. Applications under review

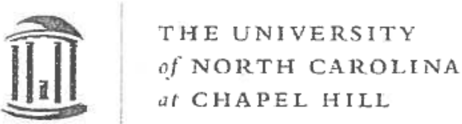
Protocol ID #	Investigator	Title
29420	BARIC RALPH	Analysis of RNA structural determinants for their impact on Zika virus replication
Approved	Mutation of the RNA secondary structure (stem loops) of the Zika virus genome while leaving coding regions in tact to asses changes in viral replication in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, Zika virus infectious clones H/PF/2013 and SPH2015	
29440	[redacted]	Mouse adaptation of BtSCoV-SHC014
Approved	Mouse adaptation of BtSCoV-SHC014 permits testing of in vivo pathogenesis and more direct comparison with mouse-adapted [redacted] To that end, recombinant BtSCoV-SHC-014 will be passaged serially in mice until a virulent strain is isolated (1- to 2-day intervals for each passage). This strain will be sequenced, and the resulting mutations compared to the pre-passage genome sequence. The newly identified mutations will then be re-incorporated into the BtSCoV-SHC-014 infectious clone. At the time of submission of this Schedule G we do not currently know what those changes/mutations will be. A subsequent Schedule G will be submitted once the passage mutations have been identified. Please note: BtSCoV-SHC-014 does not explicitly fall under the GOF pause guidelines; however, due to the virus¿ genetic relatedness to [redacted] we have received specific permission to perform this passage experiment from NIH (letter attached). If any recovery of virulence that exceeds that of [redacted] (mouse-adapted) > 1 log is observed, passage experiments will be discontinued, and we will contact the UNC IBC and NIH. **Committee comments:** The committee reviewed the NIH letter of exemption from the GOF funding pause. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli,* Coronavirus BtSCoV-SHC014	

29480		Analysis of RNA structural determinants for their impact on Zika virus replication and in vivo pathogenesis
Approved	Mutation of the RNA secondary structure (stem loops) of the Zika virus genome while leaving coding regions in tact to asses changes in viral replication in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Zika virus infectious clones H/PF/2013 and SPH2015	
29768	ANTON EVA	Radial glial development and neuronal migration
Approved	Infection of mouse neuronal brain slices with Zika-GFP in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Zika virus (Paraiba_10/2015)	
29800	LORENZO DAMARIS	Molecular Physiology of Ankyrins and Spectrins
Approved	Lentivirus mediated transduction of cDNA and siRNA in vitro. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Zika virus (Paraiba_10/2015)	
29860		Exploring Various Humanized Animal Models for the Establishment of Respiratory Virus Infections
Approved with Stipulations	The aim of this project is to determine whether respiratory viruses can replicate in humanized animal models to allow for further study. The experiments will use several different human recombinant respiratory viruses engineered to express GFP and/or Luciferase as a marker gene and nonstructural proteins obtained from viruses in the same family. Viruses will be provided b The viruses have already been propagated and are ready to be inoculated into humanized animal models. Viruses to be used are: 1. Human Parainfluenza Virus type 3 (PIV3) expressing the Green Fluorescent Protein (GFP). 2. Human PIV3 expressing GFP and the non-structural protein 1 (NS1) from RSV. 3. Human PIV3 expressing GFP and the non-structural protein 2 (NS2) from RSV. 4. Human PIV3 expressing GFP and both non-structural proteins 1 and 2 (NS1 and NS2) from RSV. 5. RSV expressing GFP. 6. RSV expressing the NS1 and NS2 genes derived from Pneumonia Virus of mice. 7. Human metapneumovirus (hMPV) 8. Human Rhinovirus (HRVs) Human parainfluenza viruses or Respiratory Syncytial Viruses will be used as gene delivery vectors to co-express either RSV NS1/NS2 or PVM NS1/NS2 in human airway epithelium. **Committee comments:** This lab has an SOP in place for research with humanized mice infected with various human pathogens at ABSL2+. IBC approval is contingent upon approval of a schedule F (biohazard form) and amending the lab SOP to include the agents listed in this application along with their associated hazards. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, human parainfluenza type 3, respiratory syncytial virus, human metapneumovirus and human rhinovirus	

29903	JONES ALAN	Plant Transformation
Approved	Agrobacterium mediated transformation of Arabidopsis and rice plants with the following genes: Regulator of G Signaling, Subunits of the heterotrimeric G protein complex, With No Lysine Kinases. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-1P, *E. coli, Agrobacterium tumefaciens*	
29920		Design of live attenuated vaccine candidates for Porcine Epidemic Diarrhea Virus (PEDV)
Approved	Creation of mutant Porcine Epidemic Diarrhea virus (PEDV) as vaccine candidates. All animal studies will be performed by a collaborator at The PI possesses a USDA permit for this research and the lab has been inspected by the USDA. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli,* Porcine Epidemic Diarrhea virus	
29925		The role of ORF8 in viruses
Approved	To understand the divergent biological role(s) of ORF8 in from human, bat, and civet strains. Parts of the manipulations described below involve replacement of the ORF8 gene with corresponding orthologs from various animal coronaviruses (Civet (SZ16 and/or HCS6103), SHC, and HKU3). These constructs will then be assembled into viruses and tested in vitro and in vivo. Because these constructs will involve the exchange of with animal (less virulent) genes, we do not anticipate an increase in replication or virulence. However, if we observe changes in either replication (increased titer >1 log) or virulence (increased disease and/or decreased LC50 > 1 log), we will cease experiments immediately and contact both UNC IBC and NIH. Because these manipulations involve a virus that falls under GOF regulations, the appropriate letter conferring permission from the NIH has been attached to this application. **Committee comments:** The committee reviewed the NIH letter of exemption from the GOF funding pause. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-3, *E. coli* animal coronaviruses (Civet (SZ16 and/or HCS6103), SHC, and HKU3	
Tabled	Phase I clinical trial **Committee comments:** The application was tabled and will be up for review at the November 2nd IBC meeting where greater attendance from research faculty is expected. the IBC's human gene transfer expert, is in conflict as a Co-PI on the study. The IRB will be contacted to encourage their participation. **Community** III-C, BSL-2,	

1. Subcommittee approval of exempt recombinant DNA: 0
2. Schedule H report: 13
3. Next IBC meeting date: 11/3/16 at 3:30 PM via conference call.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
November 2, 2016, 3:30 PM
Conference Call

Members Present: Doug Cyr, Fred Sparling, Peggy Cotter, Barbara Savoldo, Aravinda de Silva, Rita Tamayo, Daniel Eisenman,

Members Absent: Sandra Bradshaw, Matthew Lawrence, Amy Sims, Judy Nielsen, Craig Fletcher, Mary Beth Koza

Guests:

Open Meeting

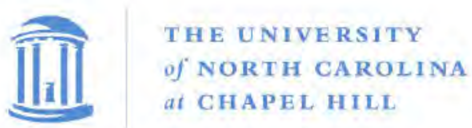
1. Applications under review

Protocol ID #	Investigator	Title
Approved	Phase I clinical trial. **Committee comments:** presented the study including the disease, recombinant DNA technology utilized as well as the facility spoke about the risks associated with the recombinant DNA manipulations as well as the extent to which are currently utilized in clinical trials. The committee agreed the containment and safety practices are appropriate for the experimental design. The committee concurred with the Investigators assessment that NIH RAC review is not recommended. **Community member comments:** None III-C, BSL-2	
29940		Translational advancement of somatostatin gene therapy for temporal lobe epilepsy
Approved	AAV mediated transduction of cells in vitro. Cells will be injected into rats intrachranially. Animal procedures will be performed outside of biosafety cabinets. **Committee comments:** Add gloves to list of PPE. The containment and safety practices are appropriate for the experimental design. **Community member comments:** None III-D, BSL-2, *E. coli*, AAV	
30161		apoE exon deletion gene knockout rat
Approved	Genome editing of the ApoE gene in rat embryos. **Committee comments:** The containment and safety practices are appropriate for the experimental design. **Community member comments:** None III-D, BSL-1	

30162	[redacted]	hCETP trangenic ZDF rat
Approved	Genome editing of the CETP gene in rat embryos. **Committee comments:** The containment and safety practices are appropriate for the experimental design. **Community member comments:** None III-D, BSL-1	

1. Review minutes from the October 25, 2016 meeting. Minutes were approved
2. Subcommittee approval of exempt recombinant DNA: 0
3. Schedule H report: 13
4. Next IBC meeting date: 12/7/16 at 3:30 PM in BW 9001.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
December 14, 2016, 3:30 PM

Members Present: Doug Cyr, Amy Sims, Peggy Cotter, Aravinda Desilva, Barbara Savoldo, Fred Sparling, Craig Fletcher, Judy Nielsen, Mary Beth Koza, Jessica Poole

Members Absent: Sandra Bradshaw, Barry McLamb, Rita Tamayo

Guests: [redacted]

Open Meeting

1. Applications under review

ID	PI	Project Title
	[redacted]	[redacted]
Approved	[redacted] proposed a Phase I clinical trial to [redacted] **Committee comments:** The presentation by [redacted] team and submitted documents informed the IBC that use of the proposed technology has been previously approved by the NIH RAC and FDA. The IBC approved the proposal and made the judgement that this proposal does not require RAC review and the technology has already been approved by the RAC. The proposal is now being submitted for FDA and IRB approval. **Community member comments:** None III-C, BSL-2, [redacted]	
29680	[redacted]	Nanoparticle-based Immune Modulators in Cancer Therapy and Vaccines-OVA plasmid
Approved	**Summary:** This study will involve cloning of the ovalbumin gene into pCDNA3, the assembly of pCDNA-OVA into nanoparticles and subsequent introduction into C57BL/BL mice. pCDNA-OVA will be introduced to mice via injection into different locations and the immune response will be evaluated. The nanoparticle design was recently published: Pavot V, Rochereau N, Resseguier J, Gutjahr A, Genin C, Tiraby G, *et al.* Cutting edge: New chimeric NOD2/TLR2 adjuvant drastically increases vaccine immunogenicity. *J Immunol* 2014, **193**(12): 5781-5785. **Committee comments:** The proposed containment and safety practices are appropriate for the experimental design. **Community member comments:** None III-D, BSL-1, *E. coli*	

30083		Nanoparticle-based Immune Modulators in Cancer Therapy and Vaccines-GFP plasmid
Approved with Stipulations	This study will involve the assembly of pSELECT-NGFP into nanoparticles and subsequent introduction into C57BL/BL mice. pSELECT-NGFP will be introduced to mice via injection in different locations and the immune response will be evaluated. **Committee comments:** The committee requested a copy of the vector maps that will be used. The proposed containment and safety practices are appropriate for the experimental design. **Community member comments:** None III-D, BSL-1, *E. coli,*	
30084		Nanoparticle-based Immune Modulators in Cancer Therapy and Vaccines-Luciferase plasmid
Approved with Stipulations	This study will involve cloning of the ovalbumin gene into pCDNA3, the assembly of pCDNA- into nanoparticles and subsequent introduction into C57BL/BL mice. A Luciferase expression plasmid will be introduced to mice via injection in in different locations and the immune response will be evaluated. **Committee comments:** The committee requested that the lab provide the identity of the luciferase expression plasmid and provide a vector map. The proposed containment and safety practices are appropriate for the experimental design. **Community member comments:** None III-D, BSL-1, *E. coli*	
30322		AAV directed expression of FVIII or FIX in dogs with hemophilia
Approved with Stipulations	Blood clotting factors FVIII and FIV will be cloned and assembled into AAV vectors and then introduced into dogs that have hemophilia. Expression of FVIII and FIV will be followed to determine if the treatment suppresses defects common to hemophilia. **Committee comments:** The lab needs to clarify that the propagation of the vector will be performed at a BSL-2. Animal work will be conducted at a BSL-1. **Community member comments:** None III-D, BSL-2, *E. coli,* AAV	
30421		LentiGuide-Puro
Tabled	Use of CRISPR technology to mutate mouse genes that are involved in bladder and kidney cancer. Genes will be introduced into the kidney and bladder of mice via replication incompetent lentivirus and outcomes will be followed. **Committee comments:** Protocol lacks sufficient details for general public to understand proposed experiments. The committee decided to table the proposal and request accurate title, description of target genes, details about lentivirus production, and vector maps. **Community member comments:** None III-D, BSL-2, *E. coli,* lentivirus	
30464		Epigenetic regulation of GABA(A)-Rs by ethanol
Approved with Stipulations	Experiments are proposed to examine how the role that post-translational modification of GABA(A)-R subunit is impacted by chronic exposure of ethanol in rats. Different forms of the a defective CAS9 gene will be cloned into a lentiviral vector and the lentiviral vector will be used to infect neurons of rats. **Committee comments:** The committee stated that all BSL-2 work must be conducted in a BSC. **Community member comments:** None III-D, BSL-2, lentivirus	

<table>
<tr><td>30492</td><td></td><td>Cortical circuits underlying the processing of biologically meaningful sounds.</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Mice will be injected with AAV virus prepared in a UNC AAV vector core that express tagged cargos under control of different promoters and changes in cargo gene expression will be monitored to study sound processing.

Committee comments: It is not clear what cargo will be expressed. Please provide a list of the constructs that will be expressed from within AAV that includes the promoter and cargo combinations that are to be inserted and the expression plasmid employed. The committee also requested the approved IACUC protocol number. The proposed containment and safety practices are adequate for the experimental design.

Community member comments: None

III-D, BSL-1, AAV</td></tr>
<tr><td>30541</td><td></td><td>Driving Selective Excitatory or Inhibitory Neurotransmission through Cre-inducible DIO/FLEx-AAVs</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Express genes in mouse brain using a Cre inducible DIO/FLEx-AAVs viral system to study neurotransmission that is triggered by activation of cargos by blue or red light. The expressed ion channel Chronos and C1V1 will be activated by blue or red light and impacts on neuronal signaling will be measured.

Committee comments: The lab needs to provide an approved IACUC protocol number. The proposed containment and safety practices are adequate for the experimental design.

Community member comments: None

III-D, BSL-1, AAV</td></tr>
<tr><td>30561</td><td></td><td>Detecting Evoked Excitatory Neurotransmission through joint Optical and MR Imaging</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">The goal is to use AAV to introduce genes that express light activated channel proteins that induce neurotransmission as a system that permits optical study of brain function.
The Opsin C1V1 and GCaMP6 will be expressed in brains of mice via an AAV system to study signaling that occurs in response to brain activation. Mice will be injected with AAV and optic fibers implanted in mouse brains will be used to measure changes in brain activity.

Committee comments: The lab needs to provide an approved IACUC protocol number. The proposed containment and safety practices are adequate for the experimental design.

Community member comments: None

III-D, BSL-1, AAV</td></tr>
<tr><td>30562</td><td></td><td>Driving Selective Excitatory or Inhibitory Neurotransmission through Cre-inducible DIO/FLEx-AAVs</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Introduce AAV that express C1V1 or GCaMP6 into brains of mice and then use mice a tools to study neurotransmission. Genes will be integrated into host DNA via use of the Cre-inducible DIO/FLEx-AAV system. AAV will be propagated in HEK293 cells.

Committee comments: The committee feels that this one is very similar to 30541. The lab was asked to clarify the difference between the two. The proposed containment and safety practices are adequate for the experimental design.

Community member comments: None

III-D, BSL-1, AAV</td></tr>
</table>

<table>
<tr><td>30615</td><td>[redacted]</td><td>Pathogenesis and therapy of liver human-specific and associated infections in novel humanized mouse model</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Immunocompetent mice will be transduced with AAV that express a replication-defective form of Hepatitis B virus. Mice will provide a model where chronic expression of HBV proteins mimics the conditions of humans that are infected with HBV that is controlled by reverse transcriptase inhibitors.

Committee comments: The lab needs to provide an approved IACUC protocol number. The proposed containment and safety practices are adequate for the experimental design.

Community member comments: None

III-D. BSL-2, E. coli, AAV, HBV</td></tr>
<tr><td>30704</td><td>[redacted]</td><td>Characterizing the roles of MERS-CoV ORFs in viral pathogenesis and fidelity</td></tr>
<tr><td>Approved</td><td colspan="2">A study to investigate the role of MERS-COV accessory proteins ORF3, ORF4a, ORF4B, and ORF5 on pathogenesis is proposed. Virus with mutated forms of these proteins will be generated and the extent to which said mutations attenuate viral life cycles will be evaluated. This work on MERs is not deemed at a GOF research and the [redacted] group has approval from the NIH to perform such work on MERS and this is documented via a letter from the NIH.

Committee comments: The proposed containment and safety practices are appropriate for the experimental design.

Community member comments: None

III-D, BSL-3, E. coli, MERS-CoV</td></tr>
<tr><td>30782</td><td>[redacted]</td><td>Mouse models of neuroadapted chikungunya virus</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">CHIKV virus will be used to infect mice of different ages and allowed to serially replicate and forms that show enhanced ability to invade the CNS will be isolated, sequenced and then studied in Vero cells, mouse fibroblasts and mouse neurons. Mutations that enhance CHIKV replication will be collected for further study of their ability to enter the nervous system.

Committee comments: The committee stated that all BSL-3 work must be conducted in a BSC.

Community member comments: None

III-D, BSL-3, E. coli, CHIKV</td></tr>
<tr><td>30826</td><td>[redacted]</td><td>Gene transfer and therapy using recombinant defective adenovirus vectors</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Introduce AAV vectors that carry cargo of therapeutic interest to cells. Virally transduced cells will be introduced into mice. Viruses employed are replication defective and include AAV and a AD5. Cargos are numerous and their identity is provided. Viral particles will be introduced into mice with standard injection protocols.

Committee comments: The lab needs to provide all maps of viruses mentioned in the protocol. The proposed containment and safety practices are adequate for the experimental design.

Community member comments: None

III-D, BSL-2, AAV, Ad5</td></tr>
</table>

2. Review minutes from the November 2, 2016 meeting.
3. Biosafety Staffing
    a. Positions will be posted after the new year.
4. RO/ARO Training Updates
    a. Mary Beth Koza and Jessica Poole attended the RO Workshop in Maryland December 6-8, 2016.
    b. The purpose of this workshop was a training opportunity to increase the knowledge of the RO/ARO on select agent regulations and updates to the program.
5. Subcommittee approval of exempt recombinant DNA: 3

    PI: Lillie Searles Title: Pre MRNA Metabolism. III-E

    PI: Henrik Dohlman Title: Second-site suppressor mutations of G alpha i. III-F

    PI: Ralph Baric Title: Role of BAI1 in phagocytosis. III-F
6. Schedule H report: 16
7. Next IBC meeting date: January 4, 2017 at 3:30 PM in BW 9001.

Adjourn.

INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
February 1, 2017, 3:30 PM
Burnett Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Peggy Cotter, Aravinda Desilva, Barbara Savoldo, Rita Tamayo, Frederick Sparling, Judith Nielsen, Mary Beth Koza, and Jessica Poole

Members Absent: Barry McLamb, Craig Fletcher

Ad hoc Members (not requested to be present): Ann Matthysse, Stanley Lemon

Guests: [redacted]

Open Meeting

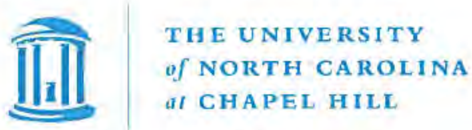
1. Review Minutes from January 11, 2017 meeting.
2. Applications under review

ID	PI	Project Title
31961	[redacted]	DREADD-Mediated Inhibition of Cortical Signaling to the Central Nucleus of the Amygdala
Approved	**Summary:** The purpose of the experiment is to study how cortical neurons regulate the central nucleus of the Amygdala via inhibiting signaling events with inhibitors of Gi-coupled signaling that are delivered to rat brains via AAV5. The gene insert is a Gi-coupled DREADD (hMD4) fused to a mCherry fluorescent reporter protein and has been packaged inside an AAV5 viral vector. Additionally, a gene insert containing only an eGFP fluorescent reporter has similarly been packaged within an AAV5 vector and will serve as a control virus. These viral vectors will be used to infect neurons in rats. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-1, AAV	
31841	RALPH BARIC	Generation of a cDNA infectious clone system for Zika virus: Dakar Strain
Approved	**Summary:** The purpose of the experiment is to develop an experimental system to study the biology of the Zika virus strain that was originally identified as being infectious to humans. ZIKV infectious cDNA clones will be maintained across four plasmids, each containing 2-5 kB of the approximately 10.7 kB viral genome. The structural proteins precursor membrane (prM0 and envelope € are located at genome positions of approximately 500-2400. The lab will mutate putative and known antibody-interaction residues in these proteins when they are identified. Full-length genomic RNA will be in vitro-transcribed from assembled full-length cDNA generated from in vitro ligated fragments. RNA will be electroporated into Vero, C636, and/or BHK cells to recover recombinant virus. **Committee comments:** The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-D, BSL-2, ZIKV	

31761	[redacted]	Oncogenic Potential of EBV latency and transforming genes in mice
Approved	**Summary:** This experiment will determine oncogenic potential of EBV and EBV latent genes through studies in cells and mice. Mammalian cell lines infected with Epstein-Barr Virus (EBV) or the non-infected control cell line will be injected into mice. The latent genes of interest will be cloned into a plasmid or viral vector to transfect mammalian cell in vitro, which will then be injected in mice. **Committee comments:** This should be a III-D instead of a III-E. Judy brought up that the protocol will be expiring this year. The proposed containment and safety practices are adequate for the experimental design. **Community member comments:** None III-E, BSL-2, EBV	
26919	[redacted]	Engineering Temperate Bacteriophage for Sustained Secretion of Protein Therapeutics or Immunogens by Mucosal Commensals
Tabled	**Summary:** Temperate bacteriophage present in naturally occurring strains of commensal bacterial organisms (e.g. SM1 phage derived from Streptococcus Mitis, phiADH phage derived from Lactobacillus Gasseri, etc.) will be used as the template for genetic modification. Bacteriophage will never be derived from host strains that have any known pathogenicity. Genomes of template bacteriophage will be modified by in-vitro natural transformation of exogenous, non-pathogenic genes generated by PCR amplification. Genetically modified phage will be induced by UV light and used to transduce additional strains of non-pathogenic commensal bacterial populations. In vivo transduction will be evaluated by oral gavage of engineered bacteriophage into gnotobiotic mice. **Committee comments:** The PI should specify the antibiotic resistant genes and other genes to be used as cargos. Vector maps need to be included. The PI needs to be more specific in regards to question 18 (Major Action). The procedures will need to be performed within a biological safety cabinet. **Community member comments:** None **III-D, BSL-2,**	

3. National Science Advisory Board for Biosecurity Meeting (1/7/2017): New Policy Guidelines for GOF – Doug
    a. More information can be found at: http://osp.od.nih.gov/office-biotechnology-activities/event/2016-01-07-130000-2016-01-08-220000/national-science-advisory-board-biosecurity-nsabb-meeting
    b. Doug presented PowerPoint presentation
    c. Jessica will send Power Point presentation to the committee.
    d. Jessica will send the GOF link and agenda to the committee.
4. FSAP Amendments - Jessica Poole
    a. Effective Date 2/21/2017
    b. Jessica will send out a summary of the changes to the select agent labs on campus, create a presentation on the changes to share with the committee, and send the new regulation and guidance documents on inactivation to the committee
    c. Major Changes:
        i. Addition of specific requirements that must be followed for the inactivation of select agents
        ii. Regulatory language concerning security, training, incident response, and records were clarified
        iii. Changes to toxin permissible limits
        iv. New provisions to biosafety sections
        v. Clarification that each registered entity must comply with the regulations for select agent and toxins listed on the registration regardless of whether the entity is in actual possession of the select agent or toxin
5. Mary Beth informed the committee of the proposed [redacted] which is being programed for animal research and high containment work.
6. Subcommittee approval of exempt recombinant DNA: 1
    PI: Katherine Warner Title: DNA Cloning in Small Scale Cell Cultures III-F
7. Schedule H report: 16
8. Next IBC meeting date: March 1, 2017 at 3:30 PM in BW 9001.

Adjourn.



Meeting Minutes
April 5, 2017 3:30 PM
Burnett Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Barbara Savoldo, Tori Baxter, and Jessica Poole
Members Absent: Barry McLamb, Frederick Sparling, Peggy Cotter, Aravinda Desilva, Rita Tamayo, Craig Fletcher, Judy Nielsen, and Mary Beth Koza
Ad hoc Member (not requested to be present): Stanley Lemon and Ann Matthysse
Guests: [redacted]

Open Meeting

1. Review minutes from the March 1, 2017 meeting.
2. Applications under review

ID	PI	Project Title
	[redacted]	[redacted]
Approved	**Summary:** [redacted] can be safely administered to adult and pediatric subjects with [redacted] The safety of [redacted] will be investigated in adult subjects initially before these same doses are tested in pediatric subjects using the 3+3 design. This trial was previously approved by the IBC and more recently the FDA. The amended version seeks [redacted] **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None	
	[redacted]	[redacted]
Approved	**Summary:** The purpose of this research study is to see how safe [redacted] A clinical trial was approved by the IBC in March of 2016 that includes [redacted] The amended version of the protocol, which [redacted] in new study arms is up for consideration. [redacted] **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None	

28213	[redacted]	Manipulation of Zika virus envelope to impact stability and neurotropism
Approved	**Summary:** Differences in the various structural loops of the envelope between the emergent Zika virus and dengue strains may be critical for both stability and neurotropism. This experiment seeks to determine if changes to all or combination of these loops reduce stability or neurotropism of Zika mutants relevant to wildtype virus. The Zika cDNAs are maintained as 4 separate cDNA cassettes in plasmids that are propagated in E. coli. The mutations will be created in the relevant plasmid (A), and mutant virus cDNAs are assembled in vitro, transcribed, and electroporated into cells. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, *E. coli*, Zika	
33022	[redacted]	Mouse adaptation of Zika Virus strains
Approved	**Summary:** The purpose of the experiment is to adapt infectious clone systems in several strains of Zika Virus (ZIKV) for development of a mouse model of pathogenesis. ZIKV infectious cDNA clones will be maintained across four plasmids, each containing 2-5 kB of the approximately 10.7 kB viral genome. Full-length genomic RNA will be in vitro-transcribed from assembled full-length cDNA generated from in vitro ligated fragments. RNA will be electroporated into Vero, C636, and/or BHK cells to recover recombinant virus. This ZIKV virus will be used for in vitro characterization (growth kinetics, antibody neutralization, cell infectivity, maturation state, etc.) as well as in vivo mouse experiments. These in vivo studies aim to develop an accurate mouse model of human ZIKV infection. ZIKV strains to be utilized include MR766, PF2013, Dakar 41519, and SPH2015. Mutations of interest include NS3 and NS4b proteins. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, *E. coli*, Zika	
33042	[redacted]	Use of a cDNA infectious clone system for Zika virus to examine natural strain variation
Approved	**Summary:** The [redacted] lab is interested in evaluating the genetic and functional variability of ZIKV structural and non-structural proteins. In particular, ZIKV recombinant viruses with 3'UTR variations will be generated via site-directed mutagenesis and commercial in vitro gene synthesis (BioBasic). The mutant DNA will be introduced into the relevant clone cDNA fragments, propagated in E. coli, assembled as full-length cDNA, transcribed to RNA and electroporated into Vero, BHK, or C636 cells. Viruses harboring the mutations will be recovered and characterized using growth in cell culture, ELISA, and neutralization assays. Selected mutants will be evaluated for antigenicity, pathogenicity, and attenuation in vivo. Infectious clones already approved for use would be utilized in these experiments: H/PF/2013 (isolated in French Polynesia in 2013) and two Brazilian 2015 isolates (BeH810915 and SPH2015). Single-nucleotide variation between the PF2013 isolate and the Brazilian 2015 strains has been theorized to play a role in small flaviviral RNAs (sfRNAs). This natural variation is of interest to better understand the current and ongoing Zika outbreak in South and Central America. In addition to in vitro characterization including but not limited to virus propagation, titration, ELISA binding, neutralization, antigen depletions, and blockade of binding assays (all under appropriate BSL2 conditions), we plan to use these recombinant ZIKV in murine models of lethal and non-lethal disease to assess the role that particular genotypic variation of the virus plays in the immune response to viral infection. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, *E. coli*, Zika	

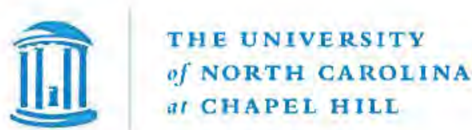
33002	[redacted]	**Etiology of Affective Disorders and Addiction**
Approved with Stipulations	**Summary:** The purpose of the experiment is to understand the neural mechanisms of chronic alcohol exposure and related disorders. The experiment will involve injecting pHSV-siMCP1 (MCP1 siRNA) and pHSV-siCTL-N1 (scrambled siRNA) into rat brains. **Committee Comments:** The committee would like the PI to change the title to make it more descriptive. The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, pHSV	
33402	[redacted]	**Therapeutic Vaccine for Walnut, Pecan, and Peanut Allergies**
Approved	**Summary:** The recombinant DNA used for this project is intended to be used as DNA vaccines for the therapeutic treatment of allergies. These DNAs will consist of several plasmids expressing peanut, walnut or pecan allergens, the immune-adjuvant cytokine IL-12 and the immunosuppressive immunomodulators IL-10, TGF-beta, IL-27, IL-33, IL-35, PD-L1, RALDH2, COX-2, iNOS and gp130 (individually). The insert genes are cloned into plasmids which will be injected into thigh muscles of mice in vivo. The insert gens are each of the major walnut and pecan allergens, called Jug r 1, 2, and 4 (from walnut) and Car I 1,2, and 4 (from pecan) and Ara h 1-13 (from peanut). These plasmids will be administered to walnut or pecan allergic mice by intramuscular injection. Additionally, IL-12, IL-10, TGF-beta, IL-27, IL-33, IL-35, RALDH2, COX-2, iNOS, gp130, and PD-L1 adjuvants with known immunologic suppressive properties will be one of the insert genes. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2,	
32902	**Daniel Crona**	**Infecting cells with Lenti virus with Cas9 payload**
Tabled	**Summary:** The purpose of the experiment is to use Cas9 construct and lentivirus to knock out genes in human and mouse cell lines. CRISPR Cas9 constructs will be transfected or transduced into cells in vitro to cause deletions of targeted genes. **Committee Comments:** The committee would like the PI to change the title to make it more descriptive. The committee would also like the PI to describe the genes that they are knocking out, the payloads, and the targets. The category should be changed to a III-E, not a III-F. **Community Member Comments:** None III-F, BSL-2, *E. coli,* lentivirus	
33082	[redacted]	**Isolating sources of cochlear potentials**
Approved with Stipulations	**Summary:** The purpose of the experiment is to introduce adeno-associated virus (AAV) into the cochlea of gerbils to transfect sensory and non-sensory cells and affect function. The insert gene will be cloned into an adeno-associated virus vector which will be utilized to transduce sensory and non-sensory cells of the gerbil cochlea in vivo after microinjection. **Committee Comments:** The committee would like the title changed to include the genes of interest. The committee would also like the PI to state whether he is making or purchasing the AAV. If the PI is making the AAV, then the research needs to be done at BSL-2. **Community Member Comments:** None III-D, BSL-1, *E. coli,* AAV	

33145	[redacted]	**Strategies for Personalized Gene Repair using I22I Severe Hemophilia-A as a Model**
Approved with Stipulations	**Summary:** The purpose of the experiment is to use gene editing technology to repair an intron 22 inversion (I22I) in the factor VIII gene that causes hemophilia A in dogs. Blood outgrowth endothelial cells (BOECs) will be isolated from dogs with hemophilia A due to an I22I, gene edited with a SpCAS9 nuclease gene and CRISPR single-guide-RNA to correct the intron 22 inversion, and the gene-edited BOECs re-infused into the dogs. **Committee Comments:** The committee has requested that question 5 be reworded to remove the run-on sentence. They would also like the PI to clarify the method for introducing the CRISPR/Cas9 into the cells. **Community Member Comments:** None III-D, BSL-2, *E. coli,*	
32642	[redacted]	**Generation of a zebrafish (Danio rerio) model of kidney development and disease**
Approved	**Summary:** The purpose of the experiment is to facilitate cell-type specific manipulation of gene expression in the podocytes of the fish kidney, as well as label the podocytes with fluorescently tagged protein. Using CRISPR/Cas9, the lab will introduce a Cre transgenic into the podocin locus to drive Cre expression in the podocytes of the kidney. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The committee has requested that the PI be reminded that he needs an IACUC protocol before the experiment can begin. **Community Member Comments:** None III-D, BSL-1, *E. coli*	
32942	[redacted]	**Genetic manipulation of rat neurons via opsins or DREADDs**
Approved	**Summary:** The purpose of this experiment is to manipulate activity of specific neurons by expressing genes that encode opsins or DREADDs or fluorescent proteins. The insert gene will be cloned into a viral vector which will be directly injected into rats where the virus will transduce cells in vivo. These genes allow for the selective manipulation of specific groups of neurons in the brain. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1, AAV	
32984	[redacted]	**Improving T cells immunity for Immunotherapy studies #3 Updated with some new molecules**
Approved	**Summary:** The purpose of the genetic medications are to improve the function, persistence and specificity of tumor and virus-specific T cells, to improve the immunogenicity of tumor cells and to elucidate immunoregulatory pathways in vitro studies and in vivo murine models. The sources of the DNAs to be cloned are murine and feline retrovirus vectors, human lentivirus vectors. The genes to be introduced into these vectors are derived from human or murine cells. They include immune modifying genes, like cytokines, chemokines, receptors, transcription factors and apoptotic molecules. These genes were clones in the previous lab of the PI in [redacted] Most were synthesized de novo from sequence information, some were derived from a biological source, some were purchased from a company. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, *E. coli*, retrovirus, lentivirus	

28187	[redacted]	**Novel nanoparticle Platform for the Delivery of Vaccines and Adjuvants-VRP vaccination**
Approved with Stipulations	**Summary:** The aim of this experiment is to induce antibody responses to recombinant nucleic acid targets introduced by Venezuelan equine encephalitis (VEE) replicon particles (VRP). All of these genes of interest are human in origin and are involved in innate immune signaling. The lab will express Human Nod-Like Receptor (NLRs). They are a family of innate immune receptor proteins that share a common architecture and function. These proteins are immunomodulatory and typically recognize either pathogen associated molecular patterns or cell damage associated molecular patterns (PAMPs/DAMPs). Additionally, the lab will express the human AIM2 (absent in melanoma) protein which is also an innate immune receptor. The VRP vaccination strategy is commonly used strategy to generate immune sera against novel antigens. The lab would like to use the VRP system to make antibodies to the various NLR proteins and AIM2. The VEE replicon used in this system has been directly obtained from the lab of [redacted] here at UNC. To our knowledge this is not a fully competent VEE but instead a replicon system in which the key viral structural genes have been replaced by our genes of interest (NLRs/AIM2). **Committee Comments:** The committee would like the vector maps attached and for the reference to be deleted. The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, VEE	
32842	**William Zuercher**	**CRISPR/Cas9 lentivirus screening**
Approved with Stipulations	**Summary:** The purpose of this experiment is to transfect cells with lentivirus containing gRNA's with CRISPR/Cas9. HEK293 cells will be transfected with pLKO.1 vector containing CRISPR/Cas9. The lab will be creating frame shifts or modifications to the genes of interest. **Committee Comments:** The committee would like target of the lentivirus and the cellular process to be included in the title. They would also like the PI to be more descriptive and would like to know what the lab is modifying and the genes of interest. The Schedule G should be a III-E not a III-F. **Community Member Comments:** None III-F, BSL-2, *E. coli*,	

3. Subcommittee approval of exempt recombinant DNA: 1
   Miroslav Styblo Mechanisms of Arsenic-Induced Diabetes Mellitus III-F
4. Schedule H report: 24
5. Next IBC meeting date: May 3, 2017 at 3:30 PM in BW 9001.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
May 3, 2017 3:30 PM
Burnett Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Barbara Savoldo, Rita Tamayo, and Jessica Poole

Members Absent: Barry McLamb, Frederick Sparling, Peggy Cotter, Aravinda Desilva, Craig Fletcher, Judy Nielsen, Tori Baxter, and Mary Beth Koza

Ad hoc Members (not requested to be present): Ann Matthysse and Stanley Lemon

Guests: [redacted]

Open Meeting

1. Review minutes from the April 5, 2017 meeting.
2. Applications under review

ID	PI	Project Title
33662	[redacted]	**Generation of Bat [redacted]-like coronaviruses expressing the Uganda Spike**
Approved	**Summary:** [redacted] Uganda has been sequenced from bats and is approximately 77% identical to [redacted]. It has not been successfully cultured, but rather exists as a sequence isolated from bats. Analysis of the receptor-binding domain suggests that it will not be able to recognize the human or mouse ACE2 receptor for entry, although it does contain some contact interface recognition sites with human and mouse receptors. However, the sequence comparisons predict that incorporation of 5 residues (436Y, L442W, K479R, T484F, V487A) from SCH014 into PDF-2386 (PDF-2386-SHC-RBD) or the 8 known mouse-adapting mutations (K411E, 436H, L472F, K411E, G473N, K479N, T484Y and V487T) into the PDF-2386 Spike (DPF-2386-MA) could confer replication of the PDF-2386 strain in mice, and perhaps, primate cells. The purpose of these experiments is to determine if these residues are necessary and sufficient to confer productive infection and replication of viruses containing the [redacted] Uganda Spike. If any chimeras are capable of using the human ACE2 receptor for docking and entry, the lab will evaluate the capacity of the viruses to replicate in primary human airway epithelial cells and Vero cells, predicting that theses chimera will demonstrate significant attenuation in growth, as compared to wildtype [redacted] The [redacted]-Uganda PDF-2386 Spike glycoprotein gene will be purchased from commercial vendors with two small adaptive cassettes that will allow for rapid insertion of the SCH014 or mouse-adapted residues into the PDF-2386 gene. The entire [redacted] Spike will first be dropped into the SHC014 genetic backbone, and recombinant viruses will be tested for their capacity to replicate in Vero cells and in mouse cells expressing the civet, mouse, human and bat ACE2 receptors. The, the Uganda-Spike RBD residues will be replaced with the five SHC014 contact interface sites (PDF-2386-SHC-RBD) or the 8 known mouse-adapted mutation set clusters (PDF-2386-MA) in the SHC014 genetic backbone. Following electroporation of full-length transcripts into cells, any viable viruses will be recovered and then tested for their capacity to replicate efficiently in Vero cells and in mouse cells expressing civet, mouse, human and bat ACE2 receptors. Viruses that can replicate efficiently in mouse cells expressing the mACE2 receptor will be evaluated for growth and pathogenic outcomes in young and then aged BALB/c mice. After demonstration of a Spike formulation that allows for efficient replication in mice and mouse cells expressing the mouse ACE2 receptor, the lab will synthetically reconstruct full-length [redacted] Uganda viruses, with mouse ACE2 enhancing mutations. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3 [redacted] *E. coli*	

<table>
<tr><td>33663</td><td></td><td>Transgenic mice expressing the human tau protein</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The purpose of the experiment is to generate mouse models of Alzheimer's disease. The inserted tau gene will be cloned into a transgenic targeting plasmid which will be injected into ES cells.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-E, BSL-1,</td></tr>
<tr><td>33963</td><td></td><td>Reporter HIV Vectors</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of the experiment is study HIV infection in humanized mice using HIV reporter vectors that express one or two fluorescent proteins (EGFP and/or mCherry fluorescent proteins). The vectors will first be used to transduce human CD34+ cells (1-10X10^6) and then the transduced cells will be used to transplant immunodeficient NOD/SCID IL2R-/- common gamma chain -/- (NSG) mice via tail vein injection (200ul volume).

Committee Comments: The committee has requested that the PI provide a more descriptive title. They suggested the title to be "HIV Reporter vectors to study HIV infection in humanized mice." The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, HIV</td></tr>
<tr><td>33962</td><td></td><td>HIV-specific lentiCRISPR Vectors for HIV Eradication</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The purpose of this experiment is to evaluate HIV-specific lentiCRISPR Vectors that express the cas9 protein and HIV-specific single guide RNAs for administration into humanized mice. The mice will not be directly exposed to the lentivirus vectors. The vectors will first be used to transduce human CD34+ cells (1-10X10^6) and then the transduced cells will be used to transplant immunodeficient NOD/SCID IL2R-/- common gamma chain -/-(NSG) mice via tail vein injection (200 ul volume)

Committee Comments: The committee has requested that the PI describe the targets for CRISPR. The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, lentivirus, HIV</td></tr>
<tr><td>33062</td><td>SCOTT HAMMOND</td><td>Small RNA Molecules</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: Small RNA molecules will be produced for the purpose of studying micro-RNA expression pathways. The insert gene will be cloned into a viral vector, which will be utilized to transduce cells in vitro.

Committee Comments: The committee has requested the PI provide a more descriptive title. They suggested the title to be "Small RNA molecules to study micro-RNA expression pathways." The category needs to be changed from a III-F to a III-E. The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-F, BSL-2, E. coli</td></tr>
</table>

<table>
<tr><td>33182</td><td>[redacted]</td><td>Tcell and monocyte-specific alpha (1,3) fucosyltransferase-dependent selectin ligand contributions to atherogenesis</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The purpose of this experiment is to study the contribution of certain fucosyltransferases to the recruitment of white blood cell subsets into the developing atherosclerotic plaque in mice. A mouse containing a loxP-flanked mus FucT-VII gene will be created by homologous recombination. Cre will be expressed in a mouse as a transgene driven by the mus monocyte-specific c-fms promotor. The c-fms-Cre transgenic mouse will be bred with the loxP-FucT-VII mouse to achieve monocyte specific deletion of FucT-VII. The mouse with monocyte-specific deletion of FucTVII will be crossed with LDLR (-/-) mice to assess the effects of FucTVII loss on atherosclerosis.

Committee Comments: The committee has requested that the PI fix the typo in the title. This experiment needs to be categorized as a III-E not a III-F. The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-F, BSL-1, E. coli</td></tr>
<tr><td>33822</td><td>[redacted]</td><td>USE of LNA/GapmeR technology to silence gene expression in vivo</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The purpose of these experiments are to allow the selective silencing of genes by use of antisense oligonucleotides that are complementary to the target mRNA, which will allow temporary gene silencing in adult mice. The LNA/GapmeRs are chemically modified antisense oligonucleotides that are synthesized de novo from sequence information to target specific mRNA transcripts. The LNA/GapmeRs are reconstituted in ultrapure water, diluted in sterile saline and injected subcutaneously into the mice at 20 mg/kg. the GapmeR binds to its complimentary mRNA in vivo reducing expression of the transcript.

Committee Comments: This experiment needs to be categorized as a III-E, not a III-F. The committee has determined that the PI needs to answer "yes" to question 3, since the experiment will be using animals. The PI will also need to fill out section III, since animals will be used in the experiment. The committee has also requested that the PI list the target genes.

Community Member Comments: None

III-F, BSL-1,</td></tr>
<tr><td>32293</td><td>[redacted]</td><td>Generation of LentiGuide-Puro virus for In vivo CRISPR in mice</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The purpose of this experiment is in vivo CRISPR of genes involved in bladder and kidney cancer will be performed to generate mouse models of cancer and determine their functional effects on cancer phenotype. LentiGuide-Puro will be injected in anesthetized mice at no greater than 100uL (kidney) and 500 ul (bladder). Kidney injections will be done either with US guidance or surgically with direct visualization, while bladder injections will be done as a procedure with catheterization of the bladder.

Committee Comments: The committee has requested that the PI provide a list of target genes. The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-E, BSL-2, E. coli, lentivirus</td></tr>
<tr><td>33622</td><td>[redacted]</td><td>Study of stabilized variants of LPL</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The purpose of this experiment is to determine if stabilizing changes to LPL can improve plasma triglyceride levels in mice. The inserted gene will be cloned into a plasmid, used to make virus, then intravascular or intramuscular injected into mice using various doses ranging from 1E9 to 1E10 for IM injections and 1E10 to 1E11 for IV injections.

Committee Comments: The committee has requested that the PI type out LPL in the title so that it is more descriptive. The containment and safety practices are adequate for the experimental design.

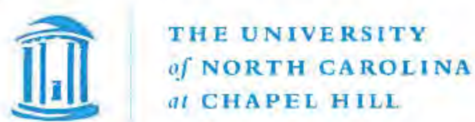
Community Member Comments: None

III-D, BSL-2, E. coli, HEK293</td></tr>
</table>

33442	[redacted]	Genetic Targeting of GABA Neurons in Wildtype Mammals
Approved with Stipulations	**Summary:** The purpose of this experiment is to validate a novel viral approach for transgene delivery in a molecularly-defined neuronal subclass. The insert gene will be cloned into an AAV plasmid, packaged in AAV2/9, and will be intracranially microinjected into rodent brain parenchyma at a volume of 1-2 microliters. The viral concentration will be 1-4X10E12vg/ml. The brain microinjections will be performed as a seterotactic surgery under 1.5-2% isoflurane as per standard lab procedure. **Committee Comments:** The committee has requested that the PI provide a list of target genes. The containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1, AAV	

3. CDC Regulations Updates – Jessica
    a. EHS is working closely with the labs as the CDC give us additional guidance on the new regulations
    b. EHS attended a webinar that discussed inactivation of select agents
4. Subcommittee approval of exempt recombinant DNA: 1
   PI: Brian Kuhlman   Design of Protein-Protein Interactions   III-F
5. Schedule H report: 10
6. Next IBC meeting date: June 7, 2017 at 3:30 PM in BW 9001.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
June 7, 2017 3:30 PM
Burnett Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Aravinda Desilva, Barbara Savoldo, Rita Tamayo, Judy Nielsen, Mary Beth Koza, and Jessica Poole

Members Absent: Barry McLamb, Frederick Sparling, Amy Sims, Peggy Cotter, Craig Fletcher, and Tori Baxter

Ad hoc Members (not requested to be present): Ann Matthysse and Stanley Lemon

Guests:

Open Meeting

1. Review minutes from the May 3, 2017 meeting.
2. Applications under review

ID	PI	Project Title
34246 35068		**Building Recombinant H1N1 Influenza Virus using Kawaoka Laboratory Infectious Clone: 2017 Renewal**
Approved	**Summary:** This is a renewal from a previously approved Schedule G that is expiring. There are two schedule Gs for this experiment. They are the exact same, but a unique number is needed for each room that the work is being performed in. The ab has obtained th laboratory's set of plasmids to build an infectious clone of the 2009 H1N1 Swine influenza virus CA04 as well as the mouse-adapted version of CA04. These viruses, once constructed, will be used in comparison wit nd/or MERS-CoV in infections of mouse models of respiratory disease. There are 12 plasmids, each containing a portion of the influenza genome required to rescue infectious H1N1 CA04 2009 influenza virus, either wild-type or mouse-adapted. The plasmids are amplified in E. coli, isolated, and transfected into 293 T cells, and virus is recovered from the supernatant. **Committee Comments:** The lab contacted the committee and said that this should be a BSL-2, not a BSL-3. The Schedule G was changed online to reflect the correct information. The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, H1N1 Swine Influenza, *E. coli*	
34262 35063		**infectious clone with and without mouse-adapted mutations: 2017 renewal**
Approved	**Summary:** This is a renewal from a previously approved Schedule G that is expiring. There are two schedule Gs for this experiment. They are the exact same, but a unique number is needed for each room that the work is being performed in. The lab has generated an infectious clone of the human coronavirus and have identified 6 mutations that confer pathogenesis in a mouse model. These genomes will be maintained and propagated as control viruses and as backbone genomes for introduction of other mutations (described in individual Schedule Gs). The sequence-verified viral genomes have been cloned as 6 separate cDNA cassettes, which are propagated in plasmid form in E. coli. The 6 mouse-adapted mutations were introduced into the wild-type genome via PCR. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None	

<table>
<tr><td></td><td colspan="2">III-D, BSL-3, E. coli</td></tr>
<tr><td>24263
35061</td><td></td><td>Accessory Open Reading Frame and Accessory Gene Deletions in Infectious Clone: 2017 Renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: This is a renewal from a previously approved Schedule G that is expiring. There are two schedule Gs for this experiment. They are the exact same, but a unique number is needed for each room that the work is being performed in. The purpose of this experiment is to generate infectious clones with deletions of specific ORFs/genes to determine their functions. It is believed that these genes encode functions that are immune modulatory or important for virus fidelity and that their deletion will have neutral or slightly attenuated effects on replication and pathogenesis in vitro and in vivo. The cDNA is maintained as 6 separate cDNA cassettes in plasmids that are propagated in E. coli, Mutations in the accessory ORFS are created in the relevant plasmids and mutant viral cDNAs are assembled in vitro, transcribed, and electroporated into cells. A series of infectious clone constructs will be generated each containing a deleted open reading frame either single deletions or in combination. All of these open reading frames can be deleted without loss of titer in tissue culture cells but there are phenotypes in infected mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-3, E. coli</td></tr>
<tr><td>34282
35064</td><td></td><td>Recombinant alphavirus expression vectors for BSL2 use: VEE Vaccine strain 3526: 2017 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: This is a renewal from a previously approved Schedule G that is expiring. There are two schedule Gs for this experiment. They are the exact same, but a unique number is needed for each room that the work is being performed in. The purpose of this experiment is to maintain the Venezuelan Equine Encephalitis Replicon system (VEE-VRP) for use as a protein expression vector system in transfection studies. This construct will be in the 3526-packaging background, which is approved for BL2 usage. This expression system will be used to produce proteins from Norovirus, Coronaviruses, ·Flaviviruses, and Influenzaviruses. Constructs expressing specific proteins will be described in other applications.

Committee Comments: The lab noted that "Animals will be inoculated via footpad injection with 10-20 uL of VRP inoculum." The lab's protocol is approved only for 10uL. The lab will need to change the schedule G to state that "animals will be inoculated via footpad injection with 10 uL of VRP inoculum."

Community Member Comments: None

III-D, BSL-2, VEE, E. coli</td></tr>
<tr><td>34302</td><td></td><td>Identification and characterization of novel bat norovirus and calicivirus</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The purpose of this experiment is to characterize the capsid of a novel bat norovirus and calicivirus. NoVs and caliciviruses have been previously identified in murine, bovine, porcine, and canine animals, but not in bats. The reactivity of these novel bat NoV and calicivirus capsids will be compared across an antibody panel to determine cross-reactivity and evaluate zoonotic infection potential. The A10 bat calicivirus and bat norovirus capsid sequences will be integrated individually into the pVR21 expression plasmid and propagated in E. coli.

Committee Comments: The lab noted that "Animals will be inoculated via footpad injection with 10-20 uL of VRP inoculum." The lab's protocol is approved only for 10uL. The lab will need to change the schedule G to state that "animals will be inoculated via footpad injection with 10 uL of VRP inoculum."

Community Member Comments: None

III-D, BSL-2, pVR21 plasmid, E. coli</td></tr>
</table>

34322 30567		**Investigation of the role of ion channels in pathogenesis and immunity**
Approved	**Summary:** There are two schedule Gs for this experiment. They are the exact same, but a unique number is needed for each room that the work is being performed in. The purpose of this experiment is to investigate the role of ion channels in Ion channels and potential ion channels have been identified in MERS-CoV (envelope), 229E (4a), PEDV (ORF3), and OC43 (nsp 12.9). Ion channels function in viral release from the cell and immune responses to viral infection. The lab aims to characterize how ion channels impact pathogenesis and immunity in vitro and in vivo and if these effects are specific. Instead of deleting ion channels, ion channels from less virulent CoVs will be substituted into the in place of ion channels. Since ion channels are thought to aid in viral release and ion channels from less virulent strains will be used, these experiments do not fall under gain-of-function prohibitions. Replication and virulence will be monitored through viral passage/tittering in cell culture and weight loss/tittering in murine models. cDNAs are maintained as 6 cDNA cassettes in plasmids that are propagated in E. coli. Mutations are created in the relevant plasmid(s) and mutant viral cDNAs are assembled in vitro, transcribed, and electroporated into cells. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3, *E. coli*	
34403 35066		**Characterizing the roles of MERS-CoV and nsp15 proteins in viral pathogenesis and fidelity**
Approved	**Summary:** There are two schedule Gs for this experiment. They are the exact same, but a unique number is needed for each room that the work is being performed in. The purpose of this experiment is to understand how MERS-CoV and proteins influence pathogenesis in vivo and in vitro. Building off the wild-type MERS-CoV and clones and the mouse-adapted versions of MERS and clones, mutations are proposed in the nsp15 protein. It is anticipated that these mutations may result in MERS and attenuation, allowing us to assess the influence of these viral proteins on disease pathogenesis in vivo and virus fidelity in vitro. These mutations are anticipated to be replication-neutral or attenuating to both viruses and not likely to yield viruses with enhanced virulence. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3 MERS-CoV, *E. coli*	
34142	**JONATHAN BERG**	**Functional assessment of genetic variants using high-throughput assays**
Approved with Stipulations	**Summary:** The purpose of this experimental is functional assessment of genetic variants using high-throughput assays. The lab will be developing high-throughput functional assays for genes of interest, introducing certain genetic variants into the cDNA, and assessing whether those variants alter the function of the gene product. The insert gene will be cloned into a plasmid using standard techniques of molecular biology and bacterial cloning. The plasmid will be transfected into yeast cells for in vitro binding assays and cloned into cultured mammalian cells for in vitro functional assays. The fluorescent reporter plasmid will be cloned into a viral vector, which will be used to transduce mammalian cells in vitro. **Committee Comments:** The committee has requested that the PI provide a more descriptive title and purpose. The aim/purpose sections need to explain what a high throughput assay is, what mutations, and the use of lentivirus in yeast. **Community Member Comments:** None III-D, BSL-2, lentivirus	

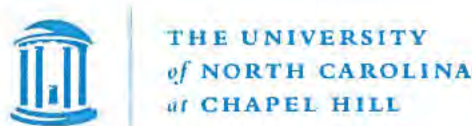
35242		Use of AAV vectors to elucidate the location and movements of a FLAG-HMGB1 fusion protein in brain
Approved	**Summary:** The purpose of this experiment is to elucidate the location and movements of a FLAG-HMGB1 fusion protein after injection into various regions of the mouse brain and subsequent experimental treatments historically performed with mice. The HMGB1 gene is cloned into a viral vector, which will be utilized to transduce cells in vitro. The virus will ultimately be injected into various regions of the mouse brain. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, AAV	
32902	**DANIEL CRONA**	**Investigating the role of candidate genes in sorafenib toxicity**
Approved	**Summary:** CRISPR Cas9 constructs (pSpCas9(BB)-2A-Puro(Px459) V2.0, developed by the Zhang Lab at Broad Institute) with targeted guide RNA. Gene specific guides will knock out candidate genes by causing frame shifts/stop codons. Candidate genes: Radixin (Rdx), Ferredoxin 1 (Fdx1), Arhgap20 and Sex comb on midleg homolog 1 (Scmh1). The use of lentivirus is required to infect the CRISPR Cas9 construct into Mef cells. Primary Mef cells are difficult to transfect (Human cells will be transfected. No virus will be used on human cells). CRISPR Cas9 constructs will be transfected or transduced into cells in vitro to cause deletions of targeted genes. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, Lentivirus	
33702	**ARAVINDA DESILVA**	**Using chimeric West Nile/dengue and West Nile/Zika viruses in serological assays of Flavivirus infection and vaccination**
Approved with Stipulations	**Summary:** The purpose of this experiment is to use chimeric Flaviviruses WNV/DENV and WNV/ZIKV, (engineered, produced and characterized by the CDC, Fort Collins), in in vitro antibody detection assays, to better characterize the quality of the antibody responses to DENV and ZIKV natural infection and vaccination. The chimeric viruses will be used to deplete human serum samples of Flavivirus cross-reactive antibodies and therefore improve the specificity of the current serologic assays. Researchers at UNC will not manipulate the recombinant nucleic acid encoding these chimeric viruses. They will only grow the chimeric viruses in cell culture to amplify the virus stock and then purify the chimeric viruses to be used in in vitro depletion and neutralization assays. The DENV-like chimeras and ZIKV-like chimeras present authentic DENV and ZIKV envelopes but replicate significantly faster than the wild-type 9wt) DENVs and ZIKV. **Committee Comments:** The CDC had to file a minor action to the NIH in order to work with these chimeras at a BSL-2. This minor action is only valid for that particular PI at the CDC. In order for the lab to work with this as a BSL-2 at UNC, the IBC needs to submit a request to the NIH. EHS is going to work with the lab to get the necessary paperwork completed. The lab has agreed not to receive the samples from the CDC until the IBC grants final approval, which is contingent on the NIH approval. **Community Member Comments:** None III-D, BSL-2, ZIKV, DENV, West Nile	
34582		**Utilization of recombinant HCMV strains with reporter genes**
Tabled	**Summary:** The main purpose of this experiment is to monitor HCMV infection in mice. Recombinant strains with reporter genes (GFP, RFP, or Luc) will help to monitor and/or track viral replication. All the recombinant HCMV strains will be provided by collaborators. The reporter genes will be inserted by recombination under an HCMV ORF promoter. The viruses will be grown in fibroblast cells and stocks will be prepared. The mice will be either infected with cell free and/or cell associated recombinant HCMV. **Committee Comments:** The PI needs to answer "yes" to question 3, which asks if recombinant or synthetic nucleic acid molecules will be introduced into whole plants or animals. Once this question is marked "yes", the	

	PI will need to fill out section III. **Community Member Comments:** None III-D, BSL-2, HCMV	
34182	[redacted]	**Use of LNA/GapmeR technology to silence gene expression in vivo**
Approved	**Summary:** The purpose of the experiment is to allow the selective silencing of genes by use of antisense oligonucleotides that are complementary to the target mRNA. This allows temporary gene silencing in adult mice. The LNA/GapmeRs are chemically modified antisense oligonucleotides that are synthesized de novo from sequence information by the company Exiqon to target specific mRNA transcripts. The oligonucleotides will be synthesized to target expression of ZFP30 and BPIFB1, along with any future targets the lab may identify. Exiqon will provide the best target sequence and the lab will perform a pilot experiment to determine optimal dosage and time points for gene silencing in mice. The LNA/GapmeRs will be reconstituted in sterile saline and directly injected into mice subcutaneously. **Committee Comments:** The committee has requested that "in vivo" in the title be changed to "in mice." The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-E, BSL-1	
26917	[redacted]	**Engineering Temperate Bacteriophage for Sustained Secretion of Protein Therapeutics or Immunogens by Mucosal Commensals**
Tabled	**Summary:** The purpose of the experiment is to develop genetically modified bacteriophage that can transduce genes of interest into commensal bacterial populations. In these studies, the lab will transduce bacteria both in vitro and in vivo (gnotobiotic mice). Temperate bacteriophage present in naturally occurring strains of commensal bacterial organisms will be used as the template for genetic modification. Bacteriophage will never be derived from host strains that have any known pathogenicity. Genomes of template bacteriophage will be modified by in-vitro natural transfection of exogenous, non-pathogenic genes generated by PCR amplification. Genetically modified phage will be induced by UV light and used to transduce additional strains of non-pathogenic commensal bacterial populations. In vivo transduction will be evaluated by oral gavage of engineered bacteriophage into gnotobiotic mice. All work with recombinant viruses will be completed in a BSC. All waste will be autoclaved out of the animal facility. **Committee Comments:** The lab needs to provide an IACUC number. **Community Member Comments:** None III-D, BSL-2,	
34402	[redacted]	**Opioids and Animal Models of PTSD and Neural mechanisms of Opioid Immune Conditioning**
Approved	**Summary:** The purpose of this experiment is DREADD receptors in rats using mcherry and mcitrine markers. The DREADD receptors will allow for astrocyte and neuron activation and inhibition in specific brain regions of rats using a designer drug to investigate specific cell function in both fear conditioning and opioid conditioning. DREADD viral vectors will be infused at a volume of 0.5-1.0 uL bilaterally directly into rat brains during surgery where rats are fully anesthetized **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1, AAV	

34062	TIMOTHY MORAN	Role of neuropilins in airway inflammation
Approved	**Summary:** The purpose of this experiment is to perform knock-down experiments of neuropilin-1 (NRP1) and neuropilin-2 (NRP2) in primary mouse cells, immortalized mouse cell lines, immortalized human cell lines, and primary human cells to explore the roles of neuropilins in immune cell biology and the pathogenesis of airway inflammation. The insert DNA will be cloned into a viral vector, which will be utilized to transduce cells in vitro to knockdown expression of NRP1 and NRP2 via the CRISPR-Cas9 system. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus	
34782	[redacted]	**DREADD-mediated Activation of Cortical Signaling to the Central Nucleus of the Amygdala**
Approved	**Summary:** The purpose of the experiment is to determine how cortical pyramidal neurons affect the activity of the Central Nucleus of the Amygdala (CeA), a prominent target of cortical neurons, by using Gq-coupled stimulatory DREADDs to specifically potentiate the activity of CeA-projecting cells. The gene insert is a Gq-coupled DREADD (hM2D) fused to an mCherry fluorescent reporter protein and has been packaged inside an AAV5 viral vector. This viral vector will be used to infect neurons in rats. Rats will be used to test the hypothesis that cortical pyramidal cells project to central nucleus of the amygdala and exhibit ethanol-induced adaptations in GABAergic signaling. Rats will be used for slice electrophysiology, drinking studies, behavioral testing, and protein expression analysis. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1, AAV	
34002	[redacted]	**Role of Fcgamma receptors, auto-antibodies and progression of atherosclerosis**
Tabled	**Summary:** The purpose of the experiment is to determine the role of Fcgamma receptors and scavenger receptor in the initiation and progression of atherosclerosis. Genes of interest will be cloned into a plasmid (mammalian expression vector). Plasmid DNA will be transfected into mammalian cells in vitro. The insert gene will be cloned into a lentiviral or AAV vector and cells will be transduced with replication-defective viruses. Specifically, cells such as CHOK1 and COS-7, RAW .264, RAW-Blue, THP-1, and HEK293 cells will be used to overexpress the recombinant transgenes. In some assays, genes will be silenced using siRNA specific for Fcgamma receptors and scavenger receptors. Bone marrow cells, T lymphocytes, and B-lymphocytes will be isolated from genetically modified mice (knockout mice). Bone marrow cells, T- or B-lymphocytes will be injected intravenously or intraperitoneally into recipient mice. **Committee Comments:** This should be a III-D, not a III-F. The PI needs to answer "yes" to question 3, which asks if recombinant or synthetic nucleic acid molecules will be introduced into whole plants or animals. Once this question is marked "yes", the PI will need to fill out section III. **Community Member Comments:** None III-E, BSL-2, AAV, lentivirus	

3. Mary Beth has been asked to serve on a Biosecurity committee in Washington D.C.
    a. Roadmap for Biosecurity Policy Implementation Expert
4. Subcommittee approval of exempt recombinant DNA: 60 See attached report
5. Schedule H report: 13
6. Next IBC meeting date: July 12, 2017 at 3:30 PM in BW 4050.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
July 12, 2017 3:30 PM
Burnett Womack 4050

Members Present: Doug Cyr, Amy Sims, Sandra Bradshaw, Tori Baxter, and Jessica Poole.

Members Absent: Barry McLamb, Frederick Sparling, Peggy Cotter, Aravinda Desilva, Barbara Savoldo, Rita Tamayo, Craig Fletcher, Judith Nielsen, and Mary Beth Koza

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests:

Open Meeting

1. Review minutes from the June 7, 2017 meeting.
2. Applications under review

ID	PI	Project Title
35757		**Study of circRNA expression patterns via delivery into mice using AAV vectors**
Approved with Stipulations	**Summary:** The purpose of the study is to design and optimize AAV vector cassettes expressing circular RNAs. Ultimately, the lab seeks to study their potential as therapeutic reagents in mice. The gene and intronic sequences will be cloned into viral vectors, which will be used to transduce cells in vitro. The viral vectors will also be injected into mice. **Committee Comments:** The committee had concerns with the lab performing IV injections with a volume of 200 uL. The lab's approved IACUC protocol also had a volume of 200 uL listed. The vet contacted the lab to get an explanation to why they are requesting to use 200 uL. The lab explained that the major limiting factor they face is the inability to concentrate AAV vectors beyond a particular copy number. To complicate this further, each AAV preparation behaves differently from a formulation perspective and the risk of generating a precipitated product is high at low volumes/high concentration, which is detrimental for the study and the animal. The lab has determined that up to 250uL IV bolus dose of saline is well tolerated in mice. Most AAV preparations that the lab uses are stable at 1e11 to 1e12 particles (optimal dose range) in 200uL. The vet is going to have the lab submit an IACUC amendment detailing some extra monitoring parameters for these IV injections **Community Member Comments:** None III-D, BSL-2, *E. coli*	
35543		**Generation of chimeric viruses containing the mouse-adapted Spike Y436H substitution**
Approved with Stipulations	**Summary:** The lab has previously generated chimeric bearing the spike coding sequence from like viruses discovered in bats (SHC014, WIV1, WIV16) inserted into our mouse adapted (MA15) infectious clone. These chimeric viruses can infect mice, but replicate poorly and cause minimal to no disease, similar to the epidemic Serial passage of in mice to generate MA15 resulted in 5 mutations necessary for disease in mice, including a Y436H mutation within the spike. Sequence alignments between and WIV1 or WIV16 reveal 92% and 97% amino acid identity, respectively, with both WIV1 or WIV16 containing a Y at residue 436, similar to the epidemic The goal of this experiment is to generate chimeric WIV1 Y436H MA15 and WIV16 Y436H- MA15 to determine if the Y436H substitution can allow for rapid mouse adaptation of coronaviruses. We have also generated a full-length infectious clone of WIV1 (Schedule G 12279) and have approval to make the Y436H substitution in full-length WIV1 (Schedule G 28193). The Y436H substitution	

<table>
<tr><td></td><td colspan="2">will be inserted into existing WIV1 and WIV16 infectious clone plasmids. Chimeras will then be constructed [redacted] backbone for fragments A-D and fragments E and F from WIV1 or 16, each with the Y436H substitution) to then generate infectious viral RNA.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: The committee requested that the lab provide a simplified purpose so that members of the community can understand what the lab is proposing to do.

III-D, BSL-3 [redacted], E. coli</td></tr>
<tr><td>35542</td><td>[redacted]</td><td>Manipulation of the dengue virus envelope to impact stability and neurotropism</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: Differences in the structural loops between dengue virus and recently emergent Zika virus may be critical for stability and neurotropism. One particular difference between the envelopes of the two viruses is within the CD-loop, with Zika virus containing one additional amino acid, allowing the loop to extend further into the 5-fold vertex of symmetry on the virion. Previous work in the lab has shown that shortening the CD-loop in Zika virus, mimicking the length found in dengue virus, results in structural and temperature instability and attenuation in a mouse model of infection. This project seeks to determine if the extension of the CD-loop of dengue virus serotype 4 (DENV4) will confer greater stability to the DENV4 virion. The dengue virus cDNAs are maintained as 4 separate cDNA cassettes in plasmids that are propagated in E. coli. The mutations will be created in the relevant plasmid (A), and mutant virus cDNAs are assembled in vitro, transcribed, and electroporated into C6/36 or Vero-81 cells.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, dengue, E. coli</td></tr>
<tr><td>35603</td><td>[redacted]</td><td>Flpo Strain</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of the experiment is to produce mice with ubiquitous expression of a codon-optimized Flp recombinase (Flpo) for efficient removal of FRT-flanked sequences. The Flpo gene will be cloned into a plasmid, which will be injected into embryos or targeted into embryonic stem cells to produce animals expressing the Flpo protein.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The committee requested that the PI provide a more descriptive title and suggested "Production of a Flpo Mouse Strain."

Community Member Comments: None

III-E, BSL-1, E. coli</td></tr>
<tr><td>35861</td><td>[redacted]</td><td>Utilization of recombinant HCMV strains with reporter genes</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The main purpose of this experiment is to monitor HCMV infection in mice. Recombinant strains with reporter genes (GFP, PRF, or Luc) will help monitor and/or track viral replication. All of the recombinant HCMV strains will be provided by collaborators. The reporter genes will be inserted by recombination under an HCMV ORF promoter. The viruses will be grown in fibroblast cells and stocks will be prepared. The mice will be either infected with cell free and/or cell associated recombinant HCMV. The animals will be exposed by the following routes: intra-lung, i.p. or i.v. For each route, the animals will receive up to 5e6 TCIU cell-free virus or 1e6 PFU of cell-associated virus in up to 100 uL volume.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The lab answered question 6 in section III "no", because the agent that they are using is a replication competent virus not a viral packing or expression system; therefore, there is no need for a higher containment level. The committee has requested that the lab provide a vector map.

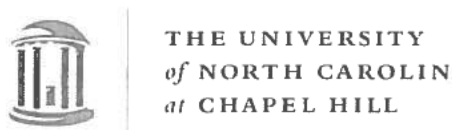
Community Member Comments: None</td></tr>
</table>

	III-D, BSL-2 HCMV	
35282	[redacted]	**Generation of a Crhr1-iCre Rat by th**[redacted]
Approved	**Summary:** The purpose of this experiment is to generate a transgenic rat that expresses Cre recombinase under the Crhr 1 promoter. The insert gene will be cloned into a viral vector, which will be utilized to transduce cells in vitro. The cell will ultimately be injected into rats. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1,	
35902	[redacted]	**Safer 5th generation lentiviral vectors with reduced viral sequence**
Approved with Stipulations	**Summary:** The aim of the proposed experiment is to generate safer lentiviral vectors, which are less likely to recombine, mobilized or affect host gene expression upon integration to target cell chromatin. Specifically, the experiment is based on two lines of improvements: a) Reduction in the parental viral sequence in the reverse-transcribed vector genome. This includes elimination of either/and of the HIV-1 packaging signal, Rev response element (RRE), part of the 5' HIV-1 U5, and part of the HIV-1 Poly-A in the 3'LTR. In several vectors, an exogeneous nuclear export sequence will replace the parental RRE. b) To minimize vector effects on host gene expression a tandem sequence of up to 1kb will be incorporated to the vector cassette. Rare inadvertent host transcription through the vector cassette will generate double stranded RNA structures which will activate the PKR pathway and silence undesired protein expression. The parental HIV-1 packaging signal (or parts of it including the primer binding site) and/or the RRE will be deleted from the vector cassette and cloned downstream to the 3'LTR (and upstream to an exogenous poly-A sequence). In some vectors, the parental 3' poly-A sequence will be mutated. In some vectors A deletion of up to 30 bp will be made in 5' U5 region. In some vectors, an exogenous (non-lentiviral) nuclear export sequence will replace the parental RRE (either between the LTR's or downstream to the 3' LTR). A tandem of non-coding sequence of up to 1kb (either from host cells, viruses or reporter genes) will be incorporated between the LTR's. Lentiviral vector (HIV-1) based will be injected intraperitoneally to either immune-competent or immune deficient mice. The vector will be injected in PBS up to 200 microliters. The vector dose will comprise up to 250 micrograms of p24gag. The maximal amount of vector will be up to 5x10e10 IU per mouse (as tittered on 293T cells). Vector titer will be up to 8x10e11 IU/mL (as tittered on 293T cells). **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The committee requested that the lab provide an IACUC protocol number before the animal work begins. The committee also requested that the lab provide the entire reference for question 6. **Community Member Comments:** None III-D, BSL-2, lentivirus, *E. coli*	
35962	[redacted]	**Molecular mechanisms of Zika virus pathogenesis**
Approved	**Summary:** The purpose of the experiment is to generate molecular clones of Zika virus. These clones will be used to generate mutant viruses to test hypotheses about the molecular mechanisms by which Zika virus causes disease, particularly interactions with antibodies and viral determinants of pathogenesis. Zika virus is closely related to dengue virus, for which multiple molecular clones have been made and for which antibody epitopes are well-described. Zika virus and dengue virus clones will be used to generate viruses with chimeric envelope proteins (e.g. incorporating known dengue virus epitopes into Zika virus, or vice versa) to better define antibody epitopes in Zika virus. The lab will also generate mutant viruses to test hypotheses about viral determinants of pathogenesis, for example the role of envelope glycosylation in neuroinvasion or mechanisms that restrict infection in mice. In other studies, the lab will use a reporter virus system where plasmids expressing the structural proteins of DENV or ZIKV are co-transfected with a replicon plasmid which includes the non-structural proteins of a lineage II West Nile virus strain and a GFP reporter in place of the viral structural proteins. This results in the production of reporter virus particles, which are structurally similar to viral particles and can be used in cell culture assays to study viral entry and neutralization, but which do not replicate. Mice will be infected with clone-derived virus by subcutaneous inoculation in the footpad (approximately 1000 FFU in 50 uL) or intracranially (approximately 1000 FFU in 25 uL).	

	Committee Comments: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, DENV, ZIKV, *E. coli*
36002	[redacted] **Role of Fcgamma receptors, auto-antibodies and progression of atherosclerosis**
Approved	**Summary:** The purpose of the experiment is to determine the role of Fcgamma receptors and scavenger receptor in the initiation and progression of atherosclerosis. Genes of interest will be cloned into a plasmid (mammalian expression vector). Plasmid DNA will be transfected into mammalian cells in vitro. The insert gene will be cloned into a lentiviral or AAV vector and cells will be traduced with replication-defective viruses. Specifically, cells such as CHOK1 and COS-7, RAW.264, RAW-Blue, THP-1, and HEK293 cells will be used to overexpress the recombinant transgenes. In some assays, genes will be silenced using siRNA specific for Fcgamma receptors and scavenger receptors. These studies will be done only in vitro using primary cells or cell lines. None of the primary cells or cell lines will be used for in vivo experiments in mice. Bone marrow cells, T lymphocytes, and B-lymphocytes will be isolated from genetically modified mice (knockout mice) or wild type mice. These cells will be isolated aseptically. Bone marrow cells, T- or B-lymphocytes will be injected intravenously or intraperitoneally into recipient mouse. None of these cells will be exposed to recombinant DNA including siRNA, or viral particles before injecting into mice. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, AAV, lentivirus
35642	[redacted] **B-cell targeted tolerance to FVIII in hemophilia A dogs**
Approved	**Summary:** The goal of this experiment is to use cell-based therapy to ablate anti-factor VIII antibodies in hemophilia A dogs. Treg cells will be engineered to express immunodominant epitopes of coagulation factor VIII (FVIII) and reinfused into the donor hemophilia A dog to monitor anti-factor VIII antibody response. The insert gene will be cloned into a viral vector, which will be utilized to transduce cells in vitro. The cell will ultimately be injected into hemophilia A dogs. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2

1. Subcommittee approval of exempt recombinant DNA: 1
   PI: Ralph Baric Category: III-F Title: Utilizing the BioID platform for the purification and rapid identification proteins that interact with viral ORFs of interest (ORFeome)
2. Schedule H report: 27
3. Next IBC meeting date: August 2, 2017 at 3:30 PM in BW 9001.

Adjourn.



THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
September 6, 2017 3:30 PM
Burnett Womack 9001

Members Present: Doug Cyr, Amy Sims, Aravinda Desilva, Barbara Savoldo, Mary Beth Koza and Jessica Poole

Members Absent: Sandra Bradshaw, Barry McLamb, Frederick Sparling, Peggy Cotter, Craig Fletcher, Judy Nielsen and Tori Baxter

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests:

Open Meeting

1. Samuel Lai Lab – Explanation of their Schedule G 26917
   - from the Lai lab explained the proposed experiment to the IBC. The lab is wanting to create a transgene in E. coli plasmid. The phage will be integrated into chromosome of the virus. The lab is requested to use erythromycin. There will be 2 cargos in the same phage. The lab explained that this is novel research. The committee requested several items for the lab to provide: flow chart of what the lab is proposing to do and literature supporting the research. The IBC informed the lab that they might seek NIH guidance for this research and that containment requirements might be higher.
2. Review minutes from the August 2, 2017 meeting.
3. Applications under review

ID	PI	Project Title
Approved With Stipulations	**Summary:** The purpose of this phase Ib/II trial is to determine whether **Committee Comments:** The committee requested that the drug combination be explained in more detail in the cover letter and the abstract. A new cover letter and abstract were supplied on 9/18/2017. The committee had no additional concerns with the study. **Community Member Comments:** None	

Approved	**Summary:** The proposed clinical study is a two-part, Phase 2, multi-center, open-label study of **Committee Comments:** The committee had no concerns with this study. **Community Member Comments:** None	
36849		**Transmembrane Proteolytic Induction and Thoracic Aneurysms**
Approved	**Summary:** The purpose of this experiment is to examine the role of membrane type-1 matrix metalloproteinase (MT1-MMP) phosphorylation in vivo. The lab will use a novel double-floxed inverted open reading frame (DIO) approach, in which adeno-associated viruses (AAV) will be used to introduce FGP-tagged MT1-MMP phosphor-site mutants (T567A, T567E) into aortic cells of mice to direct MT1-MMP localization in vivo. This will be accomplished by introducing FGP-MT1-MMP gene mutants in reverse orientation relative to their 5' end and flanked by oppositely oriented loxP and lox2272 sites, to Col1A2-CreERT2-positive and floxed-MT1-MMP positive mice, in which endogenous MTI-MMP will be knocked out with Tamoxifen treatment. The virus will then be directly injected into the adventitia of the descending thoracic aorta by blebbing, at the time of Thoracic Aortic Aneurysm induction. As the virus infects the aortic cells transmurally, in the absence of Cre activation, the phosphor-site transgenes will not be inverted into the correct orientation, and no transcription will occur. However, upon a second Tamoxifen treatment, Cre activation will catalyze the inversion of the GFP-MT1-MMP phosphor-site mutants into the correct orientation, and the viral vector will drive transcription. Mice will be anesthetized with 2% isoflurane and intubated. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, AAV	
36862		**Transmembrane Proteolytic Induction of Thoracic Aortic Aneurysms_2 (Lentivirus)**
Approved	**Summary:** The purpose of this experiment is to use Lentiviral constructs to deliver microRNA precursors to mice. The aim of the studies is to demonstrate that restoration of microRNA-133a in vivo can attenuate thoracic aortic aneurysm formation and progression. The insert gene will be cloned into a lentiviral vector and delivered to murine thoracic aortic fibroblasts and tissue in vitro and mice in vivo. Mice will be exposed via a single tail vein injection containing 100 uL of 1z10^9 pfu lentivirus. Mice will be anesthetized and intubated with 2% isoflurane. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus,	

37202		Characterization of bat and swine coronavirus HKU2
Approved with Stipulations	**Summary:** HKU2 is a -like alphacoronavirus that has been previously reported in bats. Previous attempts to characterize this virus and its pathogenesis have been limited due to its inability to grow in cell culture. Recent reports have identified an HKU2 virus within swine in China, suggesting the virus's capacity to grow in another host and providing another alternative to culture the virus in vitro. The purpose of this experiment is to determine if novel cell types (e.g., porcine cells) or culture conditions (e.g., trypsin addition) could aid in the culture and characterization of this virus. A reporter virus expressing RFP in place of HKU2 accessory proteins NS3 and/or ORF7a will also be constructed to ensure virus production. The coronavirus cDNAs are maintained as several separate cDNA cassettes in plasmids that are propagated in E. coli. KHU2 cDNAs are maintained as 6-7 cDNA cassettes in plasmids that are propagated in E. coli. Mutations are created in the relevant plasmids(s) and mutant viral cDNAs are assembled in vitro, transcribed, and electroporated into cells. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The committee requested that the lab provide a line item list of the viral particles that will be generated. **Community Member Comments:** None III-D, BSL-3, *E. coli*, HKU2	
37122		Generation of icMERS and icMERS-Uganda expressing RFP
Approved	**Summary:** The lab proposes to generate fluorescence-capable viral mutants expressing the spike protein from a Uganda MERS-like CoV in the MERS-CoV backbone. Synthetically produced spike protein from Uganda MERS-like CoV will be ligated into the MERS-CoV clone. Viable viruses will be characterized for replication in vitro and altered pathogenesis in vivo. Based on modeling structures, the Ugandan spike chimeric viruses are not expected to be replication competent and no enhanced pathogenesis is expected. The overarching goal of the project is to characterize replication competency and develop reagents to characterize novel zoonotic strains of CoVs. To facilitate visualization of virus infection via fluorescence microscopy, the lab wants to introduce tomato RFP into the infectious clone systems of MERS-CoV and MERS-CoV containing the Uganda spike. The lab will replace the viral ORF5 coding sequence with the coding sequence of tomato RFP. Replication and virulence will be monitored through: 1) viral passage in cell culture and tittering and 2) weight loss and tittering in animals. If the viruses show signs of enhanced replication or virulence over WT, the lab will cease working with them and notify the IBC. Animals will be inoculated intranasally with 50 UL of viral inoculum. Inoculation titer will range from 10^2-5X10^6 PFU. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3,	
36282		**Calcium Imaging in Behaving Animals**
Approved	**Summary:** Calcium imaging is a way of visualizing neuronal activity over time. This experiment will allow the lab to observe the activity of different cell populations in awake and behaving animals, while they are performing various tasks and/or receiving various rewards. The AAV viral vector containing the GCaMP6 gene will be introduced into the rat brain in vivo. AAV vectors will be injected into rat brain during a survival surgery via a Hamilton syringe pump. The virus concentration will be 5x10^11 to 1x10^13 cfu/mL, and 0.1-1 uL will be used per site, 1-4 sites per animal. Animals will be anesthetized with ketamine/xylazine or isoflurane. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1, AAV	

37005		**Chemogenetic manipulation of microglia to elucidate their role in alcohol-induced neuropathology**
Approved	**Summary:** The purpose of this experiment is to use AAV to inhibit microglia activation to identify microglia involvement in alcohol-induced neuropathology. In vitro, ex vivo and in vivo injection into various regions of the rat brain and subsequent experimental treatments will be performed with rats. The hM4D(Gi)-mCherry and the control gene (pAAV-EGFP) will be cloned into a viral vector, which will be utilized to transfect microglia in vitro and in ex vivo basal forebrain and HPC slice culture. Rats will be anesthetized using isoflurane. Virus (2uL) will be delivered bilaterally into the basal forebrain and hippocampus. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, AAV	
37106		**Utilization of luminescent Neisseria gonorrhoeae**
Approved ***** ID changed in system to 37567*****	**Summary:** The purpose of this experiment is to monitor Neisseria gonorrhoeae infection in mice. Neisseria gonorrhoeae with luminescent bioreporter luxCDABE will be generated by a collaborator and will be used to monitor infection in mice. Mice will be exposed to luminescent Neisseria gonorrhoeae vaginally or by penile inoculation. For vaginal exposure, 10^6 CFU units in 20 uL RPMI will be administered to mouse vagina. For penile inoculation 1-^6 CFU in 2 uL of RPMI will be administered on the top of the meatus urethra. Mice will be anesthetized with pentobarbital Nembutal or with 2,2,2-Tribromoethanol. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2	
37182		**Utilization of recombinant Influenza strains with reporter genes**
Tabled	**Summary:** The main goals of these experiments are to develop an in vivo animal model for Influenza and to use this model to test the efficacy of novel antivirals. The mice will be infected with the recombinant reporter viruses. Following Influenza exposure, mice will be bled longitudinally to measure levels of virus and immune cells in peripheral blood. For mice infected with Influenza-mCherry or Influenza-GFP, groups of mice will be euthanized and necropsied up to 28 days post-exposure. Tissues will be collected and processed for downstream analyses such as flow cytometry and real-time PCR. We will also monitor infection longitudinally utilizing IVIS. For this, mice will be imaged every 3 or 4 days post infection with IVIS. After the experimental time points, mice will be euthanized, and necropsied. Tissues will be collected and processed for downstream analyses such as flow cytometry and real-time PCR. All the recombinant Influenza strains will be produced and provided by collaborators. Influenza A and Influenza B with mCherry or GFP being expressed of the NS subgenomic fragment. Virus stocks will be prepared in Madin-Darby canine Kidney (MDCK) cells and aliquoted and stored at -80C until use. The animals will be exposed by the following routes: intra-lung, i.p or i.v. For each route, the animals will receive up to 5e6 TCIU cell-free virus in up to 200 ul volume via I.P., I.v. or intra-lung injection. **Committee Comments:** The committee requested that the lab provide the specific strains of H1N1 and H3N2 that they are requesting to use. The committee also requested EHS to check and make sure that their SOP has information regarding flu shots, since they are requesting to work with influenza. **Community Member Comments:** None III-D, BSL-2,	

36683	[redacted]	Injection of pseudorabies virus (PRV) for neural circuit tracing
Approved with Stipulations	**Summary:** The purpose of this experiment is the mapping of specific neural circuits using the pseudorabies virus (PRV-152) as a retrograde tracer. PRV 152 and PRV 614 are derivatives of the attenuated PRV strain Bartha. Each one has an insertion of an expression cassette in the PRV Bartha gG gene. The cassette insertion in PRV 152 has the CMV promotor transcribing the eGFP gene. The cassette insertion in PRV 614m has the CMV promoter driving the mRFT gene. Virus will be infused into target brain sites at a rate of 0.12-4 microliters over a period of up to 20 minutes using a Hamilton micro syringe controlled by a pump. Virus will be injected into the ventral wall of the stomach in a series of injections with separate injections sites (no more than 5 uL total volume). **Committee Comments:** The committee requested that EHS Biosafety group verify containment level. According to the BMBL, pseudorabies virus is a risk group 2. The committee also requested that DLAM and IACUC be contacted to confirm that animals will be housed in proper containment. The rats will be housed in a BSL-2 cubicle in Thurston Bowles after they have been injected with the virus. The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, pseudorabies virus (PRV)	
36702	[redacted]	Safe lentiviral vector based anti-HIV-1 vaccine
Approved	**Summary:** The goal of the proposed studies is to develop an effective lentiviral viral vector-based anti-HIV-1 vaccination protocol. Specifically, the lab proposes to employ integration competent and integration defective lentiviral vectors (ICLV and IDLV) to deliver codon optimized HIV-1 gp120 (p96ZM651gp120-opt) and gp140 (p96ZM651gp140-opt) envelop protein cDNA to immune competent mice. The above codon-optimized cDNAs will be either fused or not to 2-repeats of the human IgG FC region (2FC). Vector particles will be pseudotyped with VSV-G, Syncytin A, or Syncytin B envelop proteins. A Pol II promoter will drive expression of the above immunogens. Vector particles will be administered to immunocompetent mice via IM, IP, IV or SC injection. The vectors will be delivered in PBS with maximal volume of 250 microliters per injection. Maximal vector titers will be less than 2x10e12 IU/ml. Each vector dose will comprise less than 250 micrograms of p24gag. Each animal will be treated with up to 4 doses of vectors. Serum samples from treated animals will be collected. Titers of inhibitory serum antibodies directed to standard lentiviral vectors pesudotyped with the corresponding full length (gp160) envelope protein (p96ZM65160-opt) will be determined in vitro on 293T cells. The targeted lentiviral vectors will carry reporter/selection marker genes (GFP, RFP, GFP-Blasticidin fusion, or puromycyin resistance) under the control of a Pol II promoter. The above parental envelop proteins will be obtained from the NIH AIDS reagent program. Vector particles will be generated in the laboratory of [redacted] All vector preps for in vivo usage will be tested for replication competent retroviruses by three independent safety assays. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus	
37163	[redacted]	Safer 5th generation lentiviral vectors with reduced viral sequence including opposite orientation
Approved	**Summary:** This schedule G is an addition to the recently approved one (35902). Specifically, this Schedule G adds vectors comprising internal expression cassettes in opposite orientation to the LTRs. Some vectors will include an intron. The aim of the proposed experiment is to generate safer lentiviral vectors, which are less likely to recombine, mobilize or affect host gene expression upon integration to target call chromatin. Specifically, the experiments are based on two lines of improvements: a) Reduction in the parental viral sequence in the reverse-transcribed vector genome. This include elimination of either/and of the HIV-1 packaging signal, Rev response element (RRE), part of the 5' HIV-1 U5, and part of the HIV-1 Poly-A in the 3'LTR. In several vectors, an exogeneous nuclear export sequence will replace the parental RRE. B" To minimize vector effects on host gene expression a tandem sequence of up to 1kb will be incorporated to the vector cassette. Rare inadvertent host transcription through the vector cassette will generate double stranded RNA structures which will activate the PKR pathway and silence undesired protein expression. Lentiviral vector (HIV-1) based will be injected intraperitoneally to either immune-competent or immune deficient mice. The vector will be injected in PBS up to 200 microliters. The vector dose will comprise up to 250 micrograms	

	of p24gag. The maximal amount of vector will be up to 5x10e10 IU per mouse (as tittered on 293T cells). Vector titer will be up to 8x10e11 IU/mL. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus	
36962		**Insertion of human BORCS7/AS3MT genes**
Approved with Stipulations	**Summary:** The purpose of this study is to introduce the expression of human BORCS7/AS3MT genes into the mouse in order to define targets for the treatment of inflammatory disorders. The insert gene will be cloned into a generic plasmid construct which will be electroporated into ES cells in vitro. The cells will ultimately be injected into mice to generate transgenic mice. The vector used for the introduction of the human transgene carries an ampicillin gene which allows for the growth of the plasmid in E. coli. The vector also carries the hypoxantine phosphoribosyltransferase minigenes (HPRT) gene. This restores hprt activity to mammalian cells lacking this activity, serving as a selectable marker for DNA integration. The introduction of the HPRT gene and human transgenes pose no danger to the mouse itself or the environment. Mice will receive blastocyst microinjection of the ES cells to create transmitting chimeras and chimera breeding for germline transmission. The procedure will be carried out by the **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The committee requested that the lab explain the biological activity of the insert genes in the Schedule G. **Community Member Comments:** None III_D, BSL-1, *E. coli*	
36982		**Insertion of human FCGRT gene**
Approved with Stipulations	**Summary:** The purpose of this study is to introduce the expression of the human FCGRT gene in the mouse in order to define targets for the treatment of inflammatory diseases. The insert gene will be cloned into a generic plasmid construct which will be electroporated into ES cells in vitro. The cells will ultimately be injected into mice to generate transgenic mice. The vector used for the introduction of the human transgene carries an ampicillin gene which allows for the growth of the plasmid in E. coli. The vector also carries the hypoxantine phosphoribosyltransferase minigenes (HPRT) gene. This restores hprt activity to mammalian cells lacking this activity, serving as a selectable marker for DNA integration. The introduction of the HPRT gene and human transgenes pose no danger to the mouse itself or the environment. Mice will receive blastocyst microinjection of the ES cells to create transmitting chimeras and chimera breeding for germline transmission. The procedure will be carried out by the **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The committee requested that the lab explain the biological activity of the insert genes in the Schedule G. **Community Member Comments:** None III-D, BSL-1	
26917		**Engineering Temperate Bacteriophage for Sustained Secretion of Protein Therapeutics or Immunogens by Mucosal Commensals**
Tabled	**Summary:** The purpose of the experiment is to develop genetically modified bacteriophage that can transduce genes of interest into commensal bacterial populations. In these studies, the lab will transduce bacteria both in vitro and in vivo (gnotobiotic mice). Temperate bacteriophage present in naturally occurring strains of commensal bacterial organisms will be used as the template for genetic modification. Bacteriophage will never be derived from host strains that have any known pathogenicity. Genomes of template bacteriophage will be modified by in-vitro natural transfection of exogenous, non-pathogenic genes generated by PCR amplification. Genetically modified phage will be induced by UV light and used to transduce additional strains of non-pathogenic commensal bacterial populations. In vivo transduction will be evaluated by oral gavage of	

<table>
<tr><td></td><td colspan="2">engineered bacteriophage into gnotobiotic mice. All work with recombinant viruses will be completed in a BSC. All waste will be autoclaved out of the animal facility.

Committee Comments: A representative from the lab spoke to the committee regarding the proposed research. The committee informed the representative that the lab needs to explain exactly what they are wanting to do in a flow chart. The committee also requested the lab to provide some literature on procedures and the history of research that has been completed. The committee agrees that this research might need a higher containment level and that they might need to seek NIH guidance on this proposed experiment.

Community Member Comments: None

III-D, BSL-2,</td></tr>
<tr><td>**36382**</td><td></td><td>**Pathogenic mechanisms of rubella virus**</td></tr>
<tr><td>**Approved with Stipulations**</td><td colspan="2">**Summary:** The purpose of this experiment is to prepare rubella virus stocks from an infectious clone in order to study rubella virus replication, pathogenesis, and immune control in cell culture and in mice. Virus stocks will be prepared from the wild-type clone virus. Mice will be infected with 10^1-10^5 infectious units in 10-100uL by intranasal, subcutaneous, intraperitoneal, and intravaginal routes. Mice will be anesthetized by isoflurane.

Committee Comments: This research needs to be listed as a category III-D, not a III-F. The committee requested that EHS work with the lab to create a vaccine SOP for rubella so that lab members can get vaccinated. There was currently a SOP for work with microorganisms where vaccine is recommended. Rubella was added to this SOP and finalized on 9/18/2017. The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-F, BSL-2, *E. coli,*</td></tr>
<tr><td>**36462**</td><td>**Stanley Lemon**</td><td>**Chimeric human rhinovirus (HRV 14) expressing human hepatitis A virus (HAV) pX sequence**</td></tr>
<tr><td>**Tabled**</td><td colspan="2">**Summary:** The aim of this experiment is to determine whether insertion of the HAV pX sequence into the P1 polyprotein coding region of HRV14 confers on distantly related HRV14 picornavirus the capacity to be released nonlytically from infected cells as quasi-enveloped virions. The lab proposes to modify a human rhinovirus 14 molecular clone by inserting the pX sequence of human hepatitis A virus in two different configurations, one in which HAV-pX is expressed as an insert between the VP1 capsid protein and 2A protein sequences, and another in which pX will replace the 2A sequence of HRV. In both cases the insert would be flanked downstream by an HRV14 3C protease cleavage site To accomplish this, the pX sequence will be cloned inot a plasmid containing an infectious cDNA copy of the HRV14 genome. RNA transcribed from the resulting construct will be transfected into mammalian cell in vitro under BSL-2 conditions.

Committee Comments: The committee is concerned with combining a human rhinovirus with a sequence of human hepatitis A virus. They believe that this is going to create a protective barrier for the rhinovirus. They have requested that the lab provide a safety plan to see if the new virus is infectious and resistant.

Community Member Comments: None

III-D, BSL-2, *E. coli,* HRV14</td></tr>
<tr><td>**37602**</td><td></td><td>**Cardiac morphogenesis in Zebrafish**</td></tr>
<tr><td>**Approved**</td><td colspan="2">**Summary:** The purpose of the experiment is the generation a transgenic line to modulate intraciliary calcium. The insert gene will be cloned into a plasmid, which will be injected into zebrafish embryos in vivo. The vector will be propagated in and purified from *E. coli* and linearized by restriction enzyme digestion prior to injection into zebrafish. The zebrafish will be anesthetized by tricane.

Committee Comments: This schedule G was reviewed and approved by the committee on 8/28/2017, due funding deadline and request from IACUC. The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

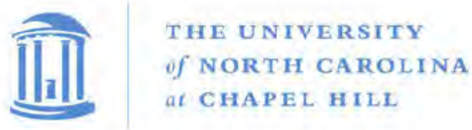
III-E, BSL-1, *E. coli*</td></tr>
</table>

36764	Lawrence Ostrowski	Investigation of Read-through Nonsense Mutations as a Treatment for Primary Ciliary Dyskinesia
Approved with Stipulations	**Summary:** The purpose of this experiment is to test and compare the ability of currently available agents (e.g., ataluren, geneticin, escin) to promote read-through of the SPAG1 founder mutation (c.2014>T [p. Gln672*]) in a reporter construct specifically in ciliated human airway epithelial cells. Lentiviruses expressing a reporter construct containing the FOXJ1 promoter, Renilla luciferase gene, WT or mutant SPAG1 gene sequence, and the Firefly luciferase gene, will be utilized to transduce human primary airway epithelial cells in vitro. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The committee requested that the lab explain what SPAG1 is in the Schedule G. **Community Member Comments:** None III-D, BSL-2, lentivirus	
36763	Lawrence Ostrowski	Determining the role of PCD genes, SPAG1 and GAS2L2, in airway epithelial cells
Approved with Stipulations	**Summary:** The purpose of the experiment is to knock out the SPAG1 gene or the GAS2L2 gene in their primary airway epithelial cell culture model using lentiviral transduction of CRISPR/Cas9 gene editing technology, followed by cilia functional studies. Lentiviruses expressing the Cas9 nuclease and guide RNAs for SPAG1 or GAS2L2 or a scrambled guide RNA will be utilized to transduce cells in vitro. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The committee requested that the lab explain what SPAG1 and GAS2L2 is in the Schedule G. **Community Member Comments:** None III-D, BSL-2, lentivirus	
37762	Scott Randell	AAV1 rescue of F508-del
Approved with Stipulations	**Summary:** The purpose of the study is to perform basic preclinical studies to develop a gene therapy for Cystic Fibrosis. The lab would like to test an AAV1 vector expressing a truncated version of CFTR. They would like to see if the vector can transduce primary human airway epithelial cells and then will measure CFTR function. The insert gene, CFTR, is packaged into an AAV1 viral vector which will be utilized to transduce cells in vitro. The function of CFTR will be measured in human primary cell cultures. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The PI answered "yes" to question 3 regarding animal work. Then in Section III wrote "N/A" for all answers. The committee requested the PI to answer "no" to question 3, since he is not working with animals. This will delete section III on the Schedule G. **Community Member Comments:** None III-D, BSL-2, AAV	
37822	[redacted]	Engineering Smart Cells for Cystic Fibrosis Therapy
Approved	**Summary:** Stem cell therapy may be one approach to help all people with cystic fibrosis (CF). In one strategy patient cells can be extracted and genetically corrected followed by reintroduction into the airway epithelium. However, the corrected cells face the challenge of re-entering the endogenous epithelium that also serves an important barrier to protect the host. The underlying hypothesis of this proposal is that "smart" cells that actively seek stem cell niches will help overcome this challenge. One of the lab's strategies is to transiently express receptors that impart enhanced directed migration to the cells to increase engraftment. The lab plans to conduct experiments in which mouse tracheal epithelial stem cells will be transduced with a replication deficient adenoviral vector that expresses the fluorescent protein EGFP. These cells will be re-instilled back into the mouse trachea and will be evaluated in a non-survival surgery procedure to track possible cell engraftment for up to a maximum of three hours. Animals will be deeply anesthetized with isoflurane or with injected Ketamine, Xylazine, or Acepromazine **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, adenovirus	

36902	Ronald Swanstrom	Understanding of HIV-1 Life Cycle
Approved	**Summary:** The purpose of the study is to analyze functions of genes encoded in the HIV-1 genome. The insert gene containing either partial or whole HIV-1 genome will be cloned into a plasmid, which will be transfected into mammalian cells in vitro. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2,	
36765		**Tet2(H1803R) Knock-in Mouse**
Approved with Stipulations	**Summary:** The purpose of the experiment is to make a new knock-in mutation, H1803R, to the Tet2 gene in mice. The germline knockout of Tet2 in mice is viable, so it is expected that the knock-in would also be viable. The point mutation H1803R of the mouse Tet2 gene will be knocked in gene will be inserted into C57BL/6 mice by CRISPR/Cas pronuclear injection. Single-cell mouse embryos will be microinjected with Cas9 protein, in vitro transcribed CRISPR guide RNA and oligonucleotide designed to insert the desired amino acid change. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The committee requested that the PI provide a description of what the Tet2 gene is in the Schedule G. **Community Member Comments:** None III-E, BSL-1	

4. Incident Report – Dr. Pylayeva-Gupta's Lab – Jessica Poole
   - Jessica updated the committee on the incident that occurred on July 21, 2017. This incident was reported to the NIH. The PI completed all of the proposed recommendations.
5. BSL-3 SOP
   - Both SOPs were reviewed and
6. Clinical Trial – Adverse Event Report –
   - updated the committee on the adverse event that was report to the IBC for It was determined that the symptoms that the individual was having was due to progression of the disease and not the study itself.
7. Subcommittee approval of exempt recombinant DNA:
   a. PI: Michael Bressan Title: Developmental Patterning of the Sinoatrial Node III-F
   b. PI: Yuhua Wang Title: Development of lipid nanoparticle for gene therapy against PD-L1 in the orthotropic colorectal tumor microenvironment
8. Schedule H report: 15
9. Next IBC meeting date: October 4, 2017 at 3:30 PM in BW 9001.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
December 6, 2017 3:30 PM
BW 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Aravinda Desilva, Barbara Savoldo, Tori Baxter, Garry Coulson, Jessica Pool

Members Absent: Barry McLamb, Frederick Sparling, Peggy Cotter, Craig Fletcher, Judy Nielsen, Mary Beth Koza

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests:

Open Meeting

1. –
2. Review minutes from the November 08, 2017 meeting.
   - The committee reviewed and approved the minutes.
3. Applications under review

	PI	Project Title
Approved with Stipulations	**Summary:** This study involves adoptive transfer Previous Phase I trial with showed no attributable adverse events and none of the patients developed A current phase I/II trial currently being conducted at has shown no dose limiting toxicities in the phase 1b portion of the trial at the maximum dose tested of For these studies, a RAC-approved was used to The current proposed study aims to build on this previous . Studies have shown that This study is a single center, open-label Phase I clinical trial designed to determin the safety of escalating doses of Patients will receive . The study will enroll up to who will be	

	given the option to complete patient reported outcomes (PRO) questionnaires during the study. **Committee Comments:** While the safety of [redacted] has been established in prior [redacted], the safety of [redacted] has not been clearly established. The Committee has requested the PI provide information (previous trial data and/or research publications) supporting the clinical precedent for safe use of [redacted] in human trials. **Community Member Comments:** None III-C
	[redacted]
Approved through *ad hoc* review	**Summary:** Please note this study is identical to [redacted] approved by the IBC 10/9/2017, except that this protocol is using a LOWER dose [redacted] **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-C
40277	[redacted] **Characterizing nsp12 (RdRp) point mutations and drug sensitivity mutants in highly pathogenic coronavirus**
Approved	**Summary:** The goal of these studies is to introduce two mutations into nsp12 RNA-dependent RNA polymerase (RdRp) from different MERS-CoV strains and assess whether these mutations affect sensitivity to the Gilead Science nucleoside analog inhibitor (GS-5734). **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL3, MERS-CoV
40278	[redacted] **MERS – CoV RNA secondary structure mutants based on SHAPE analysis**
Approved	**Summary:** The aim of this experiment is to evaluate the role of RNA secondary structures in MERS-CoV replication. Mutations will be introduced into the MERS-CoV genome based on SHAPE-derived RNA secondary structures. Affect of introduced mutations will be assessed by monitoring replication and virulence in cell culture. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None IIID, BSL3, MERS-CoV

40279		BioID for MERS – CoV genes and open reading frames in the context of whole virus
Approved	**Summary:** The purpose of this experiment is to map interactomes of proteins produced by MERS-CoV genes and open reading frames to be able to gain insight upon how they may be modulating host immune responses or contributing to viral replication. BirA tags will be added to select open reading frames and the effects of the tags will be monitored through viral passage in cell culture. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None IIID, BSL3, MERS-CoV	
39902	**Richard Cheney**	**Myosin-X and the molecular basis of filopodia function: lentiviral vectors**
Approved	**Summary:** The goal of these experiments are to understand the cell biological function of myosin-X and its functions in cells and organisms in processes such as filopodia formation, spindle orientation, signaling and cancer cell biology. Lentiviral vectors will be used to transiently or stably knock down cytoskeletal and regulatory proteins like Myo-10 in cell culture experiments, and the effects of the knockdown on the above-mentioned processed studied in various cell lines. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentiviral vectors	
40442		**An addition to Sched G ID: 38728 A safe Non-humanized mouse model for HIV-1 infection**
Approved with Stipulations	**Summary:** The purpose of this experiment is to generate chimeric HIV viruses using genes from either the NL-4 or NDK strains that either possess parental HIV-1 U3 or have been pseudotyped with MLV-U3. The resulting viruses termed NL-4-Eco/MLV-U3; NL-4-Eco/HIV-U3; NDK-Eco/MLV-U3 and NDK-Eco/HIV-U3 will be assessed in in vitro (human and mouse cell lines) and in vivo studies. **Committee Comments:** The Committee requested that the title be altered to remove "An addition to Sched G ID: 38728" and similar references to the former Schedule G throughout the submission. **Community Member Comments:** None III-D, BSL-2, replication-competent retrovirus	
40422	**Stanley Lemon**	**Hepatitis B virus (HBV) interactions with the innate immune system**
Approved	**Summary:** The goal of these studies is to establish viral replication with human hepatocyte-derived cells by transfection of plasmid DNA encoding pregenomic RNA of a replication-competent HBV genome under control of the CMV immediate-early promoter. Replication will be monitored by immune staining of cells and/or PCR. Replication-deficient lentiviral vectors will be used to knock down specific genes that may inhibit propagation of HBV in cultured cell lines. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, replication-deficient lentivirus	

39582	███	Correction of Intron 22 Inversion in Hemophilia A dogs - 2
Approved	**Summary:** The goal of this study is to use recombinant AAV vectors containing a DNA nuclease to correct Intron 22 inversion in hemophilia A dogs. Dogs will receive the AAV vector intravenously up to a dose of 10e13 vg/kg. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1, AAV	
39583	███	**Gene therapy of Hemophilia A and B-lentivirus**
Approved	**Summary:** The goal of this study is to use recombinant replication-deficient lentiviral expressing genes for coagulation factor VII, VIII, IX or von Willebrand factor to correct hemophilia A and B in dogs. Dogs will receive the lentiviral vector intravenously up to a dose of 10e11 U/ml. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. Committee requested updating the IACUC protocol number in Section III from 14-111 to 17-087. **Community Member Comments:** None III-D, BSL-2, lentiviral vector	
39702	███	**AAV directed expression of FV or FX in dogs with hemophilia**
Approved	**Summary:** The goal of this study is to use recombinant AAV vectors expressing FVIII or FIX to correct hemophilic coagulopathy dogs. Dogs will receive the AAV vector by intravenous, portal vein, hepatic artery or intramuscular routes. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, AAV	
40502	███	**Gene Therapy of Hemophila A and B**
Tabled	**Summary:** The purpose of these experiments is to use AAV vectors expressing coagulation factor VIII and IX or von Willebrand factor to correct hemophilia in dogs. **Committee Comments:** The Committee found this Schedule G to be incomplete, difficult to understand with incorrect information provided at various sections. Committee recommended the protocol be resubmitted after revision. **Community Member Comments:** None III-D, BSL-1, AAV	
39363	███	**Excitatory and Inhibitory Chemogenetic and Optogenetic Manipulations (Rats)**
Not Reviewed at PI request	**Summary:** N/A **Committee Comments:** N/A **Community Member Comments:** N/A III-D. BSL-1, AAV / HSV	

<table>
<tr><td>40023</td><td></td><td>Generation of transgenic mice of inducible caspase 3 in hepatocytes</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of these experiments is to create transgenic mice expressing mouse inducible caspase 3 in hepatocytes. The gene iCasp3 will be cloned into a BAC vector which will be used to transduceNOD-Rag1null-IL2Rgamma null mouse embryos in vitro. Resulting embryos will be seeded into female mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-1, BAC vector</td></tr>
<tr><td>40024</td><td></td><td>Generation of transgenic mice of HSV-TK in hepatocytes</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of these experiments is to create transgenic mice expressing herpes simplex virus (HSV) thymidine kinase (TK) in hepatocytes. The inducible TK gene will be cloned into a BAC vector which will be used to transduce NOD-Rag1null-IL2Rgamma null mouse embryos in vitro. Resulting embryos will be seeded into female mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-1, BAC vector</td></tr>
<tr><td>40030</td><td></td><td>Generation of transgenic mice of caspase 9 fused with FKBP in hepatocytes</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of these experiments is to create transgenic mice expressing caspase 9 fused with two binding domains of FK506 binding protein (FKBP) in hepatocytes. The inducible caspase-9 gene will be cloned into a BAC vector which will be used to transduce NOD-Rag1null-IL2Rgamma null mouse embryos in vitro. Resulting embryos will be seeded into female mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

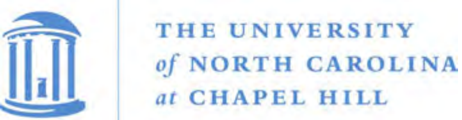
Community Member Comments: None

III-D, BSL-1, BAC vector</td></tr>
<tr><td>40282</td><td></td><td>Cancer epigenetics: Understanding chromatin modification in cancer</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The goal of this study is to express phf19, ezh2 or en1 genes in retroviral or lentiviral vectors and assess their role in chromatin modification in cancer in mouse strains such as NOD.SCID, NSG, b/6 or balb/c.

Committee Comments: This protocol involves infecting mice with transfected cell lines. However, the PI answered “NO” to the question “Will recombinant or synthetic nucleic acid molecules be introduced into whole plants or animals? The Committee recommended tabling this protocol and requested resubmission after the answer had been changed to “YES” and the corresponding Section III: GENE TRANSFER EXPERIMENTS INVOLVING WHOLE ANIMALS OR PLANTS be completed.

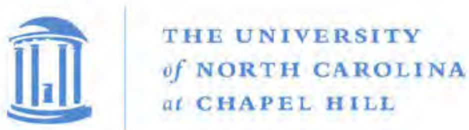
Community Member Comments: None.

III-D, BSL-2, retroviral / lentiviral vectors</td></tr>
</table>



4. Updates to Dual Use Research of Concern Policy for approval by Committee – Garry
5. Use of cell phones in the lab - Garry
6. Subcommittee approval of exempt recombinant DNA: 4
7. Schedule H report: 15
8. Next IBC meeting date: January 10, 2018 at 3:30 PM in Hooker 3100.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
January 10, 2018 3:30 PM
Hooker 3100

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Barbara Savoldo, Stanley Lemon, Judy Nielsen, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole

Members Absent: Barry McLamb, Peggy Cotter, Aravinda Desilva, Craig Fletcher

Ad hoc Members (not requested to be present): Ann Matthysse

Guests:

Open Meeting

1. **– Presentation of study proposal (**

presented a proposed study to identify genes responsible for specific disease outcomes following infection of mice. In this proposal, Collaborative Cross (CC) mice housed in will be infected with a mouse-adapted strain of . After infection with , mice will be euthanized, and select tissues and blood collected from the infected mice. All tissue and blood samples will be inactivated to render the samples non-infectious. Inactivation of samples will be performed at in accordance with the standardized and validated inactivation protocols at prior to shipping the samples to UNC. Samples will be inactivated by one of three validated procedures: i) trizol-treatment, ii) formalin-fixation, or iii) gamma-irradiation. Inactivated samples with their accompanying inactivation documents will then be shipped to UNC as non-infectious samples for downstream processing in BSL-2 containment. Trizol-treated samples will undergo chloroform extraction to isolate RNA which will be used for RT-PCR analysis. Formalin-fixed tissues will be provided to the for embedding and sectioning and returned to the lab for microscopy analysis. Irradiated blood samples will be analyzed by Stago START Hemostasis Analyzer.

Constructive and rigorous discussion regarding the proposed study was had regarding the safety of the inactivated samples, and any concerns on receiving samples that were not individually tested to confirm inactivation. It was the consensus of the attendees that the methods to inactivate the -infected samples were scientifically valid, having a long history of microbiological use, not just for but for a variety of other viral and bacterial pathogens. It was commented upon numerous times that the samples will have gone through more than one independent inactivation procedure, thus rendering the risk for these samples extremely low. The Committee was also remind that enveloped viruses like are extremely susceptible to inactivation. Furthermore, the genomic RNA from the virus is non-infectious. It was further pointed out that has an excellent reputation and track-record, and has been performing these inactivation procedures on a daily basis for a number of years. Communications from Chief of Virology, indicated that there have been zero inactivation failures using these methods for the last 8 years at . Furthermore, these inactivation protocols have been reviewed by the Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins (DSAT). Thus, the Committee overall felt confident in the safety and effectiveness of these procedures and that there existed negligible institutional risk for these samples. There were no objections voiced against the work being performed at UNC.

Regarding the formalin-fixed samples that will be transferred to the [redacted] at UNC, it was indicated that approval would be required by the Head of the core facility before submitting samples for embedding and sectioning. Further, it was suggested that section shavings be collected and discarded as is currently done for samples containing material with recombinant DNA,

Lastly, it was decided that a Communication Plan would need to be drafted in alliance with School of Public Health. The purpose of this document would be to explain the study, the inactivation procedures and the rationale supporting why these samples are non-infectious and non-biohazardous to those working with them, or anyone who might be occupationally exposed to them.

2. Review minutes from the December 06, 2017 meeting.
    - The committee reviewed and approved the minutes.
3. Applications under review

<table>
<tr><th></th><th>PI</th><th>Project Title</th></tr>
<tr><td>41242</td><td>[redacted]</td><td>Generation of mouse-adapted [redacted] chimeric viruses containing the mouse-adapted spike Y436H, S442F, T1118I and N1169D substitutions</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: Introduce substitutions into mouse-adapted chimeric [redacted] harboring spike gene from bat [redacted]-like viruses and assess which mutations are required for mouse adaptation. Replication and virulence will be monitored in vitro in tissue culture and in vivo in mouse model system.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee would like “enhanced replication” to be quantified as in previous Schedule G’s. Enhanced growth of 1-log over wildtype has been the marker in previous protocols.

Community Member Comments: None

III-D, [redacted] BSL-3</td></tr>
<tr><td>40982</td><td>[redacted]</td><td>Adaptive therapy to delay tumor resistance to immune checkpoint inhibitors</td></tr>
<tr><td>TABLED</td><td colspan="2">Summary: Use commercial lentiviral particles to transduce B16F0 murine melanoma cells to express fluorescent proteins under hEF1/TRE3G promoter, or lentiviral particles to knockdown IFN-gRa. Transduced cells will be injected into C57 and Rag-/- mice.

Committee Comments: Protocol was tabled due to answer of “NO” provided to the question asking whether recombinant molecules will be introduced into live animals.

Community Member Comments: None

III-D, lentiviral vectors, BSL-2</td></tr>
</table>

<table>
<tr><td>39106</td><td></td><td>Modification of Stem Cells with Diagnostic and Therapeutic Transgenes - 2017</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: Use of 3rd generation SIN lentiviral vectors to transduce a number of tumor cell lines with a variety of diagnostic or therapeutic genes. Transduced cells are evaluated for in vitro therapeutic efficacy using therapeutic combinations of conditioned media or cell co-cultures. In vivo, these cells are transplanted in the relevant organ of C57, Nude or SCID mice to establish tumor xenografts. Tumors are then treated with different stem cells expressing therapeutic transgenes.

Committee Comments: IACUC number provided is expired. Needs to be replaced with renewed protocol ID. In Section III, the answer of “N/A: we don’t inject live virus into animals” was provided. This is not an appropriate response since viral transduced cells are introduced into animals.

Community Member Comments: None

III-D, lentiviral vectors, BSL-2</td></tr>
<tr><td>41043</td><td></td><td>Mouse Model of Adult Heart Disease</td></tr>
<tr><td>TABLED</td><td colspan="2">Summary: Murine Pck2 cDNA will be cloned into pTRE-Myc plasmid which will be injected into mouse embryos in vivo to generate Pck2 transgenic mouse line overexpressing Pck2 gene in the heart.

Committee Comments: Protocol was tabled due to answer of “NO” provided to the question asking whether recombinant molecules will be introduced into live animals.

Community Member Comments: None

III-E, BSL-1</td></tr>
<tr><td>41062</td><td></td><td>Mouse Model of Adult Heart Disease</td></tr>
<tr><td>TABLED</td><td colspan="2">Summary: shRNA against rbfox1, Carm1, Ehmt2 or Kdm2 will be cloned into adenoviral vector rAAV9.U6::shRNA which will subsequently be injected into the anterior-dorsal subcutis of the neonatal mouse heart.

Committee Comments: Protocol was tabled due to answer of “NO” provided to the question asking whether recombinant molecules will be introduced into live animals.

Community Member Comments: None

III-D, adenoviral vectors, BSL-2</td></tr>
<tr><td>40705</td><td>Adriana Lopez</td><td>Generation of Pluripotent Stem Cells</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The purpose of this experiment is to generate pluripotent stem cells from somatic cells using a commercially available Sendai virus. Pluripotent genes OCT4, SOX2, KLF4 and CMYC will be cloned into the Sendai virus and exposed to primary fibroblast cells and blood cells.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

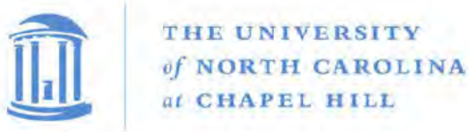
III-D, Sendai virus, BSL-2</td></tr>
</table>

40582		Vector Mediated Delivery of p450scc to Increase Steroidogenesis
APPROVED	**Summary:** The purpose of this experiment is to overexpress proteins involved in steroidogenesis in rodent neuronal cells in vitro and rodent brain in vivo using AAV2 vector to determine the role of neurosteroid production. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-E, AAV, BSL-1	
40502		Gene Therapy of Hemophilia A and B
APPROVED	**Summary:** The aim of this experiment is to express human or canine coagulation Factor VIII and/or IX and/or VWF in the liver of dogs that are deficient in these proteins using an AAV8 vector to assess correction of the hemophilic defect. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, AAV, BSL-2	
41362		Genome Editing of Sinusoidal Endothelium Stem Cells for Permanent Correction of Hemophilia A
APPROVED	**Summary:** The aim of this experiment is to use an AAV viral vector expressing the cDNA version of canine FVIII exons 23-26 to correct the intron 22 inversion that causes hemophilia A in dogs. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, AAV, BSL-2	
40842		Targeting Mechanisms of Cancer Metastasis
TABLED	**Summary:** The aim of this experiment is to explore the role of certain genes of interest in cancer, specifically lung and breast metastasis. Synthetically synthesized siRNA's or miRNA's will be cloned into plasmids or viral vectors which will be used to transduce cells *in vitro*. Some transduced cells will be injected into mice. **Committee Comments:** In Section III, the answer of "Not applicable, no vector is being used in the animal" was provided. This is not an appropriate response since viral transduced cells are introduced into animals. **Community Member Comments:** None III-D, lentiviral vectors, BSL-2	

39363	[redacted]	Excitatory and Inhibitory Chemogenetic and Optogenetic Manipulations (Rats)
APPROVED	**Summary:** The aim of this experiment is to introduced specific opsins, DREADDs or caspases into AAV vectors which will then be used to infect neuronal tissues to determine their influence over responses in the brain. Additionally, rats will be exposed to viral constructs by intracranial injection **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, AAV, BSL-1	
41222	[redacted]	Cancer Epigenetics: Understanding Chromatin Modification in Cancer
TABLED	**Summary:** The aim of these experiments is to understand the pathway of chromatin modification in cancer. Insert genes (phf19, ezh2 and en1) will be cloned into retroviral or lentiviral vectors and used to transduce common cancer cell lines of interest. These cell lines will then be xenografted into mouse strains NOD.SCID, NSG, B/6 or BALB/c. **Committee Comments:** The IACUC protocol number needs to be updated. Also, the Committee wanted clarification on whether any of the payloads represented oncogenes or tumor suppressor genes. The Committee can review out of cycle once a response is received. **Community Member Comments:** None III-D, retroviral / lentiviral vector, BSL-2	

4. Subcommittee approval of exempt recombinant DNA: 2
   PI: Sarah Cohen: Organelle Function and Dynamics
   PI: Andrew Lee: Thymidylate Synthase
5. Schedule H report: 10
6. Next IBC meeting date: 7 February 2018 – BW 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
February 07, 2018 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, , Aravinda Desilva, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole

Members Absent: Barry McLamb, Peggy Cotter, Barbara Savoldo, Craig Fletcher, Judy Nielsen

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests: [redacted]

Open Meeting

1. [redacted] – Presentation of Phase I Clinical Trial resubmission
2. Review minutes from the January 10, 2018 meeting.
   - The committee reviewed and approved the minutes.
3. Applications under review

ID	PI	Project Title
	[redacted]	[redacted]
APPROVED	**Summary:** The aim of this Phase I trial is to establish the [redacted] **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None	

	[redacted]	**AMENDMENT**. [redacted]
APPROVED	**Summary:** The aim of this amendment (Amendment #5) was [redacted] he Principal Investigator of the study was also updated from [redacted] **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None	
41703	Kristy Ainslie	Development of Amphotericin B resistant Leishmania donovani
TABLED	**Summary:** The goal of this experiment is to introduce Amphotericin B antibiotic-resistance into the parasite *Leishmania donovani* through exposure of the parasite to increasing doses of Amphotericin B **Committee Comments:** The Committee was concerned about the safety risks of potential occupational exposure to Amphotericin B *Leishmania donovani* in the lab. The Committee would like to see an SOP developed for this work covering aspects of the project from safe manipulation and culturing of the parasite, medical surveillance and post-exposure prophylaxis plans. The Committee did not feel this work warranted a Schedule G since no recombinant DNA research was being performed. The Committee would also like to see a presentation by the Ainslie lab on this proposed project. **Community Member Comments:** None III-A, BSL-2, no animals	
42002	[redacted]	CRISPR-mediated knockout in A375 xenograft study
APPROVED	**Summary:** The aim of this experiment is to test the effect of Pten knockout on tumor growth in melanoma xenografts. A375 human melanoma cells will be co-transfected with CRISPR/Cas9 knockout plasmids to abolish Pten expression in these cells. Transduced cells will then be injected into the flanks of mice and the effect of Pten knockout on tumor growth in the mice assessed. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, mice	
42360	[redacted]	Venezuelan Equine Encephalitis (VEE) BSL2 strain 3526 replicon expression of variant norovirus and sapovirus capsid genes
APPROVED	**Summary:** The aim of these experiments is to develop a series of Venezuelan equine encephalitis (VEE) replicon vector platforms expressing norovirus and sapovirus capsid genes with select mutations to help characterize these two viruses that have no currently validated cell culture or small animal models for propagation. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, VEE replicon, mice	

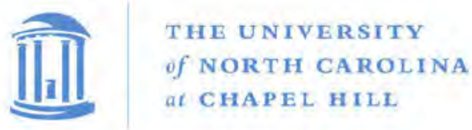
42361	[redacted]	BioID2 for MERS-CoV genes and open reading frames in the context of the whole virus
APPROVED	**Summary:** The purpose of this experiment is to use BioID2 tags to map the protein interactome of MERS-CoV to gain insight into how this virus modulates host immune responses or contributes to viral replication. BioID2 tags will be added to selected open reading frames within the context of the viral genome. Replication and virulence of tagged virus will be monitored through viral passage in cell culture and tittering. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3, no animals	
42382	[redacted]	Luciferase (lux) expressing BCG
APPROVED	**Summary:** The aim of this experiment is to produce a strain of BCG (the attenuated *Mycobacterium tuberculosis* vaccine strain) that is bioluminescent and can be used for non-invasive imaging of infected mice. Bioluminescent BCG will be produced by introducing an integrating plasmid expressing LuxABCDE from Photorhabdus luminescens into the bacterium. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, no animals	
41502	Douglas Cyr	Study of Hsp70/Hsp40 function in the ER protein quality control
APPROVED	**Summary:** The aim of this experiment is to study Hsp70/Hsp40 function in ER protein quality control by using lentiviral vectors expressing CRISPR and specific guide RNA to target genes of interest in cultured COS-7, HEK293 and lung epithelial cells. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, no animals	
41882	[redacted]	HIV-specific lentiCas9 and single guide RNAs vectors for HIV eradication
APPROVED	**Summary:** The aim of this experiment is to evaluate HIV-specific lentiviral Cas9 single guide RNA vectors in their ability to inhibit HIV replication in mammalian cells and humanized mice. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, mice	

41902	[redacted]	HIV-specific chimeric antigen receptor (CAR) T-cell vectors for HIV eradication
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to evaluate HIV-specific CAR vectors in their ability to inhibit HIV replication in mammalian cells and humanized mice. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee noted in one of the sections of the protocol it was stated that vector was being injected directly into the mouse, whereas in all other parts of the protocol it was indicated that the vector was being used to transduce cells which were being injected into the mouse. The Committee wanted this error to be corrected for consistency throughout the protocol. **Community Member Comments:** None III-D, BSL-2, mice	
42142	Eduardo Lazarowski	Abnormal nucleotide release/metabolism in dehydrated airways
APPROVED	**Summary:** The purpose of this experiment is to use lentiviral vectors expressing CRISPR-Cas9 or shRNAs to either knockdown or overexpress genes of the nucleotide release pathway in human airway epithelial cells and assess the effects of knockdown or overexpression on cell function. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus, no animals	
41702	[redacted]	Genetic analysis of chlamydial virulence
TABLED	**Summary:** The aim of this experiment is to identify and characterize genes in Chlamydia that are required for virulence and biological fitness. Genes will either be mutated or over-expressed in C. trachomatis or C. muridarum strains and effects assessed in cell culture or animal models of genital tract infection. **Committee Comments:** The Committee felt that not enough details were given regarding which genes were being targeted in the mutation and complementation analyses. The Committee would like to a more detailed list of targets for this work. The Committee would also like to see a risk analysis statement in the event a mutant, or expression strain, happens to be hypervirulent. **Community Member Comments:** None III-D, BSL-2, mice	
41722	Scott Randell	Element genomics collaboration
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to increase CFTR expression in human airway epithelial cells using lentiviral vectors expressing wildtype CFTR or inducible CFTR. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee wanted the title of the protocol to be changed to something more descriptive of the research. **Community Member Comments:** None III-D, BSL-2, lentivirus, no animals	

41602	████	Invivofectamine 3.0/USP15 siRNA complex
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to use siRNA to knock out USP15 in mice. Mice will be injected via tail vein with a mixture of Invivofectamine/siRNA and at selected time points post treatment, the mice liver responses to the siRNA mixtures will be examined. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee noticed some disparity in the volumes proposed for injecting mice compared to IACUC protocol. Wanted the volumes to be changed to be consistent with approved animal protocol. **Community Member Comments:** None III-E, BSL-1, mice	
42162	████	Plasmids containing guide RNAs
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to introduce plasmids containing guide RNA's that target the antisense transcript of the paternal allele of UBE3A in mice. Specified genes will be inserted into plasmids and electroporated in utero into the unborn pups. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee wanted verification that the proposed research with animals was covered by an IACUC protocol or pending amendment. **Community Member Comments:** None III-D, BSL-1, mice	

4. Subcommittee approval of exempt recombinant DNA: 3
   PI: Maria Azcarate-Peril: Microbiome Core Facility Research Lab (ID 71282) (III-F)
   PI: Beverly Errede: Mechanisms of Noise Regulation in Cell Fate Transitions (ID 40622) (III-F)
   PI: Beverly Errede: Spatiotemporal Modelling of Signal Transduction in Yeast (ID 406202) (III-F)
5. Schedule H report:10
6. Next IBC meeting date: 07 March 2018, Burnett-Womack (Room 9001) at 3:30pm

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
March 7, 2018 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Keith Porterfield, Amy Sims, Barbara Savoldo, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole

Members Absent: Xiao Xiao, Peggy Cotter, Aravinda Desilva, Craig Fletcher, Judy Nielsen

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests: [redacted]

Open Meeting

1. Review minutes from the February 7, 2018 meeting.
   - The committee reviewed and approved the minutes.
2. Applications under review

ID	PI	Project Title
43244	[redacted]	PAR-1 in chemotherapy
Tabled	**Summary:** The Aim of this experiment is to use plasmid-mediated CRISPR/Cas9 insertion of LoxP sites into the introns of the mouse F2rl3 gene (protease-activated receptor 4 gene) **Committee Comments:** In Section III, answers of "n/a" were provided to many of the questions related to gene transfer into whole animals. Even though the Core Facility is performing these experiments, the PI is expected to provide this information. **Community Member Comments:** None III-D, BSL-2, mice	
42562	[redacted]	Studying the biology of recombinant AAV vectors
Approved with Stipulations	**Summary:** The purpose of this experiment is to express fluorescent or luminescent reporter genes from AAV via different promoters in order to characterize the expression of various capsid engineered genes in culture and in mice. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee required that a valid IACUC number be provided in support of the animal work. **Community Member Comments:** None III-D, BSL-2, AAV, mice	

42962	[redacted]	Generation of full-length infectious clone of bat [redacted]-like coronavirus WIV16-CoV, including reporter-expressing variants
Approved	**Summary:** The Aim of this experiment is to generate a reverse genetic infectious clone system for the bat [redacted]-like coronavirus WIV16-CoV. The viral genome will be cloned as 5-7 cDNA fragments maintained in E. coli. Replication and virulence of the virus will be assessed in cell culture and in mice. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design **Community Member Comments:** None III-D, BSL-3, mice	
42886	[redacted]	Genetically modified macrophages and engineered PBM
Approved with Stipulations	**Summary:** The Aim of this experiment is to express human Glial cell-derived neurotrophic factor (GDNF) in primary macrophages from C57bl/6 mice and then inject transfected cells into mice to assess the effect of GDNF expression on mouse physiology. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee required that a valid IACUC number be provided in support of the animal work, with details on mode of administration and volumes to be administered. **Community Member Comments:** None III-D, BSL-2, mice	
42902	Camille Ehre	CFTR knockout in HT-29 MTX E12 cell line
Approved with Stipulations	**Summary:** The Aim of this experiment is to transfect CFTR-KO HT-29 cells with plasmids expressing CFTR mutants and assess function of mutant CFTR genes. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee required that clarification be made on whether plasmids or viral vectors were being used for transfection of the cells. **Community Member Comments:** None III-F, BSL-2, no animals	
42885	[redacted]	Luciferase (Lux) expressing BCG
Approved with Stipulations	**Summary:** The Aim of this experiment is to monitor Bacille Calmette Guerin (BCG) replication and dissemination in humanized mice using a reporter strain of BCG expressing luciferase (Lux). **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that BCG be spelled out in the title for sake of clarity. **Community Member Comments:** None III-D, BSL-2, mice	

<table>
<tr><td>42782</td><td>Tal Kafri</td><td>Lentiviral vector packaging cassette</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The Aim of this experiment is to generate an improved packaging cassette for lentiviral vectors. To achieve this aim, the gag region from currently used lentiviral packaging constructs will be replaced by the gag region from the NDK strain of HIV.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that a reference to “in vivo” be removed from the application since an answer of “NO” was provided to whether rDNA will be used in whole animals.

Community Member Comments: None

III-D, BSL-2, lentiviral vectors</td></tr>
<tr><td>42666</td><td></td><td>Engineering temperate bacteriophage for sustained secretion of protein therapeutics for immunogens by gut commensals</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The Aim of this experiment is to create bacteriophages that can transduce genes of interest into commensal bacterial populations in vitro and in vivo. Bacteriophages present in naturally-occurring strains of commensal bacteria will be engineered to express several DNA constructs, including RFP fluorescent reporter genes, antibiotic resistant genes (erythromycin), gluten peptidase enzyme (Kumamax) and immunogens (HSV proteins).

Committee Comments: The Committee was concerned about the transfer of antibiotic resistance to bacteriophages that had the potential to transduce unknown commensal bacteria with antibiotic resistance cassette and the possibility of horizontal transfer of the cassette to possible pathogen which might result in compromise of the antibiotic for treatment.

Community Member Comments: None

III-D, BSL-2, mice</td></tr>
<tr><td>42887</td><td></td><td>Modeling cholangiocarcinoma in mice</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The Aim of this experiment is to induce formation of cholangiocarcinoma (primary biliary tumors) in mice to better understand the cellular and molecular mechanisms of the disease. To achieve this, insert genes (Akt and NICD1) will be cloned into a piggyBAC transposon shuttle vector which will be injected into mice by tail vein injection. Tumor growth will be monitored in vivo through the use of a near-infrared reporter gene (iRFP670).

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested confirmation that volumes proposed were within approved IACUC ranges for the respective routes of administration.

Community Member Comments: None

III-D, BSL-2</td></tr>
<tr><td>42609</td><td></td><td>Sendai virus infection of airway epithelium in vitro and in vivo</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The Aim of this experiment is to generate recombinant Sendai virus (mouse parainfluenza virus 1) expressing GFP or luciferase as a genetic tool to measure the extent and duration of virus infection in vitro and in vivo.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that BCG be spelled out in the title for sake of clarity.

Community Member Comments: None

III-D, BSL-2, Sendai virus, mice</td></tr>
</table>

<table>
<tr><td>42722</td><td></td><td>Contributions of glial glutamate transport and transmission to drug abuse</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The Aim of this experiment is to express Lck membrane tagged GFP on the surface of astrocytes using the GFAP promoter. GFP expression in these astrocytes will be monitored following cocaine or saline administration in mice. AAV vectors will be used to transduce cells with the GFP plasmid.

Committee Comments: The application appeared to be scrambled, with the answers not seemingly connected to the questions. There appears to be some transposition error leading to an unreviewable protocol.

Community Member Comments: None

III-D, BSL-2, AAV, mice</td></tr>
<tr><td>42723</td><td></td><td>Protective effects of glutamate transporter GLT-1 over expression</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The Aim of this experiment is to overexpress glutamate transporter GLT-1 in astrocytes in the rat using an AAV8 vector with GLT-1 under the control of an astrocyte-specific promoter. The hypothesis is that overexpression of GLT-1 will protect against decreases in astrocyte shape after exposure to cocaine and also reduce cocaine craving.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, AAV, rats</td></tr>
<tr><td>38302</td><td>Monroe Stutts</td><td>CFTR and ENaC interactions</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The Aim of this experiment is to generate stable cell lines expressing CFTR and/or ENaC in epithelial cells. The genes will be cloned into lentiviral vectors which will be used to transduce mammalian (MDCK, CHO and HEK) and primate (COS7) cell lines in vitro.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. Although no work with animals was proposed, the PI provided an IACUC#. Approval is dependent on the PI confirming no animals are to be used and removal of the IACUC# to prevent confusion.

Community Member Comments: None

III-D, BSL-2, lentivirus, no animals</td></tr>
<tr><td>43062</td><td></td><td>Rapid identification of cocaine sensitivity genes using a novel reduced complexity cross</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The Aim of this experiment is to use Cas9-mediated allele replacement in mouse embryo's to identify genes required for cocaine sensitivity.

Committee Comments: The Committee felt this application was premature in that the genes to be replaced had not yet been identified. The Committee could not approve an application in which the target genes were not listed.

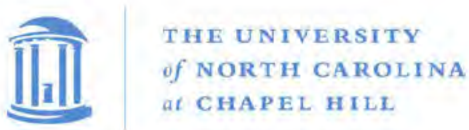
Community Member Comments: None

III-D, BSL-1, mice</td></tr>
</table>

43282	[redacted]	Study of oxygen sensing pathway in cancer
Approved with Stipulations	**Summary:** The Aim of this experiment is to clone target genes (EgIN2, FOXO3a, EgIN1, HIF and ZHX2) into lentiviral or AAV vectors which will then be used to transduce breast cancer cells or renal carcinoma cells to understand the role of these genes in oxygen sensing pathway in cancer. Transduced cells will also be injected into mice for in vivo evaluations. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that an approved IACUC# be provided. **Community Member Comments:** None III-D, BSL-2, AAV, mice	

3. Update on NIH Reportable Incidents – Garry Coulson
4. **Subcommittee approval of Schedule G Principal Investigator transfers:** 2
   PI: [redacted] Allele-specific homing endonuclease gene editing in adRP mice using self-complimentary AAV (# 42502) (III-D) (transfer of Schedule G protocol # 18895 from [redacted]
   PI: [redacted] Overcoming our clinical complications: AAV vector design for the treatment of DMD (# 42463) (III-E) (transfer of Schedule G protocol # 13989 from [redacted])
5. **Full Committee *ad hoc* approval of Schedule G's:** 3
   PI: [redacted] (# 42462) Muscle delivery of AAV vectors for gene therapy (III-D)
   PI: [redacted] (# 42482) Gene therapy with AAV vectors (III-E)
   PI: [redacted] (# 42522) Muscle delivery of AAV vectors for gene therapy (III-D)
6. **Schedule H report:** 33
7. **Next IBC meeting date:** April 11, 2018 in Hooker 3005 at 3.30pm.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
April 16, 2018 3:30 PM
Hooker 3005

Members Present: Review of Schedule G's conducted via electronic mail

Members Absent: n/a

Ad hoc Members (not requested to be present): n/a

Guests: None

Open Meeting

1. Applications under review

ID	PI	Project Title
43244		Generation of a floxed F2rl3 (PAR4) mouse
APPROVED	**Summary:** The aim of this experiment was to generate a transgenic mouse with LoxP sites inserted in the introns of F2rl3 using Cas9 system. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1, mice	
43882		Coronavirus transcription regulatory network remodeling and its effects on replication and virulence – 2018 renewal
APPROVED	**Summary:** The aim of this experiment is to explore the contribution of transcription regulatory sequences (TRSs) to coronavirus replication and virulence. An infectious clone of the betacoronovirus will be generated and TRNs systematically altered. The effect of changes to the TRNs will be assessed in vitro in cell culture and in vivo in mice. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3, mice	
43842		Characterization of Mycobacterium tuberculosis pathogenesis: roles of M. tuberculosis secreted proteins in virulence
APPROVED	**Summary:** The aim of this experiment is to characterize the role of secreted protein in Mtb virulence. This will be achieved by standard molecular methods in which desired genes are either deleted, or overexpressed in the relevant background strain. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3	

44042		Lentiviral transduction of murine fibroblasts with deactivated Cas9-VP64
APPROVED	**Summary:** The aim of this experiment is to create a stable cell line from primary murine vibroblasts that expresses a deactivated Cas9 fused to two domains of the gene activator VP64. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, mice	
43682	Martina Gentzsch	Regulation and trafficking of CFTR
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express the CFTR gene (wildtype or F508del) in the CFBE cell line to explore regulation and trafficking of CFTR inside the cell. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. Committee required changing of the classification of the experiments from III-F. **Community Member Comments:** None III-F, BSL-2, lentivirus	
43522		Ros26-LSL-Apobec3 mouse
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to generate a transgenic mouse with the murine Apobec3 gene under control of a LoxP-STOP-LoxP cassette knocked into the Rosa26 locus. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. The Committee wanted Section III of the protocol to be completed with clarification of the fate of injected embryonic cells and methods to restrain animals. **Community Member Comments:** None III-E, BSL-1	
43982		Introduction of recombinant DNA into bacterial pathogens
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to engineer Salmonella typhimurium to express flagellin under different promoters (high or low copy plasmids) and compare the effect on bacterial replication in vivo. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. The Committee wanted a valid IACUC number to be provided that supported the use of animals and more descriptive details on PPE. Lastly, Committee wanted a statement addressing the possibility of generating a hypervirulent strain during the mutation/complementation stage of the experiments. **Community Member Comments:** None III-D, BSL-2	
43403		Regulatory T cells promote alveolar epithelial repair
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express Sik1 gene in HeLa or MT-2 cells and assess how expression of these gene affects regulatory T cell function in vitro. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. The Committee requested that the IACUC number be removed if no animals being used and that the title be changed so that it was not identical to Schedule G 43422. **Community Member Comments:** None III-D, BSL-2, lentivirus	

<table>
<tr><td>43422</td><td></td><td>Regulatory T cells promote alveolar epithelial repair</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to use lentiviral particles to express short hairpin RNA sequences against Foxp3 to determine if Foxp3 knockdown affects regulatory T cell function in vitro

Committee Comments: The proposed safety and containment practices are adequate for the experimental design. The Committee requested that the IACUC number be removed if no animals being used and that the title be changed so that it was not identical to Schedule G 43403.

Community Member Comments: None

III-D, BSL-2, lentivirus</td></tr>
<tr><td>44003</td><td></td><td>Targeting mechanisms of cancer metastasis</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to explore the role of certain genes/targets of interest in cancer (specifically lung and breast) metastasis. Target genes or siRNA’s to target genes will be cloned into plasmids or viral vectors which will be used to transduce cells in vitro. Transduced cells will then be injected into mice. Alternatively, animals will be injected with nanoparticles coated in siRNA or shRNA.

Committee Comments: The proposed safety and containment practices are adequate for the experimental design. The Committee requested more details on the injection of cells into animals (route, concentration, volume).

Community Member Comments: None

III-D, BSL-2, lentivirus</td></tr>
<tr><td>43462</td><td>Dale Ramsden</td><td>DNA double strand break repair</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to express genes involved in DNA repair (e.g. DNA ligase IV, Ku, DNA polymerase theta) into viral vectors and transfect mammalian cells (human HTC116 or mouse fibroblasts) to assess the role these genes play in DNA double strand break repair.

Committee Comments: The proposed safety and containment practices are adequate for the experimental design. The Committee noted that since lentiviral vectors were being used, this should not be classified as a III-F.

Community Member Comments: None

III-F, BSL-2, lentivirus</td></tr>
<tr><td>43722</td><td>Scott Randell</td><td>Cystic fibrosis research and translational core center: Core C Cell Models Core</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is express genes (telomerase reverse transcriptase, Bmi-1) in human airway epithelial cells to assess the affect on cell life in transduced cells.

Committee Comments: The proposed safety and containment practices are adequate for the experimental design.

Community Member Comments: None

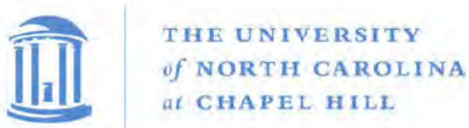
III-D, BSL-2, lentivirus</td></tr>
</table>

43723	Scott Randell	Identification of airway epithelial stem cells
APPROVED	**Summary:** The aim of this experiment is express genes (telomerase reverse transcriptase, Bmi-1) in human airway epithelial cells to assess the affect on cell life in transduced cells. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus	
43762	Scott Randell	Research and development program component II: Epithelial function in cystic fibrosis
APPROVED	**Summary:** The aim of this experiment is express genes (telomerase reverse transcriptase, Bmi-1) in human airway epithelial cells to assess the affect on cell life in transduced cells. A second aim is to knock out genes of interest using CRISPR/Cas9. A third aim is to create a dominant negative construct for expression of protein of interest. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus	
42722	[redacted]	Role of nucleus accumbens GLT-1 expression in the cellular and molecular adaptations that underlie cocaine seeking
APPROVED	**Summary:** The aim of this experiment is to assess the effects of cocaine self-administration on the structural properties of astrocytes in the rat brain, and to assess the effects of protective GLT-1 (neuroprotective glutamate transporter) overexpression on the structural properties of astrocytes. GFAP-Lck-GFP and GFAP-GLT-2 will be into AAV vectors which will be microinjected into rat brain nucleus accumbens. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, AAV, rats	
43782	[redacted]	Use of replication incompetent retroviral vectors to label cultured cells and single cells in mouse embryos
APPROVED	**Summary:** The aim of this experiment is use MMLV-derived viral vectors to carry reporter genes (EGFP and alkaline phosphatase) into infected cell cultures (HEK293T) and mouse pup embryos to study cell proliferation and differentiation. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, MMLV, mice	

43642	David Williams Jr.	Inhibition of MBD2-NuRD function
APPROVED	**Summary:** The aim of this experiment is to express small peptides from human GATAD2A and MBD2 in lentiviral vectors to assess whether binding of these peptides to MBD2-NuRD proteins induces ubiquitinylation and degradation. **Committee Comments:** The proposed safety and containment practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus	

2. Subcommittee approval of exempt recombinant DNA: 1
3. Schedule H report:
4. Next IBC meeting date: 2 May 2018 – BW 9001

Adjourn.



Meeting Minutes
June 13, 2018 3:30 PM
Hooker 3005

Members Present: Doug Cyr, Amy Sims, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole, Sandra Bradshaw, Aravinda Desilva, Barbara Savoldo, Xiao Xiao

Members Absent: Keith Porterfield, Peggy Cotter, Craig Fletcher,

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. Introduction of Xiao Xiao to the IBC Committee
2. Review minutes from the May 02, 2018 meeting.
   - The committee reviewed and approved the minutes.
3. Applications under review

ID	PI	Project Title
		PROTOCOL AMENDMENT #1.
APPROVED	**Summary:** This was an amendment to a protocol already approved by the IBC. In this amendment, changes to the protocol were made in response to FDA requirements. No changes to the vector construct or mode of administration were made. The main changes to the protocol were to the dose escalation and starting dose, and also eliminated the possibility for patients to receive a until a safe dose is determined and the data discussed with the FDA. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-C	
APPROVED	**Summary:** This study involves the use of an . **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None	

APPROVED	**Summary:** This study involves the evaluation of . **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-C	
APPROVED	**Summary:** This study involves the evaluation of **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-C	
		PROTOCOL AMENDMENT:
APPROVED	**Summary:** presented a time-urgent compassionate use protocol, , as an amendment to , to treat a patient with . The patients had previously received **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-C	

46022/47065	Ralph Baric	Gene knockout and overexpression approaches to gain insight into mammalian gene function during virus infection
APPROVED	**Summary:** The aim of this experiment is to knock out (using CRISPR/Cas9) or overexpress human genes in human cells, or mouse genes in mouse cells, to identify genes that affect virus replication. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, lentiviral vectors	
46023/47066	[redacted]	Expression of the Appalachian Ridge coronavirus spike in HKU5
APPROVED	**Summary:** The aim of this experiment is to express the Appalachian Ridge CoV spike in the HKU5 coronavirus background to evaluate the capacity of this gene from the novel CoV to promote infection and/or replication in bat, human and pig cells. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-3	
46024/47067	[redacted]	Generation of replicons from the attenuated 3526 strain of Venezuelan Equine Encephalitis (VEE) expressing the spike glycoprotein from either and HKU 2-related coronavirus or Appalachian Ridge coronavirus
APPROVED	**Summary:** The aim of this experiment is to express the spike glycoprotein from the Appalachian Ridge CoV or HKU2-related CoV in attenuated VEE-3526 (Venezuelan equine encephalitis) replicon particles. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-3, VEE, mice	
46025/47068	[redacted]	Reverse genetic clones for HKU 2 bat coronaviruses
APPROVED	**Summary:** The aim of this experiment is to design a reverse genetic clone of the bat CoV HKU 2.298, expressing native or the new HKU 2-related spike protein. The capacity of these viruses to infect and replicate in human, swine and bat cells will be assessed. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-3	

<table>
<tr><td>45682</td><td></td><td>Large animal model of steroid induced glaucoma – scAAV2.GRE.MMP1</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use AAV vectors to deliver genes of interest (e.g. MMP1) to the trabecular meshwork to assess their therapeutic potential. Viral vectors will be introduced into the sheep eye by intracameral or intravitreal injection.

Committee Comments: IACUC number needs to be updated to 18.146. The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, AAV, sheep</td></tr>
<tr><td>45862</td><td></td><td>Targeting calcification/stiffness in glaucoma with Matrix Gla. Ad-GFP-2A-iCre</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use adenoviral vectors to deliver genes of interest (e.g. GFP, iCre) to the trabecular meshwork to assess their therapeutic potential. Viral vectors will be used to flox the gene in loxP mice by intracameral or intravitreal injection.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, Adenoviral vectors, mice</td></tr>
<tr><td>45822</td><td></td><td>Lentiviral transduction of GFP</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to clone GFP into lentiviral vectors to express in cells in vitro to generate GFP-positive cell lines. Transduced cells will be injected into mice by tail-vein injection.

Committee Comments: IACUC number needs to be updated to 18.129. The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, lentiviral vectors, mice</td></tr>
<tr><td>45762/46262</td><td></td><td>Novel approaches for GVHD prevention</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to introduce GFP- or luciferase-expressing tumor cells (293T) in NSG and B6 mice by tail-vein injection and image where transduced cells implant and disseminate to.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, retroviral vectors, mice</td></tr>
<tr><td>46448</td><td></td><td>KSHV deletion viruses</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to delete genes from Kaposi sarcoma-associated herpesvirus (KSHV) to assess their importance in establishing infection using a mouse model. Deletion viruses, created using a BACmid vector system, will be injected into mice via i.p. or i.v routes.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee wanted KSHV spelled out in the title and a statement indicating what steps will be taken in the unlikely event the deletion viruses show hypervirulent phenotypes.

Community Member Comments: None

III-D, BSL-2, mice</td></tr>
</table>

46102		Investigation of the molecular cross talk between cancer and obesity
APPROVED	**Summary:** The aim of this experiment is to express GFP or luciferase in MMTVnew/HER murine breast cancer cells using a SIN retroviral expression vector (pRetroX-Tight-Puro). Transduced cells will be injected into mice by orthotopic injection into the mammary fat pad. **Committee Comments:** IACUC number needs to be updated to 18.163. Volume of cells to be injected needs to be added to the protocol. The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, retroviral vector, mice	
45466		Development of a mouse model to test natural killer (NK) cell-based immune strategies to clear latent HIV infection
APPROVED	**Summary:** The aim of this experiment is to transduce human NK cells with a retroviral vector expressing IL-15, inducible caspase 9 and NGFR. Engineered NK cells wills be assessed for an increase in cytotoxic potential and persistence in vivo in humanized mouse model system. **Committee Comments:** IACUC protocol pending approval. Approval of Schedule G is dependent on approval of IACUC protocol. The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, retroviral vector, mice	
45622		In vivo neuronal modulation via engineered G-protein coupled receptors
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express fluorescent protein (tdTomato) and mutant muscarinic receptors (hM3d or hM4D) in AAV viral vectors to assess the effect of the mutant muscarinic receptors on neuronal modulation. Mice will be injected with recombinant AAV via stereotaxic or retro-orbital routes. **Committee Comments:** The Committee wanted to see required details (volume and concetration) for the stereotaxic injections and retro-orbital routes. The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, AAV, mice	
46042		Generation of chimeric antigen modified T cells using a retroviral vector for anti-tumor therapy
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to transduce T cells with retroviral vectors expressing the single change variable fragment of an antibody to assess whether these modified T cells show enhanced tumor cell killing. **Committee Comments:** The Committee wanted the volume and dose of inoculum to be added to the protocol. Vector map should be included. The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, retroviral vector, mice	

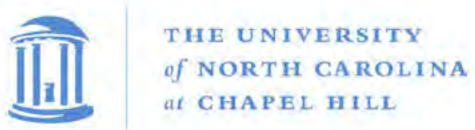
46202		KSHV deletion viruses
TABLED	**Summary:** The aim of this experiment is to delete genes from Kaposi sarcoma-associated herpesvirus (KSHV) to assess their importance in establishing infection using a mouse model. Deletion viruses, created using a BACmid vector system, will be injected into mice via i.p. or i.v routes. **Committee Comments:** The Committee noted that this was an identical submission to Dr. Damania and was unclear what the Su lab proposed to do in their lab. **Community Member Comments**: None III-D	
44826		NLRs in gastrointestinal inflammation and cancer – NLRC3 rDNA
APPROVED	**Summary:** The aim of this experiment is to express or knock down NOD-like Receptor Card-containing C3 (NLRC3) in cell lines to assess the impact on immune cells and cancer cells. Lentiviral vectors expressing the target sequences will be used to transduce immortalized and primary cells (HEK293T, T cells, FoxP3+ T cells) which will be introduced into humanized mouse models with and without cancer present. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, lentiviral vectors, mice	
45322	Yue Xiong	Retroviral Vectors of WT and R132H mutant of human IDH1 gene
APPROVED	**Summary:** The aim of this experiment is to use retroviral vectors to express the wildtype and R132H mutant form of the IDH1 gene in human cell lines. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, retroviral vectors	
45342	Yue Xiong	Lentiviral of WT and R132H mutant of human IDH1 gene
APPROVED	**Summary:** The aim of this experiment is to use lentiviral vectors to express the wildtype and R132H mutant form of the IDH1 gene in human cell lines. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, lentiviral vectors	
45362	Yue Xiong	Adenoviral (E1A deleted) and AAV vectors Ad-GFP
APPROVED	**Summary:** The aim of this experiment is to express GFP in mammalian cell lines using adenoviral and AAV vectors. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-D, BSL-2, adenoviral and AAV vectors	

45422		Myosin-X and the molecular basis of filopodia function: CRISPR mediated generation of a Myo10 KO mouse
APPROVED	**Summary:** The aim of this experiment is to use CRISPR to induce a deletion in full-length Myo10 gene in mice to help understand the function of Myo10 in neural development and cancer. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-E, BSL-1, mice	
45562		Ex281-Ex282 humanized Rho mice
APPROVED	**Summary:** The aim of this experiment is to produce a humanized mouse model for the Rho gene by replacing a 22bp segment of the mouse Rho gene with the corresponding wild-type human sequence. Mouse zygotes will be microinjected with plasmid DNA, Cas9 protein and guide RNA's. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-E, BSL-1, mice	
45563		Ex284 Gene trap mice
APPROVED	**Summary:** The aim of this experiment is to introduce an inactivating splice-acceptor polyadenylation sequence (gene trap mutation) in the mouse Ktm2d gene using CRISPR/Cas9 system. Mouse zygotes will be microinjected with the plasmid DNA construct along with the Cas9 protein and guide RNA's. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-E, BSL-1, mice	
45582		Ex285 Kdm6a floxed mice
APPROVED	**Summary:** The aim of this experiment is to introduce loxP sites into the Kdm6a gene in mice to create a Kdm6a floxed mouse. Mouse zygotes will be microinjected with the plasmid DNA construct along with the Cas9 protein and guide RNA's. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-E, BSL-1, mice	
46382		Ex286 Humanized TFRC knock-in mice
APPROVED	**Summary:** The aim of this experiment is to produce a mouse model that expresses human TFRC (transferrin receptor) from the mouse Tfrc locus. Plasmid DNA with the human TFRC construct will be electroporated into ES cells of C57BL/6N mice and microinjected into mouse blastocyst embryos to produce the chimeric mice. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-E, BSL-1, mice	

45742	[redacted]	Generation of mice with airway targeted sodium hyperabsorption
APPROVED	**Summary:** The aim of this experiment is to generate transgenic mice which overexpress the beta subunit of the epithelial sodium channel (ENaC). The gene encoding for murine ENaC, Scnn1b, will be introduced into plasmid pTG1 which will be used to generate the transgenic mice by pronuclear injection. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-E, BSl-1, mice	
45402	[redacted]	Vmat2-t2a-cre KI mouse generation using CRISPR method
APPROVED	**Summary:** The aim of this experiment is to use the CRISPR system to create a Vmat2 gene (SLC18A2) knock in mouse line to express Vmat2 in neurons of the mouse brain. A single-stranded DNA fragment of Vmat2-cre will be microinjected into each zygote. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments**: None III-E, BSL-1, mice	

4. **Update to BSL-3 plans and SOPs –** Jessica Poole gave a description of the updates to the BSL-3 [redacted]
5. **2018-2019 Goals for IBC –** Mary Beth Koza addressed the Committee and requested the Committee state a goal for the 2018-2019 year. It was decided that the Committee would perform a self-assessment audit using the NIH Self-Assessment tool.
6. **Subcommittee approval of exempt recombinant DNA:** 1
   PI: Paul Armistead **Title** The role of spliceosome mutation in carcinogenesis and neoantigen production (Schedule G ID: 44922; III-F)
   Schedule H report: 24
7. **Next IBC meeting date:** July 11, 2018 in TBD

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
September 5, 2018 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Keith Porterfield, Amy Sims, Aravinda DeSilva, Barbara Savoldo, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole

Members Absent: Peggy Cotter, Xiao Xiao

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the August 1, 2018 meeting.** Minutes approved.
2. **Presentation of by**
3. **Presentation of inactivation protocol by**
4. **Applications under review**

ID	PI	Project Title
APPROVED	**Summary:** The aim of this clinical trial is to evaluate the safety of under evaluation as a **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design **Community Member Comments:** None	

<table>
<tr><td>44304</td><td>███</td><td>Enhancing the therapeutic efficacy of the fatty acid synthase inhibitor, fasnall</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to evaluate the combinations of targeted therapies to overcome or prevent the onset of therapeutic resistance to fasnall. Lentivirus, engineered to express firefly luciferase, will be used to infect MCF10DCIS cells. Following infection, cells will be injected into mice via mammary foot pad. Tumors will be monitored by bioluminescence imaging.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Member Comments: None

III-D, BSL-2, lentivirus, mice</td></tr>
<tr><td>50162</td><td>███</td><td>Generation of the Bat Coronavirus HKU5 Infectious Clone - 2018 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: Renewal of approved protocol. The aim of this work is to develop a molecular clone for a bat MERS-like coronavirus (HKU5). The recombinant HKU5 will be evaluated for replication and pathogenesis and for feasibility as a vaccine in carious human/primate cell lines and in mice. To facilitate replication of HKU5 in cell lines, the wild-type ███ spike ectodomain will be cloned into place of the HKU5 ectodomain.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Member Comments: None

III-D, BSL-3, mice</td></tr>
<tr><td>50163</td><td>███</td><td>Generation of the MERS-CoV Infectious Clone and Deletion of Accessory ORFs - 2018 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: Renewal of approved protocol. The aim of this experiment is to create an infectious clone of MERS-CoV, with deletions in the accessory ORFs (ORFs 3, 4a, 4b and 5). These deletions will be made individually and in combination. Resulting viruses will be use to study MERS replication and pathogenesis in cell culture and in vivo.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Member Comments: None

III-D, BSL-3, mice</td></tr>
<tr><td>50164</td><td>███</td><td>Generating ███ Mutants to Improve Binding Efficiency for the 1) Civet ACE2 Receptor, 2) Bat ACE2 Receptor, or 3) Mouse ACE2 Receptor - 2018 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: Renewal of approved protocol. The aim of this experiment is to introduce mutations in the ███ spike glycoprotein to promote binding to the cellular receptor angiotensin converting enzyme (ACE2) from bat, civet or mouse. Recombinant viruses will be assess for replication and pathogenesis in cell culture and in vivo.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Member Comments: None

III-D, BSL-3, mice</td></tr>
</table>

<table>
<tr><td>50165</td><td></td><td>NSP16 Catalytic and Binding Domain Ablation in Infectious Clone - 2018 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: Renewal of approved protocol. The aim of this experiment is to create infectious clone constructs with alanine substitutions within the catalytic domain and/or non-structural protein-10 (nsp10)-binding domain of nsp16. Mutations are expected to attenuate the virus in replication in tissue culture cells and mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Member Comments: None

III-D, BSL-3, mice</td></tr>
<tr><td>50182</td><td>Ralph Baric</td><td>Generating an Infectious Clone for Human Coronavirus HKU1 (BL2) - 2018 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: Renewal of approved protocol. The aim of this experiment is to create an infectious clone of human coronavirus (HKU1) with modifications in the spike S2 domain attachment protein to promote permissiveness of infection in rodent-derived cell lines.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-D, BSL-2</td></tr>
<tr><td>50183</td><td></td><td>Introduction of Bat HKU3 mouse adaptation mutations into the parental infectious clone - 2018 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to introduce mutations into the bat coronavirus HKU3 that may facilitate improved replication in vitro and virulence in vivo.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Member Comments: None

III-D, BSL-3, mice</td></tr>
<tr><td>50202</td><td></td><td>Development of a Comprehensive Dengue Virus Molecular Clone Platform to Evaluate the Antigenic or Functional Variability of Viral Proteins - 2018</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to create full-length infectious clones of Dengue serotypes 1, 2, 3 and 4. Structural mutations (prM gene, E gene) or functional mutations (NS5 gene) will be generated via site-directed mutagenesis and in vitro synthesis. Mutants will be evaluated for antigenicity, pathogenicity and attenuation in vivo.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-D, BSL-2</td></tr>
</table>

<table>
<tr><td>49863</td><td>Keith Burridge</td><td>Role of metavinculin in actin reorganization and force transmission.</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to study the role of metavinculin in actin reorganization and force transmission. Metavinculin will be cloned into a retroviral vector which will be used to transfect mammalian cells.

Community Comments: None

III-D, BSL-2, retrovirus</td></tr>
<tr><td>50362</td><td></td><td>Ex293 Cytochrome P450 (CYP) Humanized Rat</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to create a transgenic rat expressing human Cytochrome P450 (CYP). The transgene will also include a U6-guide RNA expression cassette to facilitate enhanced transmission of the humanized allele when crossed to rats that express the Cas9 protein.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-E, BSL-1, rats</td></tr>
<tr><td>50363</td><td></td><td>Ex294 RNA helicase-Cas9 knock-in rat</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to create a transgenic rat expressing Cas9 protein under the control of regulatory elements for an RNA helicase.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-E, BSL-1, rats</td></tr>
<tr><td>49062</td><td></td><td>Adenovirus (Ad5), recombinant.</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to create a recombinant adenovirus expressing SIV gag/env/pol genes for immunization of infant rhesus macaques for vaccine studies.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee wanted clarification on who CNPRC was, and whether this research had been approved by the CNPRC biosafety/IBC office.

Community Comments: None

III-D, BSL-2, AAV</td></tr>
</table>

49082		Maternal and infant vaccination to prevent breast milk transmission of SHIV
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to create a MVA vaccine expressing HIV envelope or SIV gag as tools for development of vaccines to prevent mother to child transfer of SHIV in rhesus macaques. A chimeric SHIV virus consisting of SIV with the SIV envelope replaced by the HIV envelope will be used as challenge for this study. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee wanted clarification on who CNPRC was, and whether this research had been approved by the CNPRC biosafety/IBC office. **Community Comments:** None III-D, BSL-2, MVA	
49562		Role of miR-29 in models of Alzheimer
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to generate an AAV viral vector that overexpresses the miR-29 microRNA and to examine the outcome of miR-29 overexpression in neurons. AAV vectors expressing miR-29 will be used to infect primary mouse cortical and sympathetic neurons in cell culture and the ability of miR-29 expression to confer neuroprotection in assays of neuronal toxicity examined. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The protocol suggests animals are being used (IACUC protocol provided), but no information provided on Section III. If no animals being used, the IACUC protocol # should be removed and Section III removed. **Community Comments:** None III-D, BSL-2, AAV	
38664	Aravinda DeSilva	Using chimeric West Nile/dengue and West Nile/Zika viruses in serological assays of flavivirus infection and vaccination
APPROVED	**Summary:** The aim of these experiments is to use chimeric flaviviruses WNV/DENV and WNV/ZIKV in in vitro detection assays to characterize the quality of antibody responses to DENV and ZIKZ natural infection and vaccination. The chimeric viruses will be used to deplete human serum samples of flavivirus cross-reactive antibodies and therefore improved the specificity of the current serologic assays. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design **Community Comments:** None III-D, BSL-2	
49382	Aravinda DeSilva	Using dengue viruses with mutations in prM and E genes for the study of epitope-specific antibody responses to dengue virus
APPROVED	**Summary:** The aim of this experiment is to use recombinant infectious clones of DENV serotypes 1, 2, 3 and 4 to study DENV antigenicity and map the epitopes on the dengue virions targeted by antibodies in human and animal immune sera. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design **Community Comments:** None III-D, BSL-2	

49343		AAV-mediated Gene Therapy
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to develop effective gene therapy approaches for the treatment of mucopolysaccharidoses (MPS) and other neurogenetic diseases. Genes of interest, human alpha-N-acetylglucosaminadase (hNAGLU), human N-sulphoglucosamine sulphohydrolase (hSGSH), human iduronate-2-sufatase (hIDS), human alpha-L-iduronidase (hIDUA) or GFP will be expressed in AAV vectors which will be injected into mice via IV injection. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the title be made more descriptive and that the IACUC # be updated. Dosing of 10ml/kg should be reduced to 5ml/kg. **Community Comments:** None III-D, BSL-2	
50302		Analysis of Recombinant Influenza Ya88 strain with reporter genes
APPROVED	**Summary:** The aim of this experiment is to develop an in vivo model for influenza and to use this model to test the efficacy of novel antivirals. Recombinant influenza virus Ya88 expressing mNeonGreen or nanoLuc reporters will be propagated int 293T cells and used to infect mice. Infection will be monitored by IVIS and tissues collected for analysis by real-time PCR and flow cytometry. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, mice	
50902	Stephanie Gupton	Determining the localization and function of schiophrenia linked protein tSNARE1
APPROVED	**Summary:** The aim of this experiment is to express tSNARE1 as a myc fusion protein in a lentiviral vector which will be used to transduce neurons derived from human embryonic stems cells. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design **Community Comments:** None III-D, BSL-2	
50903		TRIM9/67 Chimeric Mice
APPROVED	**Summary:** The aim of this experiment is to create a transgenic mouse strain that expresses a chimeric TRIM9/TRIM67 protein under the control of the endogenous TRIM9 locus. The chimeric TRIM9/TRIM67 gene construct will be cloned onto a plasmid, which will be microinjected into mouse embryos along with the Cas9 protein, and guide RNA to promote insertion in the TRIM9 gene in the genome. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design **Community Comments:** None III-E, BSL-1	

50147		Rabies Virus Based Mapping of Neuronal Pathways
APPROVED	**Summary:** The aim of this experiment is to map inputs into neurochemically-defined neurons. AAV vectors expressing either TVA receptor protein (TVA950) or rabies virus envelope glycoproteins (RG) will be expressed under control of the Cre/LoxP system.Modified rabies virus (SAD916-GFP) with deletion in RG will be injected into the brain regions of mice. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design **Community Comments:** None III-D, BSL-2, AAV	
49782		Engineering antigen-specific tolerance via recombinant plasmid
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to create immunological tolerance in mice that have previously been vaccinated against the model antigen, ova protein. Plasmids encoding ova peptide, EGFP, mIL-10 and shRNA against CD80/86 will be injected into mice that have been vaccinated against the ova protein. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the inoculation stating 100uL IM be modified to state 25uL/site. **Community Comments:** None III-D, BSL-2, plasmids	
50148		Engineering a highly specific viral targeting system for systemic gene therapy applications
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to evaluate the transduction efficiency of bispecific antibody-directed lentiviruses to target and transduce specific cells in vitro and in vivo. Insert genes will be cloned into lentiviral vectors and used to transfect mammalian 293T cells. Packaged lentivirus will then be used to transduce cells in vitro, and systemically administered to mice to transduce specific cells in vivo. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the lab provide an IACUC number. **Community Comments:** None III-D, BSL-2, lentivirus	
49342		Gene Therapy with AAV Vectors
APPROVED	**Summary:** The aim of this experiment is to develop AAV vectors optimal for alpha-1 gene therapy. Human alpha-antitrypsin (hAAT) will be expressed in AAV viruses (serotypes 1-8), then delivered into mice in vivo. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the lab update the title to be more specifica and to provide an updated IACUC number. **Community Comments:** None III-D, BSL-2, AAV	

<table>
<tr><td>50082</td><td></td><td>Overcoming our clinical complications: AAV vector design for the treatment of DMD</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to create a functional dystrophin expressing vector for the treatment of DMD. An AAV vector encoding opti-mini-dys-ICP47 transgene will be directly injected into MDX mouse muscle. At different time points, phenotypic correction and CTL response to human mini-dystrophin will be detected.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the lab update the title to be more specifica and to provide an updated IACUC number.

Community Comments: None

III-D, BSL-2, AAV</td></tr>
<tr><td>49291</td><td></td><td>Mouse Model of Cardiac Maturation</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to knock down expression of rbfox1, Carm1, Ehmt2, Kdm2b in mouse neonatal heart to determine their role in cardiac maturation. shRNA against these genes will be cloned into adenoviral vector which will subsequently be injected into anterior dorsal subcutis of the neonatal mouse.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-D, BSL-2, adenovirus</td></tr>
<tr><td>49202</td><td></td><td>Chromatin regulation and remodeling during liver injury and repair</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to identify epigenetic regulators of hepatic stellate cell activation and liver fibrosis. AAV will be used to deliver CRISPR guide RNA into the mouse liver (hepatic stellate cells) through intravenous injection. Constructs will be made from a pool of CRISPR guides targeting all epigenetic regulators (~6000 gRNAs total).

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-D, BSL-2, AAV</td></tr>
<tr><td>47804</td><td>Karen Mohlke</td><td>Susceptibility to Type Diabetes and Related Quantitative Traits</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to identify regulatory elements and gene functions that influence susceptibility to type 2 diabetes or biomarkers of cardiovascular risk. Human genes, non-coding regulatory elements, or gRNAs will be expressed in plasmids, adenoviral vectors or lentiviral vectors which will be transfected/transduced into mammalian cells in vitro.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-D, BSL-2, plasmids, adenovirus, lentivirus</td></tr>
</table>

<table>
<tr><td>50242</td><td></td><td>Generation of Podocin-BioID2 mouse</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate a mouse strain that will be utilized to identify the proximity proteome of the slit diaphragm protein Podocin within the podocyte foot process. A 13xlinker-BioID2 fusion will be appended to the 3’ end of the Nphs2 (Podocin) gene at the endogenous locuc in the mouse. The 13xlinker-BioID2 with appropriate homology arms will be cloned into a plasmid which will be co-injected with Cas9:guide RNA complexes into mouse blastocysts.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-D, BSL-1, plasmids</td></tr>
<tr><td>50202</td><td>Scott Randell</td><td>Identification of Airway Epithelial Stem Cells</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use lentiviral vectors and/or plasmids to deliver cDNAs for Bmi-1 and hTERT to primary human airway cells in order to extend the life-span of the cells.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-D, BSL-2, plasmids, lentivirus</td></tr>
<tr><td>50203</td><td>Scott Randell</td><td>Molecular Therapy Core Center, Core F: Cell Culture Models</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use lentiviral vectors to express genes of interest (e.g. fluorescent proteins, alkaline phosphatase, beta-galactosidase, antibiotic markers, hTERT etc) in human airway epithelial cells.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

Community Comments: None

III-D, BSL-2, plasmids, lentivirus</td></tr>
<tr><td>49682</td><td></td><td>Rabies-Virus-based Transynaptic Retrograde Circuit Mapping</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use a Rabies-based retrograde trace technique to map neural circuits. Mice will be infected with attenuated rabies vector pRV and AAV vectors carrying TVA and RG constructs.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design

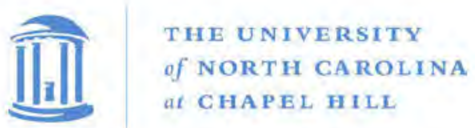
Community Comments: None

III-D, BSL-2, AAV , rabies</td></tr>
</table>

50342	Hyejung Won	Genetic basis of psychiatric disorders
APPROVED	**Summary:** The aim of this experiment is to understand how genetic variation impacts psychiatric disease susceptibility and gene regulation. Human fetal neural stem cell lines will be transduced with AAV2 and lentiviral vectors. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design **Community Comments:** None III-D, BSL-2, AAV, lentivirus	

5. **Discussion of LAI –** Aravinda DeSilva discussed a recent DENV LAI at UNC and initiatives taken in response to recommendations by EHS and NC DHHS to improve the safety of work with DENV while performing high risk activities with the virus.
6. **Changes to NIH Guidelines Human Gene Transfer Experiments** – Garry Coulson discussed the recent changes regarding NIH oversight of human clinical trials, specifically the impact to the Committee regarding less administrative responsibilities in not having to register trials with the NIH, but also some concerns about increase responsibility of the Committee since there was no longer the NIH acting as a second layer of review. Regarding approval letters for clinical trials, in the absence of further direction from the NIH or FDA, the IBC will henceforth issue final approvals for trial with language stating approval is conditionally dependent on FDA approval of IND and issuance of a “safe to proceed” letter for the IND.
7. **Discussion on SAE for** [redacted] **–** [redacted] discussed with the Committee some SAE’s associated with a single patient receiving [redacted] in this trial.
8. **Update to NIH reportable incidents 2018** – Garry Coulson discussed with the Committee that all outstanding NIH reportable incidents have been closed to the satisfaction of the NIH with no further actions.
9. **Sub-committee Approvals of Schedule G**: 3
   PI: Scott Bultman **Title:** Role of bacterial glucaronidase in reactivation of irinotecan metabolites (Schedule G 50122, III-D)
   PI: [redacted] **Title:** Roxa26-tSNARE1-Knock-in Mouse (Schedule G 50622, III-E)
   PI: Frederico Innocenti Title: Characterization of functional genetic variation in genes of the angiogenesis pathways (Schedule G 49802, III-F)
10. **Schedule H report:** 62
11. **Next IBC meeting date:** October 3, 2018. Location: Burnett-Womack 9001.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
December 5, 2018 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole

Members Absent: Keith Porterfield, Peggy Cotter, Aravinda DeSilva, Barbara Savoldo, Xiao Xiao, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests: None

Open Meeting

1. **Review minutes from the November 7, 2018 meeting.** Minutes approved.
2. **Discussion of Policy and Procedures** - Mary Beth Koza discussed new Institutional policies and procedures workflow and process and communicated to the IBC that the Biosafety Manual was slated to be entered into the new system as a policy. The Committee approved of the decision to do so.
3. **Applications under review:**

ID	PI	Project Title
51922	[redacted]	Effect of metabolism on carcinogenesis of ovarian and endometrial cancer
APPROVED	**Summary:** The aim of this experiment is to develop models to understand the development of ovarian and endometrial cancer in transgenic mice. Transgenic mice with LoxP sequences inserted into genes of interest (LKB1, p53, Brcar1 and Rb) will be injected with Adenovirus expressing Cre-recombinase resulting in the loss of expression of these genes and subsequent induction of cancer. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that Domitor be replaced with Dexdomitor as the anesthetic. **Community Member Comments:** None III-D, BSL-2, adenovirus, mice	
53822	[redacted]	Alteration of HKU4/5 Species Specificity - 2018 Renewal
APPROVED	**Summary:** The aim of this experiment is to generate viral variants of bat coronaviruses HKU4 and HKU5 that are capable of replicating in mouse models to facilitate pathogenesis studies. Mutations will be introduced into the viral spike gene in regions predicted to enhance interaction with the probable virus receptor (DPP4). Viruses that have been engineered to interact with mouse DPP4 (mDPP4) and are capable of propagating in mice will be serially passaged in mice to select for pathogenic variants. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3, coronavirus, mice	

<table>
<tr><td>53223</td><td>Dirk Dittmer</td><td>Use of guideRNA/CRISPR lentiviruses in tissue culture</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use a commercially-available library of ~30,000 small guide RNAs, together with the Cas9 enzyme, to generate specific gene knockouts in human cells to understand the function of human genes in tissue culture. Cells will be transduced with lentiviral vectors encoding the sgRNA’s and cotransfected with plasmid expressing Cas9.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, lentivirus, plasmid</td></tr>
<tr><td>53882</td><td></td><td>tg801 transgenic mice</td></tr>
<tr><td>APPROVED W/ STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to create a transgenic mouse expressing genes from Kaposi sarcoma-associated herpesvirus (KSHV) as a means to understand the function of these genes. Transgenic mice will be created by pronuclear injection of plasmid DNA containing a 12,000 bp region of the KSHV genome of interest.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested more information on the KSHV DNA region to be cloned into the plasmid, and also requested an updated IACUC number.

Community Member Comments: None

III-E, BSL-1, plasmids, mice</td></tr>
<tr><td>53942</td><td></td><td>KSHV Bacmid recombinants</td></tr>
<tr><td>APPROVED W/ STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to test the growth of genetically defined strains of KSHV in mice. Recombinant strains of KSHV will be created with desired mutations introduced into each of the 80 genes in the KSHV genome. Resulting strains will be injected into mice to assess the effect of mutation on growth,

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that a statement be provided that indicates the IBC will be contacted if any of the strains replicate more efficiently than the wildtype. The Section III and IACUC number needs to be updated.

Community Member Comments: None

III-D, BSL-2, mice</td></tr>
<tr><td>53562</td><td>Paul Eldridge</td><td>CAR.CD138 Vector/Chimeric T cells</td></tr>
<tr><td>TABLED</td><td colspan="2">Summary: The aim of this experiment is to generate viral vector and chimeric T cells for clinical studies. Synthetic DNA plasmids will be used to generate the Moloney gamma retroviral vectors expressing immunologic receptors and co-stimulatory molecules. Viral vector will be used to transduce PG13 packaging cell lines to produce the final retroviral vector which will be used to transduce patient T cells to produce CAR-T cells.

Committee Comments: The Committee wanted clarification on whether production of these viral vectors and subsequent use in clinical studies was associated with an already-approved IBC/IRB study. If so, the Committee requested IBC approval letters be provided.

Community Member Comments: None</td></tr>
</table>

<table>
<tr><td>53582</td><td>Paul Eldridge</td><td>CAR.Kappa Viral Vector/Chimeric T cells</td></tr>
<tr><td>TABLED</td><td colspan="2">Summary: The aim of this experiment is to generate viral vector and chimeric T cells for clinical studies. Synthetic DNA plasmids will be used to generate the Moloney gamma retroviral vectors expressing immunologic receptors and co-stimulatory molecules. Viral vector will be used to transduce PG13 packaging cell lines to produce the final retroviral vector which will be used to transduce patient T cells to produce CAR-T cells

Committee Comments: The Committee wanted clarification on whether production of these viral vectors and subsequent use in clinical studies was associated with an already-approved IBC/IRB study. If so, the Committee requested IBC approval letters be provided.

Community Member Comments: None

III-D, BSL-2, retroviral vectors</td></tr>
<tr><td>53583</td><td>Paul Eldridge</td><td>CAR.CD19 Viral Vector/Chimeric T cells</td></tr>
<tr><td>TABLED</td><td colspan="2">Summary: The aim of this experiment is to generate viral vector and chimeric T cells for clinical studies. Synthetic DNA plasmids will be used to generate the Moloney gamma retroviral vectors expressing immunologic receptors and co-stimulatory molecules. Viral vector will be used to transduce PG13 packaging cell lines to produce the final retroviral vector which will be used to transduce patient T cells to produce CAR-T cells

Committee Comments: The Committee wanted clarification on whether production of these viral vectors and subsequent use in clinical studies was associated with an already-approved IBC/IRB study. If so, the Committee requested IBC approval letters be provided.

Community Member Comments: None

III-D, BSL-2, retroviral vectors</td></tr>
<tr><td>53584</td><td>Paul Eldridge</td><td>CAR.GD2 Viral Vector/Chimeric T cells</td></tr>
<tr><td>TABLED</td><td colspan="2">Summary: The aim of this experiment is to generate viral vector and chimeric T cells for clinical studies. Synthetic DNA plasmids will be used to generate the Moloney gamma retroviral vectors expressing immunologic receptors and co-stimulatory molecules. Viral vector will be used to transduce PG13 packaging cell lines to produce the final retroviral vector which will be used to transduce patient T cells to produce CAR-T cells

Committee Comments:

Community Member Comments: None

III-D, BSL-2, retroviral vectors</td></tr>
</table>

53542	Eric Everett	Identification and Characterization of Genes Involved in Craniofacial Development
APPROVED W/ STIPULATIONS	**Summary:** The aim of this experiment is to investigate the actions of environmental factors, such as fluoride, on the cell response. Cells will be transfected with cDNA's of genes of interest, typically genes implicated in oral clefting or tooth enamel formation and the function of these genes assessed. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the protocol be corrected for answers provided for incorrect questions. Classification based on viral vectors should be changed from III-E to III-D. **Community Member Comments:** None III-D, BSL-2, lentivirus, plasmids	
53842	[redacted]	Ad-TGFbeta model of progressive lung fibrosis in mice
APPROVED W/ STIPULATIONS	**Summary:** The aim of this experiment is to create a murine model for lung fibrosis in mice. To understand the role of TGF beta in lung fibrosis, porcine TGFbeta 1 was expressed in replication-deficient adenoviral vector (Ad5). Adenovirus was then introduced into mice by intranasal instillation. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the IACUC number be provided, and the volume for the instillation be added. Xylazine, and not xylene, should be indicated as the anesthetic. **Community Member Comments:** None III-D, BSL-2, adenovirus, mice	
51962	[redacted]	Adeno CRE expression in mice
APPROVED W/ STIPULATIONS	**Summary:** The aim of this experiment is to express Cre-recombinase in mice. Adenoviral vectors expressing bacteriophage Cre-recombinase will be constructed and injected into mice through tail vein injection. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the volume for tail vein injection be reduced from 200uL to 100uL. **Community Member Comments:** None III-D, BSL-2, adenovirus, mice	
53904	Tal Kafri	Simple and lenti viral vectors expressing CRISPR/Cas9 and gRNA
APPROVED	**Summary:** The aim of this experiment is to create lentiviral and retroviral vectors expressing the CRISPR Cas9 and gRNAs from PolIII promotors for genome editing. Vectors will express either a single gRNA or pools of gRNA's directed to either human or rodent target sequences. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus, retrovirus	

<table>
<tr><td>54162</td><td>Bo Li</td><td>Constructing plasmids for protein expression</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to construct plasmids for expression of natural biosynthetic genes in E. coli. Genes include non-ribosomal peptide synthesis, tailoring enzymes and regulatory proteins from Pseudomonas, Burkholderia and Streptomyces spp.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. Classification of experiments should be altered from III-F to III-D.

Community Member Comments: None

III-D, BSL-2</td></tr>
<tr><td>53902</td><td>Michael Major</td><td>Mechanisms of Signal Transduction</td></tr>
<tr><td>APPROVED W/ STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to express genes of interest in mammalian cells to understand the function of these genes in cancer signaling pathways. Genes in the NRF2 pathway, WNT pathway, and other commonly altered proteins in human cancer will be expressed in a variety of human and murine cell lines through transformation with plasmids or transduction with viral vectors containing the GOI.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested a representative subset of cells and cell lines to be used be provided.

Community Member Comments: None

III-D, BSL-2, plasmids, vectors</td></tr>
<tr><td>54002</td><td>Uma Nagarajan</td><td>Characterizing the effects of Neisseria gonorrhoeae and Chlamydia trachomatis during single and co-infection of primary human fallopian tube epithelia.</td></tr>
<tr><td>APPROVED W/ STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to understand pathogenesis and gene expression profiles of N. gonorrhoeae and C. trachomatis in human primary fallopian epithelial cells either individually, or in co-infection. Naturally-occurring and recombinant bacteria expressing fluorescent markers (e.g. GFP or mCherry) will be used to infect mammalian cells.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested details on the plasmids used for creation of recombinant strains.

Community Member Comments: None

III-D, BSL-2</td></tr>
<tr><td>53422</td><td></td><td>Lineage-Specific Gene Expression</td></tr>
<tr><td>APPROVED W/ STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to use transduce cells obtained by apheresis of dogs with bleeding disorders (e.g. hemophilia A, hemophilia B and von Willebrand disease) with viral vectors to expressing coagulation Factor VIII, FIX or VWD and inject transduced cells back into the dog

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested the volume for reinfusion be provided and preferred a new title more descriptive of the research be used.

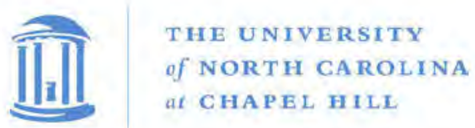
Community Member Comments: None
III-D, BSL-2, lentivirus, dogs</td></tr>
</table>

53442	███	Gene Therapy for Hemophilia
APPROVED	**Summary:** The aim of this experiment is to express canine coagulation proteins in dogs with FVIII, FIX or VWD deficiency. Canine FVIII, FIX or VWD will be expressed in recombinant adenoviral vectors which will be introduced into dogs by intramuscular or intravascular injections. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, adenovirus, dogs	
53443	███	Gene Therapy of Hemophilia A
APPROVED	**Summary: T**he aim of this experiment is to express canine coagulation proteins in dogs with FVIII, FIX or VWD deficiency. Canine FVIII, FIX or VWD will be expressed in recombinant lentiviral vectors which will be introduced into dogs by intravenous injection. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus, dogs	
53802	███	Meiotic Recombination in D. melanogaster
APPROVED W/ STIPULATIONS	**Summary:** The aim of this experiment is to create D. melanogaster strains with mutations in genes of interest (e.g. mei-9, blm) that abolish their function to assess the role of these genes in meiotic recombination. DNA plasmids containing the GOI will be sent to commercial vendors to create the modified fruit flies. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested more information on how the screen is performed and how the mutant flies created. Classification should be III-D and not III-E. **Community Member Comments:** None III-D, BSL-1, plasmids	
53642	Robert Tarran	Ion and Mucus Transport in Human Airways
APPROVED	**Summary:** The aim of this experiment is to understand the role of different membrane proteins in cell physiology. Genes of interest will be cloned into plasmids or adenoviral vectors which will be transfected/transduced into mammalian cells in vitro. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. Classification should be III-D and not III-F. **Community Member Comments:** None III-D, BSL-2, plasmids, adenovirus	

52782		Role of the Rho-GAP GRAF3 in the pathogenesis of human hypertension, Promoting chemoresistance in the heart, Smooth muscle adhesion and plasticity in coronary and outflow tract development, FAK signaling in cardiac growth and hypertrophy
APPROVED W/ STIPULATIONS	**Summary:** The aim of this experiment is to express adhesion signaling molecule proteins in cells cloning of target genes into plasmids and transfecting mammalian cells in vitro. AAV vectors may also be used to transiently express GOI in mice via intramuscular injection. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested a simpler title be used, and the volume for IM administration be amended to be in line with current recommendations. **Community Member Comments:** None III-D, BSL-2, plasmids, AAV, mice	
54122		The Role of Central Peptides in Ethanol Consumption
APPROVED W/ STIPULATIONS	**Summary:** The aim of this experiment is to understand the role of synapsin and muscarinic receptors in ethanol consumption. Genes of interest, including engineered GPCRs, will be expressed in AAV viral vectors, and introduced into mice through stereotaxic injection. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the route of administration be clearly stated, as well as the method for anesthetization. Classification should be III-D. **Community Member Comments:** None III-D, BSL-1, AAV, mice	
54123		GFP and Engineered GPCRs (DREADDs)
APPROVED W/ STIPULATIONS	**Summary:** The aim of this experiment is to understand the role of synapsin and muscarinic receptors in ethanol consumption. Genes of interest, including engineered GPCRs, will be expressed in AAV viral vectors, and introduced into mice through stereotaxic injection. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the route of administration be clearly stated, as well as the method for anesthetization **Community Member Comments:** None III-D, BSL-1, AAV, mice	
54124		AAV Expression of Cre, EGFP_Cre from hSynapsin or GFAP promoters
APPROVED W/ STIPULATIONS	**Summary:** The aim of this experiment is to express Cre-recombinase, or GFP-Cre in the brain through stereotaxic administration of AAV expressing the GOI into the brain of mice. **Committee Comments:** The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the method for anesthetization be provided. **Community Member Comments:** None III-D, BSL-1, AAV, mice	

4. **Sub-committee Approvals of Schedule G**: 0
5. **Schedule H report:** 37
6. **Next IBC meeting date:** January 9, 2019. Location: TBD

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
January 9, 2019 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Barbara Savoldo, Xiao Xiao, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole

Members Absent: Keith Porterfield, Peggy Cotter, Aravinda DeSilva, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests: None

Open Meeting

1. **Review minutes from the December 5, 2018 meeting.** Minutes approved.
2. **Goals for IBC 2019** – The Committee discussed two goals for 2019: Completion of the NIH IBC Self-Assessment and Continuing Education/Training for the IBC (invited speakers).
3. **Applications under review:**

ID	PI	Project Title
55223	[redacted]	Use of GFP, mCherry, and mLuc transfected cells
APPROVED	**Summary:** The aim of this experiment is to design targeted therapeutics for treating terminal cancers using stem cell-based therapies. Human and mouse cell lines and primary cells will be transduced with viral vectors expressing diagnostic markers (e.g. GFP, mCherry, luciferase) or therapeutic genes (e.g. TNF-alpha-related apoptosis-inducing ligand [TRAIL]). Transduced cells will then be infused into mice. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the volume for IV injection be reduced from 300uL to 100uL. **Community Member Comments:** None III-D, BSL-2, lentivirus, mice	
54443	[redacted]	Mechanisms of non-coding RNAs in breast cancer -Hector Franco-
APPROVED	**Summary:** The aim of this experiment is to understand the role of non-coding enhancer RNAs (eRNAs) in breast cancer initiation, progression and metastasis. eRNAs will be overexpressed in cell lines using lentiviral expression vectors, or knocked down using shRNA-expressing vectors. Cancer phenotypes of the perturbed cells will be evaluated by cell-based assays or mouse xenograft assays in which the cells are injected into mice via sub-cutaneous injection. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus, mice	

54676	[redacted]	Animal Models for Human Coronavirus NL63 and Recombinants - 2018 Renewal
APPROVED	**Summary:** The aim of this experiment is to determine if expression of viral attachment and other structural proteins can stimulate the production of neutralizing antibodies in mice. To determine this, exogenous proteins from measles virus, parainfluenza virus, influenza virus, human/simian immunodeficiency viruses will be expressed in place of ORF3 of the NL63 human coronavirus infectious cDNA clone. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, mice	
55062	Victoria Bautch	Transfection of constitutively active genes fused to GFP into human cell culture
APPROVED	**Summary:** The aim of this experiment is to transfect human endothelial cells with GFP-tagged proteins for overexpression experiments. Target genes for overexpression include cofilin and smad6. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. Since no animals are being used, the IACUC number should be removed. **Community Member Comments:** None III-F, BSL-2, plasmids	
40982	[redacted]	Adaptive therapy to delay tumor resistance to immune checkpoint inhibitors
APPROVED	**Summary:** The aim of this experiment is to delay tumor resistance by optimizing dosage regimen of immune checkpoint inhibitors. Commercial lentiviral particles expressing fluorescent proteins (tdTomato and ZsGreen) or shRNA to IFN-gRA will be utilized to transduce cells in vitro. Transduced cells will be injected into mice through sub-cutaneous injection. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus, mice	
54422	Matthew Hirsch	Gene Delivery to Limbal Stem Cells
APPROVED	**Summary:** The aim of this experiment is to determine lentivirus and AAV transgenic DNA transfer to human limbal stem cells. GFP reporter viruses will be obtained and incubated with stem cells and gene delivery measured by flow cytometry, histology and qPCR. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, lentivirus, AAV	

55082		Exploring mechanisms of therapeutic demethylation effects in HPV-associated head and neck cancer
APPROVED	**Summary:** The aim of this experiment is to identify the role of MMPs in 5-aza-mediated suppression of HPV-associated HNSCC metastasis. Mammalian cells will be transfected or transduced with plasmids or lentivirus vectors expressing shRNA to MMP genes. Modified cells will be injected subcutaneously into mice to analyze the effect of knockdown on tumor formation. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, plasmids, lentivirus, mice	
54402		Enhancing AAV gene therapy via bispecific fusion proteins that block anti-AAV antibodies while conferring active targeting
APPROVED	**Summary:** The aim of this experiment is to design bi-specific antibodies that can bind to AAV2 and a cell receptor (e.g. HER2 or CD3). Mammalian cells expressing anti-AAV2 bispecific antibodies targeting HER2 or CD3+ will be infected with AAV and transgene expression quantified by fluorescent microscopy or flow cytometry. Additionally, a library of human scfv antibodies will be screened against AAV2 to isolate AAV2-specific antibodies. Lastly, immunodeficient mice with HER2+ tumors or human CD3+ T-cells will be dosed with AAV-antibody complexes. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, AAV, mice	
55302		Cardiac development and regeneration in zebrafish
APPROVED	**Summary:** The aim of this experiment is to generate a transgenic line of zebrafish with modulated intraciliary calcium. A plasmid expressing arl13b and Pva1b will be constructed, linearized and injected into zebrafish embryos. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-1, plasmids, zebrafish	
55322		Cardiac development and regeneration in zebrafish
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to generate a transgenic line of zebrafish that will facilitate visualization and ablation of T cells, B cells and macrophages during cardiac regeneration. Plasmids expressing floxed GFP, diptheria toxin A selectable marker, and enhancers to a number of target genes will be constructed and injected into zebrafish embryos. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requested a unique title be provided that is different from prior protocol. The Committee also requested additional details on the use of the toxin. **Community Member Comments:** None III-D, BSL-2, plasmids, zebrafish	

<table>
<tr><td>55064</td><td></td><td>Targeting Constitutively Active G-alpha-q for the Treatment of Uveal Melanoma in animals (primary model)</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to evaluate the therapeutic efficacy of trap genes that can disrupt constitutively active Gaq signaling in uveal melanoma cell lines. Trap genes will be cloned into AAV vectors which will be used to transduce transfected cell lines OMM1.3-Fluc-eGFP, 92.1-Fluc-eGFP and OCM3-Fluc-eGFP. Transduced cells will be inoculated intra-splenically into mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, AAV, mice</td></tr>
<tr><td>55325</td><td></td><td>Targeting Constitutively Active Gαq for the Treatment of Uveal Melanoma</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to evaluate the therapeutic efficacy of trap genes that can disrupt constitutively active Gaq signaling in uveal melanoma cell lines. Trap genes will be cloned into AAV vectors which will be used to transduce uveal melanoma cell lines or injected into uveal melanoma animal model (mice).

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, AAV, mice</td></tr>
<tr><td>55326</td><td></td><td>Stable transfected uveal melanoma cell lines for bioluminescent imaging in animals</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to establish human cell lines of uveal melanoma stably expressing firefly luciferase and eGFP to be utilized in tumor establishment studies in mice. Lentiviral particles expressing Fluc and eGFP will be purchased and used to transduce uveal melanoma cells in vitro. Transduced cells will be injected intra-splenically.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, lentivirus, mice</td></tr>
<tr><td>55362</td><td></td><td>Lentivirus preparation and transduction</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to use lentivirus to introduce foreign genes into mammalian cells to modify the phenotype of target cells (T cells and tumor cells) for cancer immune therapy research. Genes of interest, including CAR, CD28 and CD3z domain, will be expressed in lentiviral vectors which will be used to transduce cells in vitro. Resulting transduced cells will be analyzed in vitro or injected into mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee request a title more specific to the proposed research be provided.

Community Member Comments: None

III-D, BSL-2, lentivirus, mice</td></tr>
</table>

<table>
<tr><td>55042</td><td>[redacted]</td><td>Analysis of virulence in Klebsiella pneumoniae, Yersinia enterocolitica, Yersinia pseudotuberculosis and Salmonella typhimurium</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to identify and characterize virulence determinants of Klebsiella pneumoniae, Yersinia enterocolitica, Yersinia pseudotuberculosis and Salmonella typhimurium. In particular, understanding how these determinants interact with the host (ie. the mouse) to cause disease. Virulence genes of interest will be cloned into plasmids for overexpression of target genes or targeted disruption of these genes. Recombinant strains of bacteria will then be analyzed in in vitro and in vivo model systems.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested information on the method of anesthesia of mice.

Community Member Comments: None

III-D, BSL-2, mice</td></tr>
<tr><td>54342</td><td>Richard Superfine</td><td>Use of Green/Red fluorescent or photoactivateable Markers for Evaluating Effects of Forces on Cells</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to determine the effect of applying forces to cells (push/pull) on specific proteins and signaling pathways. Mammalian cells and cell lines will be transfected with plasmids expressing ftractin, GFP actin or histone H2B.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-F, BSL-2, plasmids</td></tr>
<tr><td>54024</td><td>[redacted]</td><td>Novel approaches for GVHD prevention</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use tumor cells transduced with gamma retroviral vectors expressing GFP or luciferase in an in vivo murine model. Transduced cells will be injected IV into mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, retrovirus, mice</td></tr>
<tr><td>54042</td><td>[redacted]</td><td>Use of luciferase containing human and murine cancer cell lines for animal studies.</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to transduce cell lines to express luciferase as a means to monitor in vivo tumor growth intracranially. Mammalian cell lines will be transduced with lentiviral vectors expressing firefly luciferase. Transduced cells will be introduced into mice by intravenously, intracranially or intracardiac.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

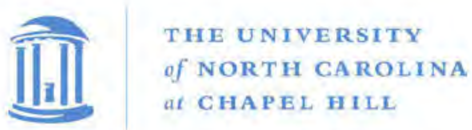
Community Member Comments: None

III-D, BSL-2, lentivirus, mice</td></tr>
</table>

54222	[REDACTED]	Introduction of p53 knockout using CRISPR/Cas9 technology with lentiviral vectors in human cancer cell lines
APPROVED	**Summary:** The aim of this experiment is to compare the anti-cancer drug and radiation responses of a p53 knockout to endogenous p53 mutant cell lines in a specific human cancer background in vitro. Lentiviral particles carrying sgRNA p53 knockout constructed will be transduced into Cas9 transfected human cancer cell lines to generate a p53 knockout line. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. Committee noted that the IACUC number provided should be removed. **Community Member Comments:** None	

4. **Sub-committee Approvals of Schedule G**: 1
   PI: [REDACTED] **Title:** Specialized Program of Research Excellence (SPORE) in Breast Cancer (ID 54682; III-D)
5. **Schedule H report:** 14
6. **Next IBC meeting date:** February 5, 2019. Location: Burnett-Womack 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes February 6, 2019 3:30 PM Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Aravinda DeSilva, Barbara Savoldo, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole

Members Absent: Keith Porterfield, Peggy Cotter, Xiao Xiao, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the December 5, 2018 meeting.** Minutes approved.
2. **Inactivation Protocol Discussion** – presented a proposal for a modification to the existing paraformaldehyde (PFA) inactivation procedure for rendering tissues from mice infected with chikungunya virus (CHIKV) uninfectious and safe to work with at BSL-2. Preliminary data for the modified protocol was presented to the Committee. The Committee had some concerns that the assay, as presented, was i) not sufficiently sensitive to detect low amounts of live virus, and ii) difficult to interpret the success of viral inactivation due to the confounding effects of the PFA on the cell monolayer. Therefore, the modified inactivation protocol was not approved in its current form.
3. **Applications under review:**

ID	PI	Project Title
56022		Expression of the human sodium iodide symporter gene (hNIS) in MERS-CoV - 2018 Renewal
APPROVED	**Summary:** The aim of this experiment is to generate a recombinant MERS-CoV that expresses the human sodium iodide symporter (hNIS) that can be used in positron tomography / computed tomography (PET/CT) studies to characterize the progression and regression of disease in infected animals. All animal experiments involving this construct will be performed at NIH **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3	

56023		Rescue of the Attenuated TRS Remodeled Phenotype with Complementing Mutation of the 5'UTR RNA Cruciform Structure
APPROVED	**Summary:** The aim of this experiment is to mutate the sequences in the putative transcription regulatory sequence (TRS) elements in the 5' UTR of CRG7 that are predicted to interact with the TRS network with the goal of rescuing wildtype replication and virulence in the attenuated CRG7 mutant strain. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3, mice	
56024		Cloning the Spike sequence of MERS-related CoV NL140422 into the MERS-CoV infectious clone
APPROVED	**Summary:** The aim of this experiment is to use the MERS-CoV reverse genetic system determine if NL140422 spike protein from MERS-like bat isolates can mediate entry into human or non-human primate cells, and determine what residues of the spike protein are critical for virus entry into cells. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-3, mice	
56025		Middle East respiratory syndrome coronavirus (MERS-CoV): incorporations of passage 35 mouse-adapted mutations into the MERS-CoV infectious clone
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to incorporate genetic changes in the MERS-CoV genome previously identified through adaptation of the MERS-CoV over 35 passages in mice. Mutations observed in the mouse-adapted strain of MERS-COV will be introduced into the MERS-CoV infectious clone system and assessed for effects virulence in vivo. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requested language be included in the protocol that states what response the lab will take should increased viral replication or virulence be observed in the recombinant virus. **Community Member Comments:** None III-D, BSL-3, mice	
56026		Expression of Angiotensin-(1-7) by Lentivirus or AAV vectors.
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to test angiotensin-(1-7) as a therapeutic for infection in mice. Angiotensin-(1-7) will be expressed in AAV or lentiviral vectors, which will be inoculated into BALB-c mice prior to challenge with **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that volumes for i.p. and i.v. administration to mice be provided **Community Member Comments:** None III-D, BSL-3, AAV, lentivirus	

<table>
<tr><td>56027</td><td>█████</td><td>Generation of mouse-adapted MERS viruses with mutations in the PLpro domain of nsp3</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate recombinant MERS-CoV strains with changes in specific amino acids of papain-like protease (PLpro) which is believed to antagonize the innate immune system. Appropriate nucleotides will be mutated in the p53 mouse-adapted MERS-CoV infectious clone backbone, and resultant viruses evaluated for altered immune susceptibility in vivo.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-3, mice</td></tr>
<tr><td>55822</td><td>█████</td><td>Recombinant Sindbis viruses expressing murine granzymes</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use recombinant Sindbis viruses (SINV) expressing murine granzymes A, B, or K to evaluate the effect of overexpression of these granzymes on the neuropathogenesis of infection and viral clearance both in vitro and in vivo.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-2, mice</td></tr>
<tr><td>56502</td><td>█████</td><td>Optogenetics in Behaving Animals</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use AAV's to express light-sensitive ion channels in neurons of rats. This will allow for temporal-and special-specific activation or inhibition of neurons in various brain pathways as rats perform behavioral tasks.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design.

Community Member Comments: None

III-D, BSL-1, AAV</td></tr>
<tr><td>56070</td><td>█████</td><td>Studying biological function of Tau using recombinant AAV vectors – AAV GFP</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to express wildtype and mutant forms of human Tau in the mouse brain or cultures cells/neurons via recombinant AAV to characterize the function of Tau.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the volume of IV administration be reduced from 200uL to 100uL.

Community Member Comments: None

III-D, BSL-2, AAV</td></tr>
</table>

<table>
<tr><td>53302</td><td>[REDACTED]</td><td>The application of murine models to study the role of dysregulated transcription factors in pediatric solid tumors</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to express various wildtype and mutant forms of chimeric transcription factors or knockdown expression using guide RNA and Cas9 of gene targets in various cell lines using lentiviral vectors or plasmids. Transduced or transfected cells may be implanted into mice.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested information regarding anesthetization of animals be corrected according to IACUC protocols.

Community Member Comments: None

III-D, BSL-2, lentivirus, plasmids, mice</td></tr>
<tr><td>49584</td><td>[REDACTED]</td><td>Evaluation of the immune response in humanized BLT mouse model</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to evaluate the ability of lentiviral vectors and integrase-defective lentiviral vectors expressing antigens to induce antigen-specific immune responses in humanized BLT (bone marrow, liver, thymus) mice. Genes of interest (GFP, HIV-1 envelope and Flu-M1) will be expressed in lentiviral vectors which will be used to immunize mice by intradermal or intramuscular routes.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee noted inconsistency with approved IACUC protocols (e.g. intradermal route not on protocol). It was also no clear whether virus, or transduced cells, were being introduced into mice. The volume for intramuscular injections needs correction to be in alignment with current IACUC recommendations.

Community Member Comments: None

III-D, BSL-2, lentivirus, mice</td></tr>
<tr><td>55522</td><td>[REDACTED]</td><td>AAV Reporter Vectors</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to evaluate the tissue and cellular tropism of different AAV serotypes in humanized mice. A variety of AAV vectors expressing GFP or luciferase will be obtained from the UNC Vector Core or commercial supplier and injected into mice either directly into the lung organoids or intravenously.

Committee Comments: The proposed containment and safety practices are adequate for the experimental design. The Committee requested a more detailed title be provided and that the IV volume be reduced from 200uL to 100uL.

Community Member Comments: None

III-D, BSL-2, AAV, mice</td></tr>
</table>

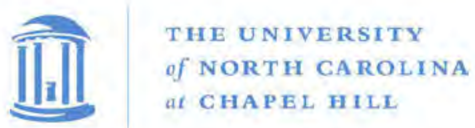
56104	[redacted]	CRISPR Screen to Identify Neutrophil Regulators of Interferon-Gamma; Signaling in Acute Lung Injury
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to use in vivo CRISPR screening to identify genes that regulate IFN gamma expression in neutrophils in the setting of S. pneumoniae infection. Purified mouse hematopoietic stem/precursor cells (HSPCs) will be infected with a library of commercially available lentiviruses containing gene-targeting sgRNA's. Transduced cells will then be implanted into irradiated recipient mice to reconstitute their immune system. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that IV volume be reduced from 200uL to 100uL. **Community Member Comments:** None III-D, BSL-2, lentivirus, mice	
54964	Silvia Kreda	Mucin secretion and mast cell degranulation in lung diseases
TABLED/ APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to transduce human airway epithelial cells and human mast cell lines with commercial retroviral vector PQCXIN expressing antarease metalloprotease. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee wanted clarification on whether just the metalloprotease was being expressed or other components of the scorpion toxin from which antarease is a part. If just the metalloprotease, approve with stipulations that this is clarified, including details on the vector. If whole toxin, then table pending further information and assessment for III-B. **Community Member Comments:** None	
56422	[redacted]	Splice switching oligonucleotides
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to test the activity of antisense oligonucleotides in preventing aberrant splicing in mRNA associated with human genetic diseases. Non-toxic oligonucleotides will be introduced into human or murine cell cultures and live mice via i.p., i.v. or intratracheal routes. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that dose/volume of nucleic acids administered to the mice be provided, as well as the method for anesthesia. **Community Member Comments:** None III-D, BSL-2, mice	
56424	Silvia Kreda	Reporter cell lines for splicing correction
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to create cell models to screen drugs/oligonucleotides that correct aberrant splicing in mRNA associated with human genetic diseases. A reporter gene (eGFP or luciferase) with a ~100bp long insertion of DNA encoding for a human splicing mutation (human CFTR or beta globin) and flanking sequence will be introduced into a retroviral vector (PQCXIP) for transduction of HeLa cells to create reporter cell line. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requests for details on the PQCXIP retrovirus. **Community Member Comments:** None III-D, BSL-2, retrovirus	

56303		AAV ITR transduction and host interactions
APPROVE	**Summary:** The aim of this experiment is to characterize the ability of AAV ITRs (inverted terminal repeats) to promote transgene expression in a mouse model. ITR-promoted Cre-recombinase will be expressed in AAV viral vectors, which will be utilized to transduce cells in vitro and in vivo. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, AAV, mice	
56122		Novel Nanoparticle Platform for the Delivery of Vaccines and Adjuvants
TABLED/ APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to test immune responses in mice to murine herpes virus (MHV68). Mice will be infected with MHV68 by intranasal or intraperitoneal route, and immune factors or viral loads analyzed at different times post-infection **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee noted that no descriptions regarding nanoparticles were provided in the protocol. If no nanoparticles used, then approved with stipulations that title be changed. If nanoparticles used, table for further information. The Committee also noted that the virus is not recombinant. **Community Member Comments:** None III-D, BSL-2, mice	
55962		Establishing MAGEA4/RAD18 As A Novel Cancer-Specific Chemotherapeutic Target
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to understand the ways in which cancer testes antigen (MAGEA4) and DNA repair genes confer tolerance of environmental, pharmaceutical and therapeutic DNA-damaging agents. DNA repair genes will be cloned into expression plasmids or viral vectors (adenovirus, lentivirus, retrovirus) which will be used to transfect or transduce mammalian cells. To induce expression of latent floxed genes (MAGEA4, Kras), mice will be treated with recombinant adenovirus encoding Cre recombinase. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requested that the IACUC number be updated, with clarification on inhalation (intranasal vs intratracheal), and clarification on whether virus into mice, or modified cells into mice. **Community Member Comments:** None III-D, BSL-2, lentivirus, adenovirus, retrovirus, mice	

55682	[redacted]	Immune Regulation and Immune diseases
APPROVED	**Summary:** The aim of this experiment is to study T cell function under normal physiology and during immune pathogenesis. Transgenes (Cre, GFP, YFP, RFP, luciferase and ovalbumin) will be expressed in mouse stem cell retrovirus (MSCV) which will be used to transduce cells in vitro. Transduced cells will be injected into mice through i.p. or i.v. administration. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. **Community Member Comments:** None III-D, BSL-2, retrovirus, mice	
56322	[redacted]	Cell therapy to treat mice with tumor labeled with OVA peptides
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to harvest T cells from OT-1 transgenic mouse spleens and transfer to mice with tumor pre-labeled with ovalbumin peptides. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee wanted clarification on mice were being restrained during IV administration of cells. **Community Member Comments:** None III-D, BSL-1, mice	
55743	Mark Zylka	Lentiviral transduction of neurons
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is use lentiviruses containing shRNAs to knock down gene targets in mouse cortical neurons. In some experiments, shRNA knockdowns will be rescued by overexpression of a shRNA-resistant construct. **Committee Comments**: The proposed containment and safety practices are adequate for the experimental design. The Committee requested a more descriptive title be provided. **Community Member Comments:** None III-D, BSL-2, lentivirus	

1. **Sub-committee Approvals of Schedule G**: 5
   PI: [redacted] **Title:** The use of AAV vectors to selectively inhibit interferon signaling in specific brain cell types (ID 56342, III-D)
   PI: Rihe Liu **Title:** Plasmid extraction from bacteria (ID 55464, III-F)
   PI: Benjamin Philpot **Title:** Role of TCF4 in Pitt Hopkins syndrome (ID 55169, III-F)
   PI: Bernard Weissman **Title:** SWI/SNF complex loss facilitates gene silencing during NSCLC development (ID 56002, III-D)
   PI: [redacted] **Title:** ARF-MDM2-P53 tumor suppression pathway knock-in mice (ID 56610, III-E)
2. **Schedule H report:** 44
3. **Next IBC meeting date:** March 6, 2019. Location: Burnett-Womack 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
May 1, 2019 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Keith Porterfield, Amy Sims, Aravinda DeSilva, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole, Eric Lewis

Members Absent: Barbara Savoldo, Xiao Xiao, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests: None

Open Meeting

1. **Review minutes from the April 3, 2019 meeting.** Minutes approved.
2. **0** – Garry Coulson presented a compassionate use request for a patient who had received
3. **Safety Report** – Garry Coulson provided an updated safety report for
4. **NIH Guidelines 2019** – Garry Coulson and Doug Cyr discussed the new NIH Guidelines that were recently release specifically some of the changes regarding administration of human gene therapy trials and the change in focus for the RAC.
5. **US National Inventory for Poliovirus Containment** – Garry Coulson discussed the poliovirus initiative driven by the CDC and the involvement of UNC in this initiative. All researchers on campus have been asked to complete an online survey to identify if they possess potentially infectious poliovirus-containing materials (PIM).
6. **Applications under review:**

ID	PI	Project Title
58219		Deletion of the coronavirus nsp2 and its effects on replication and pathogenesis
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to construct virus or MERS-CoV with deleted nsp2 gene, or a nsp2 deletion in the context of transcription regulatory sequence (TRS)-completely rewired genomes to evaluate the function of nsp2 in replication and pathogenesis. Viruses will be made using the established infectious virus clone system. Replication of virulence will be monitored in vitro in cell culture and in vivo in mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested an updated IACUC protocol number be provided. **Community Comments:** None III-D, BSL-3, mice	

<table>
<tr><td>60342</td><td></td><td>Expression of Norway rat hepacivirus core and envelope genes by Venezuelan Equine Encephalitis virus (VEEV) Replicon Particles</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to determine if Norway rat hepacivirus (NrHV) envelope proteins self-assemble into virus-like particles (VLPs) and to use them to vaccinate mice. Constructs encoding NrHV genes will be cloned into plasmids containing a modified VEEV gene (pVR21) deleted for VEEV structural genes. Plasmid will either be electroporated into cells for packaging and self-assembly of NrHV VLPs or transcribed and transfected into BHK cells to generate VRPs for injection into mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, plasmids, mice</td></tr>
<tr><td>60343</td><td>Ralph Baric</td><td>Defining the role of Dengue virus genotypic variation on the host neutralizing antibody response.</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to define the variation in dengue virus genotypes and how this variation impacts viral evasion of host immune responses. Using a dengue infectious clone, the lab will isolate 4 chimeric viruses, each containing the prM and E genes from 4 distinct isolates using standard reverse genetics. The resultant viruses will be analyzed for protein maturation and neutralization activity.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, plasmids</td></tr>
<tr><td>60345</td><td></td><td>Development of a Comprehensive Dengue Virus Molecular Clone Library Platform to Evaluate the Antigenic or Functional Variability of Viral Proteins</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to evaluate the effects of mutation of the E gene on antigenic and functional variability. Dengue serotypes 1,2, 3 and 4 carrying structural mutations in the E gene will be generated via site-directed mutagenesis and in vitro gene synthesis. Viruses harboring the mutations will be recovered and characterized in cell culture, ELISA and neutralization assays. Select mutants will be evaluated for antigenicity, pathogenicity and attenuation in vivo in mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, plasmids, mice</td></tr>
<tr><td>60346</td><td>Ralph Baric</td><td>Plasmids for Cre recombinase usage and verification</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to develop a plasmid-based system for Cre recombinase verification. Plasmids encoding Cre, and reporter genes, will be propagated in E. coli or transfected into cell lines.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

III-F, BSL-2, plasmids</td></tr>
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<tr><td>60347</td><td>Ralph Baric</td><td>Gene knock out and overexpression approaches to gain insight into gene function during virus infection (2019 Update)</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to confirm suspected host-virus interactions through gene knock out and overexpression studies in cell in culture. Replication-incompetent lentiviral vectors will be used to overexpress viral genes or knock out cellular genes of interest to determine their function in supporting viral infection.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, lentivirus</td></tr>
<tr><td>60348</td><td>Ralph Baric</td><td>Real-time plasmid constructs for calculating coronavirus replication</td></tr>
<tr><td></td><td colspan="2">Summary: The aim of this experiment is to generate plasmid constructs that can be used to evaluate coronavirus replication and processivity in a real-time assay. Orf1a and Orf1b constructs from coronaviruses of interest will be cloned into plasmids and maintained in E. coli. These constructs will not encode replication-competent genomes and no viruses will be generated. Constructs will be used as standards for real-time PCR assay.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, plasmids</td></tr>
<tr><td>60349</td><td></td><td>Mutants Removing Tertiary RNA Interactions - 2019 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to determine if pathogenic human coronaviruses use a similar mechanism to mediate translation of their RNA-dependent RNA polymerases (RdRps) as plant positive-sense, single-stranded RNA viruses. A series of single- and double-nucleotide point mutants were generated in the infectious clone which were monitored in replication in cell culture.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-3, plasmids</td></tr>
<tr><td>60350</td><td></td><td>Infectious clones of bat -like coronaviruses WIV1-CoV and SHC-014 (including reporter-expressing variants) or SARS-CoV expressing WIV1 or SHC014 Spike genes - 2019 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this study is to generate reverse genetic infectious clones of bat like coronaviruses WIV1-CoV and SHC-014, which are genetically similar to Additionally, to determine if the Spike proteins from these viruses are sufficient to confer infectivity, the Spike genes from the bat viruses will be introduced into the genome background. Replication of recombinant viruses will be monitored through viral passage in cells and infectious of mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
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	Community Comments: None III-D, BSL-3, plasmids, mice	
60351		Transposon mutagenesis of WIV16-CoV to identify genetically flexible regions of CoV genomes
APPROVED	**Summary:** The aim of this experiment is to generate a transposon mutant library spanning the WIVI6-CoV genome. The virus library will be screened in cell culture for viral fitness via passage in cell lines. Additionally, the screen will be interrogated for genes responsible for interferon antagonism or RNA replication fidelity. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-3, plasmids	
60352	Ralph Baric	Generation of phosphorylation mutants in the Zika virus envelope
APPROVED	**Summary:** The aim of this experiment is to test the importance of phosphorylated amino acids in the Zika virus envelop and their contributions to virus-host cell engagement by replacing them with amino acids that will either not be phosphorylated or possibly phosphorylated in altered amounts. The ZIKV reverse genetic clone system will be used to generate the mutants, which will be assesses for replication in cell culture. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, plasmids	
56065		TDP-43 in ALS and related neurodegenerative diseases
APPROVED	**Summary:** The aim of this experiment is to determine the role of TDP-43 in ALS and related neurodegenerative diseases. Human TDP-43 with a variety of tags, will be cloned into plasmids or lentiviral vectors for transfection or transduction of cells in vitro. TDP-43 expression plasmids will also be electroporated into mouse skeletal muscles which will be examined by histochemical and biochemical methods. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, plasmids, lentivirus, mice	
60902		Aggregated protein electroporation/expression in mouse skeletal muscle
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to study the role of microtubule-associated protein T (MAPT) and TAR-DNA binding protein of 43kDa (TDP-43) in mouse skeletal muscle. Human TDP-43 and Tau will be cloned into plasmids which will be injected into mouse muscle via electroporation. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Protocol should be III-D. **Community Comments:** None	

	III-D, BSL-1, plasmids, mice	
60163		The application of murine models to study the role of dysregulated transcription factors in pediatric solid tumors
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express reporter proteins (eg. GFP, luciferase, tdTomato) and wildtype or mutant forms of transcription factors and their targets in cells in vitro by transduction with lentiviral vectors. Transduced cells will be injected into mice through subcutaneous injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee required a list of intended target genes be provided. IACUC or WebID should be provided. **Community Comments:** None III-D, BSL-2, lentivirus, mice	
60102	Mohanish Deshmukh	Mechanism of apoptosis in mammalian cells
APPROVED	**Summary:** The aim of this experiment is to overexpress or downregulate various genes associated with regulation of apoptosis in mouse neurons, human ES cells and cell lines. Viral vectors containing constructs of interest will either be constructed using standard molecular methods, purchased from vendors or obtained from collaborators. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, lentivirus, plasmid	
60125	Mohanish Deshmukh	Transient transfection assays
APPROVED	**Summary:** The aim of this experiment is to transiently transfect mouse embryonic fibroblasts and mouse neurons with a plasmid expressing Apaf-1 to assess the effect on cell death after 48h. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-F, BSL-2, plasmid	
60142	Mohanish Deshmukh	Mechanism of apoptosis in mammalian cells
APPROVED	**Summary:** The aim of this experiment is to examine the outcome of expressing various regulators of apoptosis in mammalian cells. Plasmids and lentiviral vectors will be used to express genes of interest or shRNA to genes of interest in mammalian cells (human and mouse). **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, plasmid, lentivirus	

60782	[redacted]	Generation and characterization of mice with selected mutations in coagulation system proteins
APPROVED	**Summary:** The aim of this experiment is to generate mice with mutations in coagulation system proteins. Genes of interest will be cloned into plasmids which will be transfected into murine cells in vitro, or into mouse single cell embryos for implantation into pseudo-pregnant females. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-E, BSL-1, plasmids, mice	
60763	[redacted]	Mechanisms and Control of Cortical Network Activity
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to use AAV viral vectors to express a gene of interest (eArchT3.0) in the brains of ferrets following stereotaxic injection to assess the effects of this protein on changing neural activity. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that inoculum and concentration be provided, as well as means for anesthesia be indicated in appropriate section. **Community Comments:** None III-D, BSL-1, AAV, ferrets	
60764	[redacted]	A Control Systems Approach to Understanding Brain and Behavior
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to use AAV viral vectors to express a gene of interest (hChR2(H134R) in the brains of ferrets following stereotaxic injection to assess the effects of this protein on changing neural activity. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that inoculum and concentration be provided, as well as means for anesthesia be indicated in appropriate section. **Community Comments:** None III-D, BSL-1, AAV, ferrets	
59402	[redacted]	Generation of an allelic series within the MBD-1 gene of Collaborative Cross mice
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to generate mice with an allelic series within the MBD-1 gene. Modified MBD-1 alleles will be generated by CRISPR/Cas9 editing using synthetic DNA donor sequences. Mouse embryos will be injected with Cas9 mRNA, CRISPR targeting RNAS and donor sequences. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested an updated IACUC number be provided. **Community Comments:** None III-E, BSL-1, mice	

59202	Guochun Jiang	HIV replication
APPROVED	**Summary:** The aim of this experiment is to use lentiviral vectors to overexpress or knock down the expression of target human genes in mammalian cells to investigate their role in HIV replication. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, lentivirus	
59505		Cell mediated gene delivery
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to utilize plasmids to transfect cells in vitro with either of two reporter genes, firefly luciferase or GFP. Transfected cells, or plasmid, will ultimately be injected into mice directly in the tibialis anterior muscle. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested an updated IACUC number be provided, classification be adjusted from III-F to III-D, volume of inoculum be adjusted to <10uL and method of anesthetization be provided. **Community Comments:** None III-D, BSL-1, plasmids, mice	
60362		Lentiviral expression of luciferase
APPROVED	**Summary:** The aim of this experiment is to stably express luciferase in human pancreatic cell lines. The luciferase gene will be cloned into a lentiviral vector which will be used to transduce cells in vitro. Cells will ultimately be injected into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, lentivirus, mice	
60684		Gene Therapy of Hemophilia A
APPROVED	**Summary:** The aim of this experiment is to express coagulation FVIII in platelets of dogs that are deficient in this protein. Human FVIII will be cloned into SIN lentiviral vectors that will be used to transduce autologous peripheral blood stem cells before being reinfused back into the recipient dogs. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, lentivirus, dogs	

<table>
<tr><td>60685</td><td></td><td>Gene therapy for canine hemophilia B with an improved AAV vector</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to express coagulation FIX in platelets of dogs that are deficient in this protein. Human FIX will be cloned into AAV vectors that will be used to transduce autologous peripheral blood stem cells before being reinfused back into the recipient dogs.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, AAV, dogs</td></tr>
<tr><td>60686</td><td></td><td>Gene therapy for factor VII deficiency or hemophilia in a dog model of disease</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to express coagulation FVII, FVIIa or FVIII in platelets of dogs that are deficient in these proteins. Human coagulation proteins will be cloned into AAV vectors that will be used to transduce autologous peripheral blood stem cells before being reinfused back into the recipient dogs.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Committee requested that method of anesthetization be provided.

Community Comments: None

III-D, BSL-2, AAV, dogs</td></tr>
<tr><td>60687</td><td></td><td>Retroviral Gene Therapy of Blood Protein Deficiencies</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to express coagulation FVII, FVIIa, FVIII, FIX or von Willebrand factor in platelets of dogs that are deficient in these proteins. Target genes will be cloned into retroviral vectors that will be used to transduce autologous peripheral blood stem cells before being reinfused back into the recipient dogs.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, retrovirus, dogs</td></tr>
<tr><td>60689</td><td></td><td>AAV vectors for gene therapy of hemophilias and von Willebrand disease-1</td></tr>
<tr><td></td><td colspan="2">Summary: The aim of this experiment is to express coagulation FVII, FVIIa, FVIII, FIX (and variants), von Willebrand factor or canine tripeptidyl peptidase I in platelets of dogs that are normal or deficient in these proteins. Target genes will be cloned into rAAV vectors that will be used to transduce autologous peripheral blood stem cells before being reinfused back into the recipient dogs.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, AAV, dogs</td></tr>
</table>

<table>
<tr><td>60690</td><td>[redacted]</td><td>AAV vectors for gene therapy of hemophilias and von Willebrand disease</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to express coagulation FVII, FVIII, FIX or von Willebrand factor in platelets of dogs that are normal or deficient in these proteins. Target genes will be cloned into rAAV vectors that will be used to transduce autologous peripheral blood stem cells before being reinfused back into the recipient dogs.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, AAV, dogs</td></tr>
<tr><td>59722</td><td>Aziz Sancar</td><td>structure/Function of DNA photolyase and cryptochromes;DNA damage checkpoint and DNA repair; knockout cells</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use viral vectors to knock out genes of interest (DNA phytolyase and cryptochromes) in cells using CRISPR/Cas9 system to understand their role in DNA damage checkpoint and repair.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, lentivirus</td></tr>
<tr><td>59742</td><td>Aziz Sancar</td><td>structure/Function of DNA photolyase and cryptochromes;DNA damage checkpoint and DNA repair; Viral Vector</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use viral vectors to express genes of interest (DNA phytolyase and cryptochromes) into cells using CRISPR/Cas9 system to understand their role in DNA damage checkpoint and repair.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design

Community Comments: None

III-D, BSL-2, lentivirus</td></tr>
<tr><td>60622</td><td>Aziz Sancar</td><td>structure/Function of DNA photolyase and cryptochromes;DNA damage checkpoint and DNA repair; Plant Studies</td></tr>
<tr><td>TABLED</td><td colspan="2">Summary: The aim of this experiment is to knockout or overexpress genes involved in nucleotide excision repair in plaints using classical Agrobacterium-mediated transformation methods.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee noted that plant work was a new area for the Sancar lab to move into, and thus wanted verification of the location where this work was to be conducted and confirmation that appropriate plant containment practices and procedures were in place.

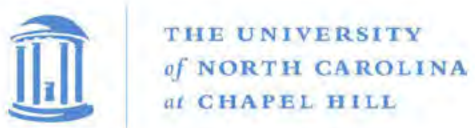
Community Comments: None

III-E, BSL-1, plasmids</td></tr>
</table>

60793	[redacted]	Recombinant Adeno-Associated Virus (rAAV) vector-based vaccination of mice
APPROVED	**Summary:** The aim of this experiment is to express transgenes of interest (IL-2, -4, -10, TGF-beta or prolactin) in mice using respective rAAV vectors to test for their capacity to block the autoimmune process of type 1 diabetes in NOD mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-1, mice	

7. **Sub-committee Approvals of Schedule G**: 6
   PI: [redacted] **Title:** Injection of Cre recombinase into mouse pups (ID 60382, III-D)
   PI: [redacted] **Title:** Injection of Cre-GFP into mouse pups (ID 60384, III-D)
   PI: [redacted] **Title:** Injection of GFP into mouse pups (ID 60385, III-D)
   PI: [redacted] **Title:** Mosaic scAAV9-mediated delivery of Cre recombinase to cortical neurons (ID 60802, III-D)
   PI: Benjamin Philpot **Title:** Genetic dissection of subplate function (ID 60802, III-D)
   PI: Yue Xiong **Title:** Retrovirus expressing GFP. RhoA and Tet2 (ID 60502, III-D)
8. **Schedule H report:** 49
9. **Next IBC meeting date:** June 5, 2019. Location: Burnett-Womack 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
May 9, 2019 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Keith Porterfield, Barbara Savoldo, Tori Baxter, Garry Coulson, Eric Lewis, Mary Beth Koza (voted in absentia), Xiao Xiao (voted in absentia)

Members Absent: Sandra Bradshaw, Amy Sims, Aravinda DeSilva, Craig Fletcher, Jessica Poole

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

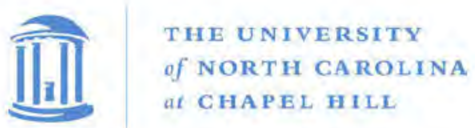
Guests:

Open Meeting

1. **Clinical Trial under review:**

ID	PI	Project Title
APPROVED	**Summary:** The aim of this clinical trial, in which UNC is a secondary site as part of a multicenter trial, is to evaluate **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The therapeutic is not novel and does not represent a first-in-human use. **Community Comments:** None III-C	

2. **Adjourn**.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
July 10, 2019 3:30 PM
GMB 2007

Members Present: Doug Cyr, Sandra Bradshaw, Aravinda DeSilva, Xiao Xiao, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole, Eric Lewis

Members Absent: Keith Porterfield, Amy Sims, Barbara Savoldo, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the June 5, 2019 meeting.** Minutes approved.
2. **Clinical Trial:**
3. **CHIV Inactivation Protocol Review** – presented an inactivation protocol for removal of brain and CNS tissue from the lab following fixation of the tissues with paraformaldehyde. Validation data for the inactivation procedure was presented to the Committee, as well as supporting documentation from two other peer institutions in support of this method. The Committee approved this inactivation procedure provided it was clearly stated in the laboratory SOP that this inactivation method applies ONLY to CHIKV-infected brain and spinal column, and tissues from mice infected with other agents still require the regular 48 hours PFA immersion before removal from the
4. **NIH Reportable Incident** – Garry Coulson discussed a recent incident in a laboratory involving an accidental needlestick exposure to a murine cell line expressing green fluorescent protein (GFP) and firefly luciferase reporter genes through retroviral transduction.

ID	PI	Project Title
APPROVED	**Summary:**	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-C	
63662	Ralph Baric	Generation of GFP and HA fusions of Coronavirus nonstructural proteins: 2019 renewal
APPROVED	**Summary:** The aim of this experiment is to track the intracellular localization of coronavirus proteins by immunofluorescence and Western blot. N- and C-terminal GFP and HA fusions of coronavirus nonstructural proteins nsp1-nsp16 will be expressed in commercial vectors and expressed in mammalian cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, plasmids	
63663		Transgenic mice expressing human dipeptidyl peptidase 4 (hDPP4) as models for MERS-CoV infection: 2019 renewal
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express the human dipeptidyl peptidase IV (DPP4) and chimeric human/mouse versions in mice from the endogenous DPP4 promoter using CRISPR-Cas9 using the . **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the volume of inoculum be provided. **Community Comments**: None III-D, BSL-2, mice	
63682		Characterization of Swine Acute Diarrhea Syndrome Coronavirus (SADS-CoV)
APPROVED	**Summary:** The aim of this experiment is to determine if novel cell types (e.g. porcine cells) or culture conditions (e.g. trypsin addition) could aid in the culture and characterization of SADS-CoV. An RFP-expressing SADS-CoV virus will created and replication monitored in cell culture and in vivo in mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-3, plasmids, mice	

63542		Production of a Transgenic Mouse Strain - BAC C4 Clones 7_O18 and 18_L6
APPROVED	**Summary:** The aim of this experiment is to produce a transgenic mouse strain expressing the C4A and C4B regions of the human genome. Bacterial artificial chromosome (BAC) harboring the unmodified C4A/C4B of the human genome will be constructed and injected into mouse embryos for integration into the genome. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-E, BSL-1, plasmid, mice	
61882	Adrienne Cox	Characterizing Ras and Rho small GTPases
APPROVED	**Summary:** The aim of this experiment is to study the signaling and other biological properties of Ras and Rho GTPases, and how these properties affect oncogenic transformation and mechanisms of responses to targeted therapeutics. shRNAs against Ras and Rho isoforms will be cloned into lentiviral vectors which will be used to transduce human cancer cells lines in vitro to knockdown exression **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, lentivirus	
62244		In vivo migration of Mesenchymal Stem Cells (MSC) from GFP (Green Fluorescent Protein) mice
APPROVED	**Summary:** The aim of this experiment is to determine the role of Thy-1 on MSC migration. Mesenchymal stem cells from commercially-obtained mice carrying GFP gene will be adoptively transferred to experimental mice (e.g. WT, Thy-1 null mice) to study their migration. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, plasmids, mice	
62245		Ad-TGFbeta model of progressive lung fibrosis in mice
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to develop a progressive model of idiopathic pulmonary fibrosis in mice. Porcine TGF-beta (wildtype and mutant variants) was cloned into plasmids and then subcloned into adenovirus derivative PJM17 (possessing an E1 deletion) before being used to transduce cells in vitro. Transduced cells were administered to mice via intranasal instillation. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that volume of intranasal administration be reduced from 62.5uL to 50uL maximum. **Community Comments**: None III-D, BSL-2, adenovirus, mice	

64075		Mayaro virus reverse genetics system
APPROVED	**Summary:** The aim of this experiment is to generate a recombinant Mayaro virus and study the role of specific viral genetic determinants in regulating viral replication and disease pathogenesis. A full-length infectious clone of Mayaro virus will be constructed in the pBR322 plasmid background. Replication of the virus in vitro and dissemination of the virus in mouse models will be assessed. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None	
64076	Mark Heise	Production of vaccine strain alphaviruses
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to use full length cDNA clones for the production of vaccine strains of chikungunya virus (CHIKV) strain 181/25 and Venezuelan equine encephalitis virus (VEE) strains V3626 or TC83. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a more specific title. **Community Comments**: None III-D, BSL-2, plasmids	
64079		Immune Evasion of Neurovirulent alphaviruses
APPROVED	**Summary:** The aim of this experiment is to test whether mutation(s) in the nsP1/nsP2 cleavage site in RRV, VEEV and CHIKV leads to attenuation in vitro. Mutations will be introduced into the cDNA infections clones of target alphaviruses and the resulting viruses tested for their ability to replicate in culture and induce type I interferon in cell culture. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-3, plasmids	
62122		Characterization of genetic interaction between Tau and Sacsin in ARSACS knockout mice model
APPROVED	**Summary:** The aim of this experiment is to study the interactions of tau kimases in Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) disease. Embryonic stem (ES) cells that have been transduced by non-viral vector expressing loxP sites flanking exon 4 of the sacsin will be purchased from an international repository and implanted into pseudopregnant recipient dams to generate a sacsin KO mouse. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. Committee requested IACUC number be provided. **Community Comments**: None III-E, BSL-1, mice	

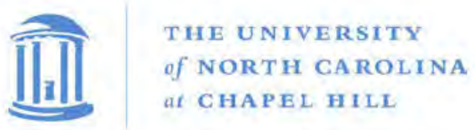
62704	[redacted]	Systemic RNA interference to reactivate p53 tumor suppression
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to test the efficacy of silencing RNAs (siRNAs) to human and mouse Bcl2L12 encapsulated in nanoparticles to reduce melanoma in a number of murine models of disease. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the volume of administration be provided in a format not expressed as "5X bodyweight". **Community Comments**: None III-D, BSL-2, mice	
62762	[redacted]	Engineering reporter B and T lymphocyte cell lines for immunotherapy studies
APPROVED	**Summary:** The aim of this experiment is to generate reporter cells lines for immunotherapy studies. Reporter genes (Ffluc, Rfluc, Gfluc. GFP, eGFP, RFP and DsRed) will be cloned into murine retroviral vectors or human lentiviral vectors, which will in turn be used to transduce B and T cells in vitro. Transduced cells will be studied in vitro and injected into mice for in vivo studies. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, retrovirus, lentivirus, mice	
49822	[redacted]	Generate fluorescently labeled human cell lines
APPROVED	**Summary:** The aim of this experiment is to make fluorescent constructs for super resolution microscopy and insert them into human and mouse cells. Genes of interest (e.g. cytoskeletal proteins) with fluorescent tags will be expressed in viral vectors which will be used to transduce mammalian cells in vitro. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, lentivirus	
63945	Timothy Moran	Role of neuropilins in airway inflammation
APPROVED	**Summary:** The aim of this experiment is to knock down expression of neuropilin-1 and -2 in primary mouse cells, mouse cell lines, human cell lines and primary human cells to explore the role of neuropilins in immune cell biology and pathogenesis of airway inflammation. sgRNA,s for NRP1 and NRP2 will be used to knockdown expression of their target genes using LentiCRISPR-Cas9 system. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, lentivirus	

63442	[redacted]	Lentiviral Gene Therapy of Inherited Bleeding Disorders in Dogs
APPROVED	**Summary:** The aim of this experiment is to express canine and human factor VII, VIIa, VIII, IX and von Willebrand factor (wildtype and variants) in dogs with inherited bleeding disorders. Genes of interest will be cloned into a SIN lentiviral vector, which will be used to transduce cells in vitro. Viral vector particles will be injected into dogs via IV injection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, lentivirus, dogs	
63482	[redacted]	BAC transgenesis of Ube3a isoform2-specific overexpressor line (Ube3a mIso2 OE)
APPROVED	**Summary:** The aim of this experiment is to generate a mouse line that over-expresses Ube3a isoform 2. The Ube3a mIso2 gene from mouse will be inserted into a BAC which will be injected into single-cell mouse embryos. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-E, BSL-1, plasmids, mice	
64342	[redacted]	Contributions of Glial Glutamate Transport and Transmission to Drug Abuse
APPROVED	**Summary:** The aim of this experiment is to perform high resolution analysis of astrocytes following cocaine self-administration. GFP will be expressed in astrocytes following injection into the brains of mice with AAV expressing GFP under the control of GFAP promoter and with Lck-tag to allow for membrane insertion of the GFP. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, AAV, mice	
63091	[redacted]	AAV ITR transduction and host interactions in astrocytes
APPROVED	**Summary:** The aim of this experiment is to characterize the ability of AAV inverted terminal repeats to promote transgene expression in astrocytes in a mouse model. AAV ITR followed by GFAP promoter to drive Cre-recombinase will be intracranially injected into Ai9 mice with floxed tdTomato. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, AAV, mice	

64002	[REDACTED]	CjCas9 and sgRNA plasmids for CFTR gene correction
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to use AAV vectors encoding Cas9 and sgRNAs to correct CFTR 508 deletion in CF mice. CFTR 508 deletion mice will be administered AAV/CFTR-Cas9 vectors via retro-orbital injection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that an IACUC number be provided, as well as the proposed volume for injection. **Community Comments**: None III-D, BSL-2, AAV, mice	

5. **Update on National Inventory for Poliovirus Containment Survey** – Garry Coulson discussed the completed results of the survey which was submitted to the CDC for review.
6. **Sub-committee Approvals of Schedule G**: 6
7. **PI**:. [REDACTED] **TITLE:** CRISPR-mediated knockout in A375 xenograft study (ID 62683; III-D)
   PI:. [REDACTED] **TITLE:** Enhancing the therapeutic efficacy of the fatty acid synthase inhibitor, fasnall (ID 62684; III-D)
   PI:. [REDACTED] **TITLE:** Pre-clinical evaluation of gene editing of TAK1 in breast cancer (ID 62702; III-D)
   PI:. [REDACTED] **TITLE:** Mechanisms of non-coding RNAs in breast cancer -Hector Franco (ID 62703; III-D)
   PI:. Jeremy Purvis **TITLE:** Developing fluorescent reporters for human genes (ID 63924; III-F)
   PI:. Miroslav Styblo **TITLE:** Environmental arsenic and diabetes (ID 63242, III-D)
8. **Schedule H report:** 30
9. **Next IBC meeting date:** August 7, 2019

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
September 4, 2019 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Amy Sims, Shawn Hingtgen, Tori Baxter, Mary Beth Koza, Garry Coulson, Jessica Poole, Eric Lewis

Members Absent: Keith Porterfield, Aravinda DeSilva, Xiao Xiao, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the August 7, 2019 meetings.** Minutes approved.
2. **Clinical Trial:** –

   PI:

ID	PI	Project Title
APPROVED	**Summary**: The aim of this multi-center trial is to evaluate the safety, tolerability and efficacy of **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-C	

61022	James Auman	Testing of CBL mutants in human Ben-Men I cells
APPROVED	**Summary**: The aim of this experiment is to investigate the effect of casitas B-lineage lymphoma protooncogene (CBL) mutation in the context of neurofibromin 2 (NF2) loss on cell biology. Lentiviral vector systems will be used to express CBL, CBL mutants or NF2 in cultured cells to examine their effects on RTK signaling and cell proliferation. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2, lentivirus	
66102		Construction of an Usutu virus infectious cDNA clone
APPROVED	**Summary**: The aim of this experiment is to construct an infectious cDNA clone of Usutu virus using standard molecular methods employed by the Baric lab for a number of other viral agents. Usuta virus fragments (A-D) will be propagated in bacterial plasmids. To assemble virus, plasmids will be cut with restriction enzymes, ligated together and electroporated into permissive cells for viral propagation. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2, plasmids	
66103		Production and use of rAAV vectors to deliver mammalian-expressing transgenes and antiviral biologics
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to express a variety of transgenes in rAAV vectors for the purpose of studying either the AAV vector itself, or the transgene. During vector production, adenoviral helper plasmid will be used. rAAV vectors will be used for transduction of mammalian cells or injection into mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. Committee requested an updated IACUC protocol number and details for intranasal anesthesia. **Community Comments:** None III-D, BSL-2, plasmids, AAV, mice	
66122	Ralph Baric	Establishment of a gRNA library for genome-wide screening of host and restriction factors for viral infections by CRISPR
APPROVED	**Summary**: The aim of this experiment is to perform genome-wide screening of host and restriction factors for viral infection or vector transduction using the lentiCRISPRv2 system. A single gRNA library of different cell types will be established and used to screen for factors that influence viral infection and vector transduction. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2, lentivirus	

<table>
<tr><td>66125</td><td>Ralph Baric</td><td>Generation of CMV promoter-driven constructs for coronavirus nonstructural and accessory proteins - 2019 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to express tagged and untagged versions of nonstructural proteins or accessory proteins from coronaviruses in mammalian cells to assay expression characteristics.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-2, plasmids</td></tr>
<tr><td>66126</td><td></td><td>Adenovirus vector-based transduced mouse model of MERS-CoV infection - 2019 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is transduce WT and DPP4 knockout mice with recombinant adenovirus (Ad5) expressing human DPP4 (or control) in order to develop a mouse model system that permits robust viral replication for MERS-CoV.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-2, adenovirus</td></tr>
<tr><td>66142</td><td>Ralph Baric</td><td>Design of Spike variant Porcine Epidemic Diarrhea Virus (PEDV) infectious clones - 2019 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to develop a full-length cDNA infectious clone of PEDV with variants in the Spike protein to identify less virulent Spike protein that can be used as potential PEDV vaccine strains. PEDV virus fragments (6-7 genome fragments) will be propagated in bacterial plasmids. To assemble virus, plasmids will be cut with restriction enzymes, ligated together and electroporated into permissive cells for viral propagation.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-2, plasmids</td></tr>
<tr><td>66143</td><td></td><td>Introduction of stop codons into coronavirus accessory open reading frame (ORF) genes to prevent expression of Coronavirus accessory proteins - 2019 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to examine the function of coronavirus accessory proteins by introducing stop codon into these genes to abrogate protein translation, while still preserving RNA structure. All manipulations will be performed using an established cDNA infectious clone of the coronaviruses of interest. Replication and virulence of derived viruses will be assayed in standard mouse model system.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-3, plasmids, mice</td></tr>
</table>

66144		Generation of Prefusion-Stabilized S2 Domain mRNAs from Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Spike (viral attachment protein) for Vaccination Studies
APPROVED	**Summary**: The aim of this experiment is to evaluate the efficacy of prefusion-stabilized spike proteins from MERS-CoV as vaccines for protection against CoV infection. mRNA's of prefusion-stabilized MERS S2 domain will be obtained from a collaborator and used to vaccinate mice prior to challenge with MERS-CoV virus. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2/3, mice	
66162		Maintenance of recombinant H1N1 influenza viruses for in vitro and in vivo infections - 2019 renewal
APPROVED	**Summary**: The aim of this experiment is to propagate H1N1 influenza viruses using infectious clones or supernatants containing virus. Viruses will be used to infect cell cultures or mice. No manipulations will be performed. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-3, plasmids, mice	
65922	Richard Boucher Jr.	Effects of hypoxia on CFTR gene transduction efficiency and efficacy in CF Airway Epithelia.
APPROVED	**Summary**: The aim of this experiment is to transduce primary human bronchiole epithelial cells with viral vectors expressing CFTR **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2, plasmids, AAV, lentivirus, PIV, adenovirus	
65962	Richard Boucher Jr.	Regions/Cell Types as Target for CFTR Therapy
APPROVED	**Summary**: The aim of this experiment is to identify region- and cell-specific responsible cell types for CFTR expression along the human airways. Human CFTR will be cloned into a variety of viral vectors which will subsequently be used to transduce cells in vitro. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2, plasmids, AAV, lentivirus, PIV, adenovirus	

<table>
<tr><td>65842</td><td>Xian Chen</td><td>The role of methyltransferase EHMT2 in epigenetic regulation and others</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to study the cellular effect when EHMT2 and Mettl3 methyltransferases are knocked down or knocked out. LentiCRISPRv2 system will be used to express sgRNA/DNA molecules for the targeted knockdown of genes of interest in tissue culture.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-2, plasmids, lentivirus</td></tr>
<tr><td>65702</td><td></td><td>Ex326 Cytochrome P450 (CYP) Humanized Rat</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to utilize the CRISPR/Cas9 system to create a humanized rat expressing human CYP (cytochrome P450) in place of its rat counterpart. The CYP transgene will be cloned into a plasmid which will be injected into rat embryos along with Cas 9protein and in vitro transcribed gRNA.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-E, BSL-1, plasmids, rat</td></tr>
<tr><td>65482</td><td></td><td>Generating mice with Cre dependent Cd276 expression</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate mice that express the cell surface marker CD276 in cells where Cre recombinase is active. The mice will also express mCherry and luciferase concurrent with the CD276. Insert genes of interest will be cloned into a plasmid which will be injected into mouse embryos before implantation in pseudopregnant dam.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-E, BSL-1, plasmids, mice</td></tr>
<tr><td>65122</td><td></td><td>Gene Targeting Retinoblastoma Using Nanoparticles</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to use gene targeting with nanoparticle technology as potential treatment for retinoblastoma (Rb). A mouse Rb cDNA library will be created using commercial vectors in human retinoblastoma cells or HEK293 cells. Plasmid DNA will be compacted to DNA nanoparticles and subliminally injected in mouse eye.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested containment be upgraded to BSL-2 to accommodate use of human cell culture and more details be provided regarding the formulation of the nanoparticle.

Community Comments: None

III-D, BSL-2, plasmids, mice</td></tr>
</table>

65123	[redacted]	Nanoparticle-mediated gene delivery for rhodopsin-associated retinitis pigmentosa
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to clone full length human rhodopsin DNA into commercial plasmids. Plasmid DNA will be compacted to DNA nanoparticles and subliminally injected in mouse eye. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested classification be adjusted from III-F to III-D and more details be provided regarding the formulation of the nanoparticle. **Community Comments:** None III-D, BSL-1, plasmids, mice	
65082	[redacted]	Generation of inducible knock down of ATG5/PC in breast and pancreatic cancer lines
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to generate an inducible knock-down of ATG5 or pyrvuvate carboxylase to determine the contribution of these proteins in treatment response in breast and pancreatic cancer. Lentiviral vectors expressing Tet-ON inducer and tetracyline regulated shRNA to ATG5 will be used to transduce cells in vitro. The cells will be characterized, and some will be injected into mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested that the concentration of transduced cells be provided in Section III. **Community Comments:** None III-D, BSL-2, lentivirus, mice	
64522	[redacted]	Roles of nuclear cGAS as a histone reader in regulating breast cancer metastasis
APPROVED	**Summary**: The aim of this experiment is to transduce MDA-MB-231 cells delete of endogenous cGAS with lentivirus expressing cGAS variants. Cells will ultimately be injected into mice to examine if cGAS mutants affect breast cancer cell metastasis. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2, lentivirus, mice	
66242	[redacted]	Molecular Physiology of Ankyrins and Spectrins
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to use rAAV as a tool to study cellular homeostasis and the mechanistic basis of cytoskeleton-associated diseases. rAAV virus containing Cre-GFP or Cre-dsRed will be purchased from UNC Vector core, and stereotactically injected into mice **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested an updated IACUC protocol number. **Community Comments:** None III-D, BSL-2, AAV, mice	

<table>
<tr><td>66282</td><td></td><td>Injection of Luciferase expressing cells into the mammary fat pad of mice</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to test the anti-tumor efficacy of new drug delivery carriers in tumored mice. Mammalian cells will be transfected with plasmids expressing GFP or luciferase. These cells will be inoculated into the mammary fat pads of mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-2, plasmids, mice</td></tr>
<tr><td>65443</td><td></td><td>Infection of neurons in mice with viral vectors</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to express transgenes in neurons in vivo for tracing the connectivity of neurons, or for imaging and manipulating the activity of neurons. Genes of interest (GFP, YFP, tdTomator, mRuby, calcium indicators etc) will be cloned into AAV or rabies vector, which will be subsequently be injected into mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested clarification on whether just AAV, or whether rabies virus vector is also being used.

Community Comments: None

III-D, BSL-2, AAV, rabies, mice</td></tr>
<tr><td>65462</td><td></td><td>Generation of novel knockin mouse lines</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to develop novel transgenic mouse lines as tools to gain genetic access to opioid-receptor expressing cells and manipulate opioid receptor expression. Transgenic mice will be generated by the ore using established methodology involving injection of plasmids expressing transgenes of interest into embryos prior to implantation in pseudopregnant dam.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-E, BSL-1, plasmids, mice</td></tr>
<tr><td>64223</td><td></td><td>Circuitry study of adult neurogenesis regulation and function</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to activate or repress neurons in vivo using rAAV or retroviruses to express channelrhodopsin-2, archaerhodopsin or muscarinic receptors in those neurons. Viral vectors, which have already been produced or purchased, will be injected into the brains of mice through stereotaxis.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested clarification on whether vectors to be used also include lentiviral and rabies vectors.

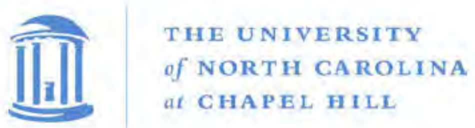
Community Comments: None

III-D, BSL-2, plasmids, retrovirus, lentivirus, rabies, mice</td></tr>
</table>

66042	[redacted]	AAV Expression of EGFP from hSynapsin promoters (hSyn.eGFP.WPRE.bGH)
APPROVED	**Summary**: The aim of this experiment is to utilize AAV vectors to express Cre, utilizing hSynapsin promoters, in site-specific regions of the brain in rodent models. Viral AAV vectors used to express Cre will be infused into the brain using stereotaxis. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-1, AAV, mice	
66043	[redacted]	AAV Packaging vector for hSyn driven KOR DREADD expression
APPROVED	**Summary**: The aim of this experiment is to utilize AAV vectors to express KOR DREADD, utilizing hSynapsin promoters, in site-specific regions of the brain in rodent models. Viral AAV vectors used to express KOR DREADD will be infused into the brain using stereotaxis. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design **Community Comments:** None III-D, BSL-1, AAV, mice	

1. **NIH Incident Report** – Garry Coulson discussed a low-risk, low-probability exposure of a laboratory worker to fixed mammalian cells expressing a reporter gene during staining of the cells.
2. **Sub-committee Approvals of Schedule G**: 5
3. **PI**:.Ralph Baric **TITLE:** Plasmids for Cre recombinase usage and verification (ID 66123, III-F)
   PI:.Ralph Baric **TITLE:** Establishment of the Sleeping Beauty transposon system for stable expression cell lines (ID 66124, III-F)
   PI:.Xian Chen **TITLE:** The role of methyltransferase EHMT2 in epigenetic regulation and others (65822, III-F)
   PI:. Martina Gentzsh **TITLE:** Expression of CFTR Protein for Antibody Production (ID 64682, III-F)
   PI:. Gregory Scherrer **TITLE:** Transformation of E. coli with plasmidic DNA (ID 65422, III-F)
4. **Schedule H report:** 42
5. **Next IBC meeting date:** October 2, 2019 Burnett-Womack 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
October 2, 2019 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Rachel Graham, Aravinda DeSilva, Tori Baxter, Garry Coulson, Jessica Poole, Eric Lewis

Members Absent: Sandra Bradshaw, Keith Porterfield, Barbara Savoldo, Xiao Xiao, Shawn Hingtgen, Craig Fletcher, Mary Beth Koza

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the September 4, 2019 meetings.** Minutes approved.
2. **Lab** – Proposal for research using (Schedule G 67055) – Presented
3. **Lab** – Proposal for research using inactivated -infected samples – Presented by Garry Coulson. The requested receipt of Trizol- or formalin-inactivated samples from from mice infected with wildtype or mouse-adapted . Samples have been inactivated using extensively tested and validated inactivation protocols that mirror the stringent protocols for inactivated -infected samples that the lab has prior approvals for from . Existing Biological Risk Assessment (BRAs) for inactivated samples should be applied to the inactivated samples. **APPROVED**.

ID	PI	Project Title
67055		Defining the role of tripartite motif gene (TRIM) family members in the host immune response to virus infection
APPROVED		**Summary**: The aim of this experiment is to use the biologically-contained clone of as a tool to understand the role of TRIM proteins in regulating immune responses to specifically how these proteins contribute to replication and virus-induced apoptosis in vitro. Since the strain is unable to replicate in cells unless they have been modified to express native VP30 protein, the deltaVP30 strain has approved by the NIH for BSL-2 containment, and has been removed from the CDC's list of select agent. The Baric lab will only work with this attenuated virus, and not the infectious clone. All work will be performed in BSL-3. No modification of the genome of this virus at UNC is proposed or permitted. The only genetic manipulations proposed by the lab are expression of VP30 in cells that either overexpress (viral vectors) or are deficient (CRISPR knockout) in TRIM genes **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. • No deltaVP30 materials may be received until an application to the NIH for a Minor Action has been approved. • Once NIH has approved the Minor Action, a Biosafety Risk Assessment (BRA) must be written for each activity with the deltaVP30 prior to receipt of samples and initiation of research project. • All work with the virus will be conducted within primary containment (e.g. BSC's) inside the BSL-3 suite following all applicable SOP's and BRA's • No virus, or materials containing the virus or viral RNA, may be distributed to any other entity outside of the Baric lab without consulting the IBC for relevant approvals.

<table>
<tr><td></td><td colspan="2">

- Attempts to clone the virus or genetically manipulate the virus are not permissible.
- Passage of the virus beyond 10 passages is restricted without prior IBC approval.
- Any anomalous or unexpected results (e.g. enhanced CPE) during in vitro passage/culture of the virus must be reported to the IBC immediately and work ceased with the virus until given approval from the IBC to proceed.
- Research approval is for in vitro activities only.

Community Comments: None

III-D, BSL-3, lentivirus
</td></tr>
<tr><td>66562</td><td>Craig Cameron</td><td>RNA-dependent RNA polymerase mechanism</td></tr>
<tr><td>APPROVED</td><td colspan="2">

Summary: The aim of this experiment is to understand the replication strategies and potential for inhibition of the virally-encoded positive-strand RNA-dependent RNA polymerases and accessory factors. Genes of interest from picornaviruses or flaviviruses will be expressed in E. coli following transfection with plasmids. Expressed proteins will be purified

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-1, plasmids
</td></tr>
<tr><td>66563</td><td>Craig Cameron</td><td>Single-cell analysis of viral infection dynamics</td></tr>
<tr><td>APPROVED</td><td colspan="2">

Summary: The aim of this experiment is to determine the parameters of the virus and host that gives rise to heterogeneity in the kinetics and magnitude of virus replication in single cells. Plasmid-encoded cDNA infectious clones for various picornaviruses will be used in in vitro transcription assays to produce transcribed RNA which will be transfected into mammalian cells for production of virus. Replication of virus will be monitored through the production of fluorescent reporter proteins expressed by the cDNA infectious clones.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-2, plasmids
</td></tr>
<tr><td>66662</td><td>Craig Cameron</td><td>Picornavirus Genome Replication</td></tr>
<tr><td>APPROVED</td><td colspan="2">

Summary: The aim of this experiment is to understand how positive-strand RNA viruses remodel the host cell and create an environment suitable for replication. Plasmid-encoded cDNA infectious clones for various picornaviruses will be used in in vitro transcription assays to produce transcribed RNA which will be transfected into mammalian cells for production of virus. Replication of virus will be monitored through the production of fluorescent reporter proteins expressed by the cDNA infectious clones.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design.

Community Comments: None

III-D, BSL-2, plasmids
</td></tr>
</table>

66682	Craig Cameron	Regulation of mitochondrial transcription
APPROVED	**Summary**: The aim of this experiment is to understand how mammalian mitochondrial transcription is regulated. Human mitochondrial transcription factors will be cloned into plasmid-expression vector that will be transfected into E. coli for expression and purification. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-F, BSL-1, plasmids	
66335	[redacted]	Optimization of oral pediatric SIV vaccines - MVA
APPROVED	**Summary**: The aim of this experiment is to express HIV/SIV genes in the MVA backbone for evaluation as a potential vaccine candidate. All animal work will be performed at collaborator. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2	
66403	[redacted]	Combination HIV_TB Vaccines
APPROVED	**Summary**: The aim of this experiment is to express SIV genes in an attenuated *M. tuberculosis* strain for evaluation as a potential vaccine candidate. All animal work will be performed at collaborator. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2	
67662	[redacted]	Investigations of RAS oncogenes and downstream signaling activities for cancer treatment
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to the role of RAS oncogenes and their downstream effectors in driving cancer growth, survival and motility. Genes of interest (eg. KRAS, NRAS, Myc) or siRNAs to these will be cloned into lentiviral or retroviral vectors which will be used to transduce cells to determine the biological effect on cells. Modified cells may be injected into mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested the volumes for administration into mice via each route be provided. **Community Comments:** None III-D, BSL-2, lentivirus, retrovirus, mice	

65782	Dirk Dittmer	Individual viral proteins influence on the p53 pathway
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to express viral genes in E. coli to understand their influence on the p53 pathway using biochemical assays. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested clarification on whether viral DNA will be provided from collaborators in the form of plasmid or genomic DNA. **Community Comments:** None III-D, BSL-2, plasmids	
65783	Dirk Dittmer	Gene knockdown by lentivirus-delivered shRNA to assay for virus fitness in tissue culture.
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to induce gene knockdown in cells to assay the influence on viral fitness and disease progression. Genes of interest (p53 pathway) will be knocked down by lentiviral expression of shRNA to those genes. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested clarification on which targets and which viruses will be used in this analysis. **Community Comments:** None III-D, BSL-2, lentivirus	
67383	[redacted]	Cortical circuits underlying the processing of biologically meaningful sounds.
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to dissect the neural circuit mechanisms underlying the sound information processing in mouse auditory cortex. Viral vectors encoding fluorescent proteins, optogenetic tools or Cre-recombinase will be injected into mouse brains. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. Committee requested IACUC number be updated. **Community Comments:** None III-D, BSL-2, CAV, mice	
66342	Ann Matthysse	Identification of genes involved in the retention of pathogenic E. coli and S. enterica by plants
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to identify bacterial genes involved in adherence or retention on plants, such as curli, pili, calcium dependent adhesins or glycoside hydrolases. Target genes from pathogenic E. coli or S. enterica will be cloned into plasmids and expressed in non-pathogenic E. coli K-12, which will be used to infect plants (e.g. tomato, lettuce). **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested Section III be completed with information on how the plants will be infected. **Community Comments:** None III-D, BSL-2, plasmids, plants	

66902		Adenovirus gene therapy for hemophilia
APPROVED	**Summary**: The aim of this experiment is to express coagulation factors or von Willebrand factor in dogs that are deficient in these proteins to correct their bleeding disorders. Respective factors will be cloned into Adenovirus vectors which will be used to transduce cells in vitro or injected into dogs. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2, adenovirus, dogs	
67342		siRNA down regulation of plasminogen in normal, hemophilia A, hemophilia B, VWD and FVII deficient dogs
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to determine if reduced levels of plasminogen correct the bleeding diathesis in dogs with inherited bleeding disorders. Synthetic siRNA targeting plasminogen for down-regulation will be encapsulated into ionizable cationic lipid nanoparticles will be injected into dogs to deliver the siRNA preferentially into the liver hepatocyte cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested details on the formulation of the nanoparticles. **Community Comments:** None III-D, BSL-1, dogs	
66223	Mark Peifer	Regulation of actomyosin structures at adherens junctions
APPROVED	**Summary**: The aim of this experiment is to understand the roles of different factors inside the c ell in actomyosin structure formation and regulation at apical adherens junctions of epithelial cells. shRNA will be cloned into lentiviral vectors which will be used to transduce mammalian cells in vitro. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-2, lentivirus	
66702		Dissecting the neural circuits of hyperarousal states
APPROVED	**Summary**: The aim of this experiment is to dissect the circuit mechanisms underlying changes in rapid arousal states across the brain. AAV viral vectors expressing fluorescent proteins, optogenetic tools, or genetically encoded calcium indicators will be injected into the mouse brain. All vectors will be obtained from collaborators or vendors. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, BSL-1, AAV, mice	

<table>
<tr><td>67422</td><td>[redacted]</td><td>Role of the Rho-GAP GRAF3 in human hypertension</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to express adhesion signaling molecules in cells. Genes of interest (GRAF and GRAF variants) will be cloned into plasmids or viral vectors (lentivirus, Adenovirus, AAV) which will be transfected into cells or injected into mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested an updated IACUC number.

Community Comments: None

III-D, BSL-2, lentivirus, AAV, adenovirus, mice</td></tr>
<tr><td>66243</td><td>[redacted]</td><td>Recombinant Adeno-Associated Virus (rAAV) vector-based vaccination of mice_Updated</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to test recombinant AAV vectors for the capacity to block the autoimmune process of type 1 diabetes in non-obese diabetic (NOD) mice. Genes of interest (e.g. murine interleukins, TGF-beta, prolactin etc) will be expressed in AAV which will be injected into mice.

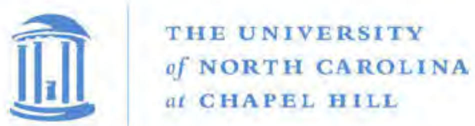
Committee Comments: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested an updated IACUC number.

Community Comments: None

III-D, BSL-1, mice</td></tr>
</table>

5. **Sub-committee Approvals of Schedule G**: 2
   PI: [redacted] **Title:** Role of the CREB Pathway in Mouse Models of Head and Neck Cancer (Schedule G 66742, III-D)
   PI: Kim Brouwer **Title:** Novel Mechanism of Drug-Induced Hepatotoxicity: Altered Transporter Phosphorylation (Schedule G 66642, III-F)
6. **Schedule H report:** 37
7. **Next IBC meeting date:** November 6, 2019 Burnett-Womack 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
December 4, 2019 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Rachel Graham, Aravinda DeSilva, Barbara Savoldo, Tori Baxter, Garry Coulson, Jessica Poole, Eric Lewis

Members Absent: Keith Porterfield, Xiao Xiao, Shawn Hingtgen, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the November 6, 2019 meetings. Approved**
2. **Clinical Trial:** **Approved with stipulations** (see below)
3. **Managed Access Program (MAP):** Managed Access Program (MAP) to provide access to or patients with out of – Garry Coulson presented this single-subject IND for the use of tisagenlecleucel that is out-of-specification for commercial release. was approved by the IBC in November 2018 for a similar trial. **Approved.**
4. **NIH Incident Report:** Garry Coulson discussed an incident in a high-containment laboratory involving a leak of possible body fluid from a bag containing infected mouse carcasses.
5. **Applications under review:**

ID	PI	Project Title
APPROVED WITH STIPULATIONS	**Summary:** The aim of this Phase 2 study is to evaluate the clinical benefit of	

	[redacted] **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The committee requested safety information from the Phase 1 studies that examined this drug. III-C, HSV-1
69349	[redacted] | Generation of a full-length infectious cDNA clone of bat MERS-like coronavirus GD/2014-422, including reporter-expressing and mouse-adapted variants
APPROVED	**Summary:** The aim of this experiment is to generate a reverse genetic system for the bat MERS-like coronavirus GD/2014-422. In addition to synthesis of the wildtype infectious clone using 6-8 plasmids, the genome of the cDNA clone will also be modified to generate reporter viruses expressing fluorescent proteins. Furthermore, amino acid substitutions in the putative viral receptor binding domain (RBD) will be introduced in order to generate a mouse-adapted version of the virus as a surrogate to MERS-CoV. Viral replication and virulence of derived strains will be assessed in cell culture and in mice models of disease. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice
70202	[redacted] | Generation of infectious cDNA clones of 2D (HKU9) coronaviruses
APPROVED	**Summary:** The aim of this experiment is to synthesize full length cDNA genomes of 2D-coronaviruses. A number of different spike genes will be derived from available GenBank sequences and introduced into the full-length cDNA genes to assess the host range of different 2D coronavirus variants. To aid in visualization, candidate viral genes may be replaced with reporter protein-coding sequences. Viral replication and virulence of derived strains will be assessed in cell culture and in mice models of disease. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice
70203	[redacted] | Generation of viral replicon particles (VRPs) from the attenuated 3526 strain of Venezuelan Equine Encephalitis (VEE) expressing the Spike glycoproteins from group 2d HKU9 coronaviruses
APPROVED	**Summary:** The aim of this experiment is to create vaccine candidates using the VEE replicon particle (VRP) system to express the spike glycoproteins from group 2D coronaviruses. VRP's will be used to vaccinate mice, and resultant sera used to assess cross-reactivity with an array of coronaviruses used in the lab. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mice

69102		K1/vIRF2 mice
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to create transgenic mice expressing K1 and vIRF2 genes from KSHV to understand the function of these genes in vivo. Target genes will be cloned into a plasmids, which will be injected into mouse embryos before implantation into pseudopregnant recipient mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that a more informative title be provided, and the IACUC number be updated to 19-282 III-E, BSL-1, plasmids, mice	
69662	Robert Dowen	The genetics of lipid metabolism in Caenorhabditis elegans
APPROVED	**Summary:** The aim of this experiment is to express target genes (e.g. GFP, mCherry, Cas9, CRE recombinase, MosI transposase) in *C. elegans* for expression. Genes of interest will be cloned into plasmids, which will be microinjected into C. elegans for *in vivo* expression. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids	
69907	Paul Eldridge	CAR.CSPG4 Retroviral Vector/Chimeric Antigen T cells
APPROVED	**Summary:** The aim of this experiment is to produce retroviral vector for use in generation of chimeric antigen T cells for clinical studies. Genes of interest SFG.iC9.2A.scFv763.hCD8a.CD28z will be cloned into Mo-MuLV retroviral vector. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, retroviral vector	
68887		Purified AAV1.Camk2a.GCaMP6f.WPRE.bGHpA vector
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to image calcium in neurons of the mouse cortex using an rAAV vector expressing the ultrasensitive protein calcium sensor injected into the mouse neocortex. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a more descriptive title, in addition to information on the dose to be injected, and whether the AAV virus is replication-deficient. III-D, BSL-1, AAV, mice	
68888	Sylvia Fitting	LentiBrite PSD95-GFP Lentiviral Biosensor
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to use commercially available lentiviral vectors to provide bright fluorescence and precise localization to enable cell analysis of PSD dynamics in primary mouse cell cultures (neurons, astrocytes, microglia) transduced with the lentivirus. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a definition be provided for PSD. III-D, BSL-2, lentivirus	

<table>
<tr><td>69623</td><td>[redacted]</td><td>Over expression and knockout of non-coding RNAs in breast cancer cell lines for mouse xenograft assays</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to over-express or knockout target non-coding RNA's in breast cancer cell lines to determine the effects on cancer cell proliferation and metastasis. Engineered cell lines will be use for mouse xenograft assays to measure tumor growth ability and metastasis of these cell lines. Mammalian expression vectors wills be used for overexpression of target non-coding RNA's, whereas CRISPR-based techniques will be used for knockout.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Committee requested that the IACUC number be updated.

III-D, BSL-2, plasmids, mice</td></tr>
<tr><td>70602</td><td>[redacted]</td><td>Analysis of genes in avirulent [redacted] (BSL2 level)</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to identify and characterize virulence determinants of [redacted] including how these genes are transcriptionally regulated under various conditions. Genes of interest will either knocked out, or overexpressed, in the attenuated BSL-2 strain of [redacted] (CD1- or pgm-), or close relatives Y. enterocolitica and Y. pseudotuberculosis, using standard plasmid-based molecular methods. Recombinant bacteria will be assessed for growth in primary mouse macrophages or human neutrophils.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids</td></tr>
<tr><td>69082</td><td>William Goldman</td><td>Analysis of virulence in Histoplasma capsulatum</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to identify and characterize virulence factors of the respiratory pathogen H. capsulatum, or closely-related fungi P. brasiliensis and B. dermatitidis. DNA plasmids are used to generate targeted mutants, complement a mutant, or modify/silence gene expression.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids</td></tr>
<tr><td>69003</td><td>[redacted]</td><td>Analysis of virulence in fully virulent [redacted]</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to identify and characterize virulence determinants of [redacted] including how these genes are transcriptionally regulated under various conditions. Genes of interest will either knocked out, or overexpressed, in fully-virulent strains of [redacted] using standard plasmid-based molecular methods. Recombinant bacteria will be assessed for growth and virulence in primary mouse macrophages or human neutrophils, and in in vivo mouse models of infection.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-3, plasmids, mice</td></tr>
</table>

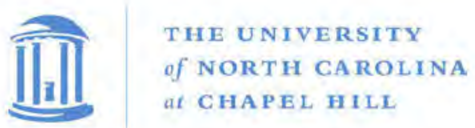
69382	William Goldman	Analysis of virulence in Histoplasma capsulatum
APPROVED	**Summary:** The aim of this experiment is to identify and characterize virulence factors of the respiratory pathogen H. capsulatum, or closely-related fungi *P. brasiliensis* and *B. dermatitidis*. DNA plasmids are used to generate targeted mutants, complement a mutant, or modify/silence gene expression. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids	
69802		AAV Gene Delivery in Pregnant Mice
APPROVED	**Summary:** The aim of this experiment is to determine if rAAV vectors alter the fetal development when administered to pregnant mice. AAV serotype 2 vectors expressing luciferase will be administered to pregnant mice through IV injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, AAV, mice	
64202		Delivering Genes into dividing cells via pBABE Retroviral Plasmid Vector
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to clone human/mouse genes into cell line models using the pBABE retroviral vector to study the effects of over-expression or ablation of a specific gene in cell line model. Modified cells may ultimately be injected into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the proposed volume of administration be adjusted to fit within current accepted IACUC limits. III-D, BSL-2, retroviral vectors, mice	
64242		Expression of Cre Recombinase in mice via adenoviral vector
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express Cre recombinase in mice using adenoviral vector Ad5CMVCre, purchased from another institution. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the proposed volume of administration be adjusted to fit within current accepted IACUC limits. III-D, BSL-2, adenoviral vectors, mice	
64243		Delivering shRNA constructs via pLKO Retroviral Plasmid Vector
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to silence specific genes in cell lines using lentiviral vector expressing shRNA directed to target genes. Transduced cells may be injected into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the proposed volume of administration be adjusted to fit within current accepted IACUC limits. III-D, BSL-2, lentiviral vectors, mice	

68942		Bispecific antibody formats for cancer imaging and therapy
TABLED	**Summary:** The aim of this experiment is inoculate luciferase or GFP-expressing cancer cell lines into mice for cancer imaging. **Committee Comments:** The Committee could not unambiguously determine what the PI was proposing to do in this protocol. III-D, BSL-2	
69482		Idua vectors for peptide enhanced AAV transduction of the brain
APPROVED	**Summary:** The aim of this experiment is to study peptide-based enhancements of blood brain barrier penetration and AAV transduction of the brain following systemic delivery in a mouse model of MPS 1 disease. An expression cassette for the mouse Idua gene will be expressed in AAV vectors which will be used to transduce the brain and other tissues of mice in vivo after systemic injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, AAV, mice	
69742		Testing the effect of Sox9 levels on the intestinal stem cell
APPROVED	**Summary:** The aim of this experiment is to create a mouse model in which Sox9 can be inducibly expressed throughout the intestine. Sox9 and the tet-on promoter will be cloned into a plasmid which will be injected into mouse embryos. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
69602		Chromatin regulation and remodeling during liver injury and repair
APPROVED	**Summary:** The aim of this experiment is to identify epigenetic regulators of hepatic stellate cell activation and liver fibrosis. AAV will be used to deliver CRISPR guide RNAs to the mouse liver through intravenous injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, AAV, mice	
68622	Lillie Searles	Pre-mRNA Metabolism
APPROVED	**Summary:** The aim of this experiment is to elucidate the role of the Drosophila suppressor of sable (su(s)) gene product in nuclear RNA metabolism. Variants of the Su(s) gene or reporter genes subject to regulation by Su(s) will be cloned into plasmids that will be transfected into cultured Drosophila cells. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids	

69722	Rita Tamayo	Identification and Characterization of Virulence Genes of Intestinal Pathogens-Aad9
APPROVED	**Summary:** The aim of this experiment is to create aad9-based vectors expressing c-di-GMP and ppGpp metabolic enzymes, putative c-di-GMP and ppGpp receptors, and predicted C. difficile adhesins. Plasmids will be introduced into C. difficile by conjugation with resulting strains assessed in a variety of in vitro assays. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids	
68922		Recombinant Adeno-Associated Virus (rAAV) vector-based vaccination of mice
APPROVED	**Summary:** The aim of this experiment is to test the capacity of packaged rAAV vectors to block the autoimmune process of type 1 diabetes in non-obese diabetic (NOD) mice. A number of transgenes of interest will be expressed in rAAV vectors which will be injected directly into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, AAV, mice	
69402		Plasmid DNA (pDNA)- based Vaccination
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to determine the therapeutic efficacy of administering pDNAs encoding cytokines and antigens to regulate immune responses in mice. Genes of interest will be cloned into a plasmid which will be administered to mice for pDNA vaccination. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested volume of IM administration be adjusted to fit within approved limits. Committee also indicated no current approvals for intradermal injection for protocol 19-200. III-D, BSL-1, plasmids, mice	
69503		Decipher catalytic dependent roles of TET3
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to generate knock-in mice that express full-length TET3 but with a mutation that compromises the catalytic activity to help understand the role of catalytic activity on TET3 function. Plasmid DNA encoding sequences for Tet3 gene will be cloned into a plasmid, which will be injected into single-cell mouse embryos. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that TET3 be defined in the protocol. III-D, BSL-1, plasmids, mice	

6. **Sub-committee Approvals of Schedule G**: 0
7. **Schedule H report:** 48
8. **Next IBC meeting date:** January 8, 2020 TBD

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
January 8, 2020 3:30 PM
MHRC 3100

Members Present: Doug Cyr, Keith Porterfield, Rachel Graham, Barbara Savoldo, Garry Coulson, Eric Lewis

Members Absent: Monica Dodson, Xiao Xiao, Shawn Hingtgen, Craig Fletcher, Tori Baxter, Jessica Poole

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the December 4, 2019 meetings.** Approved
2. **Inactivation of CoV-infected samples for removal from BSL-3.** presented validation data for inactivation of tissue culture cellular lysates infected with /MERS-CoV for removal from the BSL-3, and inactivation of RNA on nylon membranes for Northern blotting. The aim for both procedures was to demonstrate that neither replication-competent virus nor viral RNA capable of mediating generation of progeny virions are present in these samples, thus rendering the samples inactivated and non-select. Data provided using cytopathic effect (CPE) and plaque assays to quantify viability suggest that the methods described are successful in inactivating any infectious material. Since no LOD was indicated in the provided data, the Committee requested additional experiments performed (e.g. RT-PCR) to exclude the possibility of residual viable virus present undetectable using the cellular CPE and plaque assays.
3. – Garry Coulson presented this single-patient compassionate use protocol using was approved by the IBC in 2018 for a Phase I trial using this therapeutic. **Approved.**
4. **Applications under review:**

ID	PI	Project Title
71242		Investigating MERS-CoV novel ORF8C function
APROVED	**Summary:** The aim of this experiment is to investigate if ORF8C has any effect on MERS-CoV replication and pathogenesis both in vitro and in vivo. Knockout viruses in which ORF8C has been ablated by modifying wobble position codons and removing start codons will be constructed using standard infectious clone methodology in which the cDNAs will be maintained as 7 separate cDNA cassettes in plasmids. Plasmids will be propagated in E. coli, RNA will be electroporated into BHK-21 or Vero cells and used to infect Calu3 cells. Viruses will also be inoculated into mice intranasally. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	

71243	[redacted]	Investigating MERS-CoV Accessory ORF function in vivo
APROVED	**Summary:** The aim of this experiment is to investigate how accessory ORFs effect MERS-CoV pathogenesis in vivo. A panel of knockout viruses in which target accessory ORF has been ablated by modifying wobble position codons and removing start codons will be constructed using standard infectious clone methodology in which the cDNAs will be maintained as 7 separate cDNA cassettes in plasmids. Plasmids will be propagated in E. coli, RNA will be electroporated into BHK-21 or Vero cells and used to infect Calu3 cells. Viruses will also be inoculated into mice intranasally. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	
71562	[redacted]	Vero E6 cell passaging of wildtype and ExoN(-) [redacted]
APROVED	**Summary:** The aim of this experiment is to passage [redacted] ExoN(-) in Vero E6 cells to increase fitness of the attenuated virus in vitro and in vivo. Once a more-fit [redacted] ExoN(-) passage has been identified, it will be tested in vivo in mice and sequenced to identify mutations contributing to enhanced fitness. Mutations will be introduced into the [redacted] ExoN(-) to test identified mutations for their role in viral fitness and pathogenesis.. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	
71222	[redacted]	Production of a PhiC31 Mouse Strain
APROVED	**Summary:** The aim of this experiment is to produce mice with ubiquitous expression of a codon-optimized PhiC31 recombinase for efficient removal of att-flanked sequences. The PhiC31 gene will be cloned into a plasmid which will be injected into embryo's or embryonic stem cells to produce animals expressing the PhiC31 protein. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
71731	[redacted]	Ex337 Sycp1-Cas9 Mouse
APROVED	**Summary:** The aim of this experiment is to produce mice with Cas9 and eGFP expression from the Sycp1 locus. The desired transgene will be cloned into a plasmid which will be microinjected into mouse fertilized embryos along with Cas9 protein and synthetic guide RNA (gRNA) to promote insertion into the proper site of the mouse genome. Injected embryos will be surgically implanted into pseudopregnant recipient mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	

71732		Ex338 Ddx4-Cas9-DHFR Mouse
APROVED	**Summary:** The aim of this experiment is to produce mice with a modified Cas9 sequence flanked by DHFR domains expressed from the Ddx4 locus. The desired transgene will be cloned into a plasmid which will be microinjected into mouse fertilized embryos along with Cas9 protein and synthetic guide RNA (gRNA) to promote insertion into the proper site of the mouse **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
71733		Ex339 Spo11-Cas9 Mouse
APROVED	**Summary:** The aim of this experiment is to produce mice with Cas9 and eGFP expression from the Spo11 locus. The desired transgene will be cloned into a plasmid which will be microinjected into mouse fertilized embryos along with Cas9 protein and synthetic guide RNA (gRNA) to promote insertion into the proper site of the mouse genome. Injected embryos will be surgically implanted into pseudopregnant recipient mice **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
70302		Image-guided, ultrasound-enhanced long-term intracranial drug delivery
APROVED	**Summary:** The aim of this experiment is to use human glioblastoma cells lines already modified through stable expression of fluorescent and bioluminescent markers by means of lentiviral vector for tracking cancer in vitro and in vivo. Modified cells will be administered to mice and tracked over time. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentiviral vector, mice	
71422		Tyro3 Floxed Mouse
APROVED	**Summary:** The aim of this experiment is to produce a mouse strain with a floxed allele of Tyro3. CRISPR/Cas9 will be used to insert loxP sites flanking key exons of Tyro3 by embryo microinjection. Embryos will be microinjected with plasmid DNA along with Cas9 protein and guide RNA designed to stimulate insertion of the loxP sites at the correct sites in the genome **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
71102	Sylvia Fitting	Use of recombinant Tat (from HIV-1 IIIB) protein
APROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to expose in vitro primary cell cultures to the recombinant Tat protein (from HIV-1 IIIB) to assess changes at the cell level, including structural and functional changes, such as morphology, ion homeostasis, and excitability. Commercially-purchased Tat protein will be added to primary mouse cell cultures and structural/functional changes assessed. **Committee Comments:** The proposed containment and safety procedures are adequate for the	

	experimental design. Committee wanted clarification whether the recombinant Tat protein will be purchased, or whether it will be made in the lab. No classification	
71462		Abrogation of airway epithelial
APROVED	**Summary:** The aim of this experiment is to optimize gene delivery into airways in vivo. AAV or Ad vectors expressing reporter genes and the human CFTR will be constructed and administered to rabbits via direct instillation. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, AAV, Ad, rabbits	
71202		Safer 5th generation lentiviral vectors with reduced viral sequence including opposite orientation
APROVED	**Summary:** The aim of this experiment is to generate safer lentiviral vectors, which are less likely to recombine, mobilize or affect host gene expression upon integration to target cell chromatin. Vectors will be constructed with reduced parental viral sequence (including elimination of HIV packaging signals, Rev responsive elements, 5' U5 and part of Poly-A in the 3'LTR. Additionally, vectors will be created with tandem sequences up 10 1kb to trigger PKR pathway leading to silencing of undesired protein expression. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentiviral vectors, mice	
71203	Tal Kafri	Employing shRNA Lenti-Vectors on Human and Mouse Cells
APROVED	**Summary:** The aim of this experiment is to use shRNA expression cassettes to knockdown gene expression in human and mouse cell lines to determine the effects of knockdown on cellular function. Lentiviral vectors expressing the shRNAs will either be constructed by the lab or purchased from commercial vendors. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentiviral vectors,	
70802		Ceramide signaling in the regulation of cellular response to folate stress
APROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to investigate the function of ceramide synthase 6 (CerS6) in response to folate stress mediated by p53. CerS6 will be cloned into plasmids for transfection into mammalian cells in vitro. Alternatively, the sequence will be cloned into adenoviral vector for transduction of cells. Cells found to stably express the gene of interest will be injected into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested clarification on whether the volumes proposed were IACUC approved volumes. III-D, BSL-2, adenoviral vector, mice	

71705		Mouse infections with non-recombinant BSL2 viruses
APROVED	**Summary:** The aim of this experiment is to evaluate the role of host immune factors, such as interferons, in controlling viral pathogenesis. A variety of knockout, knockin or conditional knockout mice will be infected with viral pathogens (BSL-2) to evaluate virulence, viral replication and immune responses. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, mice	
71062	Chengwen Li	Variety of AAV/Cas9 constructs to test CFTR 508del correction
APROVED	**Summary:** The aim of this experiment is to develop an AAV delivered CRISPR/Cas9 system to target CFTR 508del mutations commonly found in patients with cystic fibrosis. Viral constructs will be made in which the guide RNAs target the human genome sequence near or at the CFTR 508 deletion. Guide RNAs and CjCas9 will be cloned into an AAV ITR plasmid and will be transfected with an mRFP+eGFP reporter plasmid into HEK293 cells. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, AAV	
71063	Chengwen Li	Lenti/CRISPR/Cas9 PYCARD/ASC and STING knockout cell lines
APROVED	**Summary:** The aim of this experiment is to develop two knockout cell lines, in which LentiCRISPR is used to knockdown expression of two proteins (PYCARD/ASC and STING) involved in innate immune responses to AAV. Lentiviral vectors expressing CRISPR/Cas9 will be tranduced into cells with target RNA for the genes of interest. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentiviral vector	
70663		Retrograde genetic targetting of neurons
APROVED	**Summary:** The aim of this experiment is to stereotactically inject replication-deficient HSV viral particles expressing Cre recombinase into the brains of mice. HSV particles are to be produced by collaborators at another institute. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, HSV vector, mice	
70664		Pseudorabies based mapping of neuronal pathways
APROVED	**Summary:** The aim of this experiment is to map inputs into neurochemically defined neurons. This mapping of neuronal pathways is achieved by injecting viral vectors that are Cre-dependent and depend on multiple infections and transsynaptic transport for specificity. AAV viral vectors will be generated bt viral vector core and SAD B19 produced by Falk Institute. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, AAV, mice	

<table>
<tr><td>70327</td><td>Wanda O’Neal</td><td>Knockdown and Overexpression of various genes relevant to Cystic Fibrosis</td></tr>
<tr><td>APROVED</td><td colspan="2">Summary: The aim of his experiment is to understand the effects of knocking down or over-expressing genes relevant to cystic fibrosis in cell culture. Cell lines will be generated either by transient transfection of CRISPR oligo’s or lentiviral transduction.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, lentiviral vectors</td></tr>
<tr><td>70542</td><td></td><td>D3 domain mutations to explore Muc5b Biology</td></tr>
<tr><td>APROVED</td><td colspan="2">Summary: The aim of this experiment is to mutate two cysteines responsible for multimerization of mucin in the lung to determine if prevention of multimerization results in disease or if the normal function of Muc5b can be maintained in the absence of multimerization. Mutated Muc5b gene will be cloned into plasmids which will be injected into mouse embryos along with Cas9 and sgRNAs to promote mutations at the correct site of the mouse genome.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-E, BSL-1, plasmids, mice</td></tr>
<tr><td>71362</td><td>Philip Spanheimer</td><td>Aberrant transcriptional regulation by TFAP2C as a Novel mechanism of Hormone resistance in luminal breast cancer</td></tr>
<tr><td>APROVED</td><td colspan="2">Summary: The aim of this experiment is to explore the role of regulation by the transcription factor TFAP2C in determining response to antiestrogen treatment in breast cancer. High copy plasmid vectors expressing gRNA or siRNAs corresponding to point mutations in specific promoter regions of TFAP2C will be used to generate knockout cell lines.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

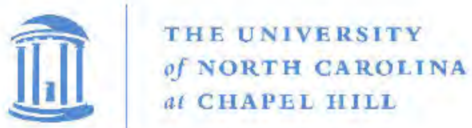
III-F, BSL-2, plasmids</td></tr>
<tr><td>70422</td><td></td><td>Luciferase expressing tumor cells for intraoperative devices to prevent tumor re0ccurrence.</td></tr>
<tr><td>APROVED</td><td colspan="2">Summary: The aim of this experiment is to use luciferase expressing tumor cells to image in vivo using luciferin in an IVIS system to better detect tumor cell burden than can be measured by hand. Cells already expressing luciferase will be implanted into mice

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-1, mice</td></tr>
</table>

5. **Sub-committee Approvals of Schedule G**: 0
6. **Schedule H report:** 27
7. **Next IBC meeting date:** February 5, 2020 Burnett-Womack 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
February 5, 2020 3:30 PM
Burnett Womack 9001

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Stan Lemon, Craig Fletcher, Garry Coulson, Eric Lewis, Cathy Brennan

Members Absent: Monica Dodson, Keith Porterfield, Xiao Xiao, Shawn Hingtgen, Tori Baxter, Jessica Poole

Ad hoc Members (not requested to be present): Ann Matthysse

Guests: [redacted]

Open Meeting

1. **Review minutes from the January 8, 2020 meetings.** Approved
2. **Proposal for research with 2019-nCoV at BSL-3:** [redacted] presented a research proposal for working with the 2019-nCoV virus under BSL-3 containment in his lab. [redacted] discussed the current knowledge on epidemiology of the disease and the current outbreak, and what is currently known about the origins of this virus given the sequence similarity to other existing coronaviruses. As a world-renowned expert for coronaviruses, [redacted] also discussed his interest in working with this novel virus in order to understand how it interacts with the host, causes diseases and how potential therapeutics may be developed using the knowledge gained from these experiments in the lab. In order to conduct these experiments, [redacted] requested approval to work with the 2019-nCoV virus, including isolates of the virus from patients and synthesized cDNA infectious clones (described in detail below). Given [redacted] extensive expertise and history in working with pathogenic coronaviruses under BSL-3 high-containment, the IBC has approved [redacted] research proposal with 2019-nCoV under strict BSL-3 containment.
3. **Applications under review:**

ID	PI	Project Title
72702	[redacted]	siRNA-mediated gene knockdown of MERS-CoV infection - 2020 Renewal
APPROVED	**Summary**: The aim of this experiment is to use siRNA-mediated knockdown of host genes in Huh-7 cells to understand how these genes affect MERS-CoV replication and pathogenesis. Synthetic siRNA molecules will be purchased from commercial vendor and transfected into cells prior to infection with MERS-CoV. Viral replication in vitro will be monitored by qRT-PCR and plaque assay. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, siRNA	
72703	[redacted]	[redacted] encoding Cre-recombinase for long-lived immune cell tracking and identification - 2020 Renewal
APPROVED	**Summary**: The aim of this experiment is to identify and characterize long-lived immune cells to determine their role post-infection and during secondary infection. A mouse strain containing a lox-P-flanked GFP sequence will be infected with a [redacted] mutant expressing Cre-recombinase, and infected cells and their progenitor cells expressing GFP tracked during and after infection.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	
72704		Generation of attenuated MERS-CoV Spike mutants - 2020 Renewal
APPROVED	**Summary**: The aim of this experiment is to determine whether certain MERS-CoV Spike protein residues are important for viral replication and pathogenesis. Recombinant MERS-CoV strains will be generated with attenuating mutations in the Spike protein. Replication and virulence will be monitored through viral passage in cell culture and titering in animals. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	
72705		Mutation and deletion of MERS-CoV ORF4a and ORF4b proteins - 2020 Renewal
APPROVED	**Summary**: The aim of this experiment is to delete or mutate MERS-CoV ORF4a and ORF4b genes to understand their role in antagonizing RNase L function in the context of infection. Using the cDNA infectious clone for MERS-CoV, point mutations will be introduced into the putative catalytic domain of ORF4b and putative nuclear localization signal within ORF4b. Effects on viral replication will be assessed in cell culture and in vivo in mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	
72706		Lentivirus-mediated identification of host factors involved in the determination of viral titer - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to understand how host genes impact immune responses or cellular pathways involved in infection. Using lentiviral vectors, host genes and non-coding RNA's involved in regulation of titer in mice will either be knocked down or over-expressed in target cell lines. The impact of upregulating vs downregulating the target gene on viral titer will be determined in *in vitro* cell culture. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids	
72707		Generation of a full-length infectious cDNA clone of the -like Wuhan Betacoronavirus, including reporter-expressing variants
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to generate a reverse genetic system for the newly described -like Wuhan betacoronavirus. The 30kb genome of the virus will be divided into 6-8 synthesized fragments which will be cloned into plasmids. Plasmids will be digested, ligated and used as template for generating full-length cDNA of the novel coronavirus genome. Infectious virus will be ultimately recovered by transfecting mammalian cell lines with the transcribed full-length RNA. Viral replication will be evaluated in cell culture and in mice, **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the viral name be updated to reflect the current naming of the virus. The Committee requested that small molecular signature be placed in the genome of the infectious clone to differentiate it from circulating wildtype viruses. III-D, BSL-3, plasmids, mice	

<table>
<tr><td>72708</td><td>███</td><td>Generating ███ mutants expressing full-length or portions of viral spike protein from the SARS-like Wuhan coronavirus</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate viral mutants of ███ expressing the spike protein from the ███-like Wuhan coronavirus. Synthetically -produced spike protein from the novel coronavirus will be ligated into the existing ███ infectious clone. Resulting viruses will be characterized for replication in vitro and pathogenesis in vivo in mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the viral name be updated to reflect the current naming of the virus. Resulting viruses using the ███ backbone must be treated as select agents.

III-D, BSL-3, plasmids, mice</td></tr>
<tr><td>72722</td><td>███</td><td>Generating BtSCoV-SHC014 mutants expressing full-length or portions of viral spike protein from the ███-like Wuhan coronavirus</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate viral mutants of BtSCoV-SHC014 bat coronavirus expressing the spike protein from the ███-like Wuhan coronavirus. Synthetically -produced spike protein from the novel coronavirus will be ligated into the existing BtSCoV-SHC014 infectious clone. Resulting viruses will be characterized for replication in vitro and pathogenesis in vivo in mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the viral name be updated to reflect the current naming of the virus.

III-D, BSL-3, plasmids, mice</td></tr>
<tr><td>72723</td><td>███</td><td>Generation of viral replicon particles (VRPs) from the attenuated 3526 strain of Venezuelan Equine Encephalitis (VEE) expressing the Spike and Nucleocapsid proteins from the SARS-like Wuhan Betacoronavirus</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to create vaccine candidates for the spike and nucleocapsid proteins of the novel ███-like Wuhan betacoronavirus using the VEE strain V3526 virus replicon particle (VRP) system. VRPs will be used to vaccinate mice, and resultant sera used to assess cross-reactivity with an array of coronaviruses currently available in the lab.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the viral name be updated to reflect the current naming of the virus.

III-D, BSL-2, plasmids, mice</td></tr>
<tr><td>72822</td><td>███</td><td>Generation of S███ chimeric virus expressing the ███-like Wuhan Betacoronavirus RNA-dependent RNA polymerase, including reporter-expressing variants</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate viral mutants of ███ in which the RNA-dependent RNA polymerase (RdRp; nsp12) protein from ███ is replaced with its homologous sequence from the ███-like Wuhan coronavirus. Synthetically -produced RdRp nsp12 from the novel coronavirus will be ligated into the existing ███ infectious clone. Resulting viruses will be characterized for replication in vitro and pathogenesis in vivo in mice.
Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the viral name be updated to reflect the current naming of the virus. Resulting viruses using the ███ backbone must be treated as select agents.
III-D, BSL-3, plasmids, mice</td></tr>
</table>

72304	[redacted]	Patterning of Cardiac Pacemaker Cell Cytoarchitecture
APPROVED	**Summary**: The aim of this experiment is to determine how the cell morphology present among cardiac pacemaker cells is patterned during embryological development. Gene expression constructs, consisting of a number of gene and promoter pairs cloned into a PiggyBac plasmid system will be transfected into cardiac tissue of chick embryos. Embryos will not be carried out past E14 stage, so no transgenic lines will be produced. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids	
72542	[redacted]	Ex345 Rosa26-Cas9-ERT2 Mouse Strain - SYNB20001
APPROVED	**Summary**: The aim of this experiment is to do a targeted insertion in the mouse Rosa26 locus of a construct containing the Cas9 sequence flanked by modified ERT2 sequences. Transgene will be cloned into a plasmid which will be microinjected into mouse fertilized embryos along with Cas9 protein and in vitro transcribed gRNA to promote insertion into the proper site of the mouse genome. Injected embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, mice	
72562	[redacted]	Ex346 Sycp1-Cas9 Rat
APPROVED	**Summary**: The aim of this experiment is to generate a transgenic rat strain with Cas9 and eGFP expression from the Sycp1 locus. Transgene will be cloned into a plasmid which will be microinjected into rat fertilized embryos along with Cas9 protein and in vitro transcribed gRNA to promote insertion into the proper site of the rat genome. Injected embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, rats	
72563	[redacted]	Ex347 Spo11-Cas9 Rat
APPROVED	**Summary**: The aim of this experiment is to generate a transgenic rat strain with Cas9 and eGFP expression from the Spo11 locus. Transgene will be cloned into a plasmid which will be microinjected into rat fertilized embryos along with Cas9 protein and in vitro transcribed gRNA to promote insertion into the proper site of the rat genome. Injected embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, rats	

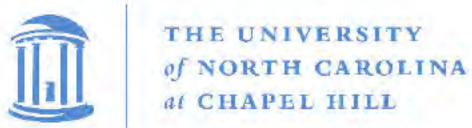
72902		Ex348 HAO1 Ex1-2 Mouse
APPROVED	**Summary**: The aim of this experiment is to create a transgenic mouse strain with human HAO1 exon 1, intron 1 and exon 2 replacing the corresponding mouse region. Transgene will be cloned into a plasmid which will be microinjected into mouse fertilized embryos along with Cas9 protein and in vitro transcribed gRNA to promote insertion into the proper site of the mouse genome. Injected embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, mice	
72922		Ex349 HAO1 Mouse
APPROVED	**Summary**: The aim of this experiment is to create a transgenic mouse strain with human HAO1 replacing the corresponding mouse region. Transgene will be cloned into a BAC plasmid which will be microinjected into mouse fertilized embryos along with Cas9 protein and in vitro transcribed gRNA to promote insertion into the proper site of the mouse genome. Injected embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, mice	
71582		Dual Reporter Imaging
APPROVED	**Summary**: The aim of this experiment is to monitor the biodistribution of 2 types of cells within one tumor via dual luciferase reporter bioluminescence. Mammalian HEK293 cells that have already been transfected with plasmid expressing firefly luciferase and nanoluc genes will be injected into mice via subcutaneous injection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mice	
71162		Macrophage-Mediated Gene Delivery in Solid Tumors
APPROVED	**Summary**: The aim of this experiment is to transfect murine cells with plasmids expressing reporter genes (Fluc or GFP). Alternatively, plasmid DNA will be injected into mice through iv injection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, mice	
70762	Tal Kafri	Generating inducible integrating and non-integrated packaging cell line
APPROVED	**Summary**: The aim of this experiment is to establish stable packaging cell lines to generate lentiviral vectors to circumvent the need for transient transfections. Furthermore, this will reduce the possibility of recombination between transiently transfected plasmids, thus enhancing the safety of lentiviral vector system.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentiviral vectors	
72182		CLG and DTG of genetically modified murine tumors
APPROVED	**Summary**: The aim of this experiment is to inject cancer cells in vitro or cells from disassociated tumors from genetically engineered mice into recipient mice **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, mice	
71982		Engineering diagnostic breast, colorectal cancer and lung cancer cells (GFP-Luc, GFP-Nluc)
APPROVED	**Summary**: The aim of this experiment is to use preexisting lentiviral-transduced cancer cell lines expressing luciferase or fluorescent protein reporters to inject into mice as diagnostic markers for cancer. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentivirus, mice	
72742		Investigate the immune response after AAV based gene therapy in germ free mice
APPROVED	**Summary**: The aim of this experiment is to investigate immune responses against AAV after AAV-based gene therapy in germ free mice. AAV vectors expressing clotting factor VIII and FVIII will be injected into mice via retro-orbital injection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, AAV, mice	
72842	Glenn Matsushima	Function of microglia during remyelination
APPROVED	**Summary**: The aim of this experiment is to determine whether microglia/macrophage are targets of phagocytosis by glia cells. Primary murine microglia or macrophages from mice with floxed genes of interest (e.g. BAX-Fl, Mertk-Fl, Axl-Fl or Lag3-Fl) will be transfected with lentivirus expressing CMV-Cre to ablate protein expression. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentivirus	
72064		Injection of luciferase- or green fluorescent protein-expressing BSL-2 cells into the mammary fat pad of mice
APPROVED	**Summary**: The aim of this experiment is to test a new biochemical toolset for investigating exosomes in mice. Human breast cancer or mesenchymal stem cells stably expressing luciferase or GFP will be injected into the mammary fat pad of mice and tracked using novel RNA-based fluorescent trackers that specifically target exosomes. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mice	

72162	[redacted]	AAV Packaging vector for viral driver of caspase-3 activation
APPROVED	**Summary**: The aim of this experiment is to use a viral vector to selectively induce cleavage of endogenous pro-caspase-3 by upstream caspases to activate caspase-3, which then induces apoptosis in a cre-dependent manner. AAV viral vectors expressing the inverted taCasp3-T2A-TEVp transgene sequence will be infused into mice by stereotaxic injection into site specific areas of the brain **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, AAV, mice	
72638	[redacted]	BSL2 bacteria for work in transgenic and knock out mice
N/A	**Summary**: The aim of this experiment is to test the efficacy of treatment with bacteria or bacterial products for the protection against injury and/or death. Mice will be administered by oral gavage with bacterial suspensions (non-transgenic bacteria) for reconstituting gut microbiome or for treating mice to test efficacy of protection provided by targeted microbiome products. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. Since BSL-1 transgenic/KO mice were being inoculated with non-genetically modified bacteria, these experiments were determined not to fall under the guidelines.	
71642	[redacted]	Use of luciferase expressing cells in mouse tumor models
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to induce tumors in mice that express luciferase. Pre-existing 344SQ mouse lung cancer cells transduced by lentiviral vectors to express luciferase will be injected into mice via subcutaneous injection or directly into the lung. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the containment level be amended from BSL-1 to BSL-2, and that a biological safety cabinet be used for administration of the cells. III-D, BSL-2, lentivirus, mice	

4. **Sub-committee Approvals of Schedule G**: 8
   PI: Erin Cox Title: Functional characterization of genetic mutations (III-F, ID 72642)
   PI: [redacted] Title: Viral infection of gene knockout mice (III-D, ID 72343 / 72386)
   PI: [redacted] Title: Immunomodulatory mechanisms in Kras-driven pancreatic cancer_Lenti (III-D, 71182)
   PI: [redacted] Title: Immunomodulatory mechanisms in Kras-driven pancreatic cancer_pLKO.1 (III-D, 71183)
   PI: [redacted] Title: Immunomodulatory mechanisms in Kras-driven pancreatic cancer_pLVTH (III-D, 71185)
   PI: Matthew Redinbo Title: Structural biology of biological macromolecules (III-F, ID 72023)
   PI: [redacted] Title: Colonizing Paneth cell reporter mice with commensal bacteria (III-D, ID 72786)
5. **Schedule H report:** 77
6. **Next IBC meeting date:** March 4, 2020 Burnett-Womack 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
March 4, 2020 3:30 PM
Burnett Womack 9001

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Keith Porterfield, Jessica Poole, Eric Lewis

Members Absent: Monica Dodson, Xiao Xiao, Shawn Hingtgen, Tori Baxter, Craig Fletcher, Garry Coulson, Cathy Brennan

Ad hoc Members (not requested to be present): Stan Lemon, Ann Matthysse

Guests: [redacted]

Open Meeting

1. **Review minutes from the February 5, 2020 meetings: Approved**
2. **Inactivation of Flaviviruses with Buffer AVL: Approved** – The [redacted] Lab validated a method that used Buffer AVL to inactivate flaviviruses (West Nile virus, Japanese encephalitis virus, and Powassan virus) in their BSL-3 lab space. Buffer AVL (e.g. from Qiagen QIAamp Viral RNA Mini Kit Cat #52906) contains a chaotropic salt (guanidine isothiocyanate, 50-100%). AVL is used per manufacturer instructions to inactivate infectious agents in serum, media, or other liquid samples prior to RNA isolation. Samples treated with AVL can be removed from the BSL-3 following standard procedures (no cytopathic effect test required). Removed samples will be stored frozen or handled within a biosafety cabinet until the addition of ethanol (per manufacturer instructions).
3. **Discussion of IBC approval for FUA: Discussed** – It was discussed during the IBC meeting and in subsequent communications that external institutions that have Facility Use Agreements (FUAs) will potentially submit their IBC protocols to the UNC IBC for review. Given the small size of operations by the FUAs, their limited/focused research programs and tendency towards lower-risk activities, it was determined that the UNC IBC has the capacity to review the FUA research. Also, given that these companies share space with UNC researchers, it is in the best interest of the UNC IBC to ensure that the FUAs are fully compliant.
4. **Applications under review:**

ID	PI	Project Title
73102	[redacted]	Adaptive transfer of transgenic T cells
APPROVED	**Summary**: The aim of this experiment is to induce type I diabetes in mice. T cells or splenocytes will be isolated from transgenic BDC2.5 mice and injected IV into SCID NOD mice. No vectors will be used in this study, mice will be purchased from Jackson Labs. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, T cells or splenocytes from transgenic mice, mice	
73789	[redacted]	Generation of attenuated MERS-CoV Spike mutants - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to generate attenuated MERS-CoV Spike mutants and examine them in mice. Recombinant MERS viruses will be generated containing combinations of these Spike mutations: R748S, A763N, F764Y, N765T.These mutations are based on conserved residues that have been identified as important residues for Spike cleavage and	

	recognition in (Belouzard et al., PNAS 106:5871). The MERS-CoV genome is maintained as six cDNA cassettes in plasmids that are propagated in *E. coli*. MERS-CoV cassettes are propagated in commercial plasmids (e.g., pCR-XL-Topo, pSMART, pUC-57). These are amplified in *E. coli* and assembled and transcribed in vitro. Assembled MERS-CoV is replication competent. Animals will be inoculated intranasally with 50 µL of viral inoculum. Inoculation titer will range from 10^2-10^5 PFU. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, MERS-CoV, mice	
73790		Generation of a full-length infectious clone of a US isolate of the novel coronavirus (2019-nCoV/SARS-CoV-2) including reporter viruses and mouse-adaptation mutants
APPROVED	**Summary**: The aim of this experiment is to generate a reverse genetic system for the newly emerged SARS-CoV-2, (aka 2019-nCoV), isolated from a US patient in Washington state. The parental wild type (WT) virus was isolated by the CDC and had been passaged three times in Vero cells (GenBank accessory number: MT020880). The lab passaged the virus one more time and will PCR amplify the genomic fragments from the 4th passage WT virus. In addition to clone the WT viral genome, to aid in the visualization and quantification of SARS-CoV-2 infection, the lab will generate reporter viruses expressing fluorescent proteins (GFP, RFP, and NanoLuc). The biology of the virus will also be examined in mice. Animals will be inoculated intranasally with 50 µL of viral inoculum. Inoculation titer will range from 1e2-1e7 PFU, depending on the resulting virus replication and virulence characteristics. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	
73382		Tg CHAT Cre Rats and DREADDS
APPROVED	**Summary**: The aim of this experiment is to analyze select neuron populations in a rodent model. AAV constructs will be injected into ChAT Cre rat brain regions. The transduced cells can then be activated or deactivated with injection of clozapine-N-oxidase (CNO), selectively controlling defined populations of neurons. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, adeno-associated virus, rats	
73523		Kaposi Sarcoma transgenic mice
APPROVED	**Summary**: The aim of this experiment is to evaluate phenotypes associated Kaposi Sarcoma associated herpesvirus protein and gene expression in transgenic mice. Prior work (IACUC 13-219.0) has shown that the proteins of a large fragment of KSHV are not reliably expressed in mice (Sin et al., Blood. 2013 121(15): 2952-63), yet a set of small RNAs, which do not encode proteins, were. The next step is to insert a bigger transgene into mice in the hopes to obtain reliable expression. The aim is to insert a transgene, which has the small RNA locus deleted. As a human virus, KSHV does not replicate in mice or rodent cells only in human cells and since the transgene is physically anchored in the mouse genome it cannot escape either. No vector is used; the naked DNA is introduced by pronuclear injection into the germline of transgenic animals. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td></td><td colspan="2">III-D, BSL-1, plasmids, mice</td></tr>
<tr><td>68042</td><td></td><td>Generation of a zebrafish (Danio rerio) model of kidney development and disease</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to facilitate cell-type specific manipulation of gene expression in the podocytes of the fish kidney, as well as label the podocytes with fluorescently tagged protein. Using CRISPR/Cas9, a Cre transgene will be introduced into the podocin locus to drive Cre expression in the podocytes of the kidney. The Cre element will be delivered in a plasmid with homology arms targeting the 3' end of NPHS2 (podocin) locus. Also, gRNA and Cas9 will be coinjected with the plasmid. The Cre element will also be substituted for tdTomato to tag the endogenous podocin protein. Plasmid and gRNA will be injected in Zebrafish embryos (~1ul).

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-1, plasmids, zebrafish</td></tr>
<tr><td>73722</td><td></td><td>Use of Venezuelan equine encephalitis virus strain ZPC738 as non-select agent challenge virus for mouse vaccination studies</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to utilize the mouse adapted VEE strain ZPC738 as a non-select agent challenge virus for testing drug and vaccine efficacy in mice. As a subtype ID, this virus strain is exempt from Select program as an “Attenuated strains of Overlap Select Agents” excluded from the requirements of 9 CFR Part 121 and 42 CFR part 73. The plasmid clone will not be manipulated. It will simply be used to generate wild type ZPC738 virus. Virus will be propagated in Vero cells, and virus stocks will be used to assess virus-neutralizing antibody responses and as in vivo challenge virus for assessing VEEV vaccines and antiviral molecules for their ability to prevent or treat VEEV-induced disease. These studies will use replication competent virus. Wild type C57Bl/6 mice will also be challenged with virus for drug and vaccine studies.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-3, plasmids, Venezuelan equine encephalitis virus, mice</td></tr>
<tr><td>73723</td><td></td><td>Fluorescent influenza viruses for in vivo tracking of infection</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal of this experiment is to utilize infectious influenza virus that expresses green fluorescent protein in virus neutralization studies. Specifically, this virus will be used in influenza microneutralization assays to quantify amounts of influenza specific neutralizing antibody elicited by inactivated influenza vaccines. The virus strain that will be used in this study is mouse adapted influenza virus A/PR/8/34 expressing GFP. This virus will be generated by collaborators at Mt. Sinai School of Medicine who have created and published on this influenza-GFP system (PMID: 20534532). Virus will be propagated in MDCK cells. All neutralization assays will also be conducted in MDCK cells.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, influenza virus</td></tr>
<tr><td>73724</td><td></td><td>Venezuelan equine encephalitis virus replicons expressing proteins from influenza A, and KSHV</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use a VEEV based replicon to express a variety of proteins and ORFs from Influenza A virus, , and Kaposi's Sarcoma Herpes virus. Briefly, full-length transcripts of pVR21 containing transgenes from the above viruses will be</td></tr>
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<tr><td></td><td colspan="2">mixed with VEE v.3526 capsid and E1-E3 helper construct transcripts and electroporated into cells under BSL2 conditions in a biosafety cabinet. Supernatants (10%) will be tested for replication competent viruses by passage in cell culture. If replicating viruses are detected, the entire prep will be decontaminated and discarded. Supernatants that pass the safety test will be used to infect cells in culture and to immunize mice for the production of antisera. The long-term goal of this project is to produce antibodies for immunoprecipitation and for immunohistochemistry with which to study the functions and interactions of previously unknown viral genes.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, Venezuelan equine encephalitis virus, mice</td></tr>
<tr><td>73842</td><td></td><td>Nonintegrating lentiviral vectors towards clinical trials</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal of this study is to enhance the safety of currently used lentiviral vectors as a means to render the vectors more suitable for human clinical trials. To this end: 1) They will test nonintegrating lentiviral vectors (which reduce the likelihood of insertional mutagenesis. 2) They will test PPT-deleted vectors which reduce illegitimate integration. 3)They will test new hFIX which reduce vector load. 3)They will use stable packaging cell lines to generate lentiviral vectors as a means to reduce the likelihood of emerging replication competent retroviruses (RCRs). 4) They will test the gp64 envelope which is less toxic than the VSV-g envelope. Lentiviral vectors expressing either the luciferase cDNA or codon optimized hFIX cDNA variants will be generated by either transient transfection or by stable packaging cell lines as described earlier by Kfri et al. (J Virol. 1999 Jan;73(1):576-84. Vector particles will be pseudotyped by either the VSV-G or the gp64 envelope. Vector particles will be concentrated by ultracentrifugation and will be tested for RCR's as described earlier by Kafri et al. (J Virol. 1999 Jan;73(1):576-84.) Vector particles will be injected IP to mice. Luciferase and hFIX expression in vivo will be determined periodically.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, lentivirus, human and mouse cell lines, mice</td></tr>
<tr><td>73142</td><td>Stan Lemon</td><td>Functional analysis of human and chiroptera hepatovirus pX sequence</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aims of this study are (1) to determine whether the hepatitis A virus (HAV) pXsequence is functionally responsible for the sorting of assembled HAV capsids into multivesicular endosomes and their subsequent secretion from cells as quasi-enveloped virions, and (2) to ascertain whether this function, if present, has been conserved over evolution and is present in both bat and human strains of HAV. Two experiments will be done: (1) Chemically-synthesized cDNA representing the HAV pX sequence from M32 virus (KT452714, recovered from a bat, E. helvum) will be fused in frame with sequence encoding a computationally designed ~200 amino acid-long protein that self-assembles as a 60-subunit nanocage dodecahedron such that pX is placed at the C-terminus of the nanocage, separated from it by a Myc tag, and with a19 amino acid sequence containing the p6 Gag myristoylation signal (from HIV-1) with a linker at the N-terminus. This construct, pEPN01-pX/M32, will be expressed under the control of the cytomegalovirus promoter in transfected 293 cells. Expression of the nanocage protein will be assessed in cell lysates, and its secretion from cells into supernatant fluids will be determined by immunoblotting of the Myc tag, as described in Voteller et al. (doi:10.1038/nature20607). Secretion of EPN01-pX/M32 will be compared with that of a comparable construct containing the pX sequence of human HM175/p16 HAV (EPN01-pX) or p6 Gag sequence of HIV (EPN01-p6) which contains known functional ESCRT-recruiting late domains (see Voteller et al.doi:10.1038/nature20607). (2) All or part of the M32 bat pX sequence (total length ~8 kDa) will be fused in frame with the polyprotein-coding sequence of</td></tr>
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	an infectious molecular clone of the HM175/p16 (human) virus such that it replaces the native HM175 pX sequence. RNA transcribed from this p16/M32 chimera will be transfected into Huh-7.5 human hepatoma cells and virus rescue/replication will be monitored by RT-qPCR and/or infectious focus assay. To determine whether the replacement of human pX sequence with bat pX sequence permits continued recruitment of capsids to endosomes and nonlytic release of virus as quasi-enveloped virions, the buoyant density profile of virus released into supernatant fluids will be determined in isopycnic iodixanol gradients. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Hepatitis A virus, human cell lines	
73702		Phenotypic characterization of HAV with modified polyprotein sequences
APPROVED	**Summary**: The aims of this study are to characterize the in vivo replication competence of hepatitis A virus (HAV) mutants with (1) ablation of protein motifs in the pX domain of VP2 that have been implicated in nonlytic release of virus from infected cells in vesicles ("quasi-enveloped virus"), and (2) with sequence encoding a T-cell epitope from Lymphocytic choriomeningitis virus (LCMV) fused to the N-terminus of the HAV polyprotein. These experiments will provide novel insight into the function of pX in the viral lifecycle and if successful will generate a tagged virus useful for monitoring T cell responses to HAV in mice. For the mouse studies, synthetic HAV RNA will be delivered to mice by 3 separate intrahepatic injections of 30 µl each using a fine needle, delivering up to a total of 100 µg RNA. Alternatively, virus (10^6-10^{10} genome equivalents) rescued from synthetic RNA in cell culture will be inoculated into mice intravenously by tail-vein injection in a volume of 200 µl. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, Hepatitis A virus, human and mouse cell lines, mice	
73442	Robert McGinty	Elucidating a role for VRK1 recognition of the nucleosome acid patch in genome-templated processes
APPROVED	**Summary**: The purpose of these experiments is to generate a human cell line transduced with wild-type, mutant, or kinase-dead VRK1 in order to investigate the biological significance of VRK1's nucleosome interaction. The insert gene will be cloned into a plasmid which will be transfected into a mammalian cell line with an envelope and packaging plasmid to generate lentiviruses. These lentiviruses will be used to infect a secondary mammalian cell line. The DNA construct for the gene of interest is the human VRK1 gene tagged at the N-terminus with EGFP. The promoter controlling expression in this plasmid is the EF-1a core promoter. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentivirus, human cell line	
73762		Subtype-specific inhibition within the cortical microcircuit
APPROVED	**Summary**: The aim of this experiment will be to determine the contribution of hyperpolarization-activated cyclic nucleotide-gated ion channels (HCNs) to the activity of inhibitory Martinotti interneurons, and resultant effects on prefrontal cortical circuit excitability. A viral vector containing an shRNA directed against HCNs (or scrambled control) and driven by the mDlx promoter will be co-injected with a virus containing Cre recombinase into the prefrontal cortex of Sprague-Dawley rats. All viruses are packaged in the replication-incompetent AAV5 virus, and animals will be handled according to BSL-2 safety standards during- and post-infection. Stereotaxic injection on anaesthetized animals into the medial prefrontal cortex according to established coordinates. Volume = 0.4uL (0.2uL shRNA, 0.2uL	

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<tr><td></td><td colspan="2">SST-Cre cocktail; minimum 10^6 injection units/mL) per hemisphere, infused with a syringe pump at a flow rate of 0.2uL per minute.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, adeno-associated virus, rats</td></tr>
<tr><td>73928</td><td>[redacted]</td><td>Study of stabilized variants of Lipoprotein lipase- mRNA version</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to determine if stabilizing changes to Lipoprotein Lipase (LPL) can improve plasma triglyceride levels in mice. The gene for an optimized version of LPL is transcribed into mRNA and then encapsulated into lipid nanoparticles and injected into mice. Animals are dosed at 0.5 mg/kg IV tail vein. This dose produces no observable toxicology.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-1, mRNA, mice</td></tr>
<tr><td>73642</td><td>[redacted]</td><td>Gene Therapy of Hemophilia and von Willebrand Disease</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to express Human or canine Coagulation Factors VIIa, VIII, IX, and VWF in animals that have inherited deficiencies of these proteins. The insert gene will be cloned into a plasmid that will be injected into dogs. Hepatic specific promoters apoEHCR and hAAT will drive transgene expression of one of the following canine or human genes: FVIIa, FIX, FVIII, VWF. These constructs will be administered to dogs (Canis familiaris) and we will monitor expression of FVIIa, FVIII, FIX or VWF in their plasma or serum.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-1, plasmids, dogs</td></tr>
<tr><td>73262</td><td>[redacted]</td><td>Sendai Virus Infection of airway epithelium in vitro or in vivo</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to use recombinant Sendai Virus expressing GFT or luciferase to monitor the extent and duration of infection in cell-lines, primary cultures of human and mouse airway epithelial cell cultures, and in vivo in the lungs/airways of mice. This is an RNA virus generated from cDNA constructs. The markers genes are driven by virus specific polymerases. For the in vivo experiments, the mice will be inoculated with virus intranasally with 30 ul of 10^8 TCID50 virus stock.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, Sendai virus, human and mouse cells, mice</td></tr>
<tr><td>72062</td><td>[redacted]</td><td>Meiotic Recombination in D. melanogaster</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to utilize CRISPR/CAS9 system to generate D. melanogaster mutants by site directed genome editing for characterization studies. The E. coli prepared with gene of interest and transferred into D. melanogaster. They will use pUC and pCAS9 based vectors propagated in E. coli with D. melanogaster genes (mei-9, blm) and driven by endogenous promoters.</td></tr>
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<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-1, plasmids, fruit flies</td></tr>
<tr><td>73942</td><td></td><td>Multimodality Therapy: B16</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this study is to assess the immune response against a specific antigen expressed on murine tumors. No new rDNA constructs will be created as transfected cells were obtained from another lab. These cells were transfected with a construct coding for ovalbumin (OVA). The B16-Ova melanoma tumor line was obtained from another lab. The cells will be injected into mice. The B16F0 cell line was obtained from the American Type Culture Collection (ATCC) (Rockville, MD) and was first characterized as a spontaneously arising melanoma in C57BL/6 mice. These cells were transfected with the constructs that code for either the truncated or full-length ovalbumin.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-1, plasmids, tumor cells, mice</td></tr>
<tr><td>71762</td><td></td><td>Crosstalk between intestinal inflammation, bone and bone proteins</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to examine crosstalk between intestinal inflammation, bone and bone proteins. Experiments will involve mice with targeted gene knockouts. Cells of interest will be isolated from recombinant mice and transferred into host recipient mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the Principal Investigator add additional information regarding their animal experiments to Section III: Gene Transfer Experiments Involving Whole Animals and Plants in their Schedule G.

III-F, BSL-1, cells from recombinant mice, mice</td></tr>
<tr><td>73162</td><td></td><td>Use of CRISPR/Cas9 vector to knockout genes in mouse cancer cell line for in vivo testing</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The purpose of the experiment is to test if murine tumor cell lines with knockouts of mouse KDM5B, KDM5C, or KDM1A will compromise tumor growth and increase sensitivity to anti-cancer drugs in mice induced with these tumors. No viral vector will be used in these experiments. Gene of interest will be cloned into a plasmid, the S. pyogenes CRISPR/Cas9 vector PX459. CRISPR/Cas9 vector will be transfected into mouse cancer cell lines in vitro. Cells with appropriate gene knockout will be used to generate new tumor cell lines used to induce tumors in mice. Tumor cells that have been genetically modified with a plasmid vector of CRISPR/Cas9 to generate gene deletions will be used to induce tumors in mice. For intracranial tumor induction, no more than 1 million cells in 5 ul of HBSS+0.5% FBS will be injected into the mice. Generally, it is 200,000 cells or less in the same volume. For mammary fat pad tumor induction, no more than 1 million cells in 100 ul in 1 Matrigel: 1 cell media will be injected into the mice.

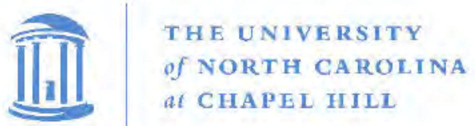
Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about the volume that will be injected into the fat pads and intracranially in the mice.

III-D, BSL-1, plasmids, mouse cancer cell lines, mice</td></tr>
<tr><td>73143</td><td></td><td>Lentiviral production of stable cell line of luciferase expression gene</td></tr>
</table>

APPROVED WITH STIPULATIONS	**Summary**: The aim of the experiment is to create a B16-OVA mouse melanoma cell line stably expressing luciferase which will be inoculated into mice via subcutaneous injection. PSPAX2, pMD2.G and luciferase vectors will be co-transfected to HEK293T cells and lentivirus containing luciferase gene were harvested and then infected to B16-OVA melanoma cells. After two-weeks of Blasticidin selection, B16-OVA cell that stably express luciferase will be tested by luminance production under plate reader and are ready for tumor inoculation (s.c. injection) into mice. A concentration of $2X10^5$ B16-OVA cells/ mice, at a volume of 10mL/kg, or 200uL (0.2mL) per 20g mouse, will be injected in the right and left back flanks of the mice. Other than that, there will be no exposure to recombinant or synthetic nucleic acids for mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about the volume that will be injected into the mice. III-D, BSL-2, plasmids, lentivirus, mice

1. **Sub-committee Approvals of Schedule G**: 2
   PI: [redacted] **Title:** Ex351 Ddx4-Cas9 Mouse (III-E, ID 74002)
   PI: Lawrence Ostrowski **Title:** Expression of tagged PCD proteins in cell culture for biochemical studies (III-F, ID 73562)
2. **Schedule H report:** 40
3. **Next IBC meeting date:** April 1, 2020 Burnett-Womack 9001

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
April 1, 2020 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Tori Baxter, Monica Dodson, Cathy Brennan, Garry Coulson, Eric Lewis

Members Absent: Xiao Xiao, Shawn Hingtgen, Stan Lemon, Craig Fletcher, Keith Porterfield, Jessica Poole

Ad hoc Members (not requested to be present): Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the March 4, 2020 meetings: Approved**
2. **: PI:**
3. **Applications under review:**

ID	PI	Project Title
74842		Generation of a full-length infectious clone of bat coronavirus RaTG13, including reporter viruses
APPROVED	**Summary**: The objective of this experiment is to generate a reverse genetic system for the bat coronavirus RaTG13 (GenBank accession # MN996532), which is the closest ancestral strain to the novel coronavirus (2019-nCoV/SARS-CoV-2). The RaTG13 and SARS-CoV-2 share 96.4% genomic identity. The aim is to use this full-length cDNA clone to study the differences in pathogenesis and host tropism between RaTG13 and SARS-CoV-2. Moreover, the RaTG13 virus may yield in a heterologous challenge model for evaluating SARS-CoV-2 vaccines and therapeutics. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, bat coronavirus, human and mouse cell lines, mice	
74882		Containing a Deletion of the Envelope (E) Coding Region - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to further characterize the nature of the attenuation, particularly concerning the immune modulators involved in the attenuation, the genetic manipulations originally published in a BAC construct will be recapitulated in our infectious clone cassette background. The mutations introduced will be contained entirely in the F plasmid (see figure). Resulting virus will be used to infect mice deficient in the interleukin 1 receptor and/or mediators of inflammasomes. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, human cell lines, mice	

<table>
<tr><td>74883</td><td>[redacted]</td><td>Enhancement of the Bat Coronavirus HKU4 binding to the human DPP4 receptor - 2020 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to examine bat coronavirus HKU4 binding to the human DPP4 receptor. HKU4 does not replicate efficiently in human/primate cell lines or in mice. To attempt to improve its replication in our experimental models, two sets of mutations will be included in the genome, both of which are predicted to enhance binding/engagement with human dipeptidyl peptidase 4 (hDPP4), the probable receptor. These mutations are all within the Spike attachment protein and include single and combinations of the following: 1) receptor binding domain: S540W, K547R, L558W; and 2) S1/S2 cleavage site: S746R, N762A.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-3, plasmids, bat coronavirus, mammalians cells, mice</td></tr>
<tr><td>74942</td><td>[redacted]</td><td>Investigating potential ORFs on the negative strand of Flaviviruses</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to look for the possibility that proteins are expressed from open reading frames (ORFs) on the negative strands of Flaviviruses such as Dengue virus (DENV) and Zika virus (ZIKV) during replication. If present, these would most likely be low abundance and expressed early in infection. For the experiments proposed here, the aim(s)will be to use reverse genetic systems to create recombinant viruses where the predicted start codons of these proteins have been altered/removed without changing the amino acid of the positive strand. If/when possible, stop codons will also be created to truncate these predicted proteins. We will then test the importance of these negative strand ORFs in vitro via growth curves and related assays, as well as in vivo using established ZIKV mouse models.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, DENV, ZIKV, mammalian and insect cells, mice</td></tr>
<tr><td>74962</td><td>[redacted]</td><td>Adaptation of the 2019-nCoV (SARS-CoV-2) infectious clone to mice expressing wild-type mouse ACE2</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The purpose of this experiment is to adapt SARS-CoV-2 so that it is able to infect mice carrying wild-type mouse ACE2 (mACE2) through serial in vivo lung passages for development of an animal model to evaluate antivirals and therapeutics and study pathogenicity in vivo. The arising mutations will be identified via sequencing and introduced into the SARS-CoV-2 infectious clone as described in a future Schedule G.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about the risk assessment for the work be added to the protocol.

III-D, BSL-3, plasmids, SARS-CoV-2, mammalian cells, mice</td></tr>
<tr><td>73062</td><td>[redacted]</td><td>Kras-driven pancreatic cancer and metastasis_pLVTH-M-GFP</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The purpose is to express Luciferase, Cre recombinase and peptide fragment of gene ovalbumin (SIY) or (SIN) in mouse cancer cells to study role of ovalbumin antigen in activation of T cell responses in vitro and in vivo.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about the plasmids and materials being used for the project.

III-D, BSL-2, plasmids, lentivirus, mouse cells, mice</td></tr>
</table>

<table>
<tr><td>73182</td><td>[redacted]</td><td>Kras-driven pancreatic cancer and metastasis_Lenti_LucS_LucOS_Cre</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The purpose is to express Luciferase, Cre recombinase and peptide fragment of gene ovalbumin (SIY) or (SIN) in mouse cancer cells to study role of ovalbumin antigen in activation of T cell responses in vitro and in vivo.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee asked for clarification of the similarity of this protocol to 73062.

III-D, BSL-2, plasmids, lentivirus, mouse cells, mice</td></tr>
<tr><td>74142</td><td>[redacted]</td><td>Adjuvant GVI3A derived from Venezuelan equine encephalitis virus (VEE) replicon particles</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The purpose of these experiments are: 1) To produce the adjuvant GVI3A (also known as nVRP), a single replication cycle, non-propagating, Venezuelan Equine Encephalitis virus (VEE)-derived replicon particle with immune enhancing properties. 2) To perform in vitro assays to test the potency of the adjuvant, and 3) To test the adjuvant in mice for its ability to enhance immunogenicity to recombinant flavivirus antigens. The proposed experiments do not include nucleic acid manipulation per se. The laboratory will obtain from Global Vaccines Inc. three plasmid DNAs needed to generate in vitro transcripts. These will be electroplated into cells to produce GVI3A single cycle replicon particles.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, Adjuvant GVI3A virus-like particles, mammalian cell lines, mice</td></tr>
<tr><td>74284</td><td>[redacted]</td><td>AAV-mediated Gene Therapy for the Treatment of Neurogenetic Diseases</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The purpose of the proposed study is to develop effective gene therapy approaches for the treatment of mucoplysaccharidoses (MPS) and other neurogenetic diseases.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Changed the title to “AAV-mediated Gene Therapy (in mice) for the Treatment of Neurogenetic Diseases”.

III-D, BSL-2, plasmids, adeno-associated virus, mice</td></tr>
<tr><td>74482</td><td>Nilu Goonetilleke</td><td>Generation of cell lines that stably express human leukocyte antigens (HLA) for use as antigen presenting cells to T cells</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal of this study is to generate a stable cell line expressing genes of interest (for example, HLA-E) that will be used as antigen presenting cells.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, lentivirus, human cell lines</td></tr>
<tr><td>71726</td><td>[redacted]</td><td>Oncogenes include NeuT, EGFP, and ampicillin resistant protein</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal of these experiments is to understand the genetic interactions between DNA damage response defects and breast cancer pathogenesis. The laboratory will analyze breast cancer development after mammary intraductal injections with RCAS avian retroviral vectors in mice harboring various DDR defects.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee suggested that the title should potentially be updated.</td></tr>
</table>

	III-D, BSL-2, plasmids, retroviral vectors, mouse cells, mice	
71729	█████	CRISPR, CRE, Luciferase, Lentivirus
APPROVED	**Summary**: The goal of these experiments is to understand the genetic interactions between DNA damage response defects and breast cancer pathogenesis. The lab will use lentiviral vectors to gene mutations in mammalian cell lines and mice. Genes that will be targeted are components of the DNA damage response. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee suggested that the title should potentially be updated. III-D, BSL-2, plasmids, lentiviral vectors, human and mouse cell lines, mice	
71730	█████	Retroviral constructs
APPROVED WITH STIPULATIONS	**Summary**: The goal of these experiments is to understand the genetic interactions between DNA damage response defects and breast cancer pathogenesis. The lab will use retroviral vectors to generate cell lines that express various oncogenes of interest. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee suggested that the title should potentially be updated. Also, the Committee requested that the Principal Investigator add additional information regarding their animal experiments to Section III: Gene Transfer Experiments Involving Whole Animals and Plants in their Schedule G. III-D, BSL-2, plasmids, retroviral vectors, mammalian cell lines	
75222	█████	Generation of a full-length infectious cDNA clone of the SARS-like 2019-nCoV (Wuhan) Betacoronavirus, including reporter-expressing variants
APPROVED	**Summary**: The objective of this experiment is to generate a reverse genetic system for the newly described ███-like Wuhan Betacoronavirus (2019 SARS-CoV2 or nCoV). In addition to synthesis of a WT viral genome, to aid in the visualization and quantification of SLCoV-WUH infection, the laboratory will generate reporter viruses expressing fluorescent proteins. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, SARS-CoV-2, mammalian cells, mice	
75223	█████	Generation of a full-length infectious clone of the novel coronavirus (2019-nCoV/SARS-CoV-2) including reporter viruses and mouse-adaptation mutants
APPROVED	**Summary**: The objective of this experiment is to generate a reverse genetic system for the newly emerged SARS-CoV-2, (aka 2019-nCoV). The parental wild type (WT) virus was isolated by the CDC and had been passaged three times in Vero cells (GenBank accessory number: MT020880). The ████ passaged the virus one more time and will PCR amplify the genomic fragments from the 4th passage WT virus. (Please note that the virus may acquire additional mutations during cell culture passaging. All the mutation will be tracked and reported in the future). A silent mutation will be introduced into a conserved region in nsp12 as a genetic marker. The laboratory will also generate reporter viruses expressing fluorescent proteins (GFP, RFP, and NanoLuc) as well as mouse-adapted mutants. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, SARS-CoV-2, mammalian cells, mice	

74082	[redacted]	Use of pancreatic cancer cell lines from KPC background in cancer research
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to use cell lines derived from Kras mut, TRP53 mut, transgenic mice for cancer research. These cell lines have already been developed by collaborators, thus the laboratory will not use any transgenic animals. These cell lines are derived from spontaneous pancreatic tumors in mice bearing mutations of Kras and TRP53. Pancreatic cancer cell lines will be delivered subcutaneously into the flank C57Bl6 mice. All injections will occur in 100ul PBS containing 50,000-500,000 cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. The Committee also requested that the Principal Investigator add additional information regarding their animal experiments to Section III: Gene Transfer Experiments Involving Whole Animals and Plants in their Schedule G. III-D, BSL-1, mouse cells, mice	
74603	[redacted]	Delineation of the role of breast and pancreatic cancer metabolism in cancer outcomes via crispr knockout of key metabolic enzymes
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to knock out expression of metabolic genes in breast and pancreatic cancer cell lines of mouse origin. Mouse Pcx, Pck1, Ldha, and Phgdh will be knocked out using recombinant Cas9 protein, and sgRNA purchased from Thermo Fisher. This will be a knockout and thus will not be regulated by a promotor. Breast cancer cell lines will be delivered to the 4th or 9th mammary fat pad of C57 mice, pancreatic cancer cell lines will be delivered subcutaneously on the left or right flank of C57 mice. All injections will occur in 100ul PBS containing 50,000-500,000 cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. Changed title to "Delineation of the role of breast and pancreatic cancer metabolism in cancer outcomes via crispr knockout of key metabolic enzymes". III-D, BSL-1, sgRNA constructs, mouse cells, mice	
74624	[redacted]	Generation of C57Bl6 C3TAG tumor cell lines
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to generate of C57Bl6 C3TAG tumor cell lines. Triple negative breast cancer is the most deadly kind of breast cancer. The C3TAg mice have been shown to be an excellent model of triple negative breast cancer, but the BALB/ C genetic background is resistant to obesity, another focus of study of the laboratory. The laboratory have established C57BL/6 mice transgenic for C3TAG by back-crossing BALB/C C3TAg mice with C57Bl6. C57Bl6 mice are highly valuable in the study of obesity response in cancer as they become readily obese on a high fat diet. To eliminate the need for transgenic animals, and to enable treatment studies. Cell lines derived from C57Bl6.C3TAG mice will be transplanted into wild type C57BL/6 mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information on the tumor cell lines that would be used in the study. III-D, BSL-1, mouse cells, mice	
74350	[redacted]	Gene Therapy with Adeno Associated Viral Vectors expressing Luciferase and Dystrophin
APPROVED WITH STIPULATIONS	**Summary**: The goal of the project is to develop an AAV vector optimal for delivery and with high expression level of mini DMD gene in different muscles of the mouse. Utilize AAV vector as a vehicle for delivery of the Optimized mini-Dystrophin gene under the control of three	

	different promoters; CMV(human cytomegalovirus) immediate-early promoter and tMCK and dMCK (human muscle specific promoters) Optimized mini Dystrophin will be packaged into capsid from AAV serotypes 2; 2/5; 8 and 9 then injected by murine tail vein or IP. Expression of DMD protein will be tested in different muscles, including heart muscle. 100uL of rAAV vectors (1e9-1e12 vg) will be injected by murine tail vein, IP or retro-orbital injection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. III-D, BSL-2, plasmids, adeno-associated virus, mice	
74362		Tumor Specific Therapy using an AAV Vector to Delivery an Immunotoxin
TABLED	**Summary**: The aim of this project is to explore tumor specific therapy using AAV vector to delivery immunotoxin linked to tumor specific receptor. In the experiment, we will explore the killing effect of immunotoxin on tumor cells using AAV vector and conjugation with tumor specific receptor. Since immunotoxin AAV vector cannot be produced in293 cells due to immunotoxin toxicity on 293 cells when it is expressed, we will use the split vector strategy. First we introduce an intron into toxin gene (PE or DT) to make pTR/IL4DT-intron and pTR/Tac-PE-intron. Next we will use PCR approach to split the pTR/IL4DT-intron or pTR/Tac-PE-intron into two separate constructs through the middle of the intron, which will generate one construct containing the promoter, IL4 or Tac, part of PE or DT upstream and part of intron, and second construct containing part of intron, part of PE or DT downstream and poly A. When two AAV vectors cotransduce target cells, the complete immunotoxin construct will be formed through AAV TR recombination between AAV vectors and produce immunotoxin to kill target cells containing the tumor specific receptor. **Committee Comments**: The protocol was tabled at this meeting. The Committee requested more information including the toxicity of the immunotoxin that would be produced during the study. III-D, BSL-2, plasmids, adeno-associated virus, immunotoxin, mice	
74382		Gene Therapy with an Adeno Associated Viral Vector encoding a Site Specific Endonuclease
APPROVED	**Summary**: The experiments are designed to harvest primary mouse cells for ex vivo gene editing studies. The laboratory will test for double strand break repair of DNA by homologous recombination. The mouse model is transgenic for a detective GFP reporter that will serve as the target for correction. They will not generate a transgenic mouse. The intended repair is of the mouse chromosome (which contains a defective GFP gene), the 2 vectors are used for site specific double-strand break and as a GFP repair molecule. All planned experiments will be performed in primary cells ex vivo. Homologous recombination between the repair construct and the mouse chromosome is desired and should be stimulated by I-SceI endonuclease. All intended experiments will be performed ex vivo. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, adeno-associated virus, mouse cells, mice	
74402		Gene Therapy with an Adeno Associated Viral Vector encoding Thymidine Kinase
APPROVED	**Summary**: The aim of the experiment is to target tumor cells via activation of thymidine kinase (TK). After xenograftment of CS1 cells into Scid/Nod mice subcutaneously, AAV1829/TK vector will be injected into tumor mass and gancyclovir will be used intraperitoneally, TK activation by gancyclovir will kill the tumor cells. rAAV containing the therapeutic TK transgene will be administered to the mouse via intravenous injection of 100 micro liters of vector at 1e9 viral particles per microliter for a total of1e11vg.	

<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, adeno-associated virus, human cell lines, mice</td></tr>
<tr><td>74922</td><td>[redacted]</td><td>Study of stabilized variants of Lipoprotein lipase</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of the experiment is to determine if stabilizing changes to LPL can improve plasma triglyceride levels in mice. The inserted gene will be cloned into a plasmid, used to make virus, then injected into mice. Human lipoprotein lipase, controlled by the Chicken beta actin promoter. Other factors affecting LPL, specifically human GPIHBP1 and human syndecan 1 may be tested in conjunction with human lipoprotein lipase. There will be separated by an IRES. The plasmid pTRs-CBH with LPL, along with additional helper plasmids containing genes, are needed for Adeno-associated viral production. 5 uL of recombinant, non-replicating adeno-associated viral vectors packaging our cassette will be delivered through intravascular or intramuscular injection into mice using various doses ranging from 1E9 to 1E10 for IM injection and 1E10-1E11 for IV injection.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about the injection routes and volumes that will be injected into the mice.

III-D, BSL-2, plasmids, adeno-associated virus, mice</td></tr>
<tr><td>74862</td><td>[redacted]</td><td>Expression of ciliary proteins in airway epithelial cells in cultured cells and in vivo</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of the experiment is to examine expression of ciliary proteins in airway epithelial cells in cultured cells and in vivo. The goal is to correct genetic deficiency in the disease Primary Ciliary Dyskinesia. The laboratory will be testing different nanoparticle formulations to deliver the missing, normal protein as a modified mRNA. To test the formulations, the laboratory may initially use RNA encoding reporter genes (e.g., EGFP, Tomato Red). RNA encoding reporter genes or a normal ciliary protein will be synthesized by commercial vendors, incorporated into lipid based nanoparticles, and delivered to cells in culture or intranasally to mice. Mice will be anesthetized using isoflurane as per our IACUC approved protocol and 10-30ul of synthetic RNA containing solution will be slowly administered to the nares to be spontaneously inhaled. Concentration will vary, but will typically be ~ 1 microgram/microliter.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about the nanoparticles that will be used during this study.

III-F, BSL-1, plasmids, mRNA, mouse cells, mice</td></tr>
<tr><td>74102</td><td>[redacted]</td><td>Influenza Virus H1N1 inoculation of Hamster Airways</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of the experiment is to study gene-deleted hamsters in an attempt to identify better models of respiratory virus infection. The laboratory will use parainfluenza viruses and Respiratory Syncytial Virus in hamsters. The laboratory will also attempt to perform comparative studies using influenza virus. The laboratory will use is a recombinant H1N1 2009 obtained from [redacted] (UNC). This is a recombinant of the wild-type virus with no additional transgene insertions. The hamsters will be intranasal inoculated with 100 microliters of a 10e6 pfu/ml.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about the influenza virus that will be used in this study.</td></tr>
</table>

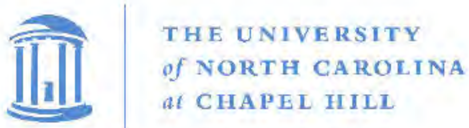
	III-D, BSL-2, recombinant influenza virus, hamsters	
73162	[redacted]	Use of CRISPR/Cas9 vector to knockout genes in mouse cancer cell line for in vivo testing
APPROVED WITH STIPULATIONS	**Summary**: The purpose of the experiment is to examine if murine tumor cell lines with knockouts of mouse KDM5B, KDM5C, or KDM1A will compromise tumor growth and increase sensitivity to anti-cancer drugs in mice induced with these tumors. Gene of interest will be cloned into a plasmid, the S. pyogenes CRISPR/Cas9 vector PX459. CRISPR/Cas9 vector will be transfected into mouse cancer cell lines in vitro. Cells with appropriate gene knockout will be used to generate new tumor cell lines used to induce tumors in mice. No viral vector will be used in these experiments. Tumor cells (1x10^4) that have been genetically modified with a plasmid vector of CRISPR/Cas9 to generate gene deletions will be used to induce tumors in mice following subcutaneous injection in a total volume of 100uL. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about the route and volume that will be injected into the mice. III-D, BSL-1, plasmids, mouse cancer cell lines, mice	
75202	[redacted]	Lentiviral production of stable cell line of luciferase expression gene
APPROVED	**Summary**: The aim of the experiment is to create additional mouse tumor (MC38 colon adenocarcinoma, CT26 colon carcinoma and 4T1 mammary carcinoma) cell lines stably expressing luciferase which will be inoculated into mice via subcutaneous injection. PSPAX2, pMD2.G and luciferase vectors will be co-transfected to HEK293T cells and lentivurus containing luciferase gene were harvested and then infected to MC38, CT26, and 4T1 cells. After two-weeks of Blasticidin selection, MC38, CT26, and 4T1 cells that stably express luciferase will be tested by luminance production under plate reader and are ready for tumor inoculation into mice (s.c. injection at 2×10^5 of the luciferase-expressing tumor cells, at a volume of 10mL/kg, or 200uL (0.2mL) per 20g mouse, on their right and left back flanks). **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentivirus, human and mouse cell lines, mice	

1. **Sub-committee Approvals of Schedule G**: 4

PI: [redacted] **Title:** Transfusion of transgenic platelets (III-E, ID 74262)
PI: [redacted] **Title**: Ex352-SYNB20003-pSYCP1-Cas9-R26-Mouse (III-E, ID 74302)
PI: [redacted] **Title:** Mutant CalDAGGEF-1 Chimeric Mice (III-D, ID 74642)
PI: Benjamin Vincent **Title:** Use of B16F10-OVA as a Peptide Vaccination Model System (III-F, ID 74122)

2. **Schedule H report:**
3. **Next IBC meeting date:** May 6, 2020 Web-Conference Call

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
May 6, 2020 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Craig Fletcher, Tori Baxter, Keith Porterfield, Cathy Brennan, Garry Coulson, Eric Lewis

Members Absent: Xiao Xiao, Shawn Hingtgen, Monica Dodson, Jessica Poole

Ad hoc Members (not requested to be present): Stan Lemon, Ann Matthysse

Guests: [redacted]

Open Meeting

1. **Review minutes from the April 1, 2020 meetings: Approved**
2. **NIH Reportable Incident** –A recent incident in a laboratory involving a potential exposure to a recombinant SARS-CoV-2 following a mouse bite was discussed.
3. **Applications under review:**

ID	PI	Project Title
76579	[redacted]	Recombinant monoclonal antibody expression for RNA viral proteins - 2020 renewal
APPROVED WITH STIPULATIONS	**Summary**: This protocol will be used to generate monoclonal antibodies targeting specific viral proteins for use as laboratory reagents (immunohistochemistry, western blot, immunoprecipitation, ELISA, etc.). Plasmids encoding human and murine antibody constant regions with variable regions specific for viral proteins (including all expressed proteins from coronaviruses, flaviviruses, and noroviruses) will be transfected into 293, CHO, and NIH3T3 cells, and antibodies will be produced from these transfected cells. Viruses will include flaviviruses (dengueviruses 1-4 and zikavirus), norovirus (all currently and previously circulating strains), and coronaviruses ([redacted], MERS-CoV, SARS-CoV-2, HKU3, HKU4, HKU5, and [redacted]-and MERS-like bat coronaviruses). Purified monoclonal antibody proteins (not genetic material) will be used in murine infection systems to assess protective capacity. Human IgG, IgA, IgM, IgE, IgD and Murine IgG, IgA, IgM, IgE, IgD antibodies will be produced. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information about names of the viruses as well as clarification for genome expression. III-F, BSL-2, plasmids, human and mouse cell lines	
76580	[redacted]	Combination of nsp14 and nsp16 attenuating mutations in the [redacted] and MERS-CoV infectious clones 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to evaluate the synergistic attenuating capacity of combining two individually attenuating mutations and to characterize a potential [redacted] vaccine candidate with inactivations of two RNA-editing enzymatic activities. A virus with inactivated exonuclease (nsp14) and 2'-O-methyltransferase (nsp16) enzymes will be produced. Individually, these mutations are attenuating in cell culture and in mouse models. Additionally, these mutations are stable upon passage both in cells and in animals and do not revert to wt	

<table>
<tr><td></td><td colspan="2">phenotypes or genotypes. Therefore, there is an anticipation that the combination virus will also be attenuated. Therefore, this virus will not fall under the gain-of-function limitations.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-3, plasmids, [redacted] MERS-CoV, mammalian cell lines, mice</td></tr>
<tr><td>76581</td><td>[redacted]</td><td>Viral pathogenesis of [redacted] strains with mutations in the papain-like proteinase domain that prevents ubiquitination - 2020 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The nsp3 region of [redacted] contains both a papain-like protease activity and a deubiquitinating (DUB) activity. Evidence garnered from in vitro studies indicate that the DUB activity may be critical for viral pathogenesis in vivo through its action on host innate immune molecules, such as interferon stimulatory gene 15 (ISG15). The laboratory proposes to generate 3 sets of mutant viruses that will help us to understand if the DUB activity does indeed influence viral pathogenesis. The mutant sets will include: i) F70A alone; ii) FMQP mutant which comprise F70A +M209A + Q233E + P248G; and iii) FHM mutant which comprise F70S + H74A + M209A. This will result in the production of three mutant virus strains, each harboring one of the three mutant sets. All production of [redacted] viruses and experiments therein will be executed within the BSL3 environment. Pathogenesis of these viruses will then be compared to wild-type [redacted]. Mice will be infected intranasally with each viral strain, mice will be monitored/weighed daily for [redacted] related disease symptoms, and tissues will collected at various time points post-infection to assess viral infection by plaque assay, RNA expression, and immunohistochemistry. Additionally, host innate and adaptive immune responses to each of the viral strains will be assessed. Previous work in another lab suggests that an active deubiquitination domain in [redacted] functions to antagonize interferon activity through an IRF3 pathway (J Virol. 11:209). Therefore, we reasonably expect that deubiquitination inactivation mutants will be attenuated for pathogenesis.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-3, plasmids, [redacted] mammalian cell lines, mice</td></tr>
<tr><td>76582</td><td>Ralph Baric</td><td>Recombinant monoclonal antibody fragment antigen-binding (Fab) expression - 2020 Renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: This protocol will be used to generate monoclonal antibody Fabs targeting specific viral proteins for use as laboratory reagents (immunohistochemistry, western blot, immunoprecipitation, ELISA, etc.). Plasmids encoding human and murine antibody constant regions with variable regions specific for viral proteins (including all expressed proteins from coronaviruses, flaviviruses, and noroviruses) will be transfected into 293, CHO, and NIH3T3 cells, and antibodies will be produced from these transfected cells. Purified monoclonal antibody proteins (not genetic material) will be used in murine infection systems to assess protective capacity.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-F, BSL-2, plasmids, mammalian cell lines</td></tr>
<tr><td>76585</td><td>[redacted]</td><td>Coronavirus nsp14 ExoN determinants of fidelity - 2020 renewal</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: To determine if fidelity-altering mutations will alter [redacted] replication in vitro and replication and pathogenesis in vivo, fidelity-altering mutations identified in experiments performed in collaboration with [redacted] will be introduced individually and in pools into the [redacted] infectious cDNA background, in the presence and absence of inactivated nsp14 ExoN activity. Viruses will be constructed as described above.</td></tr>
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<td></td>
<td colspan="2">Viable viruses will be characterized for altered replication in vitro and altered replication and pathogenesis in vivo. These experiments will focus on interacting proteins (nsp14 and other proteins or proteins that act independently of nsp14) and their impacts on replication and pathogenesis.

The above work in has shown that introducing mutations into nsp14 attenuates the virus in vivo (Graham et al. 2012, Nature Medicine 18:1820), suggesting an impact to replication for these viruses. The goal is to make similar mutations in MERS-CoV, HKU4, HKU5, and 2019-nCoV to identify residue changes that alter replication. Based on the work done in the expectation is that the mutant library will consist of attenuating mutations. Since nsp14 is primarily responsible for fidelity, there is no expectation that changes in nsp14 will alter host range, virulence, or resistance to any antivirals that may be developed. The laboratory will generate a panel of mutants that have mutations at the nsp14 active site and surrounding residues. These fidelity-altering mutants will be introduced in pools into the MERS-CoV, HKU4, HKU5, and 2019-nCoV infectious cDNA backgrounds. Viruses will be constructed as describe above. Viable viruses will be characterized for altered replication in vitro. These experiments will focus on identifying mutations that decrease the fidelity of nsp14 and are most likely to be attenuating.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee asked that the title be adjusted.

III-D, BSL-3, plasmids, , 2019-nCoV (SARS-CoV-2), MERS-CoV, HKU4, HKU5 mammalian cell lines, mice</td>
</tr>
<tr>
<td>76587</td>
<td></td>
<td>Directed evolution of adeno-associated virus (AAV) for increase in transduction efficiency, production yield and evasion of pre-existing antibodies</td>
</tr>
<tr>
<td>APPROVED WITH STIPULATIONS</td>
<td colspan="2">Summary: The aim of this experiment is to engineer a portfolio of next generation AAV vectors for gene therapy and research usage. This may be done for the purpose of studying either the basic AAV biology or utilities as gene therapy vectors. This work proposes to create and screen for multiple AAV libraries that has distinct physical and biological profiles including but not limited to increase in transduction efficiency, distinct tissue tropism, increase in production yield and evading pre-existing antibodies. The libraries will contain randomized deletion, insertion or substitution in the AAV genome. The AAV libraries will be used to transduce tissue culture cells or injected in mice. The AAV libraries will then be propagated in the presence of human (ATCC VR-1516) or mouse adenovirus (ATCCVR-550) which serve as a helper virus for AAV replication in vitro or in vivo. For this work, plasmids will be cloned in E. coli, which may be followed by transfection in mammalian cells. Alternatively, these plasmids will be used to generate rAAV vectors, which will then be used for transduction of mammalian cells or injection into mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. The Committee also requested clarifications on the volumes that would be injected into mice.

III-D, BSL-2, plasmids, adeno-associated virus, adenovirus, mammalian cells, mice</td>
</tr>
<tr>
<td>76683</td>
<td></td>
<td>Mechanisms of Formation of Pseudoexfoliation Material on Human Surgical Lens Capsules (Ad.CLU)</td>
</tr>
<tr>
<td>APPROVED WITH STIPULATIONS</td>
<td colspan="2">Summary: The ultimate goal of these experiments is to deliver genes to the trabecular meshwork and to the lens capsules to assess the therapeutic potential of their encoded proteins. We insert the selected genes into adenoviral vectors and attempt to elucidate the molecular mechanisms that regulate intraocular pressure. The adenoviral vector will be used to overexpress the Clusterin gene into the lens capsules organ cultures and primary ocular cells. The viral vector would be potentially used to deliver its cargo into the eyes of living rats or mice by intracameral or intravitreal injections.</td>
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<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee asked for clarification that the viral work would be performed within a biological safety cabinet.

III-D, BSL-2, plasmids, adenovirus, mammalian cells, mice</td></tr>
<tr><td>75662</td><td>Russell Broaddus</td><td>Investigating the molecular functions underlying endometrial cancer</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: This study is aimed at understanding the molecular functions underlying endometrial cancer with a concentration on proteins in the epithelial adherent junction including but not limited to Beta-catenin, alpha-catenin, E-cadherin and CD73. Retrovirus and lentiviruses will be used to express wild-type and mutant versions of proteins in established mouse and human cell lines and cells from patient derived xenografts.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, retrovirus, lentivirus, human and mouse cell lines</td></tr>
<tr><td>75362</td><td></td><td>EBV-infected human mononuclear cells</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The purpose of the proposed study is to study potential tumor suppression techniques. Mice will be injected with Epstein-Barr virus (EBV)-infected human mononuclear cells is anticipated to give rise to tumors, which will then treat with antibodies and chemical inhibitors to assess their effects on tumor suppression. Human cord blood mononuclear cells will be obtained from a commercial source (StemExpress) and infected with 500-5000 GreenRaji Units of EBV for approximately 1 hour at 37°C. A minimum of 10 million cells will be intraperitoneally injected in a volume of200ul into 3-6 week old NOD.SCID mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the Principal Investigator add information regarding their animal experiments to Section III: Gene Transfer Experiments Involving Whole Animals and Plants in their Schedule G.

III-D, BSL-2, EBV, Human cord blood mononuclear cells, mice</td></tr>
<tr><td>75782</td><td>Daniel Dominguez</td><td>Modulation of Gene Expression Using Lentiviral Particles</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal of this experiment is to transduce cells with lentiviral particles for the purpose of expression of recombinant fusion proteins or expression of short hairpin RNAs to induce knockdown of endogenous genes.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, lentivirus, human cell lines</td></tr>
<tr><td>75522</td><td></td><td>The role of androgen and cofactors in prostate cancer development</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal of this project is to examine the role of androgen and cofactors in prostate cancer development. The cDNA fragment of the gene (such as YY1) will be cloned into a MSCV viral based plasmid which is then transfected into prostate cancer cell lines in vitro. In addition, gene manipulation hairpins or sgRNA for the above gene is cloned into a viral vector (such asLKO-U6 promoter-based vector or Cas9 sgRNA vector) which is utilized to transduce cells in vitro. The engineered cells will ultimately be injected into mice. About one million of cells in PBS will be mixed with Matrigel followed by subcutaneous injection into each animal. The injection volume will be 100 ul.</td></tr>
</table>

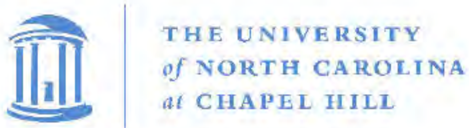
	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentivirus, human cells, mice	
76702	Martina Gentzsch	CoV Envelope protein and ion channel function
APPROVED	**Summary**: The goal of this experiment is to express the CoV E (envelope) protein in cells and assess ion channel properties. The spike protein and envelope protein sequences are both from SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2). The laboratory will use lentiviral vectors that are recombination incompetent, to generate virus particles used for transient transfection into 293T cells. The virus expressing the CoV Envelope protein will be used to infect mammalian cells to generate stable epithelial cell lines. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentivirus, mammalian cells	
75242		Generation of viral replicon particles (VRPs) from the attenuate 3526 strain of Venezuelan Equine Encephalitis (VEE) expressin the Spike and Nucleocapsid proteins from the and MERS like Coronaviruses
APPROVED	**Summary**: The aim of this experiment is to create vaccine candidates for the Spike and Nucleocapsid proteins of the 2019 SARS-CoV2 Spike and nucleocapsid proteins as experimental vaccines. This will be performed by using the Venezuelan Equine Encephalitis Virus Replicon Particle (VRP) system, packaging the replicon using the BSL2 coat glycoprotein from VEE strain V3526, a non-select BSL2 VEE strain. VRPs will be used to vaccinate mice, and resultant sera will be used to assess cross-reactivity with an array of coronaviruses currently available in our laboratory. The laboratory will use this same system to express the Spike proteins of several other closely related group 2b coronaviruses, including MERS-CoV and related viruses, as well as seasonal coronaviruses such as OC43, NL63, and 229E to test the vaccines for heterologous protection and safety in both standard (e.g. BALB/c and C57Bl/6J) and Collaborative Cross mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, VRP, mammalian cell lines, mice	
76062	Tal Kafri	COVID-19 in vitro
APPROVED	**Summary**: The aim of the experiment is to establish a reporter 293T-based cell line (expressing the human ACE2) to titer inhibitory antibodies to lentiviral vectors pseudotyped with the COVID-19 spike protein, and to generate lentiviral like particles (VLPs) and lentiviral vectors pseudotyped with the COVID-19 spike proteins. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentivirus, human cell lines	
73983		Retrograde genetic targeting of neurons
APPROVED	**Summary**: The purpose of these experiments is to map inputs into neurochemically defined neurons. This mapping of neuronal pathways is achieved by injecting viral vectors that are cre-dependent and dependent on multiple infections and transynaptic transport for specificity. The laboratory will be injecting replication deficient Herpes Simplex Viral (HSV) particles into	

	specific brain nuclei in mice using methods we have established in the lab for injection of AAV. It is important to note that this schedule G is for the use of HSV, the laboratory is already using AAV in similar experiments. This HSV is taken up by nerve terminals in the CNS and retrogradely transported back to the nucleus to produce Cre-recombinase. In the same surgery, Cre inducible AAV is injected to targeted neurons expressing Cre. This allows for pathway specific genetic targeting of neuronal populations in heterogeneous tissue. Mice will receive100-500 nl of virus in a specific brain region via hamilton syringe delivery. Virus concentrations are roughly 10XE12 GC/ml. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, HSV, mice	
76642		Interaction of virus-like particles (VLPs) with antibodies in mucus
APPROVED	**Summary**: The aim of this experiment is to study lab produced virus-like particles (VLPs) with in-house and commercial designed antibodies in mucus. VLPs free of genomic materials will be prepared by transfecting 293T cells with the plasmids encoding for VP40, NP and GP (1:1:1 ratio), with eGFP incorporated into the VP40 capsid construct. The VLPs are prepared using viral proteins from the Zaire strain responsible for the current outbreak; they have been shown to produce the same morphology and surface protein incorporation as wild type viruses (see Warfield et al (2003) PNAS 100(26):15889-15894). VLPs will be collected from culture supernatants, filter purified by sucrose gradient, and resuspended in sterile saline. Recombinant DNA will only be used in recipient 293T cells in cell culture. The purified VLPs that are produced will consist solely of lipid membrane and viral proteins (VP40, NP, and GP), with or without eGFP protein as a fluorescent tag. No DNA or RNA material will be contained in the VLPs that will be used for in vitro or in vivo experiments. The generated VLPs will be used for experiments inhuman and murine mucus samples to look for trapping of the VLPs b synthetic anti- antibodies in mucus. The VLPs will also be introduced to C57/bl6 mice via intranasal distillation to examine the effect of pre-administered antibodies on VLP trapping and clearance from the airways. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-F, BSL-2, VLPs, human cell lines, mice	
76742		AAV gene therapy for hemophilia with inhibitors
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to study the effect of different therapeutic transgene products delivered by Adeno-associated virus (AAV) vector on phenotypic correction in hemophilic mice. 100 ul of AAV8 vector encoding different transgenes at the dose of 10e9 particles to 10e13 particles will be injected into mice via retro-orbital vein. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested additional information on how the mice will be anesthetized. III-D, BSL-2, AAV, human cell lines, mice	
75902		The development of novel radiation-sensitizer based on ultra-small carbon dots
APPROVED	**Summary**: The aim of this experiment is use luciferase expression cancer cell lines to generate orthotopic non-small cell lung cancer xenograft in mice. NSCLC cell lines, including NCI-H1299, NCI-H226 (fLuc) and NCI-H460, will be stably transfected with the firefly Luciferase	

	gene expressed from the SV40 promoter. The generated cells will express fLuc will ultimately be injected into mice. The fLuc expressing cells will be harvested from in vitro cell culture flask. After centrifuge, washed with PBS twice, the cell pellet will be resuspended in PBS. About 5 x 10^5 cells in 50microliter PBS: matrigel (1:1) will be inoculated percutaneously into the lung of nude mice using a 29G syringe. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentivirus, human cell lines, mice	
75343	Shaun McCullough	Characterization of responses to air pollutant exposure within the human respiratory tract
APPROVED	**Summary**: The goal of the project is to characterize the host responses to air pollutant exposure within the human respiratory tract. Expression of exogenous genes, as well as shRNAs and dCas9/gRNAs targeting endogenous genes, will be used to determine the effects of inhaled toxicant exposures on the human respiratory tract and describe the roles of specific proteins in the response to inhaled chemical exposures. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentivirus, human cell lines	
75622	[redacted]	Desensitization of Cone Visual Signaling in Zebrafish
APPROVED	**Summary**: The laboratory has generated transgenic fish (using the Tol2kit) that express mutant zebrafish Grk7a in which the serine phosphorylation site has been mutated to alanine (S33A) or glutamic acid (S33E). The goal is to evaluate changes in visual sensitivity in these fish using noninvasive electrophysiology. Future experiments may utilize the Tol2kit to generate additional mutant transgenics, as well as conditional knockouts facilitated by transgenic CRISP/Cas systems. The volume injected into the zebrafish will not exceed 40 nl, with concentrations of 25-30 pg of recombinant DNA and 25 pg of transposase mRNA. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, zebrafish	
76902	[redacted]	Adjuvant GVI3000 derived from Venezuelan equine encephalitis virus (VEE) replicon particles [Revised]
APPROVED	**Summary**: The purpose of these experiments are: 1) To produce the adjuvant GVI3A (also known as nVRP), a single replication cycle, non-propagating, Venezuelan Equine Encephalitis virus (VEE)-derived replicon particle with immune enhancing properties. 2) To perform in vitro assays to test the potency of the adjuvant, and 3) To test the adjuvant in mice for its ability to enhance immunogenicity to recombinant flavivirus antigens. The proposed experiments do not include nucleic acid manipulation per se. The laboratory will obtain from Global Vaccines Inc. three plasmid DNAs needed to generate in vitro transcripts. These will be electroplated into cells to produce GVI3A single cycle replicon particles. Was reviewed at the previous IBC meeting held on April 1, 2020. Lab wanted to change the adjuvant that would be used in the study. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Adjuvant GVI3A virus-like particles, mammalian cell lines, mice	

4. **Sub-committee Approvals of Schedule G**: 2
   PI: [redacted] **Title:** Injection of FKBP5 silencing siRNA (III- F, ID 76827)
   PI: [redacted] **Title:** Using CRISPR technology to generate C-terminal epitope tagged Nlrc3 knock in mouse line (III-E, ID 74822)
5. **Schedule H report:** 23
6. **Next IBC meeting date:** June 3, 2020 Web-Conference Call

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
June 3, 2020 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Shawn Hingtgen, Craig Fletcher, Tori Baxter, Keith Porterfield, Cathy Brennan, Garry Coulson, Eric Lewis

Members Absent: Xiao Xiao, Monica Dodson, Jessica Poole

Ad hoc Members (not requested to be present): Stan Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Discussion of draft BSL-3 SOP and research objectives for**
2. **Clinical Trial:** PI:
3. **Review minutes from the May 6, 2020 meeting.**
4. **Applications under review:**

ID	PI	Project Title
		JC
APPROVED	**Summary**: This study is an **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-C, lentiviral vector	
75762		Radial glial development and neuronal migration
APPROVED	**Summary**: The aim of this experiment is to study the molecular and cellular mechanisms underlying progenitor development, neurogenesis, and neuronal migration in the cerebral cortex. Arl13b, TSC1/2, MEMO, and APC, Cre, shRNAs for MEMO and Arl13, or TSC linked pathway members will be cloned into plasmids and transfected into mouse cortical neurons, glial cells, or cell lines. The laboratory will express GFP, RFP, Cre, shRNAs, Arl13b, TSC1/2 or APC in developing cortical cells to visualize the patterns of proliferation, migration, and growth	

	in cultured cells. Electroporated embryonic brains will also be sliced and maintained in vitro to visualize these cellular events in cortical slices. These vectors are well-characterized for their ability to drive expression in developing mouse cortical cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mouse cells	
76922	Eva Anton	Characterization of patterns of neuronal generation, placement, and connectivity in the cerebral cortex
APPROVED	**Summary**: The aim of this experiment is to study the molecular and cellular mechanisms underlying progenitor development, neurogenesis, and neuronal migration in the cerebral cortex. FP, mRFP, Arl13b, TSC1/2, MEMO, and APC, Cre, shRNAs for MEMO and Arl13, or TSC linked pathway members will be cloned into plasmids and transfected into mouse cortical neurons, glial cells, or cell lines. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mouse cells	
77442	Richard Baker	Structural studies of membrane trafficking
APPROVED	**Summary**: The main aim of this project is to understand the molecular mechanisms of membrane trafficking, including clathrin-mediated endocytosis, vesicle coat formation, and SNARE-mediated membrane fusion. Human and yeast (S. cerevisiae) genes involved in membrane trafficking will be cloned into bacterial expression vectors (ex. pET-Duet) and used for recombinant expression of proteins in *E. coli*. Human genes involved in membrane trafficking will be cloned into insect cell expression vectors (ex. Big Bac and pFastBac systems) and used for recombinant expression in Sf9 insect cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee asked for confirmation that laboratory would more than 10 liters of culture at one time. III-D, BSL-2, plasmids, *E. coli*, Sf9 insect cells	
77443	Richard Baker	Structural studies of AP2 and the SARS-CoV-2 proteins Nsp10, Nsp14, Nsp16
APPROVED	**Summary**: The goal of this project is to perform structural studies of AP2 and the SARS-CoV-2 proteins Nsp10, Nsp14, Nsp16. SARS-Cov-2 is the viral agent responsible for the Covid-19 pandemic of 2020.The SARS-Cov-2 protein Nsp10 was recently shown to interact with the AP2 complex, a human protein complex important for clathrin-mediated endocytosis. To understand the mechanism of this interaction and its potential role in the viral life cycle, the laboratory will purify and perform structural and biochemical assays on the Nsp10/Nsp14/Nsp16 complex and its interaction with the clathrin adaptor AP2. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee asked for confirmation that laboratory would more than 10 liters of culture at one time. III-D, BSL-1, plasmids, *E. coli*	
77307		Mutation of a putative nuclear localization signal in MERS-CoV and HKU4/HKU5 CoV ORF4b proteins - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to abolish a putative nuclear localization/importation signal present in the ORF4b proteins encoded in MERS-CoV and HKU4 and HKU5 CoVs. It is	

	thought that this signal allows the ORF4b protein to translocate to the infected cell nucleus and antagonize interferon (Journal of General Virology 94:874). Therefore, abolishment of this signal will interfere with interferon antagonism and should attenuate viral replication. Therefore, these experiments will not fall under gain-of-function prohibitions. The MERS-CoV and HKU4/HKU5 cDNAs are maintained as 7 separate cDNA cassettes in plasmids that are propagated in E. coli. ORF4b is encoded in fragment F of each system. The assembled cDNA is transcribed into viral RNA via a T7 promoter. cDNA cassettes are propagated in pSMART-LC-Kan and pCR-XL-Topo, and pUC57. Reconstituted viruses are expanded into stocks in Vero cells. Animals will be inoculated intranasally with 50 µl of viral inoculum. Inoculation titer will range from 10^2-10^5 PFU. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, coronaviruses, mammalian cells, mice	
77308		Generation of Porcine Endemic Diarrhea Virus (PEDV) infectious clone tissue culture variants - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to generate a tissue culture passage variant of Porcine Epidemic Diarrhea Virus (PEDV). This virus is attenuated in piglets; thus, studies will characterize the virulence alleles defined within this variant. Passaging of pc22A virulent PEDV in tissue culture 100 times has generated an attenuated pc22a-p100 virus that causes mild disease and no mortality in piglets. The hypothesis is that the specific variants in the viral genome are responsible for this adaptation and attenuation. By altering specific nucleic acid sites in the spike and other regions of the pc22A-PEDV genome to variants seen in the pc22A-p100 attenuated genome, the laboratory aims to identify the genetic changes responsible for this adapted and attenuated phenotype. Tests of viral fitness will be conducted in vitro in cell culture and in vivo in piglets with promising infectious clone variants. Please note that all in vivo experimentation will be done in the . **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, PEDV, mammalian cells,	
77309	Ralph Baric	NFkB modulation by uncharacterized coronavirus ORFs (ORFeome) - 2020 renewal
APPROVED WITH STIPULATIONS	**Summary**: The aim of this study is to screen coronavirus ORFs (nsps 1-16 within ORF1, ORFs 2-9, hypothetical ORFs as described in the ORFeome project) for NFκB modulation activity in an in vitro screening assay. No intact virus will be used with the pNiFty2-Luc expression plasmid. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested clarification on what coronaviruses would be screened in this study. As well, the classification of the Schedule G was changed to III-E. III-E, BSL-2, plasmids, mammalian cells	
77311		SARS-CoV-2 Transcription Regulatory Network Remodeling and Its Effects on Replication and Virulence
APPROVED	**Summary**: The aim of the project is to examine the SARS-CoV-2 transcription regulatory network. Coronaviruses have a complex replication program in which the translation of downstream open reading frames (ORFs) is dependent upon the generation of a 3'-nested set of subgenomic RNAs (sgRNAs). These sgRNAs are transcribed in a discontinuous transcription mechanism regulated in part by the presence of conserved transcription regulatory sequences (TRSs), which collectively constitute the coronavirus transcription regulatory network (TRN).	

	This laboratory has previously shown that the coronavirus TRN can be completely rewired with novel core sequences (6-7 nt) without affecting virus viability. The goal of this experiment is to explore the contribution of the TRN to virus replication and virulence by replacing the TRN of the betacoronavirus SARS-CoV-2 with novel TRSs and to partially rewire the genome to determine the contribution of the transcription of accessory ORFs to virulence. The effects will be evaluated in vitro in cell cultures and in vivo in mice. Based on experiments in [redacted] the laboratory fully anticipates that the corresponding viruses in the SARS-CoV-2 background will be attenuated (PMID: 16891412, PMID: 30393776). Replication and virulence will be monitored through: 1) viral passage in cell culture and titering and B) weight loss and titering in animals. If the virus shows signs of enhanced replication or virulence over WT, the laboratory will cease working with the virus and notify the IBC. Animals will be inoculated intranasally with 50 µl of viral inoculum. Inoculation titer will range from 1e2-1e6 PFU. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, SARS-CoV-2, mammalian cells, mice	
77462	[redacted]	Antibiotic tolerance in *Staphylococcus aureus* and *Pseudomonas aeruginosa*
APPROVED WITH STIPULATIONS	**Summary**: The purpose of our research is to determine the mechanism of antibiotic tolerance of *P. aeruginosa* and *S. aureus* in vitro and in murine infection models. Previously constructed libraries of deletion mutants in both *S. aureus* and *P. aeruginosa* will be employed to characterize any genes that contribute to antibiotic tolerance. These libraries will be screened in vitro and in cell lines for survival to antibiotic challenge. Once genes of interest have been identified, the laboratory will construct clean deletions, point mutants, random mutagenesis and overexpression of these genes in order to characterize their role in resisting antimicrobial treatment. Mice will be infected with bacterial mutant strains of interest for further study. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee also requested clarifications on the volumes that would be injected into mice. III-D, BSL-2, plasmids, *P. aeruginosa*, *S. aureus*, mammalian cell lines, mice	
77302	[redacted]	Ex362-POR-2A-CreER Mouse
APPROVED	**Summary**: The aim of this experiment to produce a mouse strain with a CRE-ER expressed together with POR protein. The donor template will be a double stranded DNA (dsDNA) produced in vitro (by PCR) using a plasmid as a template. This dsDNA donor vector will be microinjected into mouse zygotes together with Cas9 protein and synthetic or in vitro transcribed guide RNAs, which will be used to cut the last intron of the POR gene to induce homologous recombination and the introduction of the correct DNA sequence right after the last exon of the POR gene. Single-cell mouse embryos are injected with picoliter amounts of the recombinant material. The embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
77303	[redacted]	Ex363-NOD-Sirp-alpha Mouse
APPROVED	**Summary**: The aim of this experiment is to replace the Sirp-alpha gene of the PIRF mouse strain with the NOD mouse variant. The donor template will be a double stranded DNA (dsDNA) containing the NOD Sirp-alpha coding sequence produced in vitro (byPCR) using a plasmid as a template. This dsDNA will be microinjected into mouse zygotes together with Cas9	

	protein and synthetic or in vitro transcribed guide RNAs to induce homologous recombination and the proper gene replacement. Single-cell mouse embryos are injected with picoliter amounts of the recombinant material. The embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
77304	[redacted]	Ex358-SYNB20004-hACE2-hFCGRT-C57BL6-Mouse
APPROVED	**Summary**: The aim of this experiment is to do a targeted insertion in the mouse Ace2 locus of a construct containing the human ACE2 sequence and an Active Genetics cassette. The transgene will be cloned into a plasmid with the pUC backbone that will be microinjected into FCGRT-KI mouse fertilized embryos along with cas9 protein and synthetically modified gRNA to promote insertion into the proper site of the mouse genome. The injected embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. Single-cell mouse embryos are injected with picoliter amounts of the recombinant plasmid at 0.5-20 ng/µl. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
77305	[redacted]	Ex359-SYNB20005-hTMPRSS2-hFCGRT-C57BL6-Mouse
APPROVED	**Summary**: To do a targeted insertion in the mouse Tmprss2 locus of a construct containing the human TMPRSS2 sequence and an Active Genetics cassette. The transgene will be cloned into a plasmid with the pUC backbone that will be microinjected into FCGRT-KI mouse fertilized embryos along with cas9 protein and synthetically modified gRNA to promote insertion into the proper site of the mouse genome. The injected embryos will be surgically implanted into the oviducts of pseudopregnant recipient mice. Single-cell mouse embryos are injected with picoliter amounts of the recombinant plasmid at 0.5-20 ng/µl. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
77306	[redacted]	Ex360-SYNB20006-hACE2-BALBc-Mouse
APPROVED	**Summary**: To do a targeted insertion in the mouse Ace2 locus of a construct containing the human ACE2 sequence and an Active Genetics cassette in the BALB/c genetic background. The transgene will be cloned into a plasmid with the pUC backbone that will be nucleofected into BALB/c ES cells along with cas9protein and synthetically modified gRNA to promote insertion into the proper site of the mouse genome. The ES cell clones with properly integrated donor will be injected into blastocysts which will be implanted in recipient females to make chimeras which will be mated to BALB/c mice for germline transmission of the targeted event. The transgene is targeted in mouse ES cells by nucleofection. The ES cells are then injected into blastocyst-stage embryos to produce chimeras **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
77310	[redacted]	Ex361-SYNB20007-hTMPRSS2-BALBc-Mouse

APPROVED	**Summary**: To do a targeted insertion in the mouse Tmprss2 locus of a construct containing the human TMPRSS2 sequence and an Active Genetics cassette in the BALB/c genetic background. The transgene will be cloned into a plasmid with the pUC backbone that will be nucleofected into BALB/c ES cells along with cas9protein and synthetically modified gRNA to promote insertion into the proper site of the mouse genome. The ES cell clones with properly integrated donor will be injected into blastocysts which will be implanted in recipient females to make chimeras which will be mated to BALB/c mice for germline transmission of the targeted event. The transgene is targeted in mouse ES cells by nucleofection. The ES cells are then injected into blastocyst-stage embryos to produce chimeras. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
77542	Douglas Cyr	Mechanism for degradation of membrane proteins by ER chaperones
APPROVED	**Summary**: The goal of this experiment is to study cellular mechanisms for selection of misfolded membrane proteins for disposal by the proteasome or lysosome. The insert gene, CFTR, GNRHR, Ora1, ATZ, will be cloned into pCDNA3.1, which will be transfected into cultured cells. Inserts are obtained via RT-PCR of DNA from human expression libraries and cloned into plasmids by after restriction digestion. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-F, BSL-2, plasmids, mammalian cell lines	
77632	[redacted]	Analysis of genetically modified cell lines in mice
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to analyze genetically modified cell lines in mice and look for altered phenotypes. Tumor cells secrete cytokines and growth factors that alter the tumor microenvironment so that the normal surrounding tissue actually promotes the growth and spread of the tumor. In addition, some of these tumor-secreted factors paralyze the normal immune response, again allowing a tumor to grow unchecked. The proposed experiments will eliminate one of several genes from the tumor cell so that when the tumor cells are implanted in the mouse, and the laboratory can determine whether that specific gene promoted tumor growth by altering the tumor microenvironment. Cas9 and guide plasmids are used to create cell lines that have a permanently disrupted gene. Genes modified included Her3, ProteinS, Gas6, Tyro3, MerTK, and Axl. Mice will be subcutaneously injected with 100 µl at a concentration of 10^6 recombinant cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. III-D, BSL-2, places, human and mouse cell lines, mice	
75603	David Lawrence	Production of stable cell lines using lentiviral transduction
APPROVED	**Summary**: The goal of this is experiment is to use lentiviral transduction to express bacterial photo-activated proteins in mammalian cells. Lentiviral packaging vectors will be obtained from collaborators on UNC's campus. Photo-activated protein DNA will be synthesized commercially. The insert gene will be cloned into a plasmid which will be transfected into mammalian cells in vitro. The insert gene will be cloned into a viral vector which will be utilized to transduce cells in vitro. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td></td><td colspan="2">III-D, BSL-2, plasmids, lentivirus, mammalian cells</td></tr>
<tr><td>77682</td><td>Pengda Liu</td><td>ACE2 protein stability control mechanisms</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this study is to identify potential molecules that destabilize ACE2 and investigate if reducing ACE2 protein expression can be used as an approach to treat/prevent infection to viruses using spike proteins. The laboratory have obtained a mammalian expression vector of the coronavirus Spike (S) protein from Dr. Neuven Krogan lab (UCSD). The laboratory will express S gene with other lentiviral packing plasmids (including delta8.9 or psPAX2) to produce pseudotyped lentiviruses with S protein expressed on surface. These pseudotyped lenti-viruses will be used to infect human cancer cell lines in vitro and will not be used in mouse studies.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, lentivirus, human cell lines</td></tr>
<tr><td>76782</td><td></td><td>Lenti-Cre for stable Cre and other gene expression</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to use lentivirus to deliver Cre expression into cells. Adeno-Cre will be used for introducing Cre recombinase into cells of interest, however this is only useful for transient Cre expression. The use of lentiviral Cre has a few advantages, such as introducing additional genes beyond Cre, or reporters for stable expression. Examples of this include using Lenti-Cre vectors in order to dually introduce Cas9-sgRNAs (Walter et al, Cancer Research, 2017) or to stably introduce a reporter into epithelial tissues (Mukhopadhyayet al, Cell Reports, 2014). When doing this work in vivo, such as done in both papers cited using intra-tracheal instillations, this will be done with the Mice will be injected subcutaneously with 10^4-10^7 Lenti-Cre in a volume of 10-100 µl total.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that for more specific details be given about the experiment. Also, previous “Lenti-Cre” title was changed to the current title.

III-D, BSL-2, plasmids, lentivirus, human cell lines, mice</td></tr>
<tr><td>77402</td><td></td><td>Surrogate Coronaviruses for BSL2 Assays</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to develop in vitro and in vivo assays using BSL2-level Coronaviruses for eventual use in SARS-CoV-2 experiments. Recombinant infectious clones of NL63-CoV and Mouse Hepatitis Virus (MHV) will be used as non-manipulated genomes or engineered to express GFP as a marker gene. These recombinant viruses will be studied in different mammalian cell lines. NL63-CoV and MHV will be inoculated by the intranasal administration route to mice and hamsters. Mice will receive 30-50 µl of 10^6 TCID50/ml and hamsters receive 100 µl of the same concentration.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, coronaviruses, mammalian cell lines, mice and hamsters</td></tr>
<tr><td>77002</td><td>Carla Ribeiro</td><td>Knockdown and Overexpression of various genes relevant to airway inflammatory responses utilizing lentivirus.</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal of this experiment is to knockdown or overexpress various genes relevant to airway inflammatory responses utilizing lentivirus. The insert gene (ERN1, ERN2 or XBP1) will be cloned into a viral vector which will be utilized to transduce cells in vitro.</td></tr>
</table>

<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

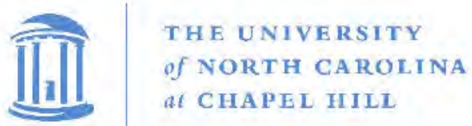
III-D, BSL-2, plasmids, lentivirus, human cell lines</td></tr>
<tr><td>76962</td><td>[redacted]</td><td>Generation of Chimeric Antigen Modified T cells for anti-tumor Therapy</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The goals of this experiment are to evaluate the ability of T cells transduced with a viral vector that expresses a single chain variable fragment of an antibody to kill tumor cells. The laboratory will also test the persistence of these cells are whether this correlates with anti-tumor activity and the type of T cells that are modified. They will isolate the heavy and light chain variable regions from the Neu antibody and clone them into a vector that contains a hinge region, a transmembrane CD8 (mouse domain) and signaling domains for CD3zeta, with the signaling domains of one or several of the following signaling proteins: CD28, CD137, ICOS, HVEM and/or OX40. The viral vector will be used to transduce mouse splenocytes/T cells or human peripheral blood mononuclear cells/T cells. These cells will be selected and expanded over 7-14 days in culture and then given intravenously to mice that have been injected with mouse and/or human tumors. The lab will inject 5-8x10^6 cells in a volume of 150 µl per mouse.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. The Committee also requested clarification on the volume and concentration of cells that would be injected intravenously into mice.

III-D, BSL-2, plasmids, lentivirus, mammalian cells, mice</td></tr>
</table>

5. **Sub-committee Approvals of Schedule G**: 1
   PI: Richard Boucher **Title:** PRR4 as a biomarker for airway submucosal glands secretion (III- D, ID 73585)
6. **Schedule H report:** 15
7. **Next IBC meeting date:** July 1, 2020 Web-Conference Call

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
July 1, 2020 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Shawn Hingtgen, Tori Baxter, Cathy Brennan, Garry Coulson, Eric Lewis

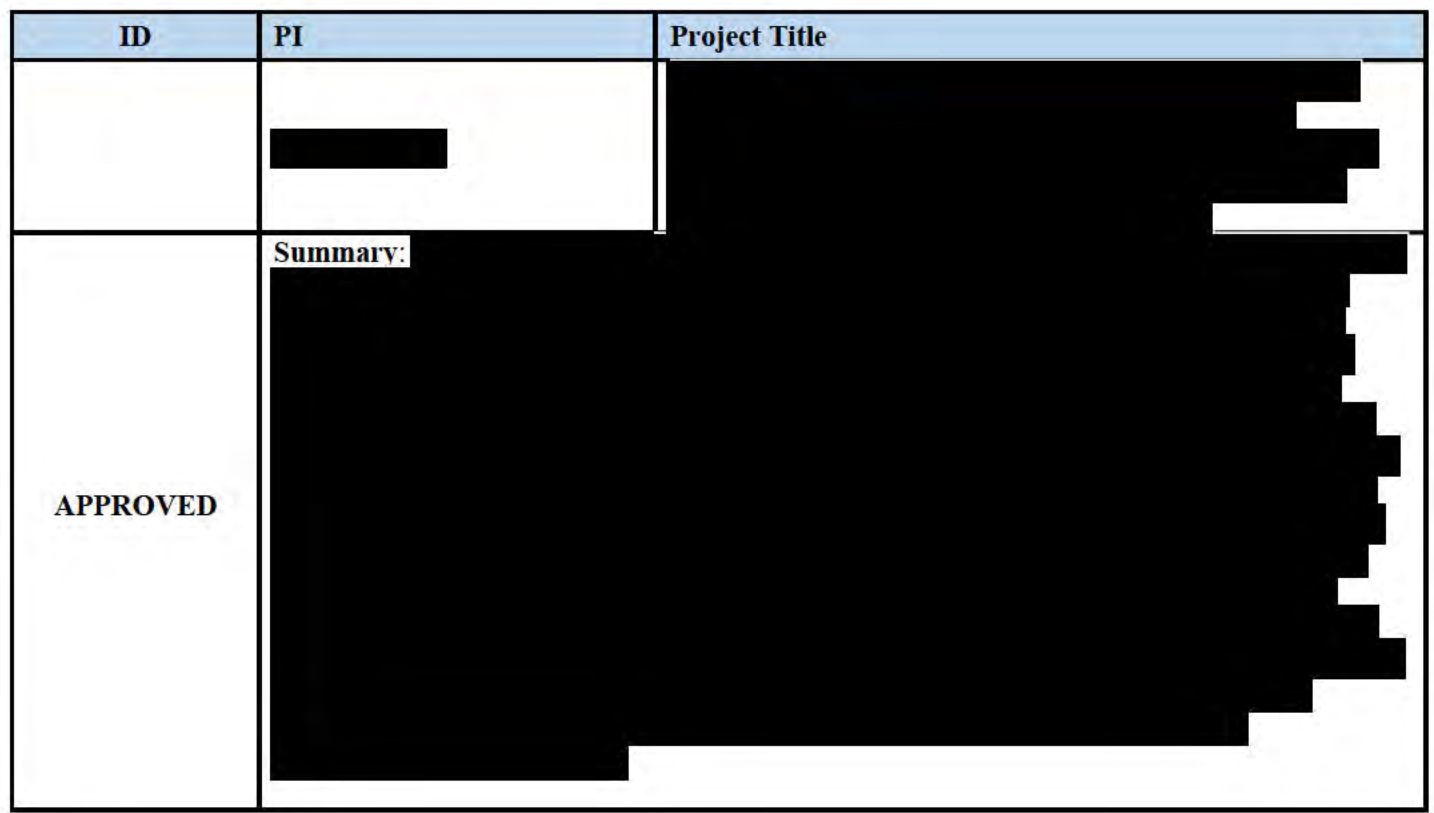
Members Absent: Craig Fletcher, Xiao Xiao, Keith Porterfield, Monica Dodson

Ad hoc Members (not requested to be present): Stan Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Clinical Trial:**
2. **Clinical Trial:**
3. **Clinical Trial:**
4. **Review minutes from the June 3, 2020 meeting.**
5. **Applications under review:**



ID	PI	Project Title
APPROVED	**Summary:**	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. III-C, Re[redacted]	
	[redacted]	[redacted]
APPROVED WITH STIPULATIONS	**Summary**: Adoptive T cell–based cellular therapies have led to remarkable advances among patients with [redacted] **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested confirmation that the adenoviral vector is replication-[redacted] deficient vector. The Committee also requested confirmation that the radioactive biohazardous waste would be handled and disposed of appropriately according to appropriate institutional policy. III-C, [redacted]	
	[redacted]	[redacted]
APPROVED	Summary: [redacted] **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-C, [redacted]	
78682	[redacted]	Recombinant Alphavirus expression vectors (based on VEE Vaccine strain 3526) expressing viral spike protein from a Ugandan MERS-like Coronavirus strain - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to develop a VEE replicon platform expressing the spike protein from a Uganda MERS-like CoV. Briefly, synthetically produced spike protein	

	from Uganda MERS-like CoV is placed within pVR21 will be mixed with VEE 3526 capsid and E1-E3 helper construct transcripts and electroporated into cells under BSL-2 conditions in a biosafety cabinet. Supernatants (~10%) will be tested for replication competent viruses by passage in cell culture as previously described by the Johnston group, noting that even a single full length recombinant VEE 3526 genome will kill the entire culture with 36-48 hrs and especially after passage. Supernatants will be concentrated by centrifugation and used in cell culture and in animal experiments. For each construct, replicon constructs will be transcribed, mixed with helper RNAs and electroporated into VeroE6 or BHK cells under BSL2 conditions in a safety cabinet, tested for the presence of replication competent viruses by serial passage in cell culture. Supernatants that pass safety testing will be concentrated and used to infect cells in culture and/or vaccinate animals at BSL-2 for antibody production. The overarching goal of the project is to develop reagents to characterize novel zoonotic strains of CoVs. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, VEE replicons, mammalian cell lines, mice	
78683		Generating MERS-CoV mutants expressing full length or portions of viral spike protein from a Ugandan MERS-like Coronavirus strain - 2020 renewal
APPROVED	**Summary**: The aim of this project is to generate viral mutants expressing the spike protein from a Uganda MERS-like CoV in the HKU5 and MERS-CoV backbones. Briefly, synthetically produced spike protein from Uganda MERS-like CoV will be ligated into the MERS-CoV or HKU5 infectious clone. Viable viruses will be characterized for replication in vitro and altered pathogenesis in vivo. Based on modeling structures, the Ugandan spike chimeric viruses are not expected to be replication competent and no enhanced pathogenesis is expected. The overarching goal of the project is to characterize replication competency and develop reagents to characterize novel zoonotic strains of CoVs. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, Coronaviruses, mammalian cell lines, mice	
78684		Introduction of mouse adaptation mutations into 2019-nCoV (SARS-CoV-2) infectious clone
APPROVED	**Summary**: The goal of the study is to develop a pathogenic model of 2019-nCoV (SARS-CoV-2) infection. The laboratory have generated a variant of SARS-CoV-2) that can utilize the murine orthologue of the human receptor, ACE2 This mouse adapted virus, can replicate in mice, but displays limited replication, rapid clearance, and causes limited disease in aged animals. To develop a pathogenic model of infection for better models of disease and medical countermeasure testing, -mouse adapted strain was serially passaged in BALB/c mice in 5 independent lineages From each of the 5 independent passage lineages, 5 plaque purified virus stocks were grown and deep sequenced. The goal of this study is to introduce the mutations that arose during in vivo passaging into the parental infectious clone as well as to test the necessity and sufficiency of each mutation. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, SARS-CoV-2, mammalian cell lines, mice	
78762		Pathogenesis of a panel of SARS-CoV-2 recombinant viruses with mutations/insertions/deletions identified in the circulating strains in human populations

<table>
<tr><td>APPROVED</td><td colspan="2">Summary: The objective of this experiment is to use a reverse genetic system to study the biological functions of mutations/insertion/deletions that are naturally occurring in the circulating SARS-CoV-2 strains that have been identified worldwide. Since the onset of the COVID-19 pandemic, many mutations/insertion/deletions have been continually accumulating in the genome of SARS-CoV-2 strains circulating throughout the world when comparing these strains with the prototype genome identified in Wuhan, China in January 2020. Evaluation of these mutations/insertions/deletions would provide a better understanding of SARS-CoV-2 adapting to the human population and benefit developments of vaccines and therapeutics.

A panel of mutant viruses will be generated by introducing one or multiple mutations/insertions/deletions separately into an infectious cDNA clone encoding a wild type US SARS-CoV-2 isolate WA1 strain (GenBank accession # MT461669). To avoid the possibility of unintentionally generating gain-of-function mutations, mutations/insertions/deletions identified from different circulated strains will not be combined and introduced into one virus. All the mutations generated in cell culture passage will also be tracked. In addition, when cloning the mutations into the WA1 genome and to aid in the visualization and quantification of SARS-CoV-2 infection, the laboratory will generate reporter viruses expressing fluorescent proteins (GFP, RFP, and NanoLuc) into the corresponding mutants.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-3, plasmids, SARS-CoV-2, mammalian cell lines, mice</td></tr>
<tr><td>77863</td><td></td><td>Xenopus genetic editing using TALEN and CRISPR-Cas9 technology</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to introduce small genetic mutations into genes of interest in Xenopus laevis and Xenopus tropicalis animals. This would enable the laboratory to identify the function of novel genes, the relevance of transcription factor domains as well as mimic human disease by introducing congenital mutations to assess their implication in cardiovascular development and disease. A TALEN repeat-variable diresidue (RVD) for each gene of interest will be cloned upstream of the FokI nuclease enzyme into a plasmid. Alternatively, the guide RNA sequence for each gene of choice will be cloned into a plasmid, as well as the Cas9 endonuclease enzyme. All synthetic capped RNA will then be transcribed from these plasmids and microinjected into fertilized Xenopus eggs. Each embryo will be injected with 2-10 nl of RNA.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that additional information on the genes that would be targeted in this experiment.

III-D, BSL-1, plasmids, Xenopus sp.</td></tr>
<tr><td>77882</td><td></td><td>Genetic editing using CRISPR-Cas technology in mice</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this project is to introduce small genetic mutations into genes of interest in mice (Mus musculus). This work is completed in collaboration with the UNC Animal Models Core. This would enable laboratory to identify the function of novel genes, the relevance of transcription factor domains as well as mimic human disease by introducing congenital mutations to assess their implication in cardiovascular development and disease. This work will be completed by the . The guide RNA sequence for each gene of choice will be cloned into a plasmid, as well as the Cas9 endonuclease enzyme. These will be injected into the pronuclei of mouse embryos.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

	III-E, BSL-1, plasmids, mice	
75962		Mouse subunit vaccine and immunogenicity study protocol
APPROVED	**Summary**: The aim of this experiment is to examine candidate *Chlamydia* T-cell protein antigens including CT443, CT043, CT368, CT144, CT338, and CT318. DNA will be PCR synthesized from *Chlamydia muridarum* or *Chlamydia trachomatis* using primers that recognize known or unknown sequences of *Chlamydia* membrane proteins. These synthesized pcr products will be cloned into *E. coli* expression plasmids to produce proteins. The recombinant proteins will eventually be injected into mice to examine the immune response. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, *E. coli*, mice (will be exposed to recombinant proteins and not rsNA)	
78802		Expression of human transferrin to enhance murine model of infection
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to examine a potential *Neisseria gonorrhoeae* infection model. Infection models in mice are limited due to specific interactions between the bacteria and its natural human host. The laboratory proposes to test whether expression of recombinant human transferrin in mice using replication deficient recombinant AAV can achieve serum levels of human transferrin equivalent to what is seen in humans and whether that prolongs infection of mouse genital tract by *N. gonorrhoeae*. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that an IACUC protocol number be provided for the work when that protocol has been completed. III-D, BSL-2, plasmids, AAV, mammalian cells, mice	
77662	Martina Gentzsch	NL63gfp Coronovirus for Ion Channel Assays
APPROVED	**Summary**: The aim of this study is to study ion channel function in vitro after a human coronavirus infection. Recombinant NL63gfp-CoV virus will be transduced into human epithelial cultures. Ion channel function will be measured in Ussing Chambers. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Coronavirus NL63, human cell lines	
78284		Modification of Stem Cells with Diagnostic and Therapeutic Transgenes-2017
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to further examine stem cell-based therapies. Due to their expansive utility, stem cell-based therapies hold the potential to redefine therapeutic approaches and provide cures for many terminal diseases. The laboratory seeks to harness the potential of stem cells to develop new and better methods for treating terminal cancers, including brain cancer. The laboratory will use an integrative approach that begins with creating specially designed targeted therapeutic proteins. The different stem cell types will be armed with the anti-cancer molecules and investigate the ability of stem cell-based therapies to improve both drug delivery and cancer cell killing using various small animal models of human brain cancer. Central to the research is the extensive integration of non-invasive imaging. Multiple imaging modalities will be used to provide real-time dynamic feedback on stem cell and tumor cell volumes and distribution, pharmacokinetics of drug delivery, and the overall effectiveness of the therapeutic approaches. The modified cells will ultimately be injected into mice.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the updated IACUC protocol numbers. They are IACUC ID numbers 19-060, 18-105, and 18-022. III-D, BSL-2, plasmids, mammalian cells, mice	
77842		Generation of MMTV-Wnt1 tumor cell lines
APPROVED	**Summary**: The aim of this project is to examine triple negative breast cancer in an animal model of disease. Triple negative breast cancer is the deadliest kind of breast cancer. The laboratory will use a mouse model of this disease to help study how it progresses and interacts with metabolism. Mammary cancer cell lines generated from MMTV-Wnt1 mice on a C57BL/6 background will be injected into wildtype female C57BL/6 mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, transgenic murine cells, mice	
78702		Targeting novel non-innate immune function of STING in treating AML
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to examine if inhibition of OTUD7B and STING activation displays a synergy in suppressing AML growth. The laboratory will use a commercially available Mx1Cre+Dnmt3aR878H/WT heterozygous mouse model and test different treatments within this model. AAVS-OTUD7B and AAVS-control viruses will be injected into Mx1Cre+Dnmt3aR878H/WT heterozygous mice by tail vein injection (100 µl in volume). The laboratory will initially try different doses of viruses and fix on optimal dose including 1e10, 1e11, and 3.16e11 total vector genomes (vg). **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The IACUC# for this work is 19-013. The Committee requested that the laboratory provide the information about the doses that will be administered to the mice. III-D, BSL-2, plasmids, AAV, murine cells, mice	
78302		Targeting Constitutively Active G-alpha-q for the Treatment of Uveal Melanoma in animals (primary model)
APPROVED	**Summary**: The aim of this experiment is to study an experimental treatment for Uveal Melanoma in an animal model of disease. The laboratory has developed a trap gene that can disrupt constitutively active Gaq signaling in uveal melanoma cell lines (92.1, OMM1.3, MP38, MP46). Trap genes are used in the laboratory and are genes of antibody or antibody-like affinity proteins that can specifically bind to the target and disrupt or trap its biological function for therapy purpose. The goal of the research is to evaluate the therapeutic efficacy of this trap gene delivered by AAV in the treatment of cancer. The MP38, MP46, OMM1.3-Fluc-eGFP, 92.1-Fluc-eGFP, OCM3-Fluc-eGFP, MP38-Fluc-eGFP, or MP46-Fluc-eGFP cells will be transduced with AAV containing decoy gene at MOI 10^4 and 10^5. Post 24-hours, cells will be washed with PBS, and collected 10^6 cells in 100 µl cells will be inoculated in the spleen on NSG mice for liver metastasis model of uveal melanoma. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, AAV, mammalian cells, mice	
77782	Ann Matthysse	Genes involved in survival and plant interaction of *Agrobacterium tumefaciens*
APPROVED	**Summary**: The aim of this study is to identify bacterial genes involved in resistance to desiccation and resistance to hydrogen peroxide. To determine the role of glycoside hydrolase	

	genes previously identified as required for virulence of the bacteria on some plant hosts. The genes or gene fragments will be cloned into *E. coli* K12. The phenotype of *E. coli* (with respect to resistance to stress or ability to carry out various enzymatic reactions) carrying the genes will be determined. Gene fragments cloned in *E. coli* will be introduced into *A. tumefaciens* to create insertion mutants in the original genes. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, *E. coli*, *A. tumefaciens*	
78582	[redacted]	Poly-primidine tract binding (PTB) protein miRNA and antisense
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to examine the modifying the level of PTB protein expression in oligodendrocytes will affect neuronal programming. A specific AAV vector will deliver and express either the miRNA or antisense that will result in the attenuation of PTB protein in rat brain oligodendrocytes. The laboratory predicts this change in PTB protein expression will result in the reprogramming of the oligodendrocytes to neurons in vivo. Either of the 2 constructs of interest will be inserted into an AAV viral vector and once infused into the rat brain will express the miRNA or antisense. Rats will receive a stereotactic infusion (1-3 microliters per infusion) into specific areas of the brain. The titer of the recombinant AAV virus will range from 5 X 10^{11} to 1 X 10^{13} viral particles per ml. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that an IACUC number is provided for the described animal work. The Committee also requested more information on how the animals will be restrained during those experiments. III-D, BSL-1, plasmids, mammalian cell line, mice	
78542	[redacted]	Modulation of neuronal function
APPROVED	**Summary**: The purpose of this experiment is to express genes in select neurons for their subsequent manipulation (opsins, Caspase, DREADDs) or identification (fluorescent proteins). The insert gene will be cloned into a viral vector which will be directly injected into mice where the virus will transduce cells in vivo. These genes allow for the selective manipulation of specific populations within the brain. Viral constructs will be directly injected into the brains of mice in very small volumes (less than 1 µl/mouse) **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, AAV, mice	
78004	[redacted]	Genomics of gene regulation in development
APPROVED WITH STIPULATIONS	**Summary**: The goal of this project is to examine how physical access to transcriptional regulatory information is controlled during specification and maintenance of cell types in *D. melanogaster* development. During the course of these studies, the laboratory will generate enhancer-driven reporter constructs (e.g. GFP, RFP or Gal4), expression constructs for epitope-tagged transcription factors, and use CRISPR/Cas9 to generate mutants by site-directed genome editing for characterization studies within *D. melanogaster*. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that more experimental details and a list of genes of interest be provided. The title of the protocol was changed to "Transcriptional regulation of cell identity in *D. melanogaster*".	

	III-E, BSL-1, plasmids, *D. melanogaster*	
78342	[redacted]	shRNA-Mediated Knockdown of Cortical Interneuron Regulatory Proteins
APPROVED	**Summary**: The aim of this experiment will utilize a miR-based shRNA knockdown strategy in vivo (rats) to explore the role of two proteins, Kv3.1 and HCN1, on the intrinsic firing activity of specific subtypes of cortical interneurons. This will use a multiplexed approach in which the double-floxed shRNA is driven by the interneuron-selective mDlx promoter and co-infected with a parvalbumin- or somatostatin-driven Cre virus to select the particular cell population. Vectors including pAAV-mDlx-HCN1-shRNA-GFP, pAAV-mDlx-Kv3.1-shRNA-GFP, pAAV-mDlx-scram-shRNA-GFP will be packaged insideAAV5 vector for infection of corticla interneurons. Additionally, pAAV-PV-Cre and pAAV-SST-Cre will be packaged inside AAV5 vector for co-infection. Anaesthetized animals will undergo stereotaxic surgery and injection of virus into brain regions of interest (specifically, prefrontal cortex and central nucleus of the amygdala). Volume of 0.4 µl -1.0 µl will be infused with a syringe pump at a rate of 0.2 µl per minute. Concentration of infused virus will be at least 10^6 infectious units/mL. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, AAV, mice	
73242	Scott Randell	Sorting primary HBECs that have been transduced with an eGFP-expressing virus
APPROVED	**Summary**: The goal of these experiments is to deliver fluorescent tags into human airway epithelial cells for tracking proliferative and differentiation behavior over time. The insert gene will be inserted into a replicated defective lentiviral vector which will be used to transduce cells in vitro. Transduced cells will be sorted for enhanced green fluorescent protein positivity, then expanded and differentiated in vitro. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentivirus, human cell lines	
78782	[redacted]	Novel cellular markers of drug-mediated calcium signaling in astrocytes
APPROVED	**Summary**: The goal of this experiment is to express genetically-encoded calcium indicators in astrocytes, to monitor calcium signaling in brain astrocytes response to experience. The laboratory will prepare 2 AAV plasmids, in which the calcium indicator CaMPARI2 will be expressed under the control of the astrocyte-specific GfaABC1D promoter, either with or without the peptide sequence Lck. Accordingly, the 2 AAV plasmids (and 2 subsequent AAVs) will be GfaABC1D-LckCaMPARI2 or GfaABC1D-cytoCaMPARI2. AAV will be microinjected directly into rat brain, in an animal protocol not yet submitted. For the animal procedure, 1 µl will most likely be microinjected per hemisphere, possibly max 2 µl. The concentration will depend on the virus from the UNC Vector Core, probably around $1x10^{13}$ particles/ml. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, AAV, rats	

6. **Sub-committee Approvals of Schedule G**: 4
   PI: [redacted] **Title:** The use of CpG in vaccination of mice (III- F, ID 79162)

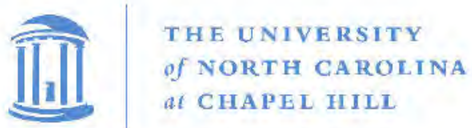
PI: Erin Heinzen **Title:** Establishing a human iPSC-derived neuronal model of SLC35A2 epilepsy to study disease mechanisms (III- F, ID 77904)

PI: Brian Diekman **Title:** Effects of swab sticks on SARS-CoV-2 detection by qPCR (III- F, ID 78882)

PI: Alecia Septer **Title:** Mechanisms of bacterial contact-dependent and -independent interactions (III- F, ID 78862)

7. **Schedule H report:** 26
8. **Next IBC meeting date:** August 5, 2020 Web-Conference Call

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
August 5, 2020 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Shawn Hingtgen, Tori Baxter, Keith Porterfield, Garry Coulson, Eric Lewis

Members Absent: Craig Fletcher, Xiao Xiao, Cathy Brennan

Ad hoc Members (not requested to be present): Stan Lemon, Ann Matthysse

Guests: None

Open Meeting

1. **Inactivation Procedure Review** – presented a inactivation validation protocol that examined the ability of Qiagen AVL Lysis Buffer to inactivate using the QIAmp Viral RNA Mini Kit (catalog 52904 or 52906). This kit is used for purification of viral RNA from plasma, serum, cell-free body fluids and cell-culture supernatants. The Committee requested additional information including clarification on sample volumes be provided about the experiment. The protocol was approved with the stipulation that required experimental details be provided and included in the protocol.
2. **Review minutes from the July 1, 2020 meeting.**
3. **Applications under review:**

ID	PI	Project Title
80022		Generation of a full-length infectious clone of pangolin SARS-2-like coronavirus, including reporter viruses
APPROVED	**Summary**: The objective of this experiment is to generate a reverse genetic system for the pangolin coronavirus (PangCoV) (GenBank accession # MT040333), which is the closest non-bat strain to the novel coronavirus (2019-nCoV/SARS-CoV-2). The PangCoV and SARS-CoV-2 share 85.3% genomic identity. The aim is to use this full-length cDNA clone to study the differences in pathogenesis and host tropism between PangCoV and SARS-CoV-2. Moreover, the PangCoV virus may yield in a heterologous challenge model for evaluating SARS-CoV-2 vaccines and therapeutics. In addition to clone the WT viral genome, to aid in the visualization and quantification of PangCoV infection, the laboratory will generate reporter viruses expressing fluorescent proteins (GFP, RFP, and NanoLuc). Viruses will also be examined in an animal model of infection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, PangCoV, mammalian cell lines, mice	
80023	Ralph Baric	Renewal for all recombinant NL63 human coronavirus (BSL2) constructs - 2020 renewal
APPROVED	**Summary**: The goal of this experiment is to examine if the human coronavirus NL63 (BSL2) can serve as a potential viral vaccine vector. This virus can target mucosal surfaces and not cause severe disease. Recombinant NL63 constructs in the lab are used for in vitro growth kinetics and characterizations.	

<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, NL63, mammalian cell lines</td></tr>
<tr><td>80024</td><td></td><td>Expression of GFP from Venezuelan Equine Encephalitis Replicon System (VEE-VRP-GFP) for use as an experimental control (3526 background, BSL2 use) - 2020 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The purpose of this experiment is to express Green Fluorescent Protein (GFP) from the BSL-2 Venezuelan Equine Encephalitis Replicon system (VEE-VRP) for use as a control in antibody production and transfection studies.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, VRPs, mammalian cell lines, mice</td></tr>
<tr><td>80025</td><td>Ralph Baric</td><td>Functional Screen of the Roles of Interferon-Stimulated Genes in CoV Infection - 2020 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of the project is to determine host interferon stimulated genes (ISGs) that directly impact coronavirus replication in either a positive or negative way. The approach takes advantage of an established GIPZ Lentiviral shRNA library that targets well-established ISGs. The experiment seeks to establish a screen that knocks down ISG function via both lentivirus transduction and select transfection approaches. Using eukaryotic selection, stable cell lines will be established. The ISG constructs and transfected/transduced cell lines will be created and maintained at BL2. The cell lines will be used in viral infection experiments at the biosafety levels indicated for the viruses.

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Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Since the project viral repertoire has been expanded since that last time this protocol was reviewed but the Committee, the Committee requested that the word “renewal” be removed from the title.

III-D, BSL-3, plasmids, Coronaviruses, mammalian cell lines</td></tr>
<tr><td>80026</td><td></td><td>Middle East respiratory syndrome coronavirus (MERS-CoV): incorporations of mouse-adapted mutations into infectious clone - 2020 renewal</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal is to incorporate genetic changes in the MERS-CoV genome that have been previously identified through adaptation of MERS-CoV in mice. Through serial passaging of MERS-CoV in a mouse model, MERS-CoV viruses have been developed that cause clinical symptoms of acute respiratory distress.

the laboratory intends to identify viral proteins that influence disease outcome. This can be accomplished by determining which MERS-CoV mutations, obtained through adaptation in mice, are central to provoking respiratory disease. To identify key mutations in MERS-CoV, the laboratory plans to incorporate various combinations of these mutations into their MERS-CoV infectious clone system. These GOF studies were part of prior approval received from NIH.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-3, plasmids, MERS-CoV, mammalian cell lines, mice</td></tr>
<tr><td>79622</td><td></td><td>Calcium Imaging in Behaving Animals</td></tr>
</table>

APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to perform calcium imaging in animals. Calcium imaging is a way of visualizing neuronal activity over time. This experiment will allow the laboratory to observe the activity of different cell populations in awake and behaving animals while they are performing various tasks and/or receiving various rewards. To perform this, an adeno-associated viral (AAV) vector containing the GCaMP6 gene will be introduced into the rat brain in vivo. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the updated IACUC protocol number. The current IACUC ID number is 20-163. III-D, BSL-1, plasmids, AAV, mice	
79482		Use of CRISPR/Cas9 System for Rodent Genetic Modification
APPROVED	**Summary**: The objective of this experiment is to use a new CRISPR/Cas9 system for rodent genetic modification in the Animal Models Core Facility. The Animal Models Core Facility produces genetically modified rodents for various clients. The CRISPR/Cas9 system is a tool for modifying the genome through direct injection in embryos, transfection of embryonic stem cells or other cell lines. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-2, plasmids, rodents	
80423		Use of AAV vectors to elucidate the location and movements of a HMGB1-GFP fusion protein in brain
APPROVED WITH STIPULATIONS	**Summary**: The goal of this experiment is to use AAV vectors to elucidate the location and movements of a HMGB1-GFP fusion protein in brain. To elucidate the location and movements of a HMGB1-GFP fusion protein after injection into various regions of the mouse brain and subsequent experimental treatments historically performed with mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the laboratory provide the information about the viral dose that will be administered to the mice. III-D, BSL-2, plasmids, AAV, mammalian cells, mice	
80442		Use of CRISPR/dCas9 tools to knock down neuronal expression of TLR4 and RAGE
APPROVED WITH STIPULATIONS	**Summary**: The goal of this experiment is to use CRISPR/dCas9 tools to knock down neuronal expression of TLR4 and RAGE in animal models. To elucidate the specific involvement of TLR4 and RAGE in alcohol-related pathogenesis. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the laboratory provide the information about the viral dose that will be administered to the mice. III-D, BSL-2, plasmids, AAV, mammalian cells, mice	
79202		Analysis of HIV co-infection with herpes viruses
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to analyze Human Immunodeficiency Virus (HIV) co-infection with herpes viruses within a mouse model of disease. HIV, Epstein-Barr virus (EBV), and Kaposi's Sarcoma-associated Herpesvirus (KSHV) are human specific pathogens that do not replicate in other species (except in non-human primates like chimpanzee, an endangered species). The mechanisms of KSHV and EBV-associated tumorigenesis in HIV patients are	

	poorly understood. The laboratory is proposing a research plan to begin elucidating details of the in vivo interactions, altered transmissibility, augmented pathologies, and altered tumorigenicity capacities that occur during co-infections in humanized mouse model. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the laboratory provide the information about the wildtype HIV strains that would be used for this experiment as well as the viral doses that will be administered to the mice. The Committee requested the updated IACUC protocol number. The current IACUC ID number is 20-235. III-D, BSL-2, plasmids, recombinant herpes viruses, mice	
80342	[redacted]	All-optical closed loop studies for next generation neural prostheses
APPROVED	**Summary**: The aim of this project is to perform all-optical closed loop studies for next generation neural prostheses. Our long-term aim is to develop neural prostheses to recover lost motor functions. Such plan entails developing two lines of research. One is to study brain circuits involved in sensory processing and motor learning. The second is to develop non-invasive interfaces with the brain via optical methods (two-photon imaging and optogenetics). More specifically, the laboratory will interface with the cerebellum to recover motor functions lost in other brain areas because of injury. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, AAV, ferrets	
80042	[redacted]	TBK1 and mTOR function in innate and adaptive immunity
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to examine TBK1 and mTOR function in innate and adaptive immunity. The laboratory proposes to overexpress, knockdown, and edit (via CRISPR-Cas9) members of the TBK1 and mTOR pathways in primary mouse cells, immortalized mouse cell lines, immortalized human cell lines, and primary human cells to study regulation of the immune response to pathogens. The laboratory will use the same methods to express mutated versions of the gene/protein generated by site directed mutagenesis to understand the function of site-specific post-translational modifications (e.g. phosphorylation and ubiquitination). **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the "GENE TRANSFER EXPERIMENTS INVOLVING WHOLE ANIMALS OR PLANTS (Section III)" section of the Schedule G protocol. III-D, BSL-2, plasmids, lentiviral vectors, human cell lines, mice	
79990	[redacted]	Ex vivo retroviral transduction and ribonucleoprotein (RNP) transfection of hematopoietic stem cells followed by transplantation
APPROVED	**Summary**: The aim of this experiment is to determine whether altering key aging-associated transcriptional and/or chromatin changes in hematopoietic stem cells (HSC) can enhance or attenuate aging phenotypes in downstream progenitor and/or effector cells. To examine this, the laboratory will use retrovirus mediated RNA over-expression and/or CRISPR-Cas9 mediated gene knockout to alter the expression level of candidate age-dynamic genes in HSCs.. Eventually the modified HSCs will be transplanted into lethally irradiated young mouse recipients to analyze their in vivo function. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design.	

	III-D, BSL-2, plasmids, retroviral vector, murine cells, mice	
80402	Tal Kafri	Improved lentitransduction
APPROVED	**Summary**: The aim of this experiment is to enhance titers and transduction of pseudotyped lentiviral vectors. In this case, the lentiviral vectors will be pseudotyped with VSV-G envelope/ COVId-D-19 Spike-RBD fusion (GS) protein. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the title potentially be updated. The title was changed to "A VSV-G envelope/ COVId-D-19 Spike-RBD fusion (GS) protein as a means to enhance lentiviral vector particle release and transduction efficiency". III-D, BSL-2, plasmids, lentiviral vector, mammalian cell lines	
80403	Tal Kafri	Using AAV vectors in vitro
APPROVED WITH STIPULATIONS	**Summary**: The goal of this study is to characterize transduction efficiency in vitro of AAV-based vectors AAV vectors (Serotypes 2-9) that will be used in different experiments. The vectors will be employed on various mammalian cell lines in vitro including rodent fibroblasts, human cells (293T, fibroblasts, Caco2, Hu7 and HepG2) and simian cells (Vero Cos).. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that additional details about the AAV experiments be provided. Also, the title of this Schedule G was changed to "Species and host factors effects on different AAV vector serotypes transduction in vitro". III-D, BSL-2, plasmids, AAV, mammalian cell lines	
76042		Mucosal trapping of SARS-CoV-2 by ACE2 receptor analogs
APPROVED	**Summary**: The goal of this experiment is to develop a bifunctional ACE2 analog capable of trapping SARS-Cov-2 in mucus. A SARS-Cov-2 S protein pseudotyped lentivirus will be used for this experiment. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, lentivirus, mammalian cells, mice	
78922		Engineering B cells with lentivirus
TABLED	**Summary**: The aim of this study is to engineer human B cells with lentivirus. The human B cells will be engineered to secrete therapeutics proteins as a cell-based platform for sustained protein delivery in vivo. **Committee Comments**: The Committee decided to table this protocol. The Committee requested that the protocol be more thoroughly rewritten and provide more details about the intended experiment.	
80002	Helen Lazear	Generating knockout cell lines using CRISPR
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to use CRISPR/Cas9 gene editing to generate cell lines genetically deficient in innate immune signaling molecules (e.g. Ifnar1, Ifnlr1) to study antiviral responses in cell culture.	

<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a more descriptive title. The title was changed to “Generate cell lines genetically deficient in innate immune signaling molecules to study antiviral responses in cell culture”.

III-D, BSL-2, plasmids, lentiviral vector, human cell lines</td></tr>
<tr><td>79602</td><td></td><td>CRISPR-edited mice expressing MAVS susceptible to HAV 3ABC cleavage (renewal)</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of the experiment is to develop a mouse model that is susceptible to Hepatitis A virus (HAV) infection. In the early stages of this project, the laboratory used CRISPR/Cas9 gene editing to produce two BL6 mouse lineages with knock-in point mutations in the Mavs gene that render the endogenously expressed murine Mavs protein susceptible to 3ABC cleavage. Two separate lineages of MAVS-vs/vs mice have been successfully established (F18 and F19). Studies in progress are now evaluating the phenotype of these mice in terms of their susceptibility to infection with wild-type HAV, their ability to signal normal IFN responses following infectious challenge with HAV and other viruses, and the ability of the HAV 3ABC protease to cleave MAVS-vs in vivo.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, oligonucleotides, mice</td></tr>
<tr><td>79542</td><td>Wanda O’Neal</td><td>Overexpression of ACE2 receptor related to Covid19 Research in airway cells</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this project is to study the effects of overexpression of the receptor ACE2 in airway cells in culture. ACE2 not only mediates the SARS-CoV-2 virus entry but also affects the pathophysiological process of virus-induced lung injury, as well as other organ damage.. Human ACE2 will be cloned into the vectors TRIPZ and PGK. TRIPZ is an inducible lentiviral vector with a TRE promoter and PGK is also a lentiviral vector with a PGK promoter. Human cell lines will be transduced with the lentiviral vectors.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, lentiviral vectors, human cell lines</td></tr>
<tr><td>79582</td><td></td><td>Generation of a podocin-GFP transgenic zebrafish line</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The goal is to generate a transgenic zebrafish line that allows fluorescent visualization (GFP) of zebrafish kidney podocytes. This will allow for easy screening of knockout zebrafish to determine if the podocytes are disrupted by gene deletion and suggest a role for the novel factor in podocyte development/maintenance. GFP will be cloned into a plasmid which will be propagated with E. coli, purified, and injected into single-cell zebrafish embryos.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-1, plasmids, zebrafish</td></tr>
<tr><td>73802</td><td>Joseph Ruiz</td><td>Development of Gene Therapeutics for Nucleotide Expansion Disorders</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The goal of this experiment is to develop gene therapeutic for long-term treatment of the nucleotide expansion disorders, myotonic dystrophy, and Huntington's Disease.. The laboratory will generate AAV particles that carry our gene therapeutic for in vitro efficacy studies using cells derived from affected patients.</td></tr>
</table>

<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested information on the therapeutic gene target.

III-D, BSL-2, plasmids, AAV, mammalian cell lines</td></tr>
<tr><td>77642</td><td>Joseph Ruiz</td><td>Development of Precision Epigenetic Gene Therapeutics for Diseases Caused by Gene Mis-regulation</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to reactivate a gene (frataxin) that is epigenetically silenced in patients with Friedreich's Ataxia. Gene therapeutics will be cloned into transposon-based vectors. Those vectors will be transfected into mammalian cells in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, plasmids, human cell lines</td></tr>
<tr><td>80362</td><td>Xiaohe Yang</td><td>shRNA lenti-viral plasmids</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to knockdown or over-express select genes within cell lines. To accomplish this, lentiviral plasmids will be transfected into mammalian cells in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a more descriptive title. The title was changed to “Use shRNA lenti-viral plasmids to modify expression of select genes within cell lines”.

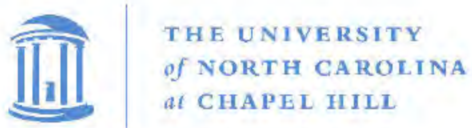
III-D, BSL-2, plasmids, lentiviral vector, human cell lines</td></tr>
<tr><td>80484</td><td>Xiaohe Yang</td><td>Gene Regulation in Breast Cancer Prevention and Experimental Therapeutics</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this project is to test functional impact of various regulators involved in growth modulation and cell death on breast cancer cells. The insert gene of interest will be cloned into a viral vector which will then be utilized to transduce cells in vitro. The cells will not be injected into mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a more descriptive title. The title was changed to “Determine if modifying gene regulation in breast cancer is a potential therapeutic strategy”.

III-D, BSL-2, plasmids, adenoviral vector, mammalian cell lines</td></tr>
</table>

4. **Sub-committee Approvals of Schedule G**: 3
   PI: John Milner **Title:** Retroviral transduction of mouse T cells (III- D, ID 79342)
   PI: John Milner **Title:** Mouse cancer cell lines expressing a transgene (III- F, ID 79502)
   PI: Chad Pecot **Title:** Evaluation of oligonucleotide therapeutics in cancer cells (III-F, ID 80003)
5. **Schedule H report:** 17
6. **Next IBC meeting date:** September 2, 2020 Web-Conference Call

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
September 2, 2020 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Tori Baxter, Keith Porterfield, Jennifer Hunter, Cathy Brennan, Garry Coulson, Eric Lewis

Members Absent: Craig Fletcher, Xiao Xiao, Shawn Hingtgen,

Ad hoc Members (not requested to be present): Stan Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Introduction of the new Associate Biological Safety Officer –**
2. **Review minutes from the August 5, 2020 meeting.**
3. **Applications under review:**

ID	PI	Project Title
81102		Investigation of the role of ion channels in and MERS coronaviruses - 2020 renewal
APPROVED	**Summary**: The objective of this study is to investigate the role of ion channels in and MERS coronaviruses. The aim is to further elucidate how ion channels affect coronavirus immunity and viral pathogenesis in vitro and in vivo. Deletion of these ion channels in both the -and MERS-CoV WT and mouse-adapted genomes should reduce viral escape from host cells, so these experiments do not fall under gain-of-function prohibitions. Replication and virulence will be monitored through viral passage/tittering in cell culture and weight loss/titering in murine models. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, Coronaviruses, mammalian cell lines, mice	
81104		Expression of human norovirus and sapovirus capsid genes in VEE replicons (3526 background) for characterization of antibody and ligand binding - 2020 renewal
APPROVED	**Summary**: The goal of this project is to study antigenic and ligand binding properties of human norovirus and sapovirus. There are no validated cell culture or small animal models for human norovirus or sapovirus propagation. To study the antigenic and ligand binding properties of these viruses, virus like particles (VLPs) composed of the capsid protein are utilized as virus surrogates. Both in vitro and in vivo experiments will be performed for this project. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, VRP, mammalian cell lines, mice	

81105	[redacted]	Expression of the human coronavirus NL63 Spike attachment protein for characterization of antibody cross-reactivity - 2020 renewal
APPROVED	**Summary:** The aim of this experiment is to create a Venezuelan Equine Encephalitis Virus Replicon (VRP) particle expressing the coronavirus NL63 spike glycoprotein as a potential vaccine platform technolog [redacted] VRP particles will be used to vaccinate mice. The resultant sera will be assessed for cross reactivity with an array of coronaviruses. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, VRPs, mammalian cell lines, mice	
81182	[redacted]	Recombinant Alphavirus expression vectors (based on VEE Vaccine strain 3526) expressing viral spike protein chimeras from coronaviruses related to SARS-CoV-2
APPROVED WITH STIPULATIONS	**Summary**: The aim of this project is to develop a VEE replicon platform expressing chimeric spike proteins from coronaviruses related to SARS-CoV-2. The overarching goal of the project is to develop reagents to characterize novel zoonotic strains of CoVs and to vaccinate animals prior to challenge with SARS-CoV-2 to determine correlates of protection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that language that confirms that the domain swaps will occur only in the context of the replicon system and not being introduced into the SARS-CoV-2 viral backbone be added to the Schedule G. III-D, BSL-2, plasmids, VRPs, mammalian cell lines, mice	
81183	[redacted]	Recombinant Alphavirus expression vectors (based on VEE Vaccine strain 3526) expressing viral spike protein from zoonotic coronaviruses related to SARS-CoV-2
APPROVED	**Summary**: The aim of this project is to develop a VEE replicon platform expressing the spike protein from zoonotic coronaviruses related to SARS-CoV-2 as a potential vaccine platform technology. The overarching goal of the project is to develop reagents to characterize novel zoonotic strains of CoVs and to vaccinate animals prior to challenge with SARS-CoV-2 to determine correlates of protection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, VRPs, mammalian cell lines, mice	
81185	[redacted]	Generation of SARS-CoV-2 viruses with ablation of the O-methyltransferase domain of nsp16
APPROVED	**Summary**: The objective of this experiment is to generate SARS-CoV-2 viruses with substitution mutations within the [redacted] nsp16 protein domain. These mutations of interest have been shown in [redacted] to attenuate progeny virus and are anticipated to be likewise attenuating in SARS-CoV-2. This virus will be examined in vitro and in vivo. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, SARS-CoV-2, mice, mammalian cell lines, mice	

81186	[redacted]	Abrogation of the expression of accessory proteins downstream of ORF1 in SARS-CoV-2
APPROVED	**Summary**: The objective of this experiment is understand the role of accessory proteins downstream of ORF1. [redacted] These proteins have largely unknown function, though some are believed to be immune modulators in the context of host infection. Evaluation of their function will be performed by deletion of these genes in SARS-CoV-2. [redacted] Ddeletion is anticipated to be attenuating in vivo, as it is in SARS-CoV infection. This virus will be examined in vitro and in vivo. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, SARS-CoV-2, mice, mammalian cell lines, mice	
81562	[redacted]	Regulation of Outflow Facility by Gene Transfer
APPROVED WITH STIPULATIONS	**Summary**: The ultimate goal of these experiments is to deliver genes to the primary human trabecular meshwork cells and to assess the therapeutic potential of their encoded proteins. The laboratory will insert the selected genes into viral vectors and attempt to elucidate the molecular mechanisms that regulate Intraocular Pressure. In this experiment, the laboratory will attempt to increase infection efficiency by mutating the coat of the virus. The viral vectors will be injected into rodent models. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee determined the Schedule G question of whether a viral vector and replication deficient is relevant for the Schedule G and should be confirmed with the laboratory. The current IACUC ID number is 20-193. III-D, BSL-2, plasmids, AAV, mammalian cells, rodents	
76942	[redacted]	Immunomodulatory mechanisms in Kras-driven pancreatic cancer and metastasis_pLVTH-M-GFP
APPROVED	**Summary**: The purpose is to express green fluorescent protein (GFP) in mouse cancer cells to track tumor cell growth in vitro and in vivo. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Lentiviral vector, human cell line, mice	
81585	[redacted]	AAV-mediated Gene Therapy (in mice) for the Treatment of Neurogenetic Diseases
APPROVED WITH STIPULATIONS	**Summary**: The purpose of the proposed study is to develop effective gene therapy approaches for the treatment of mucoplysaccharidoses (MPS) and other neurogenetic diseases. The AAV vector will be propagated in vitro then injected into a mouse model. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the Principal Investigator provide clarity on whether they will propagate AAV within their own lab or obtain purified AAV from a commercial source or from a collaborator. If the AAV is propagated within a human cell line in the lab, the Committee requested that the BSL recommendation for this project be upgraded to BSL-2. III-D, BSL-2, plasmids, AAV, human cell line, mice	
80522	[redacted]	Elucidating the Neural Circuits of Binge Drinking

<table>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The purpose of this experiment is to express genes in select neurons for their subsequent manipulation (opsins) or identification (fluorescent proteins).

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the Principal Investigator provide clarity on whether they will propagate AAV within their own lab or obtain purified AAV from a commercial source or from a collaborator. If the AAV is propagated within a human cell line in the lab, the Committee requested that the BSL recommendation for this project be upgraded to BSL-2.

III-D, BSL-1, plasmids, AAV, mice</td></tr>
<tr><td>80582</td><td>Tal Kafri</td><td>Simple and lenti viral vectors expressing CRISPR/Cas9 and gRNA</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The experiments are aimed at generating lentiviral or simple retroviral vectors expressing the CRISPR Cas9 (from a Pol II promoter) and gRNA's from Pol III promoters for genome editing (in vitro editing only).The vectors will express either a single gRNA or pools of gRNA's directed to either human or rodent target sequences.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested confirmation that genome editing will only be performed in in vitro experiments.

III-D, BSL-2, plasmids, lentiviral and retroviral vectors, human cell lines</td></tr>
<tr><td>70342</td><td>Brian Kuhlman</td><td>Design of Protein-Protein Interactions</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to test protein-protein interactions that have been first computationally designed. Proteins of interest will include mammalian IgG (or IgG fragment) Immunoglobulins and related proteins, viral non-infectious enveloped particles, and human cytoplasmic secretary proteins.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the BSL of the experiment be upgraded to BSL-2 to include proposed work with human cell lines.

III-D, BSL-2, plasmids, bacterial cells, yeast cells, mammalian cells</td></tr>
<tr><td>81330</td><td></td><td>Engineering human B cells to secrete a therapeutic protein to block SARS-CoV-2 transmission</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to engineer human B cells to secrete a proprietary therapeutic protein to block SARS-CoV-2 transmission. This is a proof of concept model. The B cells will function as a cell-based depot to achieve sustained protein delivery in vivo.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the title potentially be updated. The title was changed to “Engineering human B cells to secrete a therapeutic protein in a proof on concept model”.

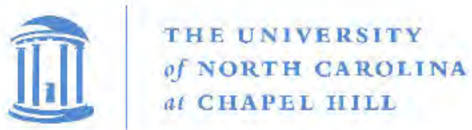
III-D, BSL-2, plasmids, lentiviral vector, human cells, mice</td></tr>
<tr><td>80674</td><td></td><td>Vector mediated delivery of p450scc to increase steroidogenesis</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to overexpress proteins involved in steroidogenesis to further determine their role in neurosteroid production. The insert gene(s) of interest will be cloned into a viral vector which will be utilized to transduce cells in vitro. The virus will ultimately be injected into rats.</td></tr>
</table>

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the BSL of the experiment be upgraded to BSL-2 to include proposed work with human cell lines. III-D, BSL-2, plasmids, AAV, human cell lines, rats	
81522	[REDACTED]	Immunomodulatory mechanisms in Kras-driven pancreatic cancer and metastasis: Ad-GFP; Ad-Cre-GFP
APPROVED	**Summary**: The goal of this experiment is to achieve expression of oncogenic form of mutant allele, which is otherwise restricted by Lox-STOP-Lox. The laboratory will achieve this using control or Cre-recombinase containing Adenovirus to induce recombination of Lox-STOP-Lox site at the mutant alleles. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, adenoviral vector, murine cells, mice	
81222	[REDACTED]	Genetic manipulation of rat neurons via opsins or DREADDs
APPROVED WITH STIPULATIONS	**Summary**: The purpose of this experiment is to manipulate activity of specific neurons by expressing genes that encode opsins or DREADDs or fluorescent proteins. The insert gene will be cloned into a viral vector which will be directly injected into rats where the virus will transduce cells in vivo. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The current IACUC ID number is 18-206. III-D, BSL-1, plasmids, AAV, mice	
81342	Bryan Roth	Sindbis Virus for use in Mammalian Evolution Chamber
APPROVED	**Summary**: The goal of this experiment is to generate a capture screen in mammalian cells capable of selecting for viral genomes carrying a desired protein coding sequence. The Sindbis viral genome will be used for this project. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Sindbis viral genome, Lentiviral vector, mammalian cell lines	

4. **Discuss NIH reportable incident –** [REDACTED] **The Committee discussed an occupational exposure involving a small splash to the eye with a recombinant *N. gonorrhoeae* bacterial strain possessing a gene deletion in the NHBA gene. The Committee discussed the incident and the recommendations for mitigating future exposures of this kind in the lab.**
5. **Sub-committee Approvals of Schedule G**: 6
   PI: Ralph Baric **Title:** Identifying the role of quantitative trait locus target BAI1 in eosinophil clearance - 2020 renewal (III- F, ID 81103)
   PI: [REDACTED] Title: Improving T cells immunity for Immunotherapy studies (renewal 2020) (III- D, ID 81462)
   PI: Ronit Fraiman **Title:** Pseudo Non-Infectious viral particles (III- D, ID 81122)
   PI: Nathaniel Hathaway **Title:** Epigenome modification research (III- D, ID 80903)
   PI: Nathaniel Hathaway **Title:** Mechanism of HP1-Mediated Heterochromatin Assembly and Durability (III-D, ID 80904)
   PI: Mark Zykla **Title:** Transfection of Plasmids containing PGK-Neo (III- F, ID 81282)
6. **Schedule H report:** 19

7. **Next IBC meeting date:** October 7, 2020 TBD

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
October 7, 2020 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Rachel Graham, Barbara Savoldo, Tori Baxter, Jennifer Hunter, Cathy Brennan, Garry Coulson, Amanda Craigen, Eric Lewis

Members Absent: Craig Fletcher, Xiao Xiao, Shawn Hingtgen, Keith Porterfield

Ad hoc Members (not requested to be present): Stan Lemon, Ann Matthysse

Guests: None

Open Meeting

1. **Review minutes from the September 2, 2020 meeting.**
2. **Updates to the IBC Charter.** The Committee reviewed changes and updates to the IBC Charter and approved the revised charter with no changes.
3. **Applications under review:**

ID	PI	Project Title
82122	[redacted]	The use of transgenic leishmania strains
APPROVED WITH STIPULATIONS	**Summary**: The purpose of this study is to monitor the infection of leishmania in mice, using strains that express luciferase. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The injection volume listed for mouse experiments was changed from '100 µl' to '50 µl'. III-D, BSL-2, plasmids, *Leishmania* species, mice	
82162	[redacted]	Generating coronavirus bearing bat coronavirus group 2D spike glycoproteins and coronavirus group 2D spike vectored vaccines - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to create an array of recombinant coronaviruses bearing group 2D spike glycoproteins in order to study if these glycoproteins are capable of supporting infection of human, primate or bat cells thus gaining insight into the host range of group 2D coronavirus. The laboratory will also create vaccine candidates for each group 2D spike protein using the Venezuelan Equine Encephalitis Virus Replicon (VRP) particle system. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, Coronaviruses, VRP, mammalian cell lines, mice	
82243	Ralph Baric	Addition of Kozak sequence and puromycin resistance cassette to the Venezuelan Equine Encephalitis viral vector pVR21 plasmid - 2020 renewal
APPROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to insert a Kozak sequence and a puromycin resistance cassette into the Venezuelan Equine Encephalitis viral vector pVR21 plasmid.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that language be is added to the protocol that confirms that the Venezuelan Equine Encephalitis viral vector that will be used for the experiment is not a select agent strain. III-D, BSL-2, plasmids, VRPs, mammalian cell lines	
82244	[redacted]	Defining the interactions of remdesivir with the [redacted] RNA-dependent RNA polymerase
TABLED	**Summary**: The aim of this experiment is to create a mutation in the SARS-CoV-2 RNA-dependent RNA polymerase that is suspected to be important for interacting with the nucleoside analog antiviral drug remdesivir. **Committee Comments**: The Committee decided to table this protocol. The Committee requested that the protocol be undergo additional review and revision by the Biosafety office before it is reviewed by the IBC again in the future.	
82245	[redacted]	Pathogenesis of a panel of mouse-adapted SARS-CoV-2 recombinant viruses with mutations/insertions/deletions identified in the circulating strains in human populations
APPROVED	**Summary**: The objective of this experiment is to study pathogenesis and biological functions of mutations/insertion/deletions that are naturally occurred in the circulating SARS-CoV-2 strains using a mouse adapted (MA) SARS-CoV-2 backbone. A panel of mutant viruses will be generated by introduced one or multiple mutations/insertions/deletions separately into an infectious cDNA clone encoding a MA backbone. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, SARS-CoV-2, mammalian cell lines, mice	
82262	[redacted]	Generating coronavirus bearing [redacted]-like bat coronavirus WIV16s spike glycoproteins and WIV16s spike vectored vaccines - 2020 renewal
APPROVED	**Summary**: For this experiment recombinant coronaviruses will be made swapping out mouse adapted [redacted] spike glycoprotein with WIV16s spike proteins. Additionally, the spike glycoprotein of WIV16s will be expressed in VEE VRPs. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, VRP, [redacted] mammalian cell lines, mice	
82263	[redacted]	Evaluating host genes in [redacted] and MERS-CoV virulence using lentiviral expression plasmids - 2020 renewal
APPROVED	**Summary**: The goal of this experiment is to use lentiviral expression vectors to overexpress or knock down host genes in vitro to evaluate the role of host genes in the virulence [redacted] [redacted] and MERS coronavirus (MERS-CoV). **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, Lentiviral vectors, Coronaviruses, mammalian cell lines	
82282	[redacted]	Identification and characterization of a novel bat norovirus capsid - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to characterize the capsid of a novel bat norovirus. NoVs have been previously identified in murine, bovine, porcine, and canine animals, but not in	

	bats. The reactivity of this novel bat NoV capsid will be compared across an antibody panel to determine cross-reactivity and evaluate zoonotic infection potential. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, VRP, mammalian cell lines, mice	
82422		TDP-43 in ALS and related neurodegenerative diseases
APPROVED	**Summary**: The goal of this experiment is to determine the role of TDP-43 in ALS and related neurodegenerative diseases. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Lentiviral vectors, mammalian cell lines, mice	
82582		A pathogenic role for tau acetylation in Alzheimer
APPROVED	**Summary**: The aim of this project is to examine the role of Tau acetylation in the pathogenesis of Alzheimer's Disease. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Lentiviral vectors, mammalian cell lines, mice	
82302	Brian Diekman	Over-expression of CHADL and a GWAS-identified variant in human chondrocytes
APPROVED	**Summary**: The purpose is to express either the normal cDNA for Chondroadherin-like (CHADL) or an 8-base pair insertion variant in primary human chondrocytes. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Lentiviral vectors, human cell lines	
82303	Brian Diekman	Genome editing base pair changes to mimic GWAS-identified variants in human chondrocytes
APPROVED	**Summary**: The purpose is to alter the DNA of primary human chondrocytes to change particular base pairs associated with an increased risk of osteoarthritis. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-F, BSL-2, plasmids, Lentiviral vectors, human cell lines	
81962		Evaluation of candidate drugs against lymphoma cell lines
APPROVED	**Summary**: The aim is to test candidate cancer drugs against cell lines that mimic aspects of cancer. These cell lines are manipulated in a number of ways that improve their utility for predicting the clinical success of the drug. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mammalian cells, mice	

81942		Generation of N. gonorrhoeae knockout mutant
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to knock out the Neisseria Heparin Binding Antigen in *Neisseria gonorrhoeae* in order to be able to test whether a vaccine that contains this protein has protective immune responses directed against this antigen using a mouse *N. gonorrhoeae* infection model. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the "GENE TRANSFER EXPERIMENTS INVOLVING WHOLE ANIMALS OR PLANTS (Section III)" section of the Schedule G protocol. III-D, BSL-2, plasmids, *N. gonorrhoeae*, mice	
82583	Mark Heise	Identification and Characterization of Alphavirus inhibitory compounds
APPROVED WITH STIPULATIONS	**Summary**: The goal of this experiment is to evaluate novel chemical compounds, such as nucleoside inhibitors and protease inhibitors, for their ability to inhibit alphavirus (i.e. Chikungunya virus) replication and protect from alphavirus-induced disease. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that a risk assessment be added to this Schedule G. III-D, BSL-2, plasmids, Chikungunya virus, mammalian cell lines	
82082	Tal Kafri	Immortalization of MEF
APPROVED	**Summary**: The aim of this experiment is to immortalized mouse and human embryo fibroblasts (MEF's and HEF's, respectively) using retroviral vectors. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Retroviral vectors, mammalian cell lines	
82462		Engineering human B cells to secrete broadly neutralizing antibodies in a proof of concept model
APPROVED	**Summary**: The aim of this experiment is to engineer human B cells to secrete broadly neutralizing antibodies against HIV, influenza, or SARS-CoV-2. This is a proof of concept model. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Lentiviral vectors, human cell lines, mice	
82622		AAV-mediated gene replacement in the ear of deaf mouse models
APPROVED WITH STIPULATIONS	**Summary**: The aim of this project is to rescue auditory function in deaf mice that are genetic models of human inherited deafness. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. III-D, BSL-2, plasmids, AAV, human cell lines, mice	
78303		Uveal Melanoma cells, viral delivery (adenovirus and luciferase cells)

<table>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The lab has developed a trap gene that can disrupt constitutively active Gaq signaling in uveal melanoma cell lines. The goal of the research is to evaluate the therapeutic efficacy of this trap gene delivered by AAV in the treatment of cancer.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. The Committee also requested that the viral dose that would be injected intraocularly into the mice.

III-D, BSL-2, plasmids, AAV, Adenoviral vector, Lentiviral vectors, human cell lines, mice</td></tr>
<tr><td>81982</td><td></td><td>Generation of GP-CD276 mice</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The purpose of this experiment is to generate a mouse model that will allow inducible expression of the glycoprotein derived from LCMV and the mouse protein CD276.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided. The Committee also requested that information including the plasmid concentration and volume that will be injected mice as well as the how the mouse will be anesthetized be provided.

III-E, BSL-1, plasmids, mice</td></tr>
<tr><td>81722</td><td></td><td>Injection of RNAs into mice</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment to assess the therapeutic efficacy of our protein-based carriers for RNA delivery.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested more information about the carrier that will be used this experiment. The title of the protocol was changed to “Injection of anti-cancer RNAs and/or anti-luciferase RNA into mice using scFv-based protein carriers”.

III-F, BSL-1, small RNA, mice</td></tr>
<tr><td>81723</td><td></td><td>Injection of luciferase- or green fluorescent protein-expressing cells into the mammary fat pad of mice</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to test the anti-tumor efficacy of new drug delivery carriers in mice with tumors.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

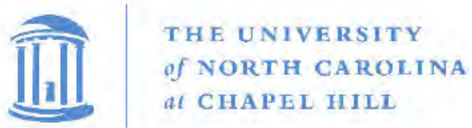
III-D, BSL-2, plasmids, mammalian cell lines, mice</td></tr>
<tr><td>81724</td><td></td><td>Injection of BSL-2 cells into the mammary fat pad of mice</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to test a new biochemical toolset for investigating exosomes in mice. The accomplish this, the lab will inject reporter human breast cancer cells into the mammary fat pad of mice and perform downstream analyses.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested more information on the human cell lines that would be used for the study and what reporters would they express. The title of the protocol was changed to “Injection of breast cancer cells into the mammary fat pad of mice for tracking exosomal RNA cargo”.</td></tr>
</table>

	III-D, BSL-2, plasmids, mammalian cell lines, mice	
81725	Juliane Nguyen	Cloning CMV vectors expressing fluorescent proteins, RNA sequences
APPROVED	**Summary:** The aim of this experiment is to create stable cell lines expressing fluorescent proteins and RNA sequences. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Lentiviral vectors, mammalian cell lines	
82042	Xiaomei Reckford	AAV gene therapy for treating neurogenetic diseases
APPROVED WITH STIPULATIONS	**Summary:** The goal of this experiment is to develop effective gene therapy to treat MPS IIIB. Potential gene therapeutics will be examined in human cell lines. This project is not related to a clinical trial nor will it create clinical trial grade products. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested Principal Investigator complete the NIH Guidelines training and provide confirmation that this work would only be performed in vitro. The title of the protocol was changed to "AAV gene therapy for treating neurogenetic diseases". III-D, BSL-2, plasmids, AAV, human cell lines	
81902	[redacted]	Use of AAV to express gene expression constructs and reporter genes in neurons and mice
APPROVED	**Summary:** The aim of this experiment is to use the adeno-associated virus to deliver reporter genes into primary mouse neuron cultures and mouse models. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, AAV, mouse cell lines, mice	
82502	[redacted]	Use of lentivirus in mice
APPROVED	**Summary:** The goal of this experiment is to use lentivirus to deliver guide RNAs into mouse models. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, Lentiviral vectors, mice	

1. **NIH Incident Report.** The Committee reviewed an incident in a BSL-2 laboratory involving potential exposure to recombinant murine parainfluenza virus obtained through a mouse bite, and the recommendations for mitigating risk of future reoccurrence.
2. **Sub-committee Approvals of Schedule G**: 2
   PI: [redacted] **Title:** Generation of a murine model that mimics a human genetic variant in Chadl with enhanced risk of osteoarthritis (III- E, ID 82062)
   PI: [redacted] **Title:** Use of AAV vector to identify cholinergic neuron transmission and fiber projections (III- D, ID 82363)
3. **Schedule H report:** 12
4. **Next IBC meeting date:** November 4, 2020 TBD

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
December 2, 2020 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Jennifer Hunter, Rachel Graham, Barbara Savoldo, Tori Baxter, Cathy Brennan, Garry Coulson, Amanda Craigen

Members Absent: Keith Porterfield, Xiao Xiao, Shawn Hingtgen, Craig Fletcher,

Ad hoc Members (not required to be present): Stan Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the November 4, 2020 meeting.**
2. **Clinical Trial:**
3. **Applications under review:**

ID	PI	Project Title
N/A		
APPROVED	**Summary:** **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-C	
83887		Design of Wild-Type, Rewired and Reporter Expressing Porcine Delta Coronavirus OH-FD22 Infectious Clones – 2020 renewal
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to study the molecular determinants of viral pathogenesis of porcine delta coronavirus using an infectious clone system of the virus. Recombinant clones may be modified to express fluorescent proteins for microscopic visualization or to introduce mutations into transcriptional networks to attenuate the virus for use in vaccine platforms. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested clarification on whether *in vivo* work is to be conducted at UNC, and if so, Section III should be completed. III-D, BSL-2, plasmids	

83888		Expression of Delta Coronavirus OH-FD22 Spike and Nucleocapsid Genes by Venezuelan Equine Encephalitis Replicon Particles - 2020 renewal
APPROVED	**Summary:** The aim of this experiment is to express spike and nucleocapsid genes from porcine delta coronavirus in a VEE replicon vector to generate replicon particles for vaccination of mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmid, mice	
84222		Introduction of mutations affecting nsp14 interactions with other replicase proteins in Betacoronaviruses
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to mutate residues in nsp14 in a number of coronaviruses to identify residues that are critical for interaction with replicase proteins. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested information on whether any of the residues for mutation are anticipated to affect activity of therapeutics targeting nsp14. The Committee requested that the protocol be amended to indicate that any enhanced replication in any of the recombinant strains be reported to the IBC. III-D, BSL-3, plasmids, mice	
84223		Expressing , SARS-CoV-2, MERS-CoV, HKU3, HKU4, HKU5, SHC014, WIV1, other Bat-CoV, Norovirus, and Dengue virus open reading frames in a Venezuelan Encephalitis Virus vector with a Kozak sequence and puromycin cassette
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express open reading frames genes from CoV's, Norovirus and Dengue virus in a VEE replicon vector to generate replicon particles for vaccination of mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested confirmation that ORF's will be expressed individually and not combined. III-D, BSL-2, plasmid, mice	
84224		Maintenance of bat coronavirus (Bat-SCoV) reporter viruses (HKU3 and HKU5) expressing reporter proteins - 2020 renewal
APPROVED	**Summary:** The aim of this renewal protocol is to maintain the use of recombinant bat coronaviruses for infection of bat cell lines to assess replication fitness of various recombinants in this system. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	
84482		Production of Multiple Resistance Marker Mouse Strain
APPROVED	**Summary:** The aim of this experiment is to produce mice expressing antibiotic resistance genes. Fibroblasts from the derived mice will be used as feeders for cell selection experiments. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	

83503		HIV-Luciferase Reporter Vectors to study HIV infection in humanized mice
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to monitor HIV infection in mice longitudinally in real time using HIV reporter vectors expressing luciferase protein supplied by a collaborator. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested clarification on the location and containment level for the IVIS imaging. III-D, BSL-2, lentiviral vector, mice	
83402	Amy Gladfelter	Phase-separation driven by WHI3 and mRNA structure
APPROVED	**Summary:** The aim of this experiment is to explore how Whi3 and mRNA interact to establish phase-separated droplets in vitro. Fungal Whi3 will be expressed in E. coli and A. gossypii (native host) for downstream analyses. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-F, BSL-1, plasmids	
83403	Amy Gladfelter	Role of septins in curvature sensing
APPROVED	**Summary:** The aim of this experiment is to determine how septins sense membrane curvature in fungi. Cell division cycle genes and other septin related genes from non-pathogenic fungi will be cloned into plasmids for expression in E. coli and non-pathogenic fungi. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-F, BSL-1, plasmids	
83405	Amy Gladfelter	FXR1-dependent localization of mRNA
APPROVED	**Summary:** The aim of this experiment is to determine how FXR1 functions in phase separation. Murine FXR1 will be cloned into plasmids and expressed in mammalian cells. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-F, BSL-1, plasmids	
83422	Amy Gladfelter	Phase separation of SARS CoV-2 RNA and nucleocapsid protein
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to determine study how SARS-CoV-2 nucleocapsid protein undergoes liquid-liquid phase separation with the viral genome. NP protein will be cloned into plasmids or lentiviral vectors for expression in E. coli or mammalian cells. Yeast artificial chromosomes (YAC) will be used for in vitro transcription of SARS-CoV genome. **Committee Comments:** The Committee requested verification that in vitro transcription will be via a cell-free system and will not be in mammalian cells. The Committee also requested confirmation that the YACs are used for maintenance of DNA plasmid of genome, and that no RNA is being transcribed in this system. Classification needs to be III-D and not III-F. III-D, BSL-2, plasmids, lentivirus	

<table>
<tr><td>84203</td><td>Tal Kafri</td><td>Improving biosafety of simple retroviral and lentiviral systems for research applications</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate retroviral and lentiviral vectors with enhanced safety profiles for use in retroviral research by pseudotyping the envelope protein.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

III-D, BSL-2, lentivirus, retrovirus</td></tr>
<tr><td>84205</td><td>Tal Kafri</td><td>Pseudotyping with SARS Spike</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to test the ability of simple retroviral and lentiviral vectors pseudotyped with SARS spike protein to transduce target cells in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested clarification on which virus.

III-D, BSL-2, lentivirus, retrovirus</td></tr>
<tr><td>84502</td><td>Samir Kelada</td><td>Use of Lentiviral shRNA or cDNA clones</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to express genes of interest, or shRNA's to genes of interest, in cells in vitro using lentiviral vectors.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Classification should be changed from III-E to III-D.

III-D, BSL-2, lentivirus</td></tr>
<tr><td>76742</td><td></td><td>AAV gene therapy for hemophilia with inhibitors</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to study the effect of different therapeutic transgene products delivered by AAV to correct hemophilic mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Committee requested that method of anesthesia in Section III be updated to reflect isoflurane and not manual restraint prior to administration.

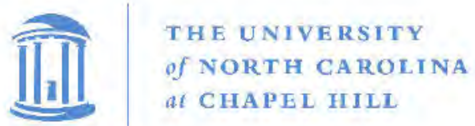
III-D, BSL-2, AAV, mice</td></tr>
<tr><td>83522</td><td></td><td>pTR-CBh-PDL1 and pTR-CBh-shRNA (amyloid precursor protein)</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to study the effect of PDL-1 or shRNA's to target gene of interest to improve symptoms in Downs syndrome and Alzheimers disease.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a clearer title reflecting the proposed work.

III-D, BSL2, AAV, mice</td></tr>
<tr><td>84023</td><td></td><td>The role of AP-1, Yap, and Stat3 in cardiac regeneration</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate transgenic zebrafish expressing wildtype, dominant negative, or constitutive active forms of genes of interest in cardiac fibroblasts or endothelial cells to examine their roles in cardiac regeneration.
Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.
III-D, BSL-1, plasmids, zebrafish</td></tr>
</table>

84462	Zachary Nimchuk	Developmental genetics in Lettuce
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to assess the consequences of gene mutation on plant development regulation. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested review by ad hoc plant specialist. III-E, BSL-1, plants	
84463	Zachary Nimchuk	Developmental genetics in Sunflower
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to assess the consequences of gene mutation on plant development regulation. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested review by ad hoc plant specialist. III-E, BSL-1, plants	
84582		Mospd3 Crispr Floxed Mouse
APPROVED	**Summary:** The aim of this experiment is to produce a mouse strain with floxed allele of Mospd3 using the Crispr/Cas9 system. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmid, mice	
84304		DMN activity and connectivity
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express fluorescent calcium sensors and opsins in mouse neurons using AAV vectors for transduction. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested that DMN be defined and Section III updated. III-D, BSL-1, AAV, mice	
84204		The Role of Central Peptides in Ethanol Consumption
APPROVED	**Summary:** The aim of this experiment is to express GFP fluorescent protein or neuropeptide Y in mouse brain using AAV vectors. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, AAV mice	

4. **NIH Incident Reports.** The Committee discussed an incident at BSL-2 involving a splash to exposed skin from a tube containing replication-deficient, self-inactivating lentiviral vector expressing reporter genes. The Committee also discussed an incident at BSL-3 in which a mouse infected with SARS-CoV-2 jumped out of a BSC, was quickly captured, and returned to the BSC.
5. **Sub-committee Approvals of Schedule G**: 0
6. **Schedule H report:** 19
7. **Next IBC meeting date:** January 13, 2021. Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
January 13, 2021 3:30 PM
Web-Conference Call

Members Present: Doug Cyr, Jennifer Hunter, Keith Porterfield, Rachel Graham, Barbara Savoldo, Tori Baxter, Cathy Brennan, Garry Coulson, Amanda Craigen

Members Absent: Xiao Xiao, Shawn Hingtgen, Craig Fletcher,

Ad hoc Members (not required to be present): Stan Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the December 2, 2020 meeting.**
2. **Applications under review:**

ID	PI	Project Title
85472		Generation of a canonical 3ClPro cleavage site at the nsp14-15 junction and combinations of the cleavage site and ExoN inactivation in group 2c Betacoronaviruses - 2021 renewal
APPROVED	**Summary:** The aim of this experiment is to introduce canonical 3ClPro cleavage site into group 2c betacoronaviruses to restore or improve cleavage at the nsp14-15 junction which may allow inactivation of the ExoN and potentially attenuate the viruses for use as vaccine candidates **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
85473		Synthesis of a Chinese variant of MERS-CoV (MERS-GD01) - 2021 renewal
APPROVED	**Summary:** The aim of this experiment is to maintain a recombinant MERS-like coronavirus to provide insights into the loci that may be important for emergence of MERS-like coronaviruses into human and other animal populations. The cDNA clone will be maintained as 7 separate cassettes in plasmids. Derived virus will be analyzed for growth in cell culture and in mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
85474	Ralph Baric	Generation of a cDNA infectious clone system for Zika virus – 2021 renewal
APPROVED	**Summary:** The aim of this renewal protocol is to maintain the use of a cDNA infectious clone system to characterize in vitro growth and pathogenesis traits and to map regions of the Zika virus particle important for antibody neutralization. The cDNA clone will be maintained as 4 separate cassettes in plasmids. Derived virus will be analyzed for growth in cell culture. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
84965		Developmental Patterning of the Sinoatrial Node
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to ectopically express genes of interest in primary cells from embryonic avian tissue.	

<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested clarification on the gene of interest.</td></tr>
<tr><td>85062</td><td></td><td>Pharmacokinetics and Pharmacodynamics of antibody-based therapy</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to evaluate antibody-target binding dynamics and antibody distribution in the local native environments (PK/PD). Nanoluc luciferase will be transduced into murine melanoma cancer cells and a murine breast cancer cell lines in vitro using commercial replication-defective lentiviral particles. Transduced cells will be injected into mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>84982</td><td></td><td>Testing live attenuated vaccines of SARS-like 2019-nCoV (Wuhan) Betacoronavirus</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to test hypothetical attenuated mutants of SARS-COV-2 in cell culture and mouse model, and if attenuation is confirmed, to test for the ability of these attenuated mutants to provide protective immunity against SARS-CoV-2.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested information on whether the attenuated mutants are hypothetically attenuated, or if there is some in vitro/in vivo data in support of their attenuation. The Committee also requested information on the location(s) of attenuating mutations and a risk assessment.</td></tr>
<tr><td>84882</td><td>Tal Kafri</td><td>Pseudotyping simple retroviral and lentiviral vectors</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to employ different envelop proteins to pseudotype retroviral and lentiviral vectors. Envelopes of interest will be cloned into viral vector plasmids which will be transfected into cells to generate viral vectors. Pseudotyped vectors will then be tested for their transduction efficiency in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>85662</td><td>Eduardo Lazarowski</td><td>Expression of recombinant mouse mucins in cultured cell lines</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate transient or stable cells lines expressing recombinant mucin using lentiviral or retroviral vectors to help understand the molecular basis of mucin biosynthesis and assembly in health and disease.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>85642</td><td></td><td>Regulation of smooth muscle differentiation</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to identify mechanism that regulate how smooth muscle cells grow, contract, differentiate and phenotypically modify. A variety of genes of interest including transcription factors, chromatin modifying enzymes, Rho signaling molecules and reporter genes will be cloned into plasmids or adenoviral vectors and expressed in cells. Additionally, transgenic mice will be created in which regulatory elements of interest will be deleted or mutated using CRISPR/Cas9.</td></tr>
</table>

<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>85751</td><td>Nathan Nicely</td><td>Generic use of recombinant nucleic acid plasmids for the purpose of recombinant protein expression</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this protocol is to cover the activities of the Protein Expression Core in which plasmids are used to express genes of interest in bacteria, mammalian cells and insect cell lines.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a statement on which toxic and/or dangerous genes/proteins would not be allowed to be expressed under this protocol (ie. biological toxins, genes from BSL-3 and select agents).</td></tr>
<tr><td>84822</td><td>Adam Palmer</td><td>Genome-wide screen for determinants of drug response</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to identify genes whose expression level affects the sensitivity of cancer cells to cancer therapies. Lentiviral vectors expressing a sgRNA library will be used to transduce mammalian cells in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested clarification on whether this a genome-wide screen or a targeted screen.</td></tr>
<tr><td>78362</td><td></td><td>Generation of Chimeric Antigen Modified T cells for anti-tumor Therapy</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to generate murine CAR-T cells using a retroviral vector and evaluate the persistence of these cells in mice and their anti-tumor activity.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee required the dose and volume for administration into the mice be provided.</td></tr>
<tr><td>84666</td><td></td><td>Generation of Chimeric Antigen Modified T cells for anti-tumor Therapy</td></tr>
<tr><td>DUPLICATE SUBMISSION</td><td colspan="2">Summary: This is a duplicate submission identical to 78362. Submission canceled.

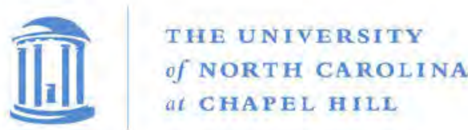
Committee Comments: N/A</td></tr>
<tr><td>85082</td><td></td><td>Batf2-Flox</td></tr>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to generate a mouse strain with a floxed Batf2 allele. CRISPR/Cas9 will be used to insert loxP sited flanking key exons of the Batf2 into mouse zygotes following microinjection.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a revised title.</td></tr>
<tr><td>84762</td><td></td><td>CBA-FIB-NPY, CBA-FIB-GFP and FIB-NPY[13-36]</td></tr>
</table>

APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to inject rAAV vectors expressing neuropeptide Y, fibronectin and GFP into site-specific areas of the mouse brain **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a revised title and definitions of acronyms in the title.	
85490	Robert Wirka	Sindbis glycoprotein to re-target lentivirus to diphtheria toxin receptor-expressing cells
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to pseudotype lentivirus with sindbis envelope glycoprotein containing the dtx receptor binding domain to assist in targeting these vectors to infect cells expressing dtx receptor. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested information on the receptor cells to verify if these were being made in the lab or purchased from a vendor.	
85002	Antonios Zannas	dCas9-guided DNA methylation editing in human fibroblasts
APPROVED	**Summary:** The aim of this experiment is to express glucocorticoid-regulated and senescence-related genes and reporter constructs in human cell lines using plasmids and lentiviral vectors. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	

1. **Sub-committee Approvals of Schedule G**: 2
   PI: Nigel Mackman **Title:** Lentiviral expression of luciferase (ID: 84682)
   PI: Kevin Slep **Title:** Functional analyses of microtubule regulators (ID: 84903)
2. **Schedule H report:** 13
3. **Next IBC meeting date:** February 3, 2021

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
February 13, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Keith Porterfield, Rachel Graham, Barbara Savoldo, Shawn Hingtgen, Tori Baxter, Catherine Brennan, Garry Coulson, Amanda Craigen

Members Absent: Xiao Xiao, Stanley Lemon, Ann Matthysse, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests: [redacted]

Open Meeting

1. **Review minutes from the January 13, 2021 meeting.**
2. **Clinical Trial:** [redacted]
3. **Applications under review**

	PI	Project Title
85962	[redacted]	SARS-2/Coronavirus expressing Spike variants from England and South African isolates
APPROVED WITH STIPULATIONS	**Summary:** The aim of this protocol is to create SARS-2 coronavirus viruses expressing spike glycoprotein variants similar to those from England and South African isolates to assess their capacity to be neutralized by current vaccines and to cause disease in animal models. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested confirmation that mutations that are unique to either the South African strain or England strain will not be mixed together in the same recombinant virus without IBC review.	
85963	[redacted]	Defining the role of genome-wide mutations on the SARS-CoV-2 B.1.1.7 variant
APPROVED	**Summary:** The aim of this experiment is to generate the SARS-CoV-2 B.1.1.7 variant to define mutations throughout the genome and evaluate their role on viral transmissibility or ability to evade immune response. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	

85964		SARS-2/Mink Coronavirus with and without nanoluciferase expression
APPROVED	**Summary:** The aim of this protocol is to create SARS-2/Coronavirus with select changes in the spike glycoprotein, which mimic those found in Danish mink farms, to test for its ability to be neutralized by current vaccines. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
85965		Use of a cDNA infectious clone system for Zika virus to examine antibody: virus interactions - 2021 renewal
APPROVED	**Summary:** The aim of this renewal protocol is to use a cDNA infectious clone system for Zika virus to examine antibody virus interactions and assess the role that particular structural antigenic patches on the virus play in the immune response to viral infection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
85542		The Role of Coronin 1B in Cell Motility
APPROVED WITH STIPULATION S	**Summary:** The aim of this protocol is to determine the role of Cronin proteins in the control of cell motility and cancer metastasis by cloning the insert gene or shRNA into a viral vector which will be utilized to transduce cells in vitro and eventually be used in vivo **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Complete Section III: Gene transfer experiment involving whole animals or plants.	
85902		Identification and Characterization of Alphavirus inhibitory compounds
APPROVED WITH STIPULATION S	**Summary:** The aim of this protocol is to evaluate chemical compounds for their inability to inhibit alphavirus replication and protect from alphavirus induced disease. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested confirmation that if a strain is created that increases replication or virulence the lab will cease the experiment and notify the IBC. The protocol was listed at BSL-2 and needs to be updated to BSL-3. III-D	
85903		Production and characterization of a full-length infectious clone of outbreak Caribbean chikungunya viruses (CHIKV).
APPROVED WITH STIPULATION S	**Summary:** The aim of this experiment is to create a full-length infectious clone of a Caribbean chikungunya virus (CHIKV) isolate and use it to identify viral determinants that regulate viral replication and virulence. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested confirmation that if a strain is created that increases replication or virulence the lab will cease the experiment and notify the IBC	
85904		Generation of a full-length infectious clone of the novel coronavirus (SHC014) including reporter viruses.

<table>
<tr><td>APPROVED WITH STIPULATIONS</td><td colspan="2">Summary: The aim of this experiment is to generate a reverse genetic system for the Group 2B Coronavirus SHC014 for use as a heterologous challenge virus for mice vaccinated against SARS-CoV-2 or other coronaviruses. Virus will be amplified in Vero cell to produce stocks and infect mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC protocol number be updated.</td></tr>
<tr><td>86062</td><td>Tal Kafri</td><td>VL30 Standard curve</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to generate a standard curve for quantifying the murine retroelement VL30 by qPCR.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>86242</td><td>Scott Magness</td><td>Defa5 human intestinal stem cell culture reporter line</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this protocol is to create a human model in which the Defa5 gene can be marked and expressed in human intestinal stem cells. Defa5 will be cloned into a plasmid which will be expressed in E. coli and mammalian cells.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>86243</td><td>[redacted]</td><td>LYZ1 mouse intestinal stem cell culture reporter line</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to create a mouse model in which the LYZI gene can be marked and expressed in mouse intestinal stem cells. LYZI will be cloned into a plasmid which will be transfected into mammalian cells in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>86462</td><td>Scott Magness</td><td>Controlling Sox9 levels in cultured intestinal stem cells</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to control the level of Sox9 expression in a human intestinal monolayer. This line contains constructs that will be cloned into plasmids and transfected into human cells in vitro.

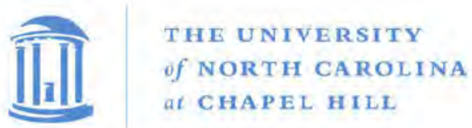
Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>86463</td><td>Scott Magness</td><td>Analyzing proliferation in intestinal cell cultures</td></tr>
<tr><td>APPROVED</td><td colspan="2">Summary: The aim of this experiment is to introduce the PIP-FUCCI dual color cell cycle reporter into mouse and human primary intestinal monolayers to track cell cycle phase changes in live, growing intestinal cells. PIP-FUCCI will be cloned into a plasmid which will then be transfected into mammalian cells in vitro.</td></tr>
</table>

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
86122	[redacted]	Functional evaluation of ploidy and driver dependent gene expression programs in cancer development and progression
APPROVED	**Summary:** The aim of this protocol is to The goal of this experiment is to (1) manipulate gene function to change tumor cell ploidy and determine how enforcing or constraining polyploidy alters tumor progression and tumor cell fate; (2) characterize clonal architecture in a ploidy specific fashion during mouse cancer progression; (3) determine how specific genes up and down regulated specifically following loss of specific tumor suppressors (e.g. p53) alter cancer progression. Short hairpins RNAs (shRNAs), single guide RNAs (sgRNAs), and neutral barcodes (unique molecular identifiers) will be cloned into retroviral and lentiviral vectors permitting constitutive and inducible expression and transduced into primary mouse pancreatic and liver cancer cell lines and organoids. Cells expressing the shRNAs and sgRNAs will be introduced orthotopically into the pancreas and liver of mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
85990	[redacted]	Serpina3n Crispr Floxed Mouse
APPROVED	**Summary:** The aim of this experiment is to produce a mouse strain with a floxed allele for Serpina3n. The Crispr/Cas9 system will be used to insert loxP sites flanking key exons of Serpina3n by embryo microinjection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
85156	[redacted]	Elucidation of pain and opioid mechanisms
TABLED	**Summary:** The aim of this protocol is to elucidate the cellular and molecular mechanisms of pain and opiods in order to create novel therapeautics to combat the opiod epidemic **Committee Comments:** Insufficient information regarding AAVs, cargos, and volumes to be injected. Term “various” was used in multiple sections.	
85922	[redacted]	AMC: Creation of Oprm1^FlpO line
APPROVED	**Summary:** The aim of this protocol is to produce an Oprm1^FlpO mouse line to study the mechanisms of action of drugs acting at the mu receptor encoding Oprm1 by inserting FlpO sequence following the Oprm1 gene. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	

4. Subcommittee approval of exempt recombinant DNA:
5. Schedule H report:

6. Next IBC meeting date: February 3, 2021.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
March 3, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Keith Porterfield, Rachel Graham, Barbara Savoldo, Tori Baxter, Catherine Brennan, Amanda Craigen

Members Absent: Xiao Xiao, Shawn Hingtgen, Stanley Lemon, Ann Matthysse, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests:

Open Meeting.

1. **Clinical Trial:**
    - The proposed containment and safety procedures are adequate for the experimental design and the clinical trial was approved.
2. **Inactivation Procedure Review:** BSL-3 facility for use at a lower containment.
    - The committee requested that the PI perform an additional validation .
3. Applications under review

	PI	Project Title
87042		Engineering syngeneic HNSCC cell lines for use in transplants of immune competent murine hosts
Approved	**Summary:** The aim of this protocol is to establish syngenic transplants of murine HNSCC cell lines to investigate the effects of modulating collagen stiffness and stability. The collagen modifying gene PLOD2 will be introduced to lentiviral vectors that enable inducible overexpression upon Doxycycline admisnistration. In addition, sgRNAs targeting PLOD2 will also be generated in lentiviral vectors that permit inducible repression of PLOD2. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
86823		Introduction of mutations conferring partial resistance to nucleoside analogues in Betacoronaviruses - 2021 renewal
Approved	**Summary:** The purpose of these experiments is to maintain constructs in which residues in nspl2 proteins have been mutated in the CoVs MERS-CoV, HKU3, HKUS, and other Betacoronaviruses (NOT including SARS-CoV-2) that may confer resistance to nucleoside analogues currently being tested for in vivo efficacy studies. The effects of these mutations will be evaluated in vitro in cell cultures and in vivo in mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the	

<table>
<tr><td></td><td colspan="2">experimental design.</td></tr>
<tr><td>86824</td><td>Ralph Baric</td><td>Creation of Dengue virus (DENV) reporter viruses in wildtype and envelope domain chimeric viruses (DENV2, DENV4, DENV4-2 EDI, DENV4-2 EDII) expressing reporter proteins</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to create a recombinant Dengue Virus expressing reporter proteins (i.e.,nanoluciferase (nLUC) tofacilitate measurement of Ab neutralization of live virus in cell lines in lab. The dengue virus infectious clones are maintained as 4 separate cDNA cassettes in plasmids that are propagated in E. coli. The mutations will be created in the relevant plasmid(s), and mutant virus cDNAs assembled in vitro, transcribed, and electroporated into C6/36 or Vero-81 cells to recover progeny virions. These DENV reporter viruses will be used in vitro to identify and describe antigenic footprint of neutralizing antibodies.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>86825</td><td>[redacted]</td><td>Construction of the SARS-CoV-2 exonuclease variant ExoN-F233L</td></tr>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: The objective of this experiment is to generate the ExoN-F233L variant to evaluate its replication, virulence, and mutation rate in vitro, in vivo, and in the presence of antibodies and therapeutics. The genomic fragments will be cloned into plasmids, which will be digested, ligated, and used as a template to generate full-length cDNA of the SARS-CoV-2

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that a risk assessment statement be made that If the recovered mutant demonstrates increased resistance to known nucleoside analogs that have been demonstrated to inhibit the parent coronavirus experiments will be paused, and the IBC will be notified.</td></tr>
<tr><td>86826</td><td>[redacted]</td><td>Adaptation of pangolin coronavirus strain GD1 to mice through serial passage</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The first purpose of this experiment is to adapt pangolin coronavirus (PgCoV) GD! so that it can infect standard laboratory mice, such as BALB/c and C57BL/6J, through serial in vivo lung passages. The aim is to development ofa pathogenic mouse model to evaluate broad-spectrum antivirals and therapeutics against SARS-2-related Co Vs, and to study the PgCo V pathogenicity in vivo.
The second purpose of this experiment is to identify mutations responding to PgCoV adapting to genetically diverse mice lines, i.e., serially passage the PgCo V in different lines of collaborative cross (CC) mice. The PgCoV has been recovered in laboratory according the existing schedule G # 80022. Since the lab has identified wild type PgCoV can replicate in mice, no additional mutation needs to be introduced into the virus before the serial passaging.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>86733</td><td>Jill Dowen</td><td>Genomics of gene regulation in development and disease</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this protocol is to study architectural proteins and their altered functions. pX330 and/or pcDNA will be used to transfect mouse and human Smcl, Smc3, Nipbl, CTCF, Stag 1, Stag2, Rad2 l, Rec8 into mouse embryonic stem cells V6.5, HEK, NIH3T3.

Committee Comments: The proposed containment and safety procedures are adequate for the</td></tr>
</table>

	experimental design.	
86562	Jonathan F Fay	Expression of recombinant proteins for structural studies
Approved	**Summary:** The aim of this experiment is to express recombinant proteins for biochemical analysis. Genes of interest (for example ABC,GPCR,SLC family of membrane proteins and membrane scaffolding proteins) will be cloned into a plasmid which will be transfected into mammalian or transformed into E.coli cells in vitro. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
86729	Jimena Giudice	Lentiviral Transduction of C2C12 and HeLa Cells with Tagged Isoforms
Approved	**Summary:** The aim of this protocol is to determine the function of muscle specific ya3 splice isoforms. The lab will tag each isoform and transduce C2Cl 2 or HeLa cells using lentivirus. Protein and RNA will be harvested from these cells to be used in downstream applications including (but not limited to) western blot, immunoprecipitation, and RNA sequencing. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
86962	Tal Kafri	Delivery of E2f7 gRNA
Approved	**Summary:** The aim of this experiment is to test the effects of knocking out E2f7 on human and mouse cells in culture. A DNA fragment encoding the U6 and the E2f7 will be cloned into lentiviral vectors either with or without the CRISPR CAS9 expression with or without selection markers including Blasticidin/GFP Puromycin/RFP Zeocin/RFP. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design.	
86623	[redacted]	Codon-optimized hepatoviruses
Approved With Stipulations	**Summary:** The aim of this protocol is to assess whether the atypical codon usage of human hepatoviruses is (1) required for replication of the viral genome in human cells, and efficient packaging of RNA into viral capsids, and (2) whether codon usage contributes to pathogenesis in a murine model of human hepatitis A. Existing infectious molecular clones of human hepatitis A virus, HMl 75-wt, HMl 75-p 16 (low pass cell culture-adapted), and Ml 75-1 Sf (high pass cell culture-adapted) will be modified by replacing sequence encoding the viral polyprotein with synthetic cDNA encoding the same polyprotein amino acid sequence but optimized for codon usage in mammalian cells. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the PI describe the restraint method for the intrahepatic injection was listed but not for the I.V. injection and fix formatting for volumes to be injected.	
86842	[redacted]	Use of DREADD receptors to stimulate astrocytes in the reward circuitry

<table>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: The aim of this experiment is to stimulate intracellular signaling in the astrocytes via virally-expressed DREADD receptors to test if it can reverse these cocaine-induced changes and inhibit drug seeking behavior. Plasmid and/or AA Vs will be obtained from Addgene. If necessary, AA V production will be performed at the UNC Viral Vector Core facility.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that PI supply IACUC protocol number.</td></tr>
<tr><td>86843</td><td></td><td>Assessment of astrocyte-neuron interactions with FRET</td></tr>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: The purpose will be to assess how cocaine use affects the interactions between neurons and astrocytes by employing Forster Resonance Energy Transfer (FRET) in acute brain slices in which one fluorophore is expressed on neuronal membranes, and one is expressed on astrocyte membranes, after cocaine use. Plasmids and/or AA Vs will be purchased from Addgene. Lab may also use GFAP-LckGFP as a negative control in astrocytes, as it should not generate a FRET signal with NAPA-N. Lab are already approved to use GFAP-LckGFP.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that PI supply IACUC protocol number.</td></tr>
<tr><td>86902</td><td></td><td>Fluorescent labeling of astrocyte mitochondria</td></tr>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: To express an AAV in rat brain which can fluorescently label astrocytes, via expression ofa tdtomato-tagged mitochrondrial protein under control ofan astrocyte-specific GFAP promoter. We will co-express a membrane-associated Lck-tdtomato, in order to visualize isolated astrocytes in which analysis mitochondria are found. These two AA Vs will be co-microinjected into rat brain, in order to determine effects of cocaine self-administration on astrocyte mitochondria. Both AAV plasmids, obtained from Josh Jackson at Drexel University, will be packaged into AAV at the UNC Viral Vector Core.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that PI supply IACUC protocol number.</td></tr>
<tr><td>87002</td><td></td><td>Neuronal tracing via local stereotaxic injection of AAVs into the mouse brain</td></tr>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: The aim of this protocol is to perform Neuronal tracing via local stereotaxic injection of AAVs into the mouse brain of transgenic or wild-type mice. Microliter volumes of AAV are injected stereotaxically (under general anesthesia) into the desired region of the mouse brain. These vectors, usually carrying a fluorescent marker, are designed to jump only one synapse at a time, allowing for precise tracing of neural networks.

Committee Comments: The proposed containment was adequate and safety procedures are adequate for the experimental design. The Committee requested that the PI provide further details of volumes of AAV's and injection site.</td></tr>
<tr><td>87022</td><td>Gregory Scherrer</td><td>Opioid receptor (OR) sensors</td></tr>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: The aim of this experiment is to create molecular constructs that enable subcellular localization of opioid receptors and similar molecules. The insert gene will be cloned into a plasmid and transformed into competent cells (E. coli, see below) in which the plasmid will be isolated and subsequently transfected into HEK293 cells.</td></tr>
</table>

<table>
<tr><td></td><td colspan="2">Committee Comments: The proposed containment was not adequate, the Committee requested changing from BSL-1 to BSL-2. Safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87023</td><td>Gregory Scherrer</td><td>Proteomic analysis of opioid receptors via proximity labeling</td></tr>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: The aim of this protocol is to examine subcellular opioid receptor interactions in a Cre-dependent manner. The insert gene will be cloned into a plasmid which will subsequently be transformed into competent cells (E. coli), then transfected into HEK293 cells.

Committee Comments: The proposed containment was not adequate, the Committee requested changing from BSL-1 to BSL-2. Safety procedures are adequate for the experimental design.</td></tr>
<tr><td>86042</td><td></td><td>CD11b-Cre</td></tr>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: The aim of this experiment is to produce a mouse strain that expresses Cre recombinase under the control of the CD 11 b promoter. The DNA construct will consist of the Cre gene downstream of the CD 11 b promoter. The mouse CD 11 b promoter will drive the expression of Cre recombinase.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the PI provide a plasmid/viral vector map. Change from to</td></tr>
<tr><td>86642</td><td>Lancce R Thurlow</td><td>Metabolism and virulence potential in MRSA</td></tr>
<tr><td>Approved With Stipulations</td><td colspan="2">Summary: The aim of this protocol is to determine how metabolism affects MRSA virulence factor expression. The insert gene will be cloned into a plasmid that will be electroporated into MRSA. The plasmid vector will integrated into the MRSA genome by recombination at the site of the gene on the plasmid. This will result in deletion of the chromosomal gene in MRSA.

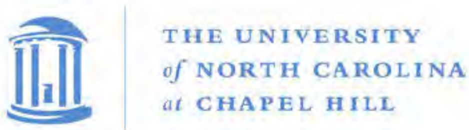
Committee Comments: The proposed containment was not adequate, the Committee requested changing from BSL-1 to BSL-2. Safety procedures are adequate for the experimental design.</td></tr>
<tr><td>86883</td><td>Yanping Zhang</td><td>ARF-MDM2-P53 Tumor Suppression Pathway</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to obtain expression of the foreign gene and the protein will be produced. The mutant p53 and MDM2 genes will be clone into the plasmid vectors and will be transfected to mammalian cells in vitro. Some of insert gene will be clone to viral vector and utilized to transduce cells in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87262</td><td></td><td>Transformation of Green or Red Fluorescent Protein into Mycobacterium abscessus</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to construct fluorescent M. abscessus to enable microscopy to visualize the Bacteria. The insert gene is clones into a plasmid that will be transformed into M.abscessus.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

4. Subcommittee approval: PI: [redacted] Title: CMV promoter followed by flox-stop-flox casset preceeding ZFP36L2-HA expression (ID: 86682)
5. Schedule H report: 34
6. Next IBC meeting date: April 7, 2021.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
April 7, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Keith Porterfield, Rachel Graham, Barbara Savoldo, Tori Baxter, Cathy Brennan, Amanda Craigen,

Members Absent: Xiao Xiao, Shawn Hingtgen, Stanley Lemon, Ann Matthysse, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the March 3, 2021 meeting.**
2. **Introduction:**
3. **Clinical Trial:**
    - The proposed containment and safety procedures are adequate for the experimental design.
4. **Clinical Trial:**
    - The proposed containment and safety procedures are adequate for the experimental design.
5. **Inactivation Procedure Review:** Inactivation of Coronavirus by Thermal Treatment (for non-select samples only
    - This inactivation validation was performed to verify
    - The proposed containment and safety procedures are adequate for the experimental design.
6. Applications under review:

	PI	Project Title
87602	Katherine Baldwin	shRNA knockdown of cell adhesion molecules in astrocytes
Approved	Summary: The aim of this experiment is to use pLKO.1 plasmid containing shRNA to produce lentivirus for the purposes of transducing rat primary astrocytes to knockdown cell adhesion molecules of interest. Specific cell adhesion molecules of interest. Specific cell adhesion molecules of interest are hepaCAM and PTPRZ1. Immunocytochemistry or biochemical experiments will be performed to assess the impact of loss of these gene functions on astrocyte development. pLKO.1 plasmid with shRNA driven by hU6 promoter will be cloned into viral vector which will be used to transduce cells in vitro. E.coli will be used to propagate DNA, HEK 293T cells will be used to produce lentivirus, primary rat and mouse cells will be transfected with plasmids or transduced with lentivirus.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
87603	Katherine Baldwin	Regulation of astrocyte development in vitro by PTPRZ1
Approved with Stipulations	Summary: The aim of this experiment is to investigate the function of PTPRZ1 in astrocyte development. Human PTPRZ1 cDNA under control of GFAP or CMV promoter will be cloned into a plasmid (pdDNA3.1). E.coli will be used to propagate recombinant DNA and rat primary astrocytes and HEK 293T cells will be transfected for experiments. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The committee requested that the proposed NIH Category listed as III-D be changed to III-F.	
87604	Katherine Baldwin	Regulation of astrocyte development in vitro by hepaCAM
Approved with Stipulations	Summary: The aim of this study is to study the molecular mechanisms through which hepaCAM controls the development of astrocyte branching complexity and astrocyte-astrocyte interactions. This involves expressing recombinant hepaCAM protein in primary rat astrocyte cultures and cell lines. The insert gene will be cloned into a plasmid which will be transfected into rat cells in vitro. Human HEPACAM cDNA under control of GFAP promotoer (for astrocyte expression) or CMV promoter (for cell line expression) will be cloned into a plasmid (pZac2.1plasmid and pcDNA3.1 plasmid). E.coli will be used to propagate recombinant DNA. Rat astrocytes and HEK 293T cells will be transfected. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
87665	[redacted]	Expressing Zika virus open reading frames in a Venezuelan Encephalitis Virus vector with a Kozak sequence and puromycin cassette - 2021 renewal
Approved	Summary: This aim of this renewal protocol is to generate Venezuelan Encephalisitis Virus vector with Kozak translation sequence and puromycin resistance replication deficient vector (VEE-pVR21-Kozak-puro). This vector is intended to enhance the expression of the genes of interest in the pVR21 expression cassette and to enable stable transfection of cell lines. Genetic elements of interest from Zika virus have been and will be clones into the vector. The constructs will then be used in vitro for protein/RNA element expression studies and polyclonal antibody generation in mice. E.coli are used to propaget the plasmid clones. VRP's are made in baby hamster kidney cells (BHK cells). VRP's will be used to vaccinate BSLB/c mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>87667</td><td></td><td>Expressing SARS-CoV-2, MERS-CoV, HKU3, HKU4, HKU5, SHC014, WIV1, other Bat-CoV, Norovirus, and Dengue virus open reading frames in a Venezuelan Encephalitis Virus vector with a Kozak sequence and puromycin cassette and vaccination of mice via microneedle delivery</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to express , SARS-CoV-2, MERS-CoV, HKU3, HKU4, HKU5, SHC014, WIV1, other Bat-CoV, Norovirus, and Dengue virus open reading frames in a Venezuelan Encephalitis Virus vector with a Kozak sequence and puromycin cassette (VEE-pVR21-Kozak-puro) and vaccination of mice via microneedle delivery

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The committee requested the amount of inoculum to be administered via microneedle patch.</td></tr>
<tr><td>87662</td><td></td><td>Ex379 Cyp21a1-I77T Mouse</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to generate a transgenic mouse strain with a point mutation in the Cyp21a1 gene. Cas9 protein, invitro transcribed guide RNA and synthetic oligonucleotide designed to introduce the desired mutation at the target site in the mouse genome will be introduced into fertilized C57BL/6 mouse embryos. The oligonucleotide is designed with a desired mutation flanked by 40-60 bp of wild-type sequence on each side. The embryos will be surgically implanted into the oviducts of pseudo pregnant recipient mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87664</td><td></td><td>Ex380 Cyp21a1 Conditional Knockout Mouse</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The purpose of this experiment is to generate a mouse strain with the loxP conditional knockout allele of the Cyp21a1 gene. Cas9 protein, in vitro transcribed guide RNAs and plasmid donor vector designed to insert the desired elements at the target sites will be introduced into fertilized C57BL/6 mouse embryos. The donor DNA construct consists of a 5’ homology arm, 5’ loxP site, one or more exons of the target gene, 3’ loxP site and 3’ homology arm in a high-copy plasmid backbone. Vector to be used is pUC. E.coli will be used to propagate the recombinant donor. The embryos will be surgically implanted into the oviducts of pseudo pregnant recipient mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

87668	████	Ex381 Rosa26-CAG-ERT2-Cre-ERT2 Mouse
Approved	Summary: The aim of this experiment is to do a targeted insertion in the mouse Rosa26 locus of a construct with the CAG promoter driving an ERT2-Cre-ERT2 fusion protein to be used for conditional gene deletion experiments. The transgene will be cloned into a plasmid with the pUC backbone that will be microinject4ed into C57BL/6 mouse fertilized embryos along with Cas9 protein and in vitro transcribed gRNA to promote insertion into proper site of the mouse genome. The injected embryos will be surgically implanted into the oviducts of pseudo pregnant recipient mice. E.coli will be used to propagate the recombinant DNA. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
87462	████	NIH3T3 cells into mice
Tabled	Summary: The aim of this experiment is to inject adult mice with mouse embryonic fibroblast cells (NIH3T3 cells) to produce tumors. These tumors will then be treated with drugs to measure drug effect on tumor growth. Committee Comments: The committee requested the principal investigator complete Section 3 for transferring cells or viruses modified with recombinant or synthetic nucleic acids into whole animals. The protocol will be reviewed by the committee again when complete.	
87582	████	Role of YY1 in castration resistant prostate cancer
Approved	Summary: The purpose of this experiment is to study the function of YY1 in prostate cancer tumorigenesis using TRAMP model. The insert gene will be cloned into a vector which will be utilized to transduce cells in vitro. The cell will be ultimately be injected into mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
87682	████	Cell line graft of carcinogen and genetically modified murine tumors
Approved With Stipulations	Summary: The aim of this experiment is to generate tumors in mice using syngeneic bladder cancer cells grown in vitro of disassociated tumors which will be implanted into the flank, bladder, kidney, lung, or liver. Committee Comments: The committee requested that the IACUC protocol associated with this Schedule G needs to be updated to ensure consistency of injection routes.	

<table>
<tr><td>87102</td><td>Scott Magness</td><td>Analyzing differentiation and proliferation in intestinal cell cultures</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to introduce red fluorescent protein into a marker for differentiated intestinal epithelial cells. An mCherry protein will be introduced into the 3’UTR of the human CHGA locus to allow for expression of a red fluorescent protein tag that marks differentiated enteroendocrine cells of the intestines.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87122</td><td>Scott Magness</td><td>Visualizing progenitor cells in intestinal cell cultures</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The purpose of this experiment is to introduce a fluorescent proten tag)GFP) at the Olfm4 locus in the cultured human intestinal epithelial cells to allow visual identification of proliferative progenitor (early transit-amplifying/stem) cells. IRES-GFP will be clones into a plasmid flanked by native sequence from the Olfm4 locus. This will be transfected along with Cas9:RNA complexes to insert the GFP sequence into the target locus by homologous recombination.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87123</td><td>Scott Magness</td><td>Visualizing enteroendocrine in intestinal cell cultures</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to introduce fluorescent protein tag (RFP) at the ChgA locus in cultured human intestinal epithelial cells to allow visual identification of enteroendocrine cells. IRES-RFP will be clones into a plasmid flanked by native sequence from the ChgA locus. This will be transfected along with Cas9:RNA complexes to insert the RFP sequence into the target locus by homologous recombination.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87124</td><td>Scott Magness</td><td>Visualizing stem cells in intestinal cell cultures</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The purpose of this experiment is to introduce a nuclear-localized fluorescent protein tag (GFP) at the Lgr5 locus in cultured human intestinal epithelial cells to allow visual identification of stem cells. IRES-NLS-GFP will be clones into a plasmid flanked by natigve sequence from the Lgr5 locus. This will be transfected along with Cas9:RNA complexes to insert the GFP sequence into the target locus by homologous recombination.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
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<table>
<tr><td>87125</td><td>Scott Magness</td><td>Visualizing goblet cells in intestinal cell cultures</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to introduce a fluorescent protein tag (BFP) at the Muc2 protein C-terminus in cultured human intestinal epithelial cells to allow visual identification of goblet cells. mTag-BFP2 will be clones into a plasmid flanked by native sequence from the Muc2 locus. This will be transfected along with Cas9:RNA to insert the BFP sequence into the target locus by homologous recombination.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87642</td><td>Scott Magness</td><td>Analyzing inflammatory signaling in intestinal cell cultures</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The purpose of this experiment is to introduce an NFkB response element fluorescent reporter into human primary intestinal monolayers to track MFkB activation in cultured intestinal stem cells as a marker for pro-inflammatory response. The synthetic insert gene will be clones into a plasmid which will then be transfected into mammalian cells in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87663</td><td></td><td>Manipulation of astrocyte cytoskeletal dynamics</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The purpose of this experiment is to use AAV to express HA-tagged Rpl22 under control of the astrocyte-specific promoter GfaABC1D, in order to immunoprecipitated translating mRNAs from astrocytes using RNA-Seq following cocaine self-administration. The amplified plasmid from Addgene will be packaged into AAV by the UNC Viral Vector Core and microinjectd into rat brain

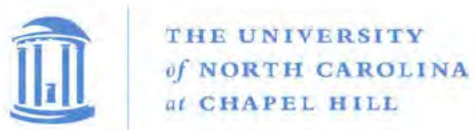
Committee Comments: The committee requested the principal investigator complete Section 3 for transferring cells or viruses modified with recombinant or synthetic nucleic acids into whole animals. The protocol will be reviewed by the committee again when complete.</td></tr>
<tr><td>87666</td><td></td><td>Ribotagging of astrocytes using ribosomal subunit Rpl22</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The aim of this experiment is to up or down regulate activity of cytoskeletal proteins Rac1 and Ezrin in astrocytes to determine the effects of manipulation of astrocyte cytoskeletal dynamics on cocaine seeking behaviors in rat. Insert genes in AAV plasmids will be amplified and packaged into AAV’s by the UNC Viral Vector Core for expression in rat brain.

Committee Comments: The committee requested the principal investigator complete Section 3 for transferring cells or viruses modified with recombinant or synthetic nucleic acids into whole animals. The protocol will be reviewed by the committee again when complete.</td></tr>
</table>

87242	Jonathan Serody	The role of spliceosome mutations in carcinogenesis and neoantigen production
Approved	Summary: The aim of this experiment is to investigate how common recurrent mutations in spliceosome gene affect RNA splicing in human tumors and lead to new tumor associated proteins and possibly neoantigens. CRISPR/Cas9 will be used to intoduce point mutations at specific spliceosome genes. The CRISPR/Cas9 plasmid vector will be constructed to have guide RNAs to target the site of the desired point mutation. Cancer cell lines with or without an HLA-A transgene will be transfected with CRISPR/Cas9 vector as well as double stranded DNA ultramer that will insert into the strand break area to introduce a point mutation. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
87962	John West	CAR.B7-H3 Chimeric T cells
Approved	Summary: The aim of this experiment is to generat CAR.B7-H3 expressing chimeric T cells by transfection of mRNA for use in downstream clinical studies. No DNA is inserted in human cells and there is no promoter involvement because the invitro transcribed mRNA is the product being delivered to the target T cells. The mRNA inserted in the cells is encoding a chimeric protein (chimeric antigen receptor) targeting human CD276 (B7-H3). Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

7. Subcommittee approval of exempt recombinant DNA:
8. Schedule H report: 33
9. Next IBC meeting date: May 5, 2021.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
May 5, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Keith Porterfield, Rachel Graham, Barbara Savoldo, Shawn Hingtgen, Tori Baxter,

Members Absent: Jennifer Hunter, Xiao Xiao, Stanley Lemon, Ann Matthysse, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests:

Open Meeting

1. **Presentation:** Studies involving pangolin coronavirus
2. **Clinical Trial:**
   - **Approved with Stipulations:** Must provide IRB number and verify PPE requirements on Appendix 10a
3. **Clinical Trial Amendment:**
   - **Approved dosage update**
4. **Inactivation Procedure Review:**
   -
   -
   -
   -
     - Validation data sufficient. The committee approved the protocol.
5. **IBC Protocol Review Procedure:** Ericka Pearce
6. **Applications under review:**

ID	PI	Project Title
88662		OVCAR3 and its derivatives (transcriptional enhancer knock outs) - Impact on ovarian cancer tumorigenesis.
Approved with Stipulations	Summary: The aim of this experiment is to inject OVCAR3 cell line and its derivatives (deletion of transcriptional enhancers of interest) to test if deletion of the enhancers impacts ovarian cancer tumor growth in mice. Committee Comments: The committee requested volume of inoculum. **The proposed containment and safety procedures are adequate for the experimental design.**	
88262	Peggy Cotter	Genetic analysis of Burkholderia thailandensis
Approved	Summary: The aim of this experiment is to use Burkkholderia thailandensis to study proteins that mediate interbacterial cooperation and competition between closely related bacteria. The goal is to construct strains that are defective, or altered, for their ability to compete or cooperate with each other.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
88263	Peggy Cotter	Genetic analysis of Bordetella bronchiseptica
Approved	Summary: The purpose of this experiment is to perform genetic analysis on Bordetella bronchiseptica to understand how filamentous hemagglutinin establish a persistent infection in the mammalian lower respiratory tract. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
88022	[redacted]	Evaluating the potential of rVSV vectors for SARS-CoV-2 vaccine development
Approved	Summary: The aim of this experiment is to evaluate the potential of recombinant vesicular stomatitis virus (rVSV) vectors for SARS-CoV-2 vaccine development. The goal is to evaluate vector replication, tropism, and antigen expression in mice implanted with human lung tissue (humanized mice). Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
88622	[redacted]	All-optical closed loop studies for next generation neural prostheses
Approved	Summary: The first goal of this experiment is to study brain circuits involved in sensory processing and motor learning. The second goal is to develop non-invasive interfaces with the brain via optical methods (two-photon imaging and optogenetics). This will interface with the cerebellum to recover motor functions lost in brain areas because of injury. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
88042	Jimena Giudice	Inhibition of gene expression using small interfering RNAs.
Approved	Summary: The aim of these experiments is to identify the physiological role of specific proteins (splicing factors, RNA binding proteins, trafficking and membrane dynamic proteins, cytoskeletal proteins, nuclear proteins, transcription factors, cytoplasmic proteins) in vitro. This will be done by inhibiting gene expression using small interfering RNAs. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
88043	[redacted]	Functional consequences of alternative splicing of clathrin light chain a (Clta), synaptosome associated protein 23kDa (Snap23), and thyroid hormone receptor interactor 10 (Trip10) trafficking genes using plasmids.
Approved	Summary: The purpose of these experiments is to identify the functional consequences of the Clta, Snap23, and Trip10 splicing isoforms in vivo and in vitro. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
88044	[redacted]	Redirection of endogenous alternative splicing using antisense oligonucleotides (morpholinos).
Approved	Summary: The aim of these experiments is to identify the functional consequences redirection of endogenous alternative splicing in vivo and in vitro using oligonucleotides (morpholinos)	

	Committee Comments: **The proposed containment and safety procedures are adequate for the experimental design.**	
87442	[redacted]	Usage of Lentivirus for cell transfection
Tabled	Summary:The aim of this experiment is to introduce reporter genes (RFP, GFP, luciferase) to human cell lines for monitoring and tracking the tumor cell growth in vitro and in mouse PDX model. Committee Comments: The committee requested project title be more descriptive and section 3 be completed.	
87923	[redacted]	Creation of HPV GEMM
Tabled	Summary: The aim of this experiment is to create HPV genetic engineered mouse models. Committee Comments: The committee requested information on gene/cargo to be used in experiment as well as inoculum amount for mice.	
88122	[redacted]	Spatiotemporal rescue of TCF4 in mice.
Tabled	Summary: The aim of this experiment is to drive GFP expression concomitant with Tcf4 expression in mice. Committee Comments: The committee requested a completed Section 3 Gene Transfer Experiments Involving Whole Animals of Plants Questionnaire.	
88123	[redacted]	Molecular Characterization of Tcf4 expression
Tabled	Summary: The aim of this experiment is to generate a spatial and time dependent re-expression of the mouse TC4 gene, as well as report TCF4 expression pattern with GFP in mice. Committee Comments: The committee requested a completed Section 3 Gene Transfer Experiments Involving Whole Animals of Plants Questionnaire.	
88064	[redacted]	Oncogenic Potential of EBV Latency and transforming genes
Approved	Summary: The purpose of this experiment is to determine the oncogenic potential of EBV and certain latent genes introduced by injection of cell lines into mice. Committee Comments: **The proposed containment and safety procedures are adequate for the experimental design.**	
88065	[redacted]	Oncogenic Potential of EBV latency and transforming genes in mice
Approved	Summary: The aim of this experiment is to determine the oncogenic potential of certain EBV latent genes and non coding RNA's Committee Comments: **The proposed containment and safety procedures are adequate for the experimental design.**	
88424	[redacted]	Functions of SAUR proteins
Approved with Stipulations	Summary: The aim of this experiment is to generate mutations in target genes in the model plant Arabidopsis thaliana. Committee Comments: The committee requested Section 3 Gene Transfer Experiments Involving Whole Animals of Plants Questionnaire be reviewed by Ad Hoc member AM (plant expert) whom was not present in meeting.	

87742	Koji Sode	Development of molecular recognition elements for biosensing technology
Subcommittee	Summary: The purpose of this experiment is to produce recombinant protein for the fabrication of molecular recognition elements for biosensing technology, which will be dedicated for the diagnosis of certain diseases such as metabolic disorders. Committee Comments: The committee requested that the NIH Category be changed from III-D to III-F and this protocol be reviewed by subcommittee since it is III-F.	
87802	Koji Sode	Development of molecular recognition elements for biomarker detection
Subcommittee	Summary: The aim of this experiment is to produce a recombinant protein for the fabrication of molecular recognition elements for biomarker detection, which will be dedicated for diagnosis and sensing of certain diseases such as mental health conditions. Committee Comments: The committee requested this protocol be reviewed by subcommittee since it is III-F	
88182	[redacted]	Site-directed infusions of Lck-tdTomato
Approved	Summary: The purpose of this experiment is to injected mice with pZax LckGFP virus in site directed infusions. The GFAP promoter allows for specific expression of the GFP in astrocytes, and the membrane tage allows for structural analysis of fine peripheral astrocytic processes. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
88183	[redacted]	Site-directed infusions of pZac LckGFP
Approved	Summary: The aim of this experiment is to inject mice with Lck-tdtomato virus in site-directed infusions in order to visualize isolated astrocytes in which analysis mitochondria are found. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
88462	[redacted]	Studying enterovirus pathogenesis in a model organism
Tabled	Summary: The purpose of this study is to understand the pathogenesis of enterovirus infections in mice to further study how host immunity modulates pathogenesis with a special focus on antibody responses. Committee Comments: The committee requested a completed Section 3 Gene Transfer Experiments Involving Whole Animals of Plants Questionnaire and to describe which part of the viral genome will be cloned into plasmids.	
88322	[redacted]	TRE-DKK1 transgenic mice
Tabled	Summary: The aim of this experiment is to create TRE-DKK1 transgenic mice. Committee Comments: This protocol is for Kannapolis which has their own IACUC but not IBC. Protocol states "pending review at NCRC IACUC committee". Need more details about the researcher and IACUC protocol	
87567	Daniel Corna	Functional genomics of the androgen receptor
Subcommittee	Summary: The aim of this experiment is to develop and validate a human cellular model that will idenift genetic markers that affect androgen receptor (AR) signaling in a variety of tumor types.	

<table>
<tr><td></td><td colspan="2">Committee Comments: The committee requested that the NIH Category be changed from III-D to III-F and this protocol be reviewed by subcommittee since it is III-F.</td></tr>
<tr><td>89142</td><td>[redacted]</td><td>Cellular immune dysfunction after burn injury</td></tr>
<tr><td>Approved via subcommittee 04/28/21</td><td colspan="2">Summary: The aim of this experiment is to generate Pseudomonas PAK mutant strains to infect mice after burn injury.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>89222</td><td>[redacted]</td><td>Neuronal tracing via local stereotaxic injection of G-deleted rabies into the mouse brain</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The purpose of this experiment is to use glycoprotein (G)-deleted rabies virus to determine the monosynaptic connection between brain regions of interest in mice.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>88542</td><td>[redacted]</td><td>Molecular Mechanisms of Inhibitory Circuit Development</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The aim of this experiment is to label proteins within the cortical neurons with fluorescent molecules to allow for confocal imaging.

Committee Comments: The committee requested a completed Section 3 Gene Transfer Experiments Involving Whole Animals of Plants Questionnaire.</td></tr>
<tr><td>86182</td><td>[redacted]</td><td>Regulation of Dendritic Spine Morphogenesis by NrCAM</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The aim of this experiment is to label proteins within the cortical neurons with Fluorescent molecules to allow for confocal imaging.

Committee Comments: The committee requested a completed Section 3 Gene Transfer Experiments Involving Whole Animals of Plants Questionnaire.</td></tr>
<tr><td colspan="3"><u>Previously Approved with Stipulations Updated</u></td></tr>
<tr><td>88948</td><td>[redacted]</td><td>NIH3T3 cells into mice_updated</td></tr>
<tr><td>Approved</td><td colspan="2">Update: Added Section 3

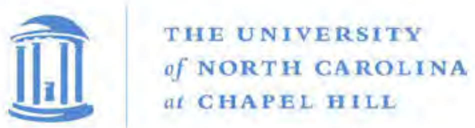
Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87666</td><td>[redacted]</td><td>Manipulation of astrocyte cytoskeletal dynamics</td></tr>
<tr><td>Approved</td><td colspan="2">Update: Added IACUC number and Section 3

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>87663</td><td>[redacted]</td><td>Ribotagging of astrocytes using ribosomal subunit Rpl22</td></tr>
<tr><td>Approved</td><td colspan="2">Update: Added IACUC number and Section 3</td></tr>
</table>

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
86902	██████	Fluorescent labeling of astrocyte mitochondria
Approved	Update: Added IACUC number and Section 3 Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
86843	██████	Assessment of astrocyte-neuron interactions with FRET
Approved	Update: Added IACUC number and Section 3 Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
86842	██████	Use of DREADD Receptors to stimulate astrocytes in the reward circuitry
Approved	Update: Added IACUC number and Section 3 Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
85903	████	Production and characterization of a full-length infectious clone of outbreak Caribbean chikungunya viruses
Approved	Update: Provided clarification on variants Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
85902	████	Identification and Characterization of Alphavirus inhibitory compounds
Approved	Update: Provided clarification on variants Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

7. Subcommittee approval: 1
8. Schedule H report: 32
9. Next IBC meeting date: June 2, 2021.

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
June 2, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Keith Porterfield, Rachel Graham, Barbara Savoldo, Amanda Craigen, Tori Baxter, Catherine Brennan, Jennifer Hunter; Shawn Hingtgen

Members Absent: Xiao Xiao, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon, Ann Matthysse

Guests:

Open Meeting

1. **Review minutes from the May 5, 2021 meeting.** Approved
2. **Clinical Trial:** Approved
3. **Applications under review:**

ID	PI	Project Title
N/A		
APROVED	**Summary:** **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-C,	
89522	-	Creation of HPV GEMM
APROVED	**Summary:** The aim of this experiment is to study the role of human papilloma virus (HPV) in cancer susceptibility using a genetically engineered mouse model (GEMM). Transgenic mice expressing the HPV genome will be created using the UNC Animal Models Core. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, viral vectors, mice	

89568		Molecular Characterization of Tcf4 expression
APROVED	**Summary:** The aim of this experiment is to develop a novel luciferase reporter mouse to visualize TCF4 expression in vivo. A new CRISPR/Cas9 system for transducing murine embryonic stem cells (ES) will be used. To create transgenics, cells will be either injected into mouse embryos or implanted in adult females. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
89602		Spatiotemporal rescue of TCF4 in mice.
APROVED	**Summary:** The aim of this experiment is to investigate the spatiotemporal expression of transcription factor 4 (Tcf4) in a novel GFP reporter mouse model. Transformed mouse ES cells will be injected into mouse embryos. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	
88462		Studying enterovirus pathogenesis in a model organism
APROVED	**Summary:** The aim of this experiment is to study how host-immune response modulates pathogenesis in a mouse model of enteroviral infection. RNA transcripts (enterovirus D68, A71) will be fluorescently tagged and transfected into human cell lines and used in the mouse infection model. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mice	
89863	Paul Armistead	Generation of cultured human cell lines that secrete HLA-E
APROVED	**Summary:** The aim of this experiment is to improve antigen detection methods for mass spectrometry. The experiment involves using a lentiviral vector to create stable cell lines which express HLA-E, a leukemia tumor antigen. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentiviral vector, plasmids	
88642		Characterization of the role of SARS-CoV-2 accessory proteins on viral pathogenesis and host cell immune response
APROVED	**Summary:** The aim of this experiment is to evaluate the roles of known 2019-nCoV accessory proteins in viral pathogenesis and host cellular responses to infection. SARS-CoV-2-MA10 strain will be used to implement ORF deletions and compared to the wildtype SARS-CoV-2-MA10 strain. Plasmids will be transfected into cells in vitro and then injected into mice.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mice	
89838		Generation and utilization of a reverse genetics-based viral replicon particle (VRP) system for Zika virus - 2021 renewal
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to study the course of Zika virus (ZIKV) in mice with viral replicon particles (VRP) created by replacing structural genes with fluorescent markers. Cell cultures of VRP will be used for downstream experiments (e.g., neutralization assays, in vivo imaging in mice). Safety tests will be performed to ensure no replication competent virus is present in the stocks. **Committee Comments:** The committee requested IV and intracranial volumes of innoculums. The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, mice	
89839		Characterization of infectious SARS-CoV-2 and SARS-CoV-2 MA10 clones containing the Brazilian variant (P.3) spike protein
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to investigate the potential effects that spike protein mutations have on infectivity and pathogenesis by comparing mutations in the SARS-CoV-2 Brazilian variant to the lab's wildtype SARS-CoV-2 infectious clone. Mutations will be introduced into pUC57 plasmids containing a nano luciferase cassette, and transfected cells will be injected into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee wanted to see language for a risk assessment added. III-D, BSL-3, plasmids, mice	
89466		Antibiotic tolerance in *Staphylococcus aureus* and *Pseudomonas aeruginosa*
APROVED	**Summary:** The purpose of this experiment is to determine mechanisms of antibiotic tolerance in *P. aeruginosa* and *S. aureus* using in vitro and in mouse infection models. Genes that contribute to antibiotic resistance will be identified by creating transposon libraries and cloned into replicative expression vectors. Bioluminescent and inducible GFP *S. aureus* will also be used in mouse infection models. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mice	
89182		Control vectors for studying the expression of human transferrin to enhance murine model of *N. gonorrhoeae* infection
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to investigate whether expression of recombinant human transferrin in mice can achieve local concentrations equivalent to what is seen in humans, and if that level of human transferrin prolongs *Neisseria gonorrhoeae* infection. Recombinant AAV expressing human transferrin and reporter genes (from UNC Viral Vector Core/VVC) will be administered to mice. Mice will be considered ABSL2 for 72 hours post inoculation.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. IACUC protocol number is expired and needs to be updated (New # is 21-024) III-D, BSL-2, AAV mice	
89627		Investigations of RAS oncogenes and downstream signaling activities for cancer treatment.
APROVEDWITH STIPULATION	**Summary:** The purpose of this experiment is to develop new cancer therapeutics by investigating the role of downstream effectors, affecting tumor growth, survival and motility. Various tumor cells will be transfected with KRAS and other oncogenes or knocked down by siRNA. CRISPR screening will be used in lentiviral vectors to identify to target genes. Tumor cells will then be either orthotopically implanted or administered subcutaneously into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The committee requested that gloves be added list of personal protective equipment in Section III question #5. III-D, BSL-2, lentiviral vectors, mice	
88946		Exploring Various Humanized Animal Models for the Establishment of Zika Virus Infection
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to establish Zika virus infection in a humanized mouse model. Virus will be obtained from lab (SchedG 25353). *E. coli* will be used to propagate plasmids containing cDNA cassettes, transfected into cells, and then injected into mice. Many routes and doses will be explored to characterize viral infection. If viral replication is observed in any of the models, other aspects of Zika virus infection will be studied. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested clarification on how the inoculum will be delivered and what concentrations will be used for each route. BSL2 enhanced precautions to include work in the BSL2+ space, work inside the biosafety cabinet, using sealed rotors or centrifuge safety cups and use of bite-resistant gloves while handling infected mice. III-D, BSL-2+, lentiviral vectors, mice	
88947		Evaluating the potential of rMV vectors for SARS-CoV-2 vaccine development
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to evaluate the potential for using measles vaccine strain (MV Schwarz) expressing a SARS-CoV-2 S-protein in vaccine development. SARS-CoV-2 spike protein will be cloned into the recombinant MV vector and injected into humanized mice. Vector replication, tropism, and antigen expression will be monitored and used to evaluate therapeutic potential. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee recommended BSL2 enhanced precautions to include work in the BSL2+ space, work inside the biosafety cabinet, using sealed rotors or centrifuge safety cups and use of bite-resistant gloves while handling infected mice. III-D, BSL-2+, rMV	

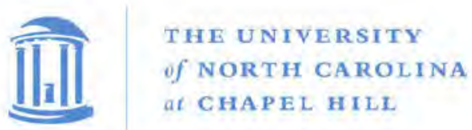
89242		Testing of vaccine efficacy against SARS-CoV2 and new viral variants
APROVED	**Summary:** The purpose of this experiment is to test the safety and efficacy of inactivated SARS-CoV-2 vaccines in a mouse adapted SARS-CoV-2 strain. Wild-type, mouse-adapted viruses expressing various fluorescent reporter genes will be tested. Risk Assessment: viral replication and virulence will be evaluated by 1) viral passage in the cell culture and tittering, and 2) weight loss, virus-induced inflammatory pathology, and viral loads. If any novel variance or enhance virulence (e.g., improved replication >1 log, enhanced disease) is observed the lab will cease the project and notify the IBC. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-3, plasmids, mice	
77182		Adenovirus delivery for uveal melanoma cell treatment
TABLED	**Summary:** The purpose of this experiment is to use replication defective adenoviral vectors to deliver therapeutic genes to melanoma cell lines and tumors. **Committee Comments:** Committee requested more information about study design (e.g., where the work will be done, clarification on the role of the company, and other details as needed). III-D, BSL-2, mice	
89265	Nathaniel Moorman	Lentiviral or retroviral mediated gene transfer into human cells
APROVED WITH STIPULATIONS	**Summary:** The purpose of the experiment is to transfer a variety of genes into human cells via replication-defective retro/lentiviruses. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested a more specific title that reflects the work being done. III-D, BSL-2, lentiviral vector	
89266	Nathaniel Moorman	Construction of mammalian expression vectors for host and viral proteins
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to identify new therapeutic targets or antivirals in human cytomegalovirus (HCMV) infection and host-pathogen interactions. Experiments will insert various HCMV genes into cells for protein expression. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested a more specific title that reflects the work being done. III-D, BSL-2, lentiviral vector, retroviral vector	

88842		Transduction of tumor cells via viral vector metastatic TNB models
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to study triple-negative breast cancer (TNBC) and determine efficacy of established conventional chemotherapy in combination with novel inhibitor drug regimens. Murine and patient-derived tumor cells will be transduced with mCherry using replication deficient lentiviral vectors. Tumor cells and xenografts will be used in the mouse model. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requests the volume of inoculum and number of cells be added to Section III-1. Updated protocol with information requested, new Schedule G ID number is 90162. III-D, BSL-2, viral vector	
89203		Delivery of sgRNA to cells using 3rd generation lentivirus delivery
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to perform CRISPR/Cas9 genome-wide screens on primary mouse neurons. A lentiviral CRISPR/Cas9 construct will be used to knock out and identify target genes of interest. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requests a more descriptive title and aim clarifying their genes of interest. III-D, BSL-2, lentivirus. CRISPR/Cas9, mice	
89204		gi-DREADD
APROVED WITH STIPULATIONS	**Summary:** The purpose of this experiment is to use Designed Receptors Exclusively Activated by Designer Drugs (DREADDs) to reversibly inhibit neurons in the primary visual cortex of mice. Visually evoked potentials will be measured in accordance with lab procedure. AAV expressing Gi-DREADD, will be administered by stereotaxic injection into the primary visual cortex and/or prefrontal cortex of mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee has requested that the title be revised to be more specific and that the vector dose is clarified in Section III-1. III-D, BSL-2, AAV, mice	
89642		Over expression of glycerol-3-phosphate acyltransferase 1 (GPAT1) in mouse hepatocytes impairs insulin signaling transduction and leads to hepatic insulin resistance
APROVED	**Summary:** The purpose of this experiment is to show the role of glycerol-3-phosphate acyltransferase 1 (GPAT1) over-expression in insulin resistance. Replication defective adenoviral vectors (UNC Core) expressing GPAT1 or EGFP will be used to transduce mouse hepatocytes. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids, mice	

89762		DMN activity and connectivity for Rats
APROVED	**Summary:** The purpose of this experiment is to study neuromodulation and brain connectivity within the default mode network (DMN) in an animal model. Rats will be intracranially injected with AAV encoding fluorescent calcium sensors and light activated modulatory proteins (opsins) to record and manipulate neuronal activity. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-1, BSL-1, AAV, rats	
88822		Gene transfer and therapy using recombinant defective Adenovirus Associated Vectors
TABLED	**Summary:** The purpose of this experiment is to identify potential new therapeutics in muscle and connective tissue disorders by using gene transfer in a mouse model. The aim is to use AAV vectors carrying fluorescently tagged target genes to measure therapeutic effects. **Committee Comments:** The committee requests clarification on the leadership of this protocol, as is not listed on the IACUC protocol, the protocol was submitted under Additionally, the IACUC protocol does not approve them for IM injections, and the volumes of the inoculum are too high and need to be revised. III-D, BSL-2, mice	
89542		Intramyocardial Injection of MSCs
TABLED	**Summary:** The purpose of this experiment is to inject murine bone marrow derived mesenchymal stem cells to the mouse heart. **Committee Comments:** The committee requests more information regarding the procedure Including a section III. III-D, BSL-1, mice	

4. **Sub-committee Approvals of Schedule G**: 6
   - **PI:** **Title:** Ex384 Canine Interleukin Mouse (III-E, ID: 8945)
   - **PI:** **Title:** Ex385 Canine Interleukin Receptor Mouse (III-E, ID: 89862)
   - **PI:** **Title:** Correction of Intron 22 Inversion in Hemophilia A dogs (III-E, ID: 90043)
   - **PI:** **Title:** BAC transgenesis of conditional humanized HERC2 over-expressor line (HERC2-roxOE) (III-E, ID: 89584)
   - **PI:** **Title:** Floxed ELOVL2-/- Knockout Mouse on a C57BL/6J background (III-E, ID: 87762)
   - **PI:** Paul Armistead **Title:** Generation of secretable HLA constructs for identifying antigens presented by leukemia cells (III-F, ID: 89802)
5. **Schedule H report:** 16
6. **Next IBC meeting date:** July 7, 2021

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
July 7, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Rachel Graham, Tori Baxter, Jennifer Hunter, Cathy Brennan

- **Members Absent:** Xiao Xiao, Craig Fletcher, Keith Porterfield, Barbara Savoldo, Shawn Hingtgen

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests:

Open Meeting

1. **Presentation:**
2. **Clinical Trial:**
   - ➢ **Approved**
   - ➢ **Approved**

3. **Applications under review:**

ID	PI	Project Title
n/a		
Approved	Summary: Committee Comments: The committee requested volume of inoculum. The proposed containment and safety procedures are adequate for the experimental design.	
n/a		
Approved	Summary:	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
92198	Maria Aleman	Modulation of iron metabolism-related genes in cells using lentiviral particles
Approved	Summary: The aim of this experiment is to modulate the expression of Poly C binding proteins and evaluate how it impacts cellular activity. Lentiviral vectors will be used to transduce human cell lines with insert genes or shRNA sequences. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
91322		Reverse Genetic Generation of Clade 3 Bat Sarbecoviruses
Approved with Stipulations	Summary: The aim of this experiment is to compare the pathogenesis and host tropism of different bat sarbecoviruses to SARS-CoV-2 using full length cDNA clones. Plasmids will be used to transmit bat CoV genome fragments to mice. Committee Comments: The committee requested mouse strain be added. The proposed containment and safety procedures are adequate for the experimental design.	
91323		SARS-CoV-2 viruses encoding the B.1.617.1 variant spike
Approved	Summary: The aim of this experiment is to express the B.1.617.1 variant spike in order to evaluate the role of this variant's mutations on viral transmissibility and immune responses. Plasmids will be transfected into cells, which may be used to infect mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
91324		SARS-CoV-2 viruses encoding the OVA CTL epitopes in accessory ORFs
Approved	Summary: The aim of this experiment is to generate SARS-CoV-2 viruses that express CTL epitopes in order to analyze antigen-specific T cell responses to SARS-CoV-2. Virus will be cultured in primary lung cells and may be used to infect mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
91342		Use of a cDNA infectious clone system for Zika virus to examine 5¿ untranslated region RNA structure mutants ¿ 2021 renewal
Approved	Summary: The aim of this experiment is to assess how mutations in the conserved regions of the 5' untranslated region of Zika virus affect viral traits (i.e., pathogenesis, immune response). Mice will be inoculated with virus via various injection sites. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
90122	Jeanette Cook	Cell cycle dynamics that ensure genome maintenance
Approved	Summary: The purpose of this project is to monitor how the different genes impact cell cycle progression and DNA replication. Selected genes will vary between experiments depending on the hypothesis. Plasmids or self-inactivating retroviruses will be used to transfect cells.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
90302		Targeting immune checkpoints and complement regulatory proteins as potential breast cancer therapy
Approved with Stipulations	Summary: The aim of this experiment is to determine whether modulating complement regulatory proteins causes triple negative breast cancer cells to become more sensitive to therapeutic intervention. CRISPRCas9 will be used to target the genes of interest in cancer cell cultures. Transformed cells will be injected into mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Committee requested that the volume of inoculum be added to Section III.	
92158		Overexpression of collateral genes via AAV injection into mouse p0-p1 pups
Approved	Summary: The aim of this experiment is to evaluate how overexpressing collateral genes in mouse pups changes the collateral number in postnatal mice. AAV constructs containing the genes of interest will be injected directly into mouse pups. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
92614	Stephanie Gupton	Generation of Trim9 ko mouse melanoma line
Approved	Summary: The aim of this experiment is to assess how deleting TRIM9 from B16 cells affects cytoskeletal regulation and motility of melanoma. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
90382		Gene Augmentation for Autosomal Dominant Retinitis Pigmentosa Using Rhodopsin Genomic Loci Nanoparticles
Approved	Summary: The aim of this experiment is to develop a novel gene therapy for retinitis pigmentosa using a nanoparticle-mediated, full-length rhodopsin gene from the genomic locus. Commercial plasmids containing the insert gene will be conjugated to nanoparticles and injected into mice. Committee Comments: The committee requested information on gene/cargo to be used in experiment as well as inoculum amount for mice.	
90703		testing vaccine efficacy against SARS-CoV2 Variants
Approved	Summary: The aim of this experiment is to evaluate how effective vaccine induced antibodies are at neutralizing SARS-CoV2 variants. Experiments will be performed in vitro. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
90702		Use of molecular clone derived viruses of the pangolin SARS-2 like coronavirus and reporter viruses
Approved	Summary: The aim of this experiment is to perform neutralization and antibody cross reactivity assays on cells infected with molecular clone derived pangolin SARS2-like coronaviruses. Committee Comments: The committee requested a completed Section 3 Gene Transfer Experiments Involving Whole Animals of Plants Questionnaire.	

91102		Generation of a full-length infectious clone of the novel coronavirus (WIV-1) including reporter viruses
Approved	Summary: The aim of this experiment is to test vaccine-induced neutralizing antibodies against Group 2B Coronavirus WIV-1 and viruses expressing reporter genes. No additional recombinant DNA manipulations will occur in the Heise lab. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
91302		Development of mWnt lung GECI tumor cells
Approved with Stipulations	Summary: The aim of this experiment is to evaluate the impact of obesity on a metMwnt lung induced with genetically encoded calcium indicator (GECI) cells. Cells will be transduced with a lentiviral vector before being injected into mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
90322	Ilona Jaspers	Evaluation of SERPINEB3 function in human airway epithelial cells
Approved	Summary: The aim of this experiment is to study the function of SERPINEB3 in human airway epithelial cell lines. A wild-type and mutated version of SERPINEB3 will be transduced into cells via lentiviral vectors. Viral particles will be generated at the Lenti-shRNA Core Facility. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The committee elevated this protocol form III-F to based on the use of lentiviral vectors.	
92199		Characterization of mRNA and pDNA based polyplexes in vivo using adjuvants as gene delivery enhancers
Approved	Summary: The aim of this experiment is to study the effect that the adjuvant pluronic 85 has on gene delivery by mRNA-lipid nanoparticles, mRNA-based polyplexes, and pDNA-based polyplexes. Luciferase-encoding mRNA and pDNA will be injected into mice and gene expression will be monitored in macrophages and dendritic cells. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
91838		pTR-TTR-FIXa for AAV Gene Therapy for Hemophilia
Approved with Stipulations	Summary: The aim of this experiment is to study the immune response to AAV gene therapy in hemophilia dog and mouse models. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Committee requested that the IACUC protocol number be provided for both dogs and mice. Committee requested that inoculum volume for dogs be reported in mL/kg (instead of vg/kg in 1-100mL). Committee also wanted clarification on the restraint methods and safety precautions designated for each animal.	
91242		Targeted mutagenesis and transgenesis in zebrafish to study specific gene functions
Approved	Summary: The aim of this experiment is to use targeted mutations, reporter genes, transgenes with specific zebrafish genes, and/or orthologous genes from other species to identify genes essential to the development and activities of innate immune cells, elucidate their functions, and document their interactions with the brain and other organ systems. All transgenic or mutagenic manipulations will be conducted in zebrafish embryos. Zebrafish genes or their orthologs may be introduced into mammalian cells in vitro as an additional method to assess proteins.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
90402		Dietary Agents that Affect Development
Approved	Summary: The aim of this experiment is to test how altering the expression of select ribosomal genes affects development in fetal alcohol syndrome models. Genetically modified zebrafish will be produced with CRISPR, and gene expression will be further modulated with morpholinos. Additionally, retinol binding protein 4 deficient mice will be exposed to ethanol on different diets and monitored for developmental sensitivities. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
91799	Jason Stein	Understanding genetic variants responsible for changes in neurodevelopmental phenotypes using CRISPR-dCas9-KRAB or VP64 domains
Approved	Summary: The aim of this experiment is to investigate how genetic variations affect neurological development, identify which variants are responsible for specific neurodevelopmental phenotypes. Once identified, the genetic variants will be tested with CRISPR constructs. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
91162		Infection of gene knockout mice by dengue viruses
Approved with Stipulations	Summary: The aim of this experiment is to test how engineered vaccine formulations (nanoparticle- and microneedle-based) perform against dengue virus infections. Immunodeficient mice will be injected with virus and monitored for disease progression. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. Committee requested the following statement be removed from Section II Question 5: "No recombinant or synthetic nucleic acids will be injected in mice."	
92400		Testing innate immune factors against orthotopic cancers when combined with targeted CAR-T immunotherapy
Approved	Summary: The aim of this experiment is to investigate how innate immune factors, in combination with chemotherapy and chimeric antigen receptor on T Cells (CAR-T), function against orthotopic cancers. Murine cancer cells will be generated with retroviral vectors and injected into mice. CAR-T cells will be generated and administered with a similar method. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

4. Subcommittee approval: 10 (2 Clinical Trial amendments, 8 Schedule G Submissions)
5. Schedule H report: 22
6. Next IBC meeting date: August 4, 2021.

Adjourn.

Meeting Minutes
August 4, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Keith Porterfield, Rachel Graham, Tori Baxter, Cathy Brennan, Amanda Craigen

Members Absent: Jennifer Hunter, Barbara Savoldo, Xiao Xiao, Shawn Hingtgen, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests: [redacted]

Open Meeting

1. **Review minutes from the July 7, 2021 meeting.**
2. **Applications under review:**

ID	PI	Project Title
93474	Paul Armistead	Generation of human leukemia cell lines expressing firefly luciferase with and without TP53 mutations
Approved	Summary: The aim of this experiment is to create human leukemia cell lines that express firefly luciferase for use in T-cell mediated cytotoxicity assays as a way to quantify cell death. A lentiviral CRISPR/Cas9 system will be used to target the TP53 gene and cells will be monitored for luciferase activity. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
93894	[redacted]	Coronaviruses that allow for antiviral activity evaluations of inhibitors to the nsp12 protein - 2021 renewal
Approved	Summary: The aim of this experiment is to evaluate antiviral activity against nsp12 RNA-depending RNA polymerase in a virulent MERS-CoV mouse model using a recombinant virus strain (developed by the lab). Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
93896	[redacted]	Incorporating attenuating mutations in the MERS-CoV mouse-adapted infectious clone to determine pathogenicity - 2021 renewal
Approved	Summary: The aim of this experiment is to evaluate how mutations in nonstructural and/or spike proteins of a mouse-adapted strain of MERS-CoV impact pathogenesis. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>93897</td><td></td><td>Mouse adaption of WIV1-CoV - 2021 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to evaluate how mutating Y436H in a mouse-adapted strain of WIV1-CoV affects binding affinity to human ACE2 receptors versus murine ACE2 receptors.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>93898</td><td></td><td>Assessment of the pathogenic effects of the SARS-CoV-2 B.1.617.2 spike protein</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to introduce mutations into the spike protein of the B.1.627.2 variant of SARS-CoV-2 and assess the effect(s) on pathogenesis and infectivity.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>93974</td><td>Ralph Baric</td><td>Use of the Dengue cDNA infectious clone system to study Zika epitopes</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to assess the antigenic and functional variability of ZIKA structural proteins prM and E by transplanting epitopes from infectious Zika clones into Dengue infectious clones and performing immunogenicity assays.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>94410</td><td></td><td>Interferon Regulatory Factor 7 Antisense - effects of brain IRF7 knockdown with siRNA on alcohol consumption</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to evaluate how decreased interferon regulatory factor 7 (IRF7) expression impacts alcohol consumption. IRF7 expression will be modulated with siRNA.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>93554</td><td>Miriam Braunstein</td><td>Utilization of BSL-2 Mycobacterium tuberculosis strains</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to study how protein secretion and localization changes when the satS gene is deleted. Experiments will be performed using two mutant strains of Mycobacterium tuberculosis.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The committee cannot finalize approval until notification is sent and recognized by the NIH's Office of Science Policy for containment lowering for work with the mutant M. tuberculosis strains.</td></tr>
</table>

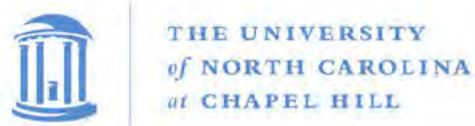
94349	[redacted]	Characterization of systemic immunodynamics in metastatic disease
Approved	Summary: The aim of this experiment is to create a mathematical model to describe immunodynamics of metastatic breast cancer during anti-PD-1 therapy. Data will be obtained from bilateral orthoptic murine models with the intention of extrapolating to human disease. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94331	[redacted]	Optogenetic (Channel- and Halo-rhodopsin; ChR2 & NpHR) and Chemogenetic (designer receptors exclusively activated by designer drugs DREADDS) Manipulation of Neural Circuits in High Fat Intake
Approved	Summary: The aim of this experiment is to characterize circuit-based interactions resulting in overeating. AAVs encoding ChR2, NpHR, or DREADDS will be injected into specific brain regions of mice. Committee Comments: The committee requested information on gene/cargo to be used in experiment as well as inoculum amount for mice.	
94318	Klaus Hahn	Design of RhoGEF biosensors
Approved	Summary: The aim of this experiment is to obtain more information regarding differences in protein distribution between normal cells and cancer cells by designing biosensors that can affirm spatiotemporal activation patterns of RhoGEFs and Rho GTPases. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94319	Klaus Hahn	Elucidating Mechanism of GTPase Regulation of the Cytoskeleton in Hematopoietic Cells
Approved	Summary: The aim of this experiment is to study how certain abnormalities in blood cells cause disease by elucidating the mechanism through which Rho-family GTPase regulate cytoskeletal changes. Cells will be transduced with AAV, then used for imaging and biochemical studies. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94320	Klaus Hahn	Designing a General Method for Creating Biosensors for Autoinhibitory Proteins
Approved with Stipulations	Summary: The aim of this project is to develop a method for designing auto-inhibitory protein biosensors to monitor activation sites. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The committee determined that this protocol falls under III-F because it does not include cell work and requests that it be recategorized prior to final approval.	

94469		Using AAVs to elucidate the neural components of goal-directed movement/dexterity
Approved with Stipulations	Summary: The aim of this experiment is to elucidate the neural pathways underlying reward-based movement in mouse models. AAVs will be used to manipulate cells in the motor circuits of the brain and/or excite neurons with protein expression. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. However, the original IACUC protocol, 20-026, was withdrawn and the current protocol number is 21-202. IACUC protocol number 21-202 only described work with a G-deleted rabies virus. The committee requires that IACUC protocol # 21-202 be appended to include the work with AAVs.	
94470		Using Rabies to elucidate the neural anatomical circuits of goal-directed movement/dexterity
Approved with Stipulations	Summary: The aim of this experiment is to elucidate the neural pathways underlying reward-based movement by using a G-deleted rabies virus to map cell-specific circuits in the brain and spinal cord. Committee Comments: The committee requested verification that all work leading up to vector injection is performed in a biosafety cabinet and only stereotaxic injection occurs on the benchtop, and that all PPE specified will be used during the surgery.	
93914		Usage of Lentivirus for cell transfection for tumor cell growth
Approved with Stipulations	Summary: The aim of this experiment is to monitor tumor cell growth with reporter genes. Committee Comments: The committee requested that their intramuscular injection volume be amended from 40uL to the approved maximum for that route of 25uL. The committee also requested that the method for anesthetizing the mice before intramuscular injection be added. Additionally, the IACUC protocol number, 22-028, was mistyped and the actual protocol number is 20-228.	
93954	Mark Heise	Analysis of RNA structural determinants for their impact on chikungunya virus replication
Approved	Summary: The aim of this experiment is to characterize how mutations in key stem loop regions affect RNA secondary structure and how the resultant structural changes impact viral replication in cell culture. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94389		Modulation of Sfrp4 and Pthlh in murine and human models of colon cancer
Approved	Summary: The aim of this experiment is to assess how colon cancer growth patterns change in response to expression modulation of Sfrp4 and Pthlh, key regulators of epithelial to mesenchymal transition. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

92244	[redacted]	Lentiviral delivery of Cre and guide RNAs to mice
Approved	Summary: The aim of this experiment is to elucidate the role of certain genes in liver disease and liver cancer through targeted gene knockout guided by a library of epigenetic regulators. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94369	[redacted]	Infusing AAV-DJ-hSyn-fDIO hMD3Gs, hMD3Gq, hMD4Gi-mCherry DREADDs into mouse brains to study neuronal projection
Approved	Summary: The aim of this experiment is to introduce a reporter virus into specific mouse brain regions to gather data on neuronal projection. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94370	[redacted]	Infusion of Cre-Dependent Retrograde Reporters, Retro Cre-Dep Flp, Flp-dependent vectors into mouse brains for study of brain regions and alcohol consumption
Approved	Summary: The aim of this experiment is to study the role of neuronal connections in modulating alcohol consumption in rodent models by infusing reporter genes and DREADDS to specific brain regions. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94429	[redacted]	Optogenetic (Channel- and Halo-rhodopsin; ChR2 & NpHR) Manipulation of Neural Circuits underlying social impairments in mouse models for ASD
Approved	Summary: The aim of this experiment is to study the connection between neuronal circuits, serotonergic modulation, and behavior in mice by using AAVs to facilitate optogenetic manipulation. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

3. **Subcommittee approval**: 4
    - Schedule G Submissions: 4
4. **Schedule H report**: 30
5. **Next IBC meeting date**: September 1, 2021.

Adjourn.



Meeting Minutes
September 1, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Rachel Graham, Barbara Savoldo, Shawn Hingtgen, Cathy Brennan, Amanda Craigen, Ilana Galex, Ericka Pearce, Rachael Turner

Members Absent: Keith Porterfield, Xiao Xiao, Craig Fletcher, Tori Baxter,

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from the August 4, 2021 meeting.**
2. **Applications under review:**

<table>
<tr><th>ID</th><th>PI</th><th>Project Title</th></tr>
<tr><td>95009</td><td>Ralph Baric</td><td>Incorporation of Luciferase into our Dengue Virus Molecular Clone Platform</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to investigate the antigenic variability of structural proteins and the functional variability of non-structural proteins in Dengue Virus (DENV). Recombinant DENV virus will be selected based on the presence of the luciferase gene. Cell cultures and binding assays will be used to characterize mutants. If selected, mutants will be tested for antigenicity, pathogenicity, attenuation, and epitope specificity.

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>95011</td><td></td><td>Assessment of the pathogenic effects of the SARS-CoV-2 C.37 spike protein</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to evaluate how spike protein mutations in the SARS-CoV-2 variant C.37 alter pathogenesis, antigenicity, sensitivity to therapeutics, and infectivity. In vitro and in vivo experiments will be performed with two types of infectious clones. Intranasal inoculation will be used to infect mice.

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design. The committee requested verification that the code #&956;L was analogous to microliters.</td></tr>
<tr><td>95029</td><td>Ralph Baric</td><td>Overexpression of the protease TMPRSS2 in LLCMK2 cells - 2021 renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to create and maintain LLCMK2 cells that overexpress the transmembrane protease TMPRSS2. Cells will be transfected with plasmids containing the human TMPRSS2 gene and selected based on the presence of a G418 resistance marker.

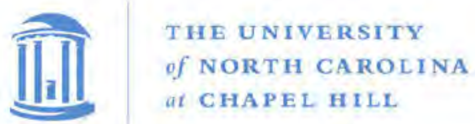
<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

94869		Optimizing antibody-based therapies through a system platform of integrating pharmacokinetics-pharmacodynamics-immunodynamics
Approved	Summary: The aim of this experiment is to characterize the pharmacokinetic and pharmacodynamic profiles of antibody-based therapies, as well as investigate the mechanism of antibody-dependent cellular cytotoxicity. Luciferase will be introduced into cells to facilitate analysis. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94909		Developing CAR-T cell therapy for medulloblastoma using endogenous mouse tumor models and single-cell transcriptomic analysis
Approved with Stipulations	Summary: The aim of this experiment is to study how T cells containing chimeric antigen receptors interact with brain tumors to direct CAR-T cell therapy design. Committee Comments: The committee requested that intracranial injection volume be added to Section III, and wanted verification that the code #&956;L was analogous to microliters. The committee found all other proposed containment and safety procedures to be adequate for the experimental design.	
95089		Use of CRISPR/Cas9 vector to knock in genes in mouse cell line for in vivo testing.
Approved	Summary: The aim of this experiment is to verify that plasmids used to knock-in genes can restore gene function, drug resistance traits, and tumor development in opposing knock-out cultures. CRISPR/Cas9 will be used to introduce the genes of interest into mouse tumor cells, and transfected cells will be injected into mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94271	Jennifer Webster-Cyriaqu	Production of stable cells to analyze the Epstein-Barr virus (EBV) promoter of the BRLF1 and BZLF1 genes
Approved	Summary: The aim of this experiment is to culture stable cells containing the BRLF1 or BZLF1 promoter/reporter construct. Transduced cells will be used to study the mechanism behind promoter activation of the BRLF1 and BRLZF1 genes focusing on the relationship between cellular levels of gene product and the EBV lytic reactivation. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94989		Retroviral transduction of mouse T cells to elucidate the role of specific genes in T cell differentiation
Approved	Summary: The aim of this experiment is to elucidate how certain immune response genes response alter T cell differentiation and/or function. Retroviral vectors containing the genes of interest (mutant or wild-type alleles) will be used to transduce cells, and selected cells will either be used for flow cytometry or injected into LCMV-infected mice for further observation. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

94950	[redacted]	Systematic in vivo characterization of disease-associated regulatory variants
Approved	Summary: The aim of this experiment is to investigate how genetic variation impacts gene regulation in humans by using a massively parallel reporter assay (MPRA). AAV-MPRA libraries will be used to select genes for transfer into mice. Vectors will contain a 20 basepair barcode for identification. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
95289	Douglas Phanstiel	Identifying Alzheimer's Disease Causal Variants Using MPRA
Approved	Summary: The aim of this experiment is to study how non-coding genetic variants associated with either increased or decreased risk of Alzheimer's Disease impact gene regulation. Data from initial experiments will be used to develop complete list of target genes. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
95369	[redacted]	Splice switching oligonucleotides aerosolized
Approved	Summary: The aim of this experiment is to investigate the potential for antisense oligonucleotides to prevent abnormal mRNA splicing patterns associated with human disease. Oligonucleotides will be administered to human cells, mouse cells, and live mice via aerosolization. The oligonucleotides used will be commercially synthesized, non-toxic, non-mutagenic, and have no known genomic effects. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
95389	[redacted]	mRNA neo-antigen vaccination for cancer immunotherapy
Approved	Summary: The aim of this experiment is to investigate the anticancer potential of neo-antigen specific mRNA vaccines. Vaccine design will vary based on computationally predicted neoantigens for the tumor models. Customized formulas will be injected in tumor bearing and healthy mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

3. **Subcommittee approval**: 6
    - Clinical Trial Amendments: 3
    - Schedule G Submissions: 3
4. **Schedule H report**: 1
5. **Next IBC meeting date**: October 6, 2021.

Adjourn.



Meeting Minutes
October 6, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Rachel Graham, Tori Baxter, Barbara Savoldo, Cathy Brennan, Amanda Craigen, Ilana Galex, Ericka Pearce, Rachael Turner

Members Absent: Keith Porterfield, Xiao Xiao, Craig Fletcher, Shawn Hingtgen,

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests: N/A

Open Meeting

1. **Review minutes from the September 1st, 2021 meeting.**
2. **Applications under review:**

<table>
<tr><th>ID</th><th>PI</th><th>Project Title</th></tr>
<tr><td>96209</td><td></td><td>Mouse adaption of BtCoV-SHC014 - 2021 renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this project is to investigate the in vivo pathogenesis of a mouse adapted BtCoV-SHC-014, and to improve ACE2 binding in a mouse infection model through targeted mutations in the Y436H gene of BtCoV-SHC-014. Optimizing a mouse-adapted strain of SHC-014 will facilitate a more direct comparison of with mouse adapted .

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>96210</td><td></td><td>The role of ORF8 in and -like viruses - 2021 renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to analyze functional and/or structural differences between ORF8 orthologs from and -like animal coronaviruses. In vitro studies will be performed in mammalian cells with recombinant virus (mouse-adapted containing bat ORF8, civet ORF8, or a reporter gene), or expression vectors containing an orthologous ORF. The viral constructs will also be used to infect mice.

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>96211</td><td>Ralph Baric</td><td>Analysis of RNA structural determinants for their impact on Zika virus replication - 2021 renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to evaluate how novel RNA secondary structures seen in zika virus (ZIKV) facilitate replication. Structural motifs will be selected based on data obtained from SHAPE-MaP and disrupted with targeted mutations in regions of the ZIKV genome encoding key stem loops. Cell cultures will be used to evaluate how viral yield, RNA synthesis, and protein expression compared to the parent strain.

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

96212		Design of live attenuated vaccine candidates for Porcine Epidemic Diarrhea Virus (PEDV) - 2021 renewal
Approved	Summary: The aim of this experiment is to design and isolate mutants for the synthesis of a live attenuated PEDV vaccine. Candidates will be developed by testing various combinations of pre-determined mutations on different full-length viral backbones. Recombinant virus will be monitored for sufficient attenuation. Promising constructs will be sent to a collaborating lab at for further study *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
96229		Infection of human organoid mice with coronavirus to evaluate tissue tropism
Approved	Summary: The aim of this experiment is to investigate how well human organoids (e.g., bone marrow, lung tissue) explanted onto mice support infection. Different human and animal coronaviruses will be used to infect organoids and monitored for pathogenesis. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
97443		CAR-T cell injections into TAM Family Receptor KO mice pre-treated with Chemotherapy agents
Approved	Summary: The aim of this experiment is to study CAR-T cell therapy in mouse models for ovarian cancer. CAR-T cells will be created from isolated splenocytes and administered to mice that have received chemotherapy. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
96487		Developing AAV gene therapy for treating MPC IIIC
Approved with Stipulations	Summary: The aim of this experiment is to develop AAV gene therapy for treating Mucopolysaccharidosis (MPS) IIIC. Constructs will be designed to restore heparan alpha-glucosaminide N-acetyltransferase (HGSNAT) activity, a core deficiency in MPS IIIC. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design, but the Committee was concerned by the proposed injection volumes. The Committee requested that the PI revise their method for the proposed animal work and asked one of its animal use experts to follow up with the investigator.	
95469		Using retroviral vectors to induce glioma development in mouse models
Approved	Summary: The aim of this experiment is to generate mouse models for glioma development to study treatments and tumor biology. Gliomas will be induced in neonatal mice with retroviral vectors designed to selectively target cells expressing the TVA receptor. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>97018</td><td>[redacted]</td><td>Modulation of FRZB in murine and human models of colon cancer</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this proposal is to develop colon cancer cell lines that stably express different levels of frizzled related protein (FRZB), a protein linked to cancer metastasis. Gene expression will be modulated with lentiviral vectors or shRNA. The cell lines will be used to study cancer growth and aggressiveness both in vitro and in vivo.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>95349</td><td>Alan Jones</td><td>Transformation of Rice and Arabidopsis</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to insert plant genes tagged with reporter genes or purification proteins into Arabidopsis and rice.

Committee Comments: Proposal was reviewed by the Committee's ad hoc plant expert prior to the meeting. The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>95409</td><td>[redacted]</td><td>Use Staphylococcus aureus infection models to study effects of bacterial proteins in cGAS/STING signaling</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to elucidate which bacterial proteins bind cyclic GMP-AMP synthase (cGAS), and how those interactions impact cGAS/STING activation and infection clearance. CRISPR/Cas9 will be used to modify gene expression in S. aureus. Cells will be infected in vitro and injected into mice for further observation in vivo.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>97015</td><td>David Margolis</td><td>Use of shRNA to knock down specific cellular genes for HIV latency reversal -2021</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to study whether select HDAC, PRC2, P-TEFb, and estrogen receptor genes play a role in HIV latency reversal, and how lower expression levels of these genes impact cellular function. shRNA will be used to knock down the genes of interest.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>97016</td><td>David Margolis</td><td>Creation of a novel HIV-1 latency model – 2021</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to generate novel cell lines and primary cell models to study HIV-1 transcription and latency. Primary cell models will be generated using a replication competent plasmid that encodes the whole HIV-1 genome and other cell lines will be transduced with lentiviral particles.

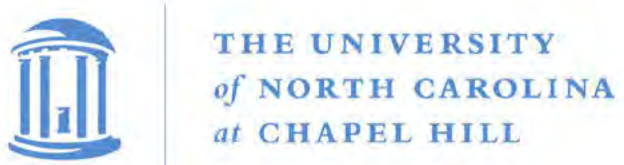
Committee Comments: The Committee requested clarification containment level and recommended the use of enhanced BLS-2 conditions instead of the proposed BSL-3.</td></tr>
</table>

97383	David Margolis	Disrupting HIV latency in cell lines - 2021
Approved	Summary: The aim of this experiment is to elucidate the mechanism of HIV reactivation by disrupting HIV latency with small molecule inhibitors. Constructs will be used to overexpress certain genes to assess 1) the efficacy of the selected latency reducing agents, and 2) the role that affected proteins have in latency reversal. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
94529	Shaun McCullough	Characterization of responses to air pollutant exposure within the human respiratory tract
Approved with Stipulations	Summary: The aim of this experiment is to use *in vitro* models of the human respiratory tract to study how select proteins respond to air pollutants. Protein expression will be modulated with lentiviral vectors and/or shRNA. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the title be revised to reflect the *in vitro* nature of this proposal.	
97555	[redacted]	AAV induction of cancer in mice
Approved with Stipulations	Summary: The aim of this experiment is to induce tumorigenesis in mice by modulating gene expression with AAV vectors, gRNA, and Cre or CRISPR/Cas9. Work may be expanded to include *in vitro* infection studies with human and/or mouse cell lines. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee found the title to be misleading, and requested it be revised to clarify that the genetic material, not the vector, will be what induces tumorigenesis.	
95529	[redacted]	Recombinant Sendai Viruses expressing additional genes from other viruses
Tabled	Summary: The aim of this experiment is to investigate how cells respond to infection with recombinant Sendai Virus (rSeV) engineered to express genes from different respiratory viruses. Constructs will be tested *in vitro* with human, mouse, and hamster cell lines; *in vivo* experiments are planned for mice and hamsters. Committee Comments: The Committee had concerns about the proposed level of containment, the use of replication competent rSeV for the vector, and the proposed work with hamsters, and wished to consult with the High Containment Committee before finalizing its decision.	
96149	[redacted]	Mouse models of Cardiac Disease - Direct Reprogramming of Cardiac Endothelial Cells
Approved	Summary: The aim of this experiment is to determine whether *in vitro* reprogramming of cardiac fibroblasts creates functional cardiac endothelial cells. Endothelial cells will be generated from isolated fibroblasts induced to overexpress certain transcription factors. Reprogrammed cells will be injected into the hearts of mice to test whether they can successfully engraft with cardiac endothelium. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

97303		Neuronal tracing via local stereotaxic injection of AAVs into the mouse brain (Updated 9-24-21)
Approved	<u>Summary</u>: The aim of this proposal is to report the Scherrer lab's inventory of AAV constructs and transgenic mouse lines. The vectors will be administered to mice locally through stereotaxic injection and used to trace neural networks. <u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.	
97423		In Utero Electroporation of Engineered Opioid Receptor Fusion Genes for Proximity Labeling
Approved	<u>Summary</u>: The aim of this experiment is to transfect plasmids containing cre-dependent, opioid receptor fusion genes into the neurons of embryonic mice (*in utero*) to induce expression of the corresponding proteins for proximity labeling. <u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.	
97323		Infusing pAAV-EF1a-fDIO-Cre into mouse brain to study neuronal projections
Approved	<u>Summary</u>: The aim of this experiment is to inject AAV vectors containing flp-dependent cre into the brains of mice via stereotaxic surgeries. The presence Cre in the regions of interest will facilitate site-specific recombination with other AAV constructs and allow further study of neural networks. <u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.	
95489		CRISPR modified mouse melanoma cells for targeting myeloid cells to overcome anti-PD-1 resistance conferred by cancer mutations
Approved	<u>Summary</u>: The aim of this experiment is to knock-out common genes associated with increased resistance in mouse melanoma cell cultures with a CRISPR/Cas9 system. The cell will be used for *in vitro* resistance studies with several antibodies or will be injected into mice for tumor growth and treatment studies. <u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.	

3. **Discussion:** Incident Report
4. **Subcommittee approval**:
   - Clinical Trial Amendments: 1
   - Schedule G Submissions: 4
5. **Schedule H report**: 25
6. **Next IBC meeting date**: November 3, 2021.

Adjourn.



Meeting Minutes
November 3, 2021 3:30 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Rachel Graham, Tori Baxter, Barbara Savoldo, Cathy Brennan, Amanda Craigen, Ilana Galex, Ericka Pearce, Rachael Turner

Members Absent: Keith Porterfield, Craig Fletcher, Shawn Hingtgen,

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests: [redacted]

Open Meeting

1. **Review minutes from the October 6th, 2021 meeting.**
2. **Applications under review:**

ID	PI	Project Title
98403	[redacted]	apoE exon deletion gene knockout rat
Approved with Stipulations	Summary: The aim of this experiment is to create a pro-atherogenic rat strain by deleting the apoE gene in Zucker Diabetic Fatty rat embryos. The apoE gene will be targeted with Cas9 or with mRNA and sgRNA. Committee Comments: The Committee noted that the provided IACUC protocol numbers were outdated and needed to be fixed prior to approval. The proposed containment and safety procedures are adequate for the experimental design.	
98506	[redacted]	Assessment of the pathogenic effects of the SARS-CoV-2 C.1.2 spike protein
Approved	Summary: The aim of this experiment is to develop an infectious clone of the C.1.2 variant of SARS-CoV-2 to study characteristics of its spike protein. Experiments testing for pathogenesis, infectivity, and neutralization will be performed *in vitro* and *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

98503		Rosa26-CAG-loxSTOPlox-TdTomato Mouse
Approved	Summary: The aim of this experiment is to insert a reporter construct into fertilized mouse embryos. Plasmids will be used in combination with Cas9 and gRNA to integrate transgene into mouse genome. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
98504		Rosa26-CAG-loxSTOPlox-EmGFP Mouse
Approved	Summary: The aim of this experiment is to insert a reporter construct into fertilized mouse embryos. Plasmids will be used in combination with Cas9 and gRNA to integrate transgene into mouse genome. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
98507		Ex393-hSIRPa-E2 Mouse
Approved	Summary: The aim of this experiment is to insert a reporter construct into fertilized mouse embryos. Plasmids will be used in combination with Cas9 and gRNA to integrate transgene into mouse genome. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
98508		Ex398-hSIRPa-cDNA Mouse
Approved	Summary: The aim of this experiment is to insert a reporter construct into fertilized mouse embryos. Plasmids will be used in combination with Cas9 and gRNA to integrate transgene into mouse genome. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>98663</td><td>[redacted]</td><td>Exploring Various Humanized Animal Models for the Establishment of Respiratory Virus Infections</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to challenge different strains of humanized mice with recombinant respiratory viruses to establish novel infection models. Possible viral constructs include rhinovirus, metapneumovirus, recombinant human parainfluenza virus, and/or recombinant respiratory syncytial virus.

<u>Committee Comments</u>: The Committee noted that IV injected volume was too high and requested that it be corrected to match the volume described in the corresponding the corresponding IACUC protocol. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>98783</td><td>Nathaniel Hathaway</td><td>General protocol for CRISPR based genetic and epigenetic screening to identify novel drug targets</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this proposal is to establish the UNC CRISPR Screening Center with the IBC. This facility will provide core services with CRISPR, Cas9, and gRNA to assist with drug discovery research. Protocol specifics will vary by investigator.

<u>Committee Comments</u>: The Committee provided a reminder to use special precautions with sharps, especially when working with 2nd generation lentiviral constructs. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>98383</td><td>[redacted]</td><td>Testing effects of SPOP inhibition with anti-checkpoint blockade in cancer treatment</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to test the efficacy of SPOP inhibition and anti-PD1 checkpoint blockade as a combination therapy for cancer. Cancer cells will be injected into mice and challenged with the proposed treatment once a tumor is established.

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>97986</td><td>[redacted]</td><td>Ribotagging of astrocytes using ribosomal subunit Rpl22</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to study molecular changes that occur after chronic heroin withdrawal in rats. AAV vectors will be used to induce expression of HA-tagged ribosomal subunit(s). HA-tagged ribosomes will be used to facilitate immunoprecipitation of target mRNA.

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

<table>
<tr><td>98423</td><td></td><td>Recombinant Sendai Viruses expressing additional genes from other viruses for in vitro studies</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to investigate how cells respond to infection with recombinant Sendai Virus (rSeV) engineered to express genes from different respiratory viruses. Constructs will be tested in vitro with human, mouse, and hamster cell lines.

<u>Committee Comments</u>: Due to the potential impacts certain transgenes could have on infectivity, the Committee required this preliminary work be performed with BSL-2 enhanced precautions. The Committee also requested specific metrics for the investigator’s risk assessment. Finally, since no work will be done in vivo for these experiments, the Committee requested removal of IACUC protocol numbers from this submission.</td></tr>
<tr><td>98424</td><td></td><td>Recombinant Sendai Viruses expressing additional genes from other viruses for in vivo studies</td></tr>
<tr><td>Tabled</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to investigate how cells respond to infection with recombinant Sendai Virus (rSeV) engineered to express genes from different respiratory viruses. In vivo experiments are planned for mice and hamsters.

<u>Committee Comments</u>: The Committee postponed review for these experiments until results for preliminary in vitro work are finalized.</td></tr>
<tr><td>98263</td><td></td><td>Mouse models of cardiac disease</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to use mouse models to assess how different transgenes impact cardiac regeneration and heart function after myocardium infarction. Retroviral vectors will be used to introduce foreign genes into mouse cells in vitro or injected directly into mouse hearts in vivo following cardiac injury.

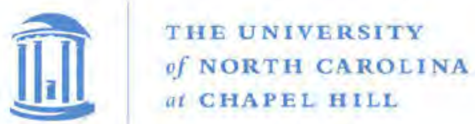
<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>98303</td><td></td><td>Using jGCaMP8 Rapid Kinetics Calcium Indicators to monitor cell-specific neuronal activity and dissect neural origins of rodent brain functional networks</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary</u>: The aim of this experiment is to use a new type of genetically encoded calcium indicator (GECI) to monitor neuronal activity in rats and mice. The GCaMP8 indicator will be encoded in AAV vectors and injected into the brains of mice and or rats.

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

98483	[redacted]	Delivery of mRNA and siRNA-based vaccines with microneedle patches
Approved	Summary: The aim of this experiment is to make microneedle-based formulations of mRNA and saRNA (self-amplifying RNA) vaccines. Mice will be evaluated for vaccine-induced immunity after receiving vaccine formulations intra/trans-dermally via microneedle patch. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
98703	[redacted]	The in Vivo role of the MDM2-MDMX interaction in p53 regulation mouse
Approved	Summary: The aim of this experiment is to observe how inducing a mutation in MDM2 that disrupts E3 ubiquitin ligase activity impacts the MDM2-MDMX interaction that regulates p53. Viral vectors (adenoviral, retroviral, lentiviral) will be used to transduce mutant genes *in vitro*, and mice will receive constructs via electroporation or injection. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

3. **Discussion:**
    a. HGT Training
    b. Incident Report
    c. HCT Meeting
    d. DURC Policy Review (for December)
4. **Subcommittee approval**:
    - Clinical Trial Amendments: 0
    - Schedule G Submissions: 4
5. **Schedule H report**: 34
6. **Next IBC meeting date**: December 1st, 2021.

Adjourn.



Meeting Minutes
December 1, 2021 3:30 PM
Hybrid Conference

Members Present: Doug Cyr, Jennifer Hunter, Rachel Graham, Barbara Savoldo, Cathy Brennan, Ilana Galex, Ericka Pearce, Rachael Turner

Members Absent: Keith Porterfield, Craig Fletcher, Tori Baxter, Shawn Hingtgen, Amanda Craigen

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Guests: [redacted]

Open Meeting

1. **Review minutes from the November 3, 2021 meeting.**
2. **Applications under review:**

ID	PI	Project Title
100100	[redacted]	Effect of metabolism on carcinogenesis of ovarian and endometrial cancer
Approved	Summary: The aim of this experiment is to induce endometrioid adenocarcinoma or serous epithelial ovarian cancer in Cre and LoxP mice. Cre-Lox systems will be manipulated with AdenoCre or estrogen exposure to induce oncogenesis. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
99994	[redacted]	Generation of bat clade 3 Sarbecoviruses and expression plasmids via reverse genetics
Approved	Summary: The aim of this experiment is to generate Bat Clade 3 sarbecoviruses *in vitro* using plasmids that encode genes or genome fragments from the selected viruses. Reconstituted virus will be used for *in vivo* pathogenesis and treatment studies. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
99996	[redacted]	Characterizing the role(s) of MERS-CoV ORFs in viral pathogenesis and fidelity - 2021 renewal
Approved	Summary: The aim of this experiment is to elucidate how specific ORF accessory proteins impact MERS-CoV pathogenesis by introducing mutations into target genes and studying reconstituted virus *in vitro and in vivo*. The described mutations are expected to be attenuating and have been approved by the NIH. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>100000</td><td>[redacted]</td><td>Generation of a recombinant SARS-CoV-2 lacking the Furin-cleavage site in the spike protein</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to study how deleting the coding sequence of the Furin-cleavage site impacts in vitro pathogenesis of SARS-CoV-2.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>100001</td><td>Ralph Baric</td><td>Role of BAI1 in phagocytosis - 2021 renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to compare how the phagocytic capacity of macrophages varies between the A/J and C57BL/6J alleles of Brain-Specific Angiogenesis Inhibitor 1 (BAI1) in vitro.

Committee Comments: The committee requested that the BAI1 acronym be written out in the title. The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>100060</td><td>[redacted]</td><td>Studying biological function of Tau and TDP43 using recombinant AAV vectors</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to study the biological role(s) that human Tau and TDP43 play in relation to neurodegenerative diseases. AAV vectors will be used to transfer the genes of interest into live mice or cell cultures.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>100040</td><td>[redacted]</td><td>Roles of loss of kinase motif BUD12 mutants in colon cancer</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to investigate how colon cancer regulation is affected when the kinase motif BUD12 is knocked out. Lentiviral vectors will be used to transduce human colon cancer cell lines, and modified cells will be injected into immune deficient mice for observation.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>99523</td><td>[redacted]</td><td>Generation of genetically manipulated murine tumor cell lines to recapitulate human disease</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to study the importance of common resistance-associated genes for controlling tumor growth. A CRISPR-Cas9 system will be used to create knockout cell lines from several types of cancer cells. The KO cells will be implanted into mice for in vivo tumor growth studies.

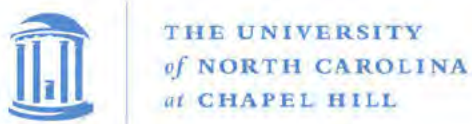
Committee Comments: The committee requested further information regarding the proposed animal work. The investigator will need to provide the relevant IACUC protocol number and amend Section III with further information regarding the methods of restraint and exposure that will be used.</td></tr>
</table>

99524		Use of replication-defective lentiviruses to transduce hematopoietic stem cells and other immune cells
Approved with Stipulations	Summary: The aim of this experiment is to study how myeloid cells regulate tumor growth by using a CRISPR-Cas9 system to delete genes of interest in hematopoietic stem cells or mature immune cells. Cells will be modified *in vitro* and injected into mice for *in vivo* tumor growth studies. Committee Comments: The committee requested the IACUC protocol # and that the investigator specify inoculum concentration and volume for the subcutaneous route. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.	
99383	Kimberly Ritola	BRAIN Initiative Viral Vectors for Neuroscience
Approved with Stipulations	Summary: The aim of this experiment is to establish a general protocol for a new viral vector core specializing in neuroscience research. AAV, rabies virus, and lentiviral vectors will be generated for use in non-human models. Target genes and promoters will vary based on end user applications. Committee Comments: The committee requested language emphasizing this is a general protocol for a core lab. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.	
100094		In vitro ILC2 Experiments
Approved with Stipulations	Summary: The aim of this experiment is to knock down the expression of target genes in ILC2 cells with a CRISPR-Cas9 system. Modified cells will be analyzed and used for *in vitro* experiments or injected into live mice undergoing bone marrow transplants. Committee Comments: The committee found the title to be misleading since the studies will involve animal work and requested that it be revised. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.	
98763		Driving Selective Excitatory or Inhibitory Neurotransmission through Cre-inducible DIO/FLEx-AAVs
Approved	Summary: The aim of this experiment is to study mouse brain activity *in vivo* by modulating neurotransmission during fMRI scans. Neurons expressing Cre-recombinase will be targeted with AAV vectors encoding opsins or DREADDs. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
99443		Use of DREADD receptors to stimulate astrocytes in reward circuitry
Approved	Summary: The aim of this experiment is to investigate whether intracellular signaling impacts alcohol consumption patterns in mice. AAV vectors encoding DREADDs will be injected via site-directed stereotaxic surgeries. The receptors will be used to modulate astrocyte activation *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

99483		Site-directed infusions of pAAV-EF1a-mCherry-IRES-Flpo
Approved	Summary: The aim of this experiment is to investigate how Flp recombinase affects binge-like alcohol consumption patterns in rodent models. AAV vectors encoding the gene of interest will be injected via site-directed stereotaxic surgeries. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
99543		Clarification of Cre-Dependent vectors into mouse brains for study of brain regions and alcohol consumption
Approved	Summary: The aim of this experiment is to use Cre-Dependent DREADDs in rodent models to study neuronal connections associated with alcohol consumption patterns. AAV vectors encoding the gene of interest will be injected via site-directed stereotaxic surgeries. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
99563		Infusing AAV-fDIO-CreGFP into mouse brain to study neuronal projections
Approved	Summary: The aim of this experiment is to study neuronal connections for specific pathways using Flp and a CreGFP fusion protein. AAV vectors encoding the gene of interest will be injected via site-directed stereotaxic surgeries. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
87423		Transfection of Cultured Cells with DNA Constructs and Retroviral Vectors
Tabled	Summary: The aim of this experiment is to evaluate the expression levels of reporter genes after transiently transfecting cells with vectors. Human neuroblastoma and keratinocyte cell lines will be used for *in vitro* transfection. Embryonic neural progenitor cells will also be transfected via *in utero* electroporation. Committee Comments: The committee wanted to verify that Steven Zeisel is still acting PI for the lab.	

3. **Discussion**
4. **Subcommittee approval**: 0
5. **Schedule H report**: 17
6. **Next IBC meeting date**: January 12th, 2021.

Adjourn.



Meeting Minutes
December 8, 2021 4:00 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Rachel Graham, Barbara Savoldo, Cathy Brennan, Ilana Galex, Ericka Pearce, Rachael Turner, Amanda Craigen

Members Absent: Keith Porterfield, Craig Fletcher, Tori Baxter, Shawn Hingtgen, Stanley Lemon, Ann Matthysse

Ad Hoc Meeting

1. **Applications under review:**

ID	PI	Project Title
100474	[redacted]	Investigating intervention strategies against the full-length SARS-CoV-2 Omicron variant
Approved	Summary: The aim of this experiment is to investigate how mutations in the SARS-CoV-2 genome impact the efficacy of current therapies and vaccines. This project will focus on synthesizing infectious clones of the Omicron variant for in vivo mouse studies and in vitro neutralization assays. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
100475	[redacted]	Modeling the effect of SARS-CoV-2 Omicron variant spike protein with in vivo and in vitro assays
Approved	Summary: The aim of this experiment is to study how mutations in the spike protein impact infectivity and pathogenesis. This project will compare the spike protein of the SARS-CoV-2 Omicron variant to infectious clones of the SARS-CoV-2 wildtype and MA10 system. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
100476	[redacted]	Characterization of infectious SARS-CoV-2 and SARS-CoV-2 MA10 clones containing the Brazilian variant (P.1) spike protein
Approved	Summary: The aim of this experiment is to study how mutations in the spike protein impact infectivity and pathogenesis. This project will compare the spike protein of the SARS-CoV-2 Brazilian variant (P.1) variant to a wildtype SARS-CoV-2 infectious clone. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

2. **Next IBC meeting date**: January 12th, 2021.

Adjourn.

Meeting Minutes
January 12, 2022 3:30 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Rachel Graham, Cathy Brennan, Ericka Pearce, Rachael Turner, Keith Porterfield, Amanda Craigen Tori Baxter, Barbara Savoldo, Ilana Galex

Members Absent: Craig Fletcher, Shawn Hingtgen

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

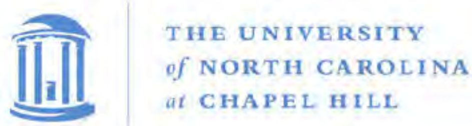
1. **Review minutes from December meetings**
   a. December 1st, 2021
   b. December 8th, 2021 (Ad Hoc)
2. **Clinical Trial Presentation and Review**
   a. [redacted]
3. **Applications under review:**

ID	PI	Project Title
100754	[redacted]	Using a CRISPR-Cas9 system to insert a R59A point mutation in murine protease-activated receptor 4 (PAR4, Factor 2 receptor like 3 [F2rl3]) to elucidate the effect of non-activatable PAR4 in heart failure.
Approved with stipulations	Summary: The aim of this experiment is to elucidate whether signal cascades triggered by PAR4 cleavage at site R59 contribute to heart failure. A CRISPR-Cas9 system will be used to introduce a point mutation at R59. The change is expected to block cleavage at that site but still allow binding/activation at other sites. Committee Comments: The committee requested more complete answers describing the animal work for all parts of Section III.	
100774	[redacted]	SARS-CoV-2 expressing Cre recombinase
Approved	Summary: The aim of this experiment is to create a mouse-adapted SARS-CoV-2 reporter virus using a Cre-Lox system. The Cre-containing mutant will be used to monitor and map infection *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

100775	[redacted]	Effect of Spike mutation on SARS-CoV-2 ACE2-independent cellular entry
Approved	Summary: The aim of this experiment is to evaluate how mutations in the SARS-CoV-2 Spike protein have impacted ACE-2-independent cellular entry by using a reverse genetic system to revert relevant P.1 Spike mutations into residues seen in WA1. Experiments will only be performed *in vitro* at this time. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
100794	[redacted]	Ha-CoV-2 and Ha-CoV-2 Variants
Approved	Summary: The aim of this experiment is to compare the cellular tropism and pathogenesis between variants of a hybrid virus-like particle (VLP), Ha-CoV-2. The VLPs consist of an alphavirus-based backbone, structural proteins from SARS-CoV-2, and a reporter gene, and will be used to infect humanized mice. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
100454	[redacted]	Mouse models of neuro-adapted chikungunya virus
Approved w/ stipulations	Summary: The aim of this experiment is to isolate and sequence neurovirulent variants of CHIKV to identify genes associated with enhanced neurological disease. Once identified, the genes of interest will be introduced into the lab's CHIKV infectious clone and used for infection studies *in vitro* and *in vivo*. Committee Comments: The committee requested further clarification on the animal work described in Section III Question 1.	
100120	[redacted]	Chemical control of AAV vector transgene expression
Approved w/ stipulations	Summary: The aim of this experiment is to investigate the therapeutic potential of a "CEMtrol system" that uses small molecules to modulate AAV transcription activity. The chemical inducer will be tested in human cells and wildtype mice to monitor efficacy, safety, and dosing. Committee Comments: The committee requested further clarification on "general facility PPE" in Section III Question 5.	
100435	Stephen Hursting	Stable Expression of the autophagy marker LC3-B in murine mammary cancer cells
Approve	Summary: The aim of this experiment is to monitor autophagic flux by comparing the relative concentrations of three proteins (LC3-B, mCherry, and GFP) in murine mammary cancer cells. A lentiviral construct will be used to transduce cells and direct constitutive expression for the three genes of interest for *in vitro* analysis. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

101017		Cortical circuits underlying the processing of biologically meaningful sounds
Approve w/ stipulation	<u>Summary:</u> The aim of this experiment is to outline the neural connections within the mouse auditory system using a modified rabies vector encoding a fluorescent reporter protein. The vector will be obtained from Salk Vector Core and used for *in vivo* studies. <u>Committee Comments:</u> The committee requested clarification on what type of facility the "surgery room" is.	
101114		Cloning and expression of recombinant proteins in E coli or mammalian cell culture for diagnostic and vaccine studies
Approved w/ stipulations	<u>Summary:</u> The aim of this experiment is to synthesize gene products from coronaviruses and arboviruses to test their potential utility as vaccine components. Diagnostic experiments will be performed solely *in vitro*. <u>Committee Comments:</u> The committee requested clarification on whether the lab will be performing *in vivo* work. If yes, the proposal will be reviewed again after Section III is filled out; if no, the committee grants conditional approval with the stipulation of minor revisions (improve title, fix typos, remove mention of mouse work)	
100574		AAV directed expression of FVIII or FIX in dogs with hemophilia
Approve w/ stipulation	<u>Summary:</u> The aim of this experiment is to use an AAV vector to increase expression levels of FVIII and FIX in dogs with hemophilia. <u>Committee Comments:</u> The committee requested the inoculum parameters for each route of exposure. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.	
100494		In Vivo neuronal Monitoring with Calcium Sensors
Approved	<u>Summary:</u> The aim of this experiment is to trace neuronal activity using a genetically encoded calcium indicator delivered into the brains of mice via AAV vector. <u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.	
89542		Intramyocardial Injection of Cells or other biomaterials
Approved	<u>Summary:</u> The aim of this protocol is to document the provision of animal injection services to requesting labs. The requestion lab will provide cells or other biomaterials for intramyocardial injection in mice. <u>Committee Comments:</u> Protocol had been tabled since the June 2021 meeting because it needed to have Section III completed. All sections are now complete, and the proposed containment and safety procedures are adequate for the experimental design.	

4. **Meeting Went into Closed Session at 4:30pm**
5. **Sub-Committee Approvals**
    a. Clinical Trial Amendments: 0
    b. Schedule G Submissions: 4
6. **Schedule H Report:** 4
7. **Next IBC Meeting:** February 2, 2022



Meeting Minutes
February 2, 2022 3:30 PM
Web Conference

Members Present: Doug Cyr, Jennifer Hunter, Rachel Graham, Cathy Brennan, Rachael Turner, Keith Porterfield, Amanda Craigen Tori Baxter, Barbara Savoldo, Ilana Galex

Members Absent: Craig Fletcher, Shawn Hingtgen

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from January meetings**
   a. January 12th, 2022 (Open Session)
   b. January 12th, 2022 (Closed Session)
2. **Applications under review:**

ID	PI	Project Title
101315		Reverse genetic construction of the Laos bat coronavirus BANAL-236
Approved	Summary: The aim of this experiment is to generate an infectious clone of the BANAL-236 virus to study pathogenesis *in vitro* using human cells and compare its viral antigens to other coronaviruses. Data will be used to supplement current efforts to generate pan-coronavirus vaccines and antiviral treatments. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
101316		Generation of BANAL-236 spike-expressing VRPs in the VEE replicon platform
Approved with Stipulations	Summary: The aim of this experiment is to develop a virus-derived replicon particle (VRP) that expresses the spike protein from the BANAL-236 coronavirus using the Venezuelan Equine Encephalitis (VEE) replicon platform. Supernatants containing VRPs will be used *in vivo* to test antibody production and immunity, and to develop a characterization system for novel zoonotic coronaviruses. Committee Comments: The Committee requested that sleeve covers and eye protection be added to the listed PPE in section III. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>101754</td><td>Matthew Hirsch</td><td>HLA-G isoform mediated immune suppression</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to develop an in vitro screening method for human leukocyte antigen G (HLA-G) isoforms and mutants to 1) elucidate their functional mechanisms and 2) evaluate their potential candidacy for ocular gene therapy.

Committee Comments: The Committee requested that the rDNA category be changed from III-D to III-E. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>101214</td><td></td><td>Exploring mechanisms of therapeutic demethylation effects in HPV-associated head and neck cancer (2022 Renewal)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to identify how demethylation impacts the role of matrix metalloproteinases (MMPs) as it relates to HPV-associated head and neck squamous cell carcinoma (HNSCC) metastasis. Lentiviral vectors will be used to deliver MMP-targeting shRNA.

Committee Comments: The Committee requested that sleeve covers, bonnets, and booties be added to the listed PPE in section III. Otherwise, the proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>101714</td><td></td><td>Neural tracing using engineered yellow fever vaccine (YFV-17D) in mice</td></tr>
<tr><td>Approved with stipulations</td><td colspan="2">Summary: The aim of this experiment is to use a YFV-17D based viral system for anterograde tracing of neural connections within the mouse auditory system using. The vector is replication deficient, and inoculum will not need modification prior to in vivo studies.

Committee Comments: The Committee was concerned about the proposed vector's infectious potential and requests that the lab contact UEOHC to discuss the special immunization program and medical surveillance SOP.</td></tr>
<tr><td>101478</td><td></td><td>In-situ Redox Imaging (Loeser R01 mice)</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to use fluorescent probes to measure the in vivo activity of reactive oxygen species within knee joints. An adenoviral vector will be used to deliver the reporter genes to mice via injection at the knee's synovial space.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>101474</td><td>Sharon Campbell</td><td>Structure and Mechanism of Cell Signaling GTPases and Cell Adhesion Proteins</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to characterize the structure and mechanism of various cell signaling proteins. Bacterial expression vectors will be used to amplify the genes of interest in E. coli.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

<table>
<tr><td>101374</td><td></td><td>Ex403-407 GAA Mice</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use a CRISPR/Cas9 system to generate a transgenic mouse strain that expresses human GAA gene. Modified embryonic stem cells will be injected into mouse embryos prior to implantation.

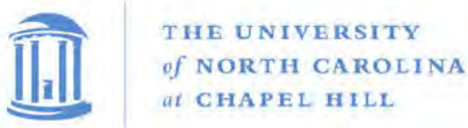
<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>101314</td><td></td><td>Rosa26-CAG-LoxSTOPLox-MEK1DN mouse</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to generate a Cre-inducible transgenic mouse that abrogates activation of ERK1/2 with a dominant negative MEK1 mutation. Mouse zygotes will be microinjected with the plasmid DNA.

<u>Committee Comments</u>: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>101414</td><td></td><td>Generation of NOD.UTX^DMD mice</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use a CRISPR/Cas9 system to generate a transgenic NOD mouse lacking UTX demethylase activity. The Cas9 reagent mixture will be injected into the pronuclei of fertilized eggs prior to implantation.

<u>Committee Comments</u>: The committee requested the containment selected on the form be changed from BSL-2 to BSL-1 to match the work described; otherwise, the safety procedures are adequate for the experimental design.</td></tr>
<tr><td>101354</td><td></td><td>Generation of NOD.Aire^GW/+ mice</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use a CRISPR/Cas9 system to generate a transgenic mouse strain without the ability to identify and eliminate self-reactive T cells. The Cas9 reagent mixture will be injected into the pronuclei of fertilized eggs prior to implantation.

<u>Committee Comments</u>: The committee requested the containment selected on the form be changed from BSL-2 to BSL-1 to match the work described; otherwise, the safety procedures are adequate for the experimental design.</td></tr>
</table>

3. **Sub-Committee Approvals:** 0
4. **Schedule H Report:** 9
5. **Next IBC Meeting:** March 2, 2022



Meeting Minutes
March 02, 2022 3:30 PM
Web Conference

Members Present: Amanda Craigen, Barbara Savoldo, Catherine Brennan, Doug Cyr, Ilana Galex, Jennifer Hunter, Rachael Turner, Rachel Graham

Members Absent: Craig Fletcher, Keith Porterfield, Shawn Hingtgen, Victoria Baxter

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from February meeting**
2. **Clinical Trials**
   a. **Presentation & Review**

   b. **Review**

3. **Applications under review:**

ID	PI	Project Title
n/a		
Approved	Summary: The aim of this clinical trial is to test the safety, tolerability, and immunogenicity of Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>n/a</td><td></td><td></td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this clinical trial is to test the efficacy

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>102294</td><td>Ralph Baric</td><td>Manipulation of Zika virus envelope to impact stability and neurotropism - 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to characterize how a set of mutations observed in the envelope structural loops of Zika virus affect stability and neurotropism. Viral variants will be propagated and characterized in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>102295</td><td>Ralph Baric</td><td>Generation of a cDNA infectious clone system for Zika virus: Dakar Strain - 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to develop an infectious clone system for the Dakar strain of Zika virus (ZIKV). The ZIKV Darkar clone will be characterized with in vitro growth and pathogenesis studies.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>102296</td><td>Ralph Baric</td><td>Use of a cDNA infectious clone system for Zika virus to examine natural strain variation - 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to evaluate a set of approved ZIKV infectious clones for genetic and functional deviations in their structural and non-structural proteins. Viral variants will be propagated and characterized in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>102359</td><td></td><td>Attenuating SARS-CoV-2 through the introduction of human micro-RNA target sites</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to repress SARS-CoV-2 replication with cellular regulatory systems by integrating micro-RNA target sites into the SARS-CoV-2 genome. CRISPR/Cas9 will be used to knockout miRNA of interest in cell lines to allow propagation of recombinant virus.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

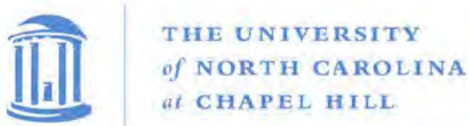
102374		Introduction of lentivirus vectors with shRNAs into mouse tumor cells
Approved with Stipulations	Summary: The aim of this experiment is to knockdown expression of different coagulation proteins in murine tumor cells using shRNA constructs. Pre-made lentiviral vectors will be used to deliver shRNA constructs to cells *ex vivo,* and modified cells will be injected into mice. Committee Comments: The Committee granted conditional approval pending the completion of Section III for the animal work referenced in Section II.	
101974		Using CRISPR/Cas9 to Generate Zebrafish Line Expressing GFP-nr4a1 Transgene
Approved	Summary: The aim of this experiment is to induce expression of GFP-linked nuclear receptor sub family 4, group A, member 1 (GFP-nr4a1) in zebrafish. A CRISPR/Cas9 system will be used to generate embryos that express the transgene of interest. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
101975		Using nppc:GFP to Monitor Endothelial Cell Activation in Zebrafish
Approved	Summary: The aim of this experiment is to monitor endothelial cell activation during cardiac regeneration with GFP expression. A cloning vector will be injected into zebrafish embryos and GFP expression will be driven by a natriuretic peptide C (nppc) enhancer. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
102334		Cancer epigenetics: Understanding chromatin modification in cancer
Approved with Stipulations	Summary: The aim of this experiment is to use cell lines with targeted gene knockouts to study how the chromatin modification pathway relates to cancer development. Lentiviral systems will be used to deliver shRNA and sgRNA constructs *in vitro*, and modified cell lines will be used for additional studies *in vivo.* Committee Comments: The Committee granted conditional approval pending additional details on the method of restraint in Section III.	
102134	Alisa Wolberg	Lentiviral plasmid transfection of hormone receptors into endothelial cells
Approved	Summary: The aim of this experiment is to determine whether the presence of hormone receptors allows endothelial cells to respond to hormone exposure *in vitro*. Lentiviral vectors will be used to deliver the genes of interest to cell culture. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

102394		Injection of KPC tumor cells into mice.
Approved	Summary: The aim of this experiment is to induce *in vivo* KPC tumor development. KPC cells will be obtained from the lab and will be generated with either a CRISPR/Cas9 system or lentiviral vector delivery of shRNA. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
102234		Yeh Lab plasmid and CRISPR protocol
Approved with Stipulations	Summary: The aim of this experiment is to use a CRISPR library to study the gene-specific effects on *in vivo* tumor growth. Genes will be selected based on differential RNA sequencing expression, and cells will be modified *in vitro* with a lentiviral iCas9 vector. Committee Comments: The Committee granted conditional approval pending the completion of Section III for the animal work referenced in Section II. Title changed to "In-vivo tumor subtypes using CRISPR."	
102358		Preclinical Studies of Complement Activation Modulation in the Treatment of Glomerulonephritis and Vasculitis Caused by Anti-neutrophil Cytoplasmic Autoantibodies (ANCA)
Approved	Summary: The aim of this experiment is to evaluate the therapeutic potential of siRNA directed against Complement Factor B in mouse models of ANCA glomerulonephritis. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
102254		Invivofectamine 3.0/ USP15 siRNA complex
Approved with Stipulations	Summary: The aim of this experiment is to study the genetic and biological impacts of *in vivo* USP15 inhibition with siRNA. Committee Comments: The Committee granted conditional approval pending additional clarification on the injection and restraint methods described in Section III.	
102277	Dougald Monroe III	Create a bifunctional antibody that will interact with canine factor IXa and canine factor X.
Approved	Summary: The aim of this experiment is to create a bifunctional antibody that interacts with canine factor IXa and X to treat hemophilia in dogs. The work proposed will be performed *in vitro* only using standard mammalian expression vectors. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>102278</td><td>Dougald Monroe III</td><td>Random mutagenesis to create a bifunctional antibody that will interact with canine factor IXa and canine factor X</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to create a bifunctional antibody that interacts with canine factor IXa and X to treat hemophilia in dogs. The work proposed will be performed in vitro using phagemid particles to test the binding affinity of different mutations.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

4. **Discussion**
    a. NIH Reportable Incident
5. **Sub-Committee Approvals**
    a. Clinical Trial Amendments: 0
    b. Schedule G Submissions: 1
        i. **PI:** [redacted] **Title:** Therapeutic mRNA and DNA Vaccines for Peanut, Walnut, Pecan, Cashew, Pistachio and Shellfish Allergies (III-D, ID: 101955)
6. **Schedule H Report:** 24
7. **Next IBC Meeting:** April 6, 2022



Meeting Minutes
June 1, 2022 3:30 PM
Hybrid Conference

Members Present: Amanda Craigen, Catherine Brennan, Doug Cyr, Jennifer Hunter, Ilana Galex, Rachael Turner, Rachel Graham

Members Absent: Barbara Savoldo, Craig Fletcher, Keith Porterfield, Matthew Hirsch, Shawn Hingten, Victoria Baxter

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

Review minutes from May 4, 2022 meeting

1. **Clinical Trials**
   a. **Presentation and Review**
   PI:

2. **Applications under review:**

Clinical Trials		
Product	**PI**	**Project Title**
HGT		
Approved	Summary: The aim of this study is to test the safety and tolerability Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><th colspan="3">Schedule G Submissions</th></tr>
<tr><th>ID</th><th>PI</th><th>Project Title</th></tr>
<tr><td>104034</td><td>Ralph Baric</td><td>Transfer of Dengue Virus epitopes into an Usutu virus infectious cDNA clone</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to develop an Usutu/EDE-Dengue chimera to characterize binding sites for cross-reactive antibodies. The chimera will also be tested as a tool for measuring the concentration of EDE-like antibodies in patient sera.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>104035</td><td></td><td>Bat- -like coronaviruses expressing the Uganda Spike - 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to evaluate the accuracy of sequence analysis data for the receptor-binding domain of by testing the binding affinity of recombinant viruses. Viruses that replicate efficiently in vitro will be used to guide the development of a full-length containing mACE2 interaction-enhancing mutations for use in vivo.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>104594</td><td></td><td>Locked nucleic acids as antiviral agents</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to design short locked nucleic acids for use as antiviral agents. The locked nucleic acid oligonucleotides will be studied in vivo and administered via inhalation.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>104614</td><td></td><td>Building Recombinant H1N1 Influenza Virus Using Kawaoka Laboratory Infectious Clone: 2022 Renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to synthesize an infectious clone of the 2009 H1N1 Swine influenza virus, CA04. The recombinant H1N1 will be used for in vivo studies of respiratory disease, and findings will be compared to the infection profiles of different coronaviruses.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

<table>
<tr><td>104615</td><td></td><td>infectious clone with and without mouse-adapted mutations: 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to maintain an infectious clone of the strain for future use as a control virus or a backbone genome. Any new mutations will be registered with the IBC.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>104616</td><td></td><td>Accessory Open Reading Frame and Accessory Gene Deletions in Infectious Clone: 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to generate infectious clones of carrying targeted deletions to study its ORFs and accessory gene functions.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>104617</td><td></td><td>Recombinant alphavirus expression vectors for BSL2 use: VEE Vaccine strain 3526: 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to use a Venezuelan Equine Encephalitis Replicon system (VEE-VRP) as a protein expression system in transfection studies. VEE-VRP will be used to express proteins from a variety of viruses (e.g., norovirus, coronaviruses). Each construct will be registered with the IBC in a separate application.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>104618</td><td></td><td>Identification and characterization of novel bat norovirus and calicivirus capsids - 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to assess the antigenic profile and zoonotic infection potential of a novel bat norovirus and calcivirus.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

104619		Investigation of the role of ion channels in pathogenesis and immunity - 2022 renewal
Approved	Summary: The aim of this experiment is to compare the ion channels and potential ion channels of different coronaviruses and evaluate their impact on pathogenesis. The infectious clone will be used as a backbone and substituted with different ion channel genes for study *in vitro* and *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
104620		Characterizing the roles of MERS-CoV and nsp15 proteins in viral pathogenesis and fidelity – 2022 renewal
Approved	Summary: The aim of this experiment is to introduce mutations into the nsp15 gene of MERS-CoV and to evaluate how the nsp15 protein impacts pathogenesis i*n vivo* and *in vitro.* Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
104934		HIV and Mtb Co-infection using humanized mice
Approved with Stipulations	Summary: The aim of this experiment is to study HIV and TB co-infection *in vivo* in NSG mice. HIV and TB will not be administered simultaneously. Committee Comments: The Committee granted conditional approval pending clarification that the work with HIV is covered appropriately in their SOP.	
104554	Aravinda Desilva	Antibody Responses to SARS-CoV-2
Approved	Summary: The aim of this experiment is to determine the SARS-CoV-2 neutralizing antibody response in the blood serum of SARS-CoV-2 vaccinated individuals and individuals who have had natural infection with SARS-CoV-2. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

104374		Regulation of IgE Antibody Responses - Recombinant Salmonella Typhimurium PnirOVA and PnirBEM
Approved	Summary: The aim of this experiment is to compare how helminth infection impacts antibody response to oral vaccination with a recombinant strain of Salmonella Typhimurium. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
104216		Studying enterovirus pathogenesis in a model organism
Approved	Summary: The aim of this experiment is to study enterovirus pathogenesis *in vivo*. This proposal amends Schedule G #88462 (approved 06/07/2021) to include coxsackievirus B3. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
104775	Bernard Weissman	The Role of Protein Kinases in NRF2-driven Lung Squamous Cell Carcinoma
Approved with Stipulations	Summary: The aim of this experiment is to develop human and mouse tumor cell lines with doxycycline-inducible expression of oncogenes. Committee Comments: The Committee granted conditional approval pending amendment to Section II Question 11 to include specific cell lines.	
104794	Bernard Weissman	The Role of Protein Kinases in NRF2-driven Lung Squamous Cell Carcinoma
Approved with Stipulations	Summary: The aim of this experiment is to develop human and mouse tumor cell lines via CRISPR-mediated knockout of tumor suppressor genes. Committee Comments: The Committee granted conditional approval pending amendment to the title to differentiate this proposal from Schedule G #104775.	

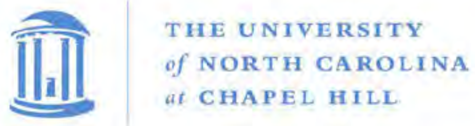
3. Sub-Committee Approvals: 11

a. **PI:** Ralph Baric **Title:** Determining how Zika NS2a affects interferon responses - 2022 renewal (III-F, ID: 104036)

b. **PI:** Ralph Baric **Title:** Expressing Civet Cat, Mouse, Bat, and Human ACE2 Constitutively in SARS-CoV Nonpermissive Cell Lines - 2022 renewal (III-F, ID: 104037)
c. **PI:** [redacted] **Title:** Generation of C3AR1 knockout mice via CRISPR (III-E, ID: 104621)
d. **PI:** [redacted] **Title:** Generation of C5 knockout mice via CRISPR (III-E, ID: 104622)
e. **PI:** [redacted] **Title:** Generation of C3AR1 knockout mice via CRISPR (III-E, ID: 104623)
f. **PI:** [redacted] **Title:** Generation of C6 knockout mice via CRISPR (III-E, ID: 104624)
g. **PI:** Patrick Brennwald **Title:** Polarized Exocytosis: Rabs, Tethers, and SNAREs (III-F, ID: 104434)
h. **PI:** [redacted] **Title:** Production of a Flpo Mouse Strain (III-E, ID: 104654)
i. **PI:** [redacted] **Title:** Visualizing cell cycle in mouse using PIPFUCCI (III-E, ID: 104674
j. **PI:** Rachel Noble **Title:** Production of or use of purchased plasmids or synthetic nucleic acids for use as positive controls for PCR, quantitative PCR, and digital droplet PCR (III-F, ID: 104575)
k. **PI:** Rachel Noble **Title:** Production of or use of purchased plasmids or synthetic nucleic acids for use as positive controls for PCR, quantitative PCR, and digital droplet PCR (III-F, ID: 104577)

5. **Schedule H report:** 15
6. **Next IBC meeting:** July 13, 2022

Adjourn.



Meeting Minutes
July 13, 2022 3:30 PM
Web Conference

Members Present: Amanda Craigen, Barbara Savoldo, Catherine Brennan, Doug Cyr, Jennifer Hunter, Keith Porterfield, Ilana Galex, Matthew Hirsch, Rachael Turner, Rachel Graham Victoria Baxter

Members Absent: Craig Fletcher, Shawn Hingtgen,

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

Review minutes from June 1, 2022 meeting

1. **Applications under review:**

<table>
<tr><th>ID</th><th>PI</th><th>Project Title</th></tr>
<tr><td>105930</td><td></td><td>Development of lipid nanoparticles for nucleic acids</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to further explore the therapeutic potential of nanoparticles by developing a lipid nanoparticle carrier system for nucleic acid delivery. Nucleic acids will encode reporter genes and/or influenza antigens.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>105514</td><td></td><td>Manipulation of the dengue virus envelope to impact stability and neurotropism: 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to elucidate whether the longer structural loops observed with Zika virus (ZIKV) contribute to the improved stability and neurotropism in comparison to dengue virus. Residues will be added to a specific structural loop motif of DENV4 to test if the mutation confers greater stability.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

105515		Generation of containing the mouse-adapted Spike Y436H substitution: 2022 renewal
Approved	<u>Summary:</u> The aim of this experiment is to incorporate a point mutation into the spike gene on a mouse adapted strain expressing the btSCoV-WIV1 viral attachment protein. The construct will be used to assess pathogenesis *in vitro* and *in vivo.* <u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.	
105516		Assessment of the Omicron B.1.1.529 BA.2 Spike variant in *in vitro* & *in vivo* model systems
Approved	<u>Summary:</u> The aim of this experiment is to characterize the pathogenicity and antigenicity profile of the Omicron B.1.1.529 SARS-CoV-2 variant. Data from this project will be used to guide development of therapeutic strategies and interventions for clinical infections. <u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.	
106214		Investigating the genetic and epigenetic control of cell cycle progression in a drosophila model
Approved with Stipulations	<u>Summary:</u> The aim of this experiment is to monitor how cell cycle progression control varies in response to mutations in endogenous proteins in a Drosophila model. Depending on the mutation, the lab will either generate the strains in-house or purchase from vendors. <u>Committee Comments:</u> The committee granted conditional approval pending the removal of non-specific language in Section II Question 8 (i.e., including, but not limited to).	
105394	Henry Earp	Creation of cell lines expressing TAM receptors
Approved	<u>Summary:</u> The aim of this experiment is to evaluate the downstream signaling effects of TAM receptor protein tyrosine kinases using lentiviral expression vectors *in vitro*. <u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.	

105814	Jack Griffith	Toxic telomere-transcribed peptide accumulation in cells
Approved	Summary: The aim of this experiment is to evaluate whether dysfunctional or uncapped telomeres lead to higher concentrations of telomeric toxic dipeptide aggregates within nuclei *in vitro*. Mutated cell lines will be generated via lentiviral vectors. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
105452		Determining the role for systemic and tumoral metabolism in CAR-T cell antitumor efficacy
Approved	Summary: The aim of this experiment is to investigate the antitumor effects of novel immune stimulating techniques. The project will focus on improving lymphocyte receptor specificity to cancer cell antigens and evaluating the impact of modulating effector molecules and transcription factors on immune cell functions. Committee Comments: The committee granted conditional approval pending clarification on anesthetic method for brain injections.	
105494		Safer 5th generation lentiviral vectors with reduced viral sequence
Approved	Summary: The aim of this experiment is to generate a novel lentiviral vector with a reduced parental viral sequence that utilizes a vector cassette containing a tandem sequence. The proposed design is intended to improve safety by limiting the potential for recombination, mobilization, and alteration of host gene expression. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
105298		Molecular mechanisms of Zika virus pathogenesis
Approved	Summary: The aim of this experiment is to investigate the molecular mechanisms associated with ZIKV infection and disease symptoms by generating ZIKV mutants and/or utilizing a reporter virus system. Committee Comments: The committee granted conditional approval pending the addition of a risk assessment statement to the proposal.	

<table>
<tr><td>105299</td><td></td><td>Recombinant herpes simplex virus</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to monitor in vivo herpes simplex virus (HSV) infection with modified HSV strains that express either reporter genes or Cre recombinase.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>104974</td><td></td><td>Rational design of AAV vectors with human hepatocyte tropism and neutralizing antibody evasion</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to optimize AAV vector transduction efficiency in human hepatocytes by testing different AAV mutants and serotypes in vivo with xenograft models.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>104774</td><td>Shanmugam Nagarajan</td><td>Role of Fcgamma receptors, auto-antibodies and progression of atherosclerosis</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiments is to elucidate the role of Fcgamma receptors and scavenger receptor in the initiation and progression of atherosclerosis. Lentiviral or AAV expression vectors will be used to express the genes of interest in vitro.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>105594</td><td>Bryan Roth</td><td>TC-83 Vaccine Strain for Directed Evolution of Therapeutic Proteins</td></tr>
<tr><td>Tabled</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to create more effective therapeutics through directed evolution in vitro.

<u>Committee Comments:</u> The committee moved to table review pending further clarification on the experimental method, target genes, and risk assessment.</td></tr>
</table>

105565	[redacted]	Evaluation of micro-array patch delivery of Measles live attenuated virus vaccines in mice
Approved	Summary: The aim of the experiment is to test the immunogenicity of live attenuated Measles vaccines *in vivo* using susceptible transgenic models. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

2. **Discussion:**
   - **a.** West Nile Virus and St. Louis Encephalitis Virus Containment Recommendations
   - **b.** WCG IBC Services for Clinical Trial Review

3. **Sub-Committee Approvals:** 6

   III-E

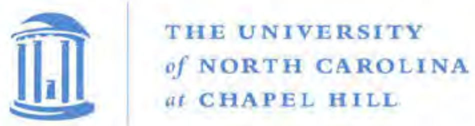
   a. **ID:** 105517 **PI:** [redacted] **Title:** Ex413 & 415 SLC7A9 Mice
   b. **ID:** 105518 **PI:** [redacted] **Title:** Ex414 & 416 SLC3A1 Mice
   c. **ID:**105614 **PI:** [redacted] **Title:** Generation of Crhr1-iCre Rat by UNC transgenic animal core

   III-F

   d. **ID:** 104914 **PI:** Sarah Cohen **Title:** Organelle function and dynamics
   e. **ID:** 105054 **PI:** [redacted] **Title:** Splice Switching Oligonucleotides (SSO) and Oligonucleotide Enhancing Compounds (OEC)
   f. **ID:** 104754 **PI:** Zhi Liu **Title:** Determining the prevalence of IgA autoantibodies in canine pemphigus foliaceus using a novel detection method

4. **Schedule H report:** 38
5. **Next IBC meeting:** August 3, 2022

Adjourn.



Meeting Minutes
August 3, 2022 1:00 PM
Web Conference

Members Present: Amanda Craigen, Barbara Savoldo, Doug Cyr, Jennifer Hunter, Ilana Galex, Matthew Hirsch, Rachael Turner, Rachel Graham

Members Absent: Catherine Brennan, Craig Fletcher, Shawn Hingtgen, Keith Porterfield, Victoria Baxter

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

Review minutes from July 13, 2022 meeting

1. **Applications under review:**

ID	PI	Project Title
106274	[redacted]	AAV Vector Injection in Mouse Cortex for Quantitative Proteomics
Approved	Summary: The aim of this experiment is to facilitate proximity labeling studies *in vivo* with AAV vectors. Vectors will be used to express reporter genes and/or recombinant proteins. Brain tissue will be collected and analyzed with quantitative proteomics. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
106454	[redacted]	Characterization of bat and swine coronavirus HKU2: 2022 renewal
Approved	Summary: The aim of this experiment is to culture alphacoronavirus HKU2 *in vitro* with novel culture conditions and cell types. If successful, HKU2 cultures will be used to characterize the virus. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

106455		Generation of icMERS and icMERC-Uganda expressing RFP: 2022 renewal
Approved	<u>Summary:</u> The aim of this experiment is to develop reagents for characterizing the *in vitro* replication competency and *in vivo* pathogenesis profile of novel zoonotic coronavirus strains. This study will focus on a recombinant infectious clone (ic) of MERS-CoV modified to express the ic-MERSC-Uganda spike protein. <u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.	
106542	Richard Boucher Jr.	Properties of Distal Airway Secretory Cells as Targets for CF Molecular Therapy
Approved	<u>Summary:</u> The aim of this experiment is to use a CRISPR-Cas9 system and GFP to identify which cell types express CFTR in primary bronchial epithelium. <u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.	
106255		Chemogeneitic manipulation of microglia to elucidate their role in alcohol-induced neuropathology
Approved with Stipulations	<u>Summary:</u> The aim of this experiment is to investigate the role of microglia in alcohol-induced neuropathology by inhibiting microglial activation with AAV vectors. Microglia inhibition will be assessed *in vitro, ex vivo,* and *in vivo.* <u>Committee Comments:</u> The committee granted conditional approval pending clarification of anesthetizing agent in S3Q3.	
105675		Utilization of recombinant HCMV strains with reporter genes
Approved with Stipulations	<u>Summary:</u> The aim of this experiment is to monitor HCMV infection progression *in vivo* through the use of recombinant HCMV reporter strains. <u>Committee Comments:</u> The committee granted conditional approval pending correction of the intralung injection volume to match what is approved in their associated IACUC protocol.	

<table>
<tr><td>106574</td><td>[redacted]</td><td>Utilization of recombinant Human Metapneumovirus (HMPV) strains with reporter genes</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to use recombinant HMPV reporter virus to evaluate the efficacy of novel antivirals in vivo.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>106575</td><td>[redacted]</td><td>HIV Reporter Vectors to study HIV infection in humanized mice</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to study HIV infection in vivo through the use of recombinant HIV reporter strains.

Committee Comments: The committee granted conditional approval pending confirmation that S2Q11 lists all humanized mouse strains, and removal of the non-specific language in S3Q1 (“various humanized mice”).</td></tr>
<tr><td>106576</td><td>[redacted]</td><td>Utilization of recombinant RSV strains with reporter genes</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to use recombinant RSV reporter virus to evaluate the efficacy of novel antivirals in vivo

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>106577</td><td>[redacted]</td><td>Utilization of luminescent Neisseria gonorrhoeae</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to study Neisseria gonorrhoeae infection in vivo through the use of recombinant N.gonorrhoeae reporter virus.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

<table>
<tr><td>106494</td><td></td><td>Biomaterial-CAR T cell Therapy for Post-surgical Brain Cancer</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to develop and optimize a hydrogel system for in vivo immunotherapy delivery. Glioblastoma animal models will be utilized to simulate the clinical scenario of tumor resection and TME-associated immunosuppression.

Committee Comments: The committee granted conditional approval pending additional revisions to Section 3 Question 3. The current answer is verbose and includes unnecessary information regarding the use of local anesthetics.</td></tr>
<tr><td>106394</td><td></td><td>Using CRISPR In Vivo to generate mouse models of bladder and kidney cancer</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to develop heterogenous mouse models of kidney and bladder cancer using a lentiviral CRISPR system. Data from the cancer genome atlas (i.e., mutation frequency, co-mutation significance) will be used to guide transgene selection.

Committee Comments: The committee granted conditional approval pending the addition of gloves and a lab coat to the PPE list in Section 3 Question 5.</td></tr>
<tr><td>106395</td><td></td><td>Using pBABE vector to express oncogenes and tumor suppressor genes to study bladder and kidney cancer biology</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to study how overexpressing oncogenes and/or tumor suppressor genes affect bladder and kidney cancer biology in vitro.

Committee Comments: The committee granted conditional approval pending the addition of gloves and a lab coat to the PPE list in Section 3 Question 5.</td></tr>
<tr><td>106215</td><td>Scott Randell</td><td>AAV1 rescue of F508-del</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this project is to develop a gene therapy for cystic fibrosis. These experiments will evaluate AAV1 vector transduction success in primary human airway epithelial cells.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

106216		Engineering Smart Cells for Cystic Fibrosis Therapy
Approved	Summary: The aim of this experiment is to improve stem cell engraftment with the endogenous epithelium with "smart" stem cells engineered to direct migration towards favorable niches. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
105594	Bryan Roth	TC-83 Vaccine Strain for Directed Evolution of Therapeutic Proteins
Approved with Stipulations	Summary: The aim of this experiment is to optimize Cas12f1 transfection efficiency in BHK21 cells. Effective transfection will be measured via EGFP activation levels. Committee Comments: The committee granted conditional approval pending additional clarification on the experimental method.	
106417	Ron Swanstrom	Understanding of HIV-1 life cycle
Approved with Stipulations	Summary: The aim of this experiment is to analyze HIV-1 gene functions *in vitro*. Committee Comments: The committee granted conditional approval pending additional clarification on the target genes.	
106054		Infusing pAAV-EF1a-fDIO-mCherry into mouse brain as a control to study neuronal projections
Approved	Summary: The aim of this experiment is to use pAAV-EF1a-fDIO-mCherry as a control virus to compare how previously approved constructs affect neuronal projections. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

105874	John West	CAR.CD30 Retroviral Vector/Chimeric Antigen T cells
Approved with Stipulations	Summary: The aim of this experiment is to produce viral vectors for CAR T-cells. The resulting CAR T-cells will be used in clinical trials sponsored by the [redacted] [redacted] Committee Comments: The committee granted conditional approval pending the removal of the Lab Safety Plan number in Section 2 Question 7.	

2. Discussion

a. New Member Announcement & Vote

3. Sub-Committee Approvals: 2

III-E

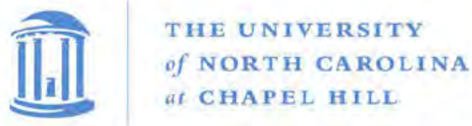
a. **ID:** 105754 **PI:** Scott Randell **Title:** Optimizing Electroporation for CF Therapy Gene Delivery, Gene Editing, and Small Airway Delivery

III-F

i. **ID:** 106634 **PI:** Shawn Gomez **Title:** BioBrick Assembly

5. Schedule H Report: 22

Next IBC meeting: September 7, 2022



Meeting Minutes
October 5, 2022 1:00 PM
Hybrid Conference

Members Present: Amanda Craigen, Catherine Brennan, Doug Cyr, Keith Porterfield, Ilana Galex, Matthew Hirsch, Rachael Turner, Rachel Graham, Shawn Hingtgen,

Members Absent: Barbara Savoldo, Craig Fletcher, Victoria Baxter

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

Review minutes from September 7, 2022 meeting

1. **Applications under review:**

ID	PI	Project Title
108675	[redacted]	Development of lipid nanoparticles for nucleic acids for immune therapy
Approved	Summary: The aim of this experiment is to develop lipid nanoparticles that function as carriers for nucleic acids. The nucleic acids will encode cytokines for *in vitro* and *in vivo* immune therapy research. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
107974	Richard Baker Jr.	Utilizing HIV VLPs to display human transmembrane proteins
Approved	Summary: The aim of this experiment is to HIV viral-like particles (VLPs) to display human transmembrane proteins, such as receptors, in cell culture. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

108815	[redacted]	A Chemically Inactivated Rabies-based Vaccine Candidate against MERS-CoV: 2022 renewal
Approved	Summary: The aim of this experiment is to test whether a chemically inactivated rabies-derived vaccine (RABV) can be used to express the MERS spike protein and elicit an effective immune response against MERS-CoV. The vaccine particles will be received from a collaborator and inactivated on-site for *in vivo* studies. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
108816	Ralph Baric	Creation of multiple nanoluciferase (nLuc) reporter viruses in West Nile virus (WNV), Zika virus (ZIKV), Japanese Encephalitis virus (JEV), and Usutu virus (USUV)
Approved with Stipulations	Summary: The aim of this experiment is to facilitate quantification of antibody neutralization by creating recombinant flaviviruses that express reporter proteins. Committee Comments: The committee granted conditional approval pending confirmation that these experiments use the attenuated vaccine strain of JEV.	
108443	Kathleen Caron	Lentiviral Transduction of LEC Enhancer
Approved with Stipulations	Summary: The aim of this experiment is to use lentiviral vectors to express rLuc tagged proteins to analyze different enhancer sequences. Committee Comments: The committee granted conditional approval pending amendment of Section II Question 5 to include information about the specific enhancer elements that are being analyzed.	
108054	[redacted]	Viral transduction in cardiomyocytes
Approved	Summary: The aim of this experiment is to use an AAV vector that specifically transduces cardiomyocytes to express different levels of Cre recombinase, SRL gene, GFP, or microRNA-871. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>108814</td><td></td><td>Innate Immunity and KSHV—Vesicular Stomatitis Virus (VSV)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to perform infection studies in vivo with recombinant and wildtype VSV strains.

Committee Comments: The committee granted conditional approval pending amendment of Section II Question 7 to include the current IACUC protocol number(s).</td></tr>
<tr><td>108694</td><td>Ronald Falk</td><td>Modulation of ANCA autoantigen gene expression using CRISPR/dCas9</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to use a CRISPR/Cas9 system to suppress the expression of proteinase 3 and myeloperoxidase.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>107854</td><td></td><td>Developing AAV gene therapy for treating MPS IIIC</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use AAV vectors to restore or remediate disfunctions in the heparan alpha- glucosaminide N-acetyltransferase (HGSNAT) gene that cause mucopolysaccharidosis (MPS) IIIC.

Committee Comments: The committee granted conditional approval pending amendment of Section II Question 7 to include the current IACUC protocol number(s) and Section III Question 1 to correct injection volume range.</td></tr>
<tr><td>108634</td><td></td><td>AAV-mediated Gene Therapy (in mice) for the Treatment of Neurogenetic Diseases-2022 revision</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to develop AAV-mediated gene therapy strategies for treating mucopolysaccharidoses (MPS) and other neurogenetic diseases.

Committee Comments: The committee granted conditional approval pending amendment of Section II Question 7 to include the current IACUC protocol number(s) and Section III Question 1 to correct injection volume range.</td></tr>
</table>

108414	[redacted]	Utilization of recombinant Influenza strains with reporter genes
Approved with Stipulations	Summary: The aim of this experiment is to engineer a reporter strain of influenza for testing novel antivirals *in vivo*. Committee Comments: The committee granted conditional approval pending amendment of Section III Question 2 to list pentobarbital instead of Nembutal.	
108534	Matthew Hirsch	Isolation of AAV Inverted Terminal Repeats From a Mutant Library for Enhanced Functions
Approved	Summary: The aim of this experiment is to use an AAV library to identify sequences with mutated inverted terminal repeats (ITRs) which exhibit enhanced potential for rAAV production and/or the ability to stimulate targeted HR in human cell lines. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
108334	[redacted]	Limbic glutamatergic circuits in ethanol self-administration
Approved with Stipulations	Summary: The aim of this experiment is to use AAV vectors that express reporter genes to facilitate study of the neurochemical and neurobiological pathways involved in alcohol-related behaviors. Committee Comments: The committee granted conditional approval pending amendment of Section II Question 11 to include specific mouse strain information, Section III Question 1 to include the inoculum concentration, and Section III Question 3 to include current protocol information.	
109139	Tal Kafri	HIV-1 gene expression in mouse cells
Approved	Summary: The aim of this experiment is to characterize HIV-1 gene expression in mouse cells with a recombinant HIV-1 based vector. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>109141</td><td>█████</td><td>Pseudotyping retroviral vectors (Update of 27773 - IM, SC and CNS injection added)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use recombinant, self-inactivating (SIN) lentiviral vectors to evaluate how different mouse proteins transduce cells or tissues in vitro and in vivo.

Committee Comments: The committee granted conditional approval pending amendment of the title, Section II Question 7 to include the current IACUC protocol number(s), Section III Question 1 to specify the injection site, and Section III Question 2 to specify the anesthetic method.</td></tr>
<tr><td>109142</td><td>█████</td><td>This is a 5-year renwal of Sch G: 16248. Role of cis elements and chromatin-modifying drugs in the lentiviral gene expression (3)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to is to improve transgene expression with lentiviral vectors by modifying cis elements in the viral genome and/or using chromatin-modifying drugs.

Committee Comments: The committee granted conditional approval pending amendment of the title and Section II Question 7 to include the current IACUC protocol number(s).</td></tr>
<tr><td>109144</td><td>█████</td><td>A safe Non-humanized mouse model for HIV-1 infection</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to develop a novel, non-humanized murine model to study HIV-1 in vivo. HIV particles will carry an ecotropic envelope that cannot mediate infection in human cells.

Committee Comments: The committee granted conditional approval pending amendment of Section II Question 7 to include the current IACUC protocol number(s) and Section III Question 1 to include IP injection volume.</td></tr>
<tr><td>108394</td><td>Premkumar Lakshmanane</td><td>Cloning and expression of recombinant proteins in E coli or mammalian cell culture for diagnostic and vaccine studies (arboviruses and coronaviruses)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to produce recombinant proteins derived from arboviruses and/or coronaviruses, and to evaluate their utility in serological assays.

Committee Comments: The committee granted conditional approval pending the inclusion of specific source arboviruses and coronaviruses.</td></tr>
</table>

108735	[redacted]	AAV gene therapy for hemophilia with inhibitors
Approved with Stipulations	Summary: The aim of this experiment is to study how therapeutic transgene products impact phenotypic correction in hemophilic mice that have pre-existing autoantibody against clotting factors (Inhibitors) Committee Comments: The committee granted conditional approval pending amendment of Section III Question 2 to specify the anesthetic method.	
108794	[redacted]	Development of an Effective Strategy to Block Nab Activity for AAV Brain Transduction
Approved	Summary: The aim of this experiment is to study the therapeutic potential that co-administration of protein M and specialized AAV vectors has in AAV pre-immunized mice. Committee Comments: The committee granted conditional approval pending amendment of Section III Question 1 to include injection volumes and Section III Question 2 to specify the restraint method for IP injections.	
108174	Douglas Phanstiel	Mechanisms and Functions of Dynamic Chromatin Looping During Differentiation
Approved	Summary: The aim of this experiment is to elucidate the mechanisms involved in chromatin loop formation and how formation of that structural motif impacts gene transcription. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

2. **Sub-Committee Review: 4**

   III-E

   i. **ID:** [redacted] **PI:** Frank Conlon **Title:** Genetic editing using CRISPR-Cas technology in Srl mice

   ii. **ID:** [redacted] **PI:** Li Qian **Title:** CRISPR/Cas9 mediated knockin of 3X Flag tag into the Carm1 C-terminal

   III-F

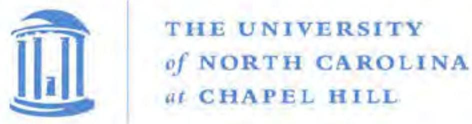
   i. **ID:** 108774 **PI:** Ronald Falk **Title:** Expression of MPO and PR3 in HEK-293 Cells

   ii. **ID:** 108494 **PI:** Andrew Lee **Title:** Chorismate Mutase

5. **Schedule H Report:** 28

6. **Next IBC meeting:** November 2, 2022

Adjourn.



IBC Meeting – Open Session Minutes
November 2, 2022 1:00 PM
Hybrid Conference

Members Present: Amanda Craigen, Catherine Brennan, Doug Cyr, Keith Porterfield, Ilana Galex, Matthew Hirsch, Rachael Turner, Rachel Graham, Shawn Hingtgen, Victoria Baxter

Members Absent: Barbara Savoldo, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Session

1. **Review minutes from October 5, 2022 meeting**
2. **Applications under review:**

<table>
<tr><th>ID</th><th>PI</th><th>Project Title</th></tr>
<tr><td>109775</td><td></td><td>Lamp3-mediated knockdown of human coronaviruses</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use siRNA constructs to knockdown Lamp3 expression in cells that will be infected with human coronaviruses.

<u>Committee Comments:</u> The committee granted conditional approval pending correction of Section II Question 15.</td></tr>
<tr><td>109776</td><td></td><td>BioID for MERS-CoV genes and open reading frames in the context of whole virus: 2022 renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to map interactions of MERS-CoV proteins in vitro via proximity labeling tags.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

109777		Characterizing nsp12 (RdRp) point mutations and drug sensitivity mutants in MERS-CoV: 2022 renewal
Approved with Stipulations	Summary: The aim of this experiment is to evaluate how an nsp12 mutation in the MERS-CoV its RNA dependent RNA polymerase (RdRp) impacts its sensitivity to certain drugs. Committee Comments: The committee granted conditional approval pending clarification on the anticipated "further downstream studies" referenced in Section II Question 2.	
109934		Assessment of the efficacy of drug and antibody interventions against the SARS-CoV-2 Omicron BA.5 variant
Approved	Summary: The aim of this experiment is to use *in vitro* and *in vivo* techniques to analyze the spike protein and assess the efficacy of monoclonal antibodies and small molecule inhibitors on the SARS-CoV-2 omicron BA.5 variant. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
109924	Richard Cheney	Myosin-X and the molecular basis of filopodia function: lentiviral vectors
Approved with Stipulations	Summary: The aim of this experiment is to investigate the function of Myosin-X(Myo10) by using lentiviral vectors to introduce reporter genes and/or modify the *in vitro* expression levels of Myo10. Committee Comments: The committee granted conditional approval pending amendment of Section II Question 8 to include a current list of anticipated target genes.	
109474	Amy Gladfelter	Transcriptional heterogeneity amongst syncytiotrophoblast nuclei
Approved	Summary: The aim of this experiment is to use lentiviral vectors with reporter genes to investigate how cell to cell fusion in the human placenta causes transcriptional changes in the syncytium. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

109714	[redacted]	Temporally restricted expression of transgenes to the mouse brain via intravenous injection of adeno associated viral vectors
Approved with Stipulations	Summary: The aim of this experiment is to use AAV vectors to deliver transgenes (Cre, STUB1 variants) *in vivo* or into *ex vivo* brain slices. Committee Comments: The committee granted conditional approval pending clarification regarding the discrepancy between the PI listed on the Schedule G (Brian Jensen) and the PI listed on the IACUC protocol (Jonathan Schisler).	
109154	[redacted]	Tumor development from transformed mouse tail fibroblast
Tabled	Summary: The aim of this experiment is to use lentiviral vectors to map factors that may affect host penetrance of tumor development. Committee Comments: The committee voted to table this protocol as insufficient information was provided for the intended animal work.	
109414	Tal Kafri	HIV-1 gene expression in mouse and human cells
Approved	Summary: The aim of this experiment is to characterize HIV-1 gene expression *in vitro* with a recombinant HIV-1 based vector. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
109594	Eduardo Lazarowski	Abnormal nucleotide release/metabolism in dehydrated airways
Approved with Stipulations	Summary: The aim of this experiment is to transfect human cells with lentiviral vectors to upregulate nucleotidase cDNA expression or deliver shRNAs targeting nucleotide release pathway genes. Committee Comments: The committee granted conditional approval pending amendment of Section II Question 8 to include more specific information. The PI withdrew their submission as it was an unnecessary renewal of an old protocol.	

109814	[redacted]	Doxycycline regulatory element for gene expression
Approved	Summary: The aim of this experiment is to assess AAV vector gene expression levels under a doxycycline regulated gene switch. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
109855	[redacted]	Capsid Shuffling/Directed Evolution of an Oligodendrocyte Precursor Cell-Preferring AAV Vector for In Vivo Neuronal Transdifferentiation
Approved with Stipulations	Summary: The aim of this experiment is to develop a novel AAV vector that exhibits a selective tropism for oligodendrocyte precursor cells *in vivo*. Committee Comments: The committee granted conditional approval pending receipt of a general construct map, amendment of the title to remove "neuronal transdifferentiation," and correction of Section II Question 7 to include the current IACUC protocol number.	
108674	[redacted]	Herpes Virus oncogenesis, latency and reactivation: Transformation by Epstein-Barr Virus
Approved	Summary: The aim of this experiment is to determine the oncogenic potential of EBV, HPV, and/or latent EBV genes; viral vectors will be used to express the latent EBV genes in cell lines. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
108934	[redacted]	Herpes Virus oncogenesis, latency and reactivation: Transformation by Epstein-Barr Virus
Approved with Stipulations	Summary: The aim of this experiment is to determine the oncogenic potential of EBV, HPV, EBV non-coding RNAs, and/or latent EBV genes; viral vectors will be used to express EBV latent genes and/or non-coding RNA. Committee Comments: The committee granted conditional approval pending amendment of Section II Question 8 to include more information about the non-coding RNAs.	

109294	[redacted]	Recombinant Influenza Virus H1N1 and H3N2 invitro inoculation of human bronchial cells
Approved with Stipulations	Summary: The aim of this experiment is to study how burn pit combustion products affect mature human bronchial cell cultures and their potential synergism with flu viruses. Two recombinant strains of influenza viruses will be used for these studies. Committee Comments: The committee granted conditional approval pending confirmation that this proposal is for *in vitro* work only and the removal of Section III.	
109494	[redacted]	In vivo screen of LUC mRNA nanoparticles
Approved with Stipulations	Summary: The aim of this experiment is to compare the activity of nanoparticle formulations *in vivo*. Nanoparticles will deliver synthetic LUC mRNA. Committee Comments: The committee granted conditional approval pending amendment of Section III Question 1 to include the inoculum concentration.	

3. **Sub-Committee Review: 7**

III-E

i. **ID:** 109874 **PI:** [redacted] **Title:** Rosa26-CAG-GFP1-10 Mouse Strain

ii. **ID:** 106442 **PI:** [redacted] **Title:** Biodistribution of Polystyrene Particles in SOD1-G93A mice

III-F

i. **ID:** 109922 **PI:** Richard Cheney **Title:** Myosin-X and the molecular basis of filopodia function: CRISPR cell lines

ii. **ID:** 109923 **PI:** Richard Cheney **Title:** Myosin-X and the molecular basis of filopodia function: PiggyBac transposase vectors

iii. **ID:** 109925 **PI:** Richard Cheney **Title:** Myosin-X and the molecular basis of filopodia function: plasmid construction and transfection

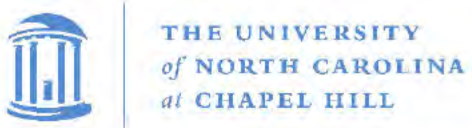
iv. **ID:** 108318 **PI:** Amy Gladfelter **Title:** Role of septins in curvature sensing

v. **ID:** 109554 **PI:** Leslie Hicks **Title:** Thimet oligopeptidase proteolytic characterization in Arabidopsis thaliana

5. **Schedule H Report:** 13

6. **Next IBC meeting:** December 14, 2022

Adjourn.



IBC Meeting Minutes
December 14, 2022 1:00 PM
Hybrid Conference

Members Present: Amanda Craigen, Ann Matthysse, Barbara Savoldo, Catherine Brennan, Doug Cyr, Keith Porterfield, Matthew Hirsch, Rachael Turner, Rachel Graham, Shawn Hingtgen, Victoria Baxter, Wil Lawson, William Bucha

Members Absent: Ilana Galex, Craig Fletcher

Ad hoc Members (not requested to be present): Stanley Lemon

Open Session

1. **Review minutes from November 2, 2022 meeting**
2. **Clinical Trials:**

   i. **Presentation**

   PI:
   Title:

   Status: Approved
   The IBC found the proposed containment and safety procedures to be adequate for the experimental design.

3. **Applications under review:**

ID	PI	Project Title
108318	Amy Gladfelter	Role of septins in curvature sensing
Approved with Stipulations	Summary: The aim of this experiment is to determine how septins sense membrane curvature in fungi (Ashbya gossypii. Aureobasidium pullulan, and Knufia petricola). Committee Comments: The committee granted conditional approval pending clarification on agrobacterium strain that will be used for the nucleic acid manipulations and addition of their daily decontamination procedures to the lab's Schedule F.	

<table>
<tr><td>110994</td><td></td><td>Reverse genetic construction of the Laos bat coronavirus BANAL-52</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to synthetically reconstruct the BANAL-52 virus to assess in vitro performance and compare antigenic profile to other circulating coronaviruses.

<u>Committee Comments:</u> The committee granted conditional approval pending amendment of the risk assessment to specify use and assessment of a baseline titer.</td></tr>
<tr><td>111054</td><td></td><td>SARS-CoV-2 expressing CD4 and CD8 epitopes from influenza nucleoproteins</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to compare antigen-specific T cell responses to SARSCoV-2 in vivo by expressing influenza nucleoprotein CD4 or CD8 epitope from SARS-2 accessory open reading frames (ORFs).

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>110695</td><td>Aravinda Desilva</td><td>Using Zika virus to identify Dengue cross-neutralizing epitopes</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to modify residues in the Zika Virus pre-membrane and envelope protein sequences.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>111435</td><td></td><td>Abrogation of Airway Epithelial Cell Barriers 2</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to optimize gene delivery to airway epithelial cells via electroporation. Three plasmid-based constructs will be assessed in vivo.

<u>Committee Comments:</u> The committee granted conditional approval pending the addition of eye/face protection to the PPE list in Section III Question 5.</td></tr>
</table>

110436	Stephanie Gupton	Uncovering the mechanisms linking netrin signaling, cytoskeletal dynamics and exocytosis in developing neurons
Approved	Summary: The aim of this experiment is to use TIRF microscopy to visualize and assess colocalization netrin receptors and signaling proteins, cytoskeletal proteins, and markers of exocytosis *in vitro*. Primary neurons will be transduced with adenovirus constructs and either imaged or lysed for biochemical assays. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
110234	[redacted]	Modulation of B7H3 in murine models of breast cancer
Approved	Summary: The aim of this experiment is to generate a murine breast cancer cell line with constitutive B7H3 to better model CAR-T cell therapy efficacy in humans. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
110074	Boa Kim	Glutaminase 1 (GLS1) overexpression to test the role of glutamine metabolism in cell function
Approved	Summary: The aim of this experiment is to overexpress GLS1 in cells via lentiviral transduction to test the role of GLS1 enzyme and glutamine metabolism in cellular function. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
111154	[redacted]	Endothelial lipid droplet metabolism in AAV8-PCSK9 induced atherosclerosis
Approved	Summary: The aim of this experiment is to test the role of endothelial cell lipid droplet metabolism in the progression of AAV-induced atherosclerosis. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
110174	Stanley Lemon	expression of tagged rhinovirus genes

Approved	Summary: The aim of this experiment is to use tagged (e.g., GFP, HA, Flag) rhinovirus proteins to assess their *in vitro* expression patterns with microscopy, immunoblots and proteomics. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
110194	Stanley Lemon	Mechanisms of replication in rhinovirus
Approved with Stipulations	Summary: The aim of this experiment is to identify and map the viral sequence(s) that define STING-dependent replication in rhinovirus proteins. RV-A and RV-B species clones will be used to evaluate STING dependence. Committee Comments: The committee granted conditional approval pending the inclusion of a risk assessment for this project.	
110214	Stanley Lemon	Rhinovirus tagging
Approved with Stipulations	Summary: The aim of this experiment is to tag rhinovirus proteins to assess their *in vitro* expression patterns with microscopy, immunoblots and proteomics. Molecular clones of RV-A, RV-B, and RV-C will be tagged within the reading frame of select non-structural genes. Committee Comments: The committee granted conditional approval pending the inclusion of a risk assessment for this project.	
110494	[redacted]	Gene Therapy of Hemophilia A and B - Lentivirus - 2022 Renewal
Approved	Summary: The aim of this experiment is to correct inherited bleeding disorders *in vivo* via gene therapy with lentiviral vectors. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

110495	[redacted]	AAV directed expression of FV or FX in dogs with hemophilia - 2022 Renewal
Approved	Summary: The aim of this experiment is to test the efficacy of AAV gene therapy to correct hemophilic coagulopathy *in vivo* via expression of FV or FX. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
110857	[redacted]	Herpes Virus oncogenesis, latency and reactivation: Transformation by Epstein-Barr Virus
Approved	Summary: The aim of this experiment is to determine the oncogenic potential of EBV genes, miRs, and lncRNAs. The EBV latent genes of interest will be cloned into a plasmid or viral vector to transfect mammalian cells for use *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
110870	[redacted]	Herpes Virus oncogenesis, latency and reactivation: Transformation by Epstein-Barr Virus (EBV)
Approved	Summary: The aim of this experiment is to determine the oncogenic potential of EBV, select EBV latent genes, and/or non-coding RNAs when introduced into a human papilloma virus (HPV) infected cell line Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
111014	[redacted]	Functional effects of cocaine self-administration on astrocytes
Approved with Stipulations	Summary: The aim of this experiment is to how calcium ion depletion within astrocytes via AAV vectors impacts cocaine seeking behavior *in vivo*. Additionally, AAV vectors will be used to express an adenosine biosensor to assess the effects of cocaine self-administration on evoked adenosine release from astrocytes. Committee Comments: The committee granted conditional approval pending additional information regarding the constructs and target genes.	

110274	[redacted]	Evaluation of the Anti-tumor Response after Bone Marrow Transplant
Approved with Stipulations	Summary: The aim of this experiment is to. We will use P815 mastocytoma cells that express luciferase to allow us to image tumor growth in mice after bone marrow transplantation Committee Comments: The committee granted conditional approval pending the addition of eye/face protection to the PPE list in Section III Question 5.	
110294	[redacted]	Tumor Studies in Mice Undergoing Bone Marrow Transplantation
Approved with Stipulations	Summary: The aim of this experiment is to. immunity To evaluate if mice after bone marrow transplant have intact anti-tumor Committee Comments: The committee granted conditional approval pending the addition of eye/face protection to the PPE list in Section III Question 5.	
110354	[redacted]	Optogenetic (Channel- and Halo-rhodopsin; ChR2 & NpHR) and Chemogenetic (designer receptors exclusively activated by designer drugs DREADDS) Manipulation of Neural Circuits underlying social impairments in mouse models for ASD
Approved with Stipulations	Summary: The aim of this experiment is to. To infuse AAVs containing either ChR2, NpHR, or DREADDS Committee Comments: The committee granted conditional approval pending clarification of the ASD acronym.	

3. **Sub-Committee Review:** 3

III-E

i. **ID:** 111034 **PI:** [redacted] **Title:** Ex447-KLKB1 Mouse

III-F

i. **ID:** 110981 **PI:** Sarah Cohen **Title:** Organelle function and dynamics

ii. **ID:** 110714 **PI:** Isis Trujillo-Gonzalez **Title:** Transfection of Cultured Cells with DNA Constructs

5. **WCG Report:** 1

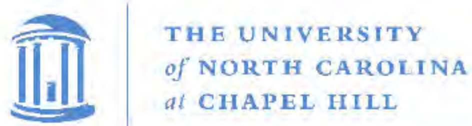
i. **PI:** [redacted]
Title: [redacted]

Status: Approved

6. **Schedule H Report:** 26

7. **Next IBC meeting:** January 11, 2022

Adjourn.



IBC Meeting Minutes
January 11, 2023 1:00 PM
Web Conference

Members Present: Amanda Craigen, Barbara Savoldo, Catherine Brennan, Doug Cyr, Ilana Galex, Keith Porterfield, Matthew Hirsch, Rachael Turner, Rachel Graham, Shawn Hingtgen, Victoria Baxter, Wil Lawson, William Bucha

Members Absent: Craig Fletcher

Ad hoc Members (not requested to be present): Ann Matthysse, Stanley Lemon

Open Session

1. **Review minutes from December 14, 2022 meeting**
2. **Applications under review:**

ID	PI	Project Title
112195	[redacted]	Assessment of the efficacy of drug and antibody interventions against the SARS-CoV-2 Omicron BA.2.12.1 variant
Approved	Summary: The aim of this experiment is to assess the efficacy potential interventions against COVID-19 and evaluate the SARS-CoV-2 omicron spike protein. Experiments will be performed with modified versions of the lab's mouse-adapted and D614G nLuc reporter SARS-CoV-2 strains that express the omicron spike protein. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
112196	[redacted]	Venezuelan Equine Encephalitis (VEE) BSL2 strain 3526 replicon expression of variant norovirus and sapovirus capsid genes: 2022 Renewal
Approved with Stipulations	Summary: The aim of this experiment is to characterize human norovirus and sapovirus using virus-like particles (VLPs) expressing their capsid proteins with variation in the predicted binding domains. VLPs will be used to evaluate the ligand and antibody binding profiles to inform susceptible population estimates and vaccine design. Committee Comments: The committee granted conditional approval pending update of the IACUC protocol number listed in Section II Question 7.	

111374		Using caspase to elucidate the functional of anatomical circuits during a goal-directed behaviour
Approved with Stipulations	Summary: The aim of this experiment is to elucidate the neural components underlying goal-directed movement by studying neural circuits related to reaching and walking movements *in vivo*. Committee Comments: The committee granted conditional approval pending amendment of Section 3 Question 5 to describe BSC use, and correction of Section II Question 10.	
111534		Lentiviral vectors to study immune responses
Tabled	Summary: The aim of this experiment is to identify key genes related to immune defense systems by manipulating T cells or hematopoietic stem cells (HSCs) with lentiviral vectors *in vivo.* Committee Comments: The committee voted to table this submission until the February 2023 meeting due to insufficient information about the intended target genes and animal work.	
111477	Nathaniel Moorman	lentiviral or retroviral mediated gene transfer into human cells to study the mechanisms of virus replication
Approved	Summary: The aim of this experiment is to study mechanisms of virus replication *in vitro* using replication-defective retro- and lentiviral vectors to modulate target genes (human genes, HCMV genes) via induced expression or shRNA delivery. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
111478	Nathaniel Moorman	Construction of mammalian expression vectors for host and viral proteins to measure the effect of over-expression on virus replication
Approved	Summary: The aim of this experiment is to use mammalian expression vectors *in vitro* to study how the over-expression of target genes (human proteins, HCMV proteins) impact viral replication. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>111479</td><td></td><td>Temporally restricted expression of transgenes to the mouse brain via intravenous injection of adeno associated viral vectors</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to replace the use of CRE transgenic mice with AAV vectors. AAV vectors will be used to deliver CRE recombinase or C-terminus of HSC70 Interacting Protein (CHIP) variants during in vivo and ex vivo studies.

Committee Comments: The committee granted conditional approval pending the addition of full DCM PPE to Section III Question 5.</td></tr>
<tr><td>111495</td><td></td><td>The role of CHIP in health and disease (adenovirus)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to study CHIP's regulatory role in cellular metabolism, stress response, and cellular degeneration. Adenoviral vectors will be used to transduce cells that are difficult to transfect via lipid-based delivery systems

Committee Comments: The committee granted conditional approval pending confirmation that animal work is not part of this proposal, and removal of the IACUC protocol number from Section II Question 7.</td></tr>
<tr><td>111254</td><td></td><td>Excitatory and Inhibitory Chemogenetic and Optogenetic Manipulations (Rats)</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to precisely modulate neuronal populations via specific opsins, DREADDs, or caspase. The chemogenetic and optogenetic receptors will be delivered in vivo with viral vectors.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>111454</td><td></td><td>mRNA-based flavivirus vaccines</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to evaluate the in vivo immunogenicity profile of mRNA-based flavivirus subunit vaccines formulated in lipid nanoparticles.

Committee Comments: The committee granted conditional approval pending the addition of full DCM PPE to Section III Question 5.</td></tr>
</table>

3. **Discussion:**

   i. Rabies Pre-Exposure Prophylaxis

   ii. Incident Reports

   iii. 2022 Summary

238 Schedule Gs	185 Full Committee Review 53 Sub-Committee Review
10 Clinical Trials	9 UNC IBC Review 1 WCG IBC Review
246 Schedule Hs	

4. **NIH Reportable Incidents:** 2

   i. **DOI 11-30-2022:** Amanda Craigen presented on a potential exposure to recombinant DNA at BSL-2 on 11/30/22 involving a finger puncture with equipment used on mice transfected with lentiviral vector modified to express the firefly luciferase under the control of a liver specific promoter (hAAT).

   ii. **DOI 12-13-2022:** Amanda Craigen presented on a potential exposure to recombinant DNA at BSL-2 on 12/13/2022 involving a spill containing recombinant vaccine strain of Chikungunya virus.

5. **WCG IBC Review:** 0

6. **Sub-Committee Review:** 4

   III-E

   i. **ID:** 111694 **PI:** [redacted] **Title:** Ex448-Ex449-Lmna-Mutant Mice

   ii. **ID:** 111494 **PI:** [redacted] **Title:** Creation of a Lox-Stop-Lox Cre-inducible Stub1 mouse using CRISPR technology

   III-F

   i. **ID:** 111294 **PI:** Rebecca Berlow **Title:** Structural and Functional Characterization of Intrinsically Disordered Proteins and Metabolic Enzymes

   ii. **ID:** 111496 **PI:** Jonathan Schisler **Title:** The role of CHIP in health and disease (plasmid DNA)

7. **Schedule H Report:** 12

8. **Next IBC meeting:** February 1, 2023

Adjourn.

Meeting Minutes
February 01, 2023 1:00 PM
Hybrid Conference

Members Present: Amanda Craigen, Barbara Savoldo, Doug Cyr, Rachael Turner, Rachel Graham, Shawn Hingten, William Bucha, Wil Lawson

Members Absent: Catherine Brennan, Craig Fletcher, Ilana Galex, Keith Porterfield, Victoria Baxter

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from January 11, 2022 meeting**
2. **Applications under review:** 14

<table>
<tr><th>ID</th><th>PI</th><th>Project Title</th></tr>
<tr><td>112197</td><td>Ralph Baric</td><td>NL63 recombinant viruses expressing nano Luciferase (NLuc) or Cre recombinase</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to generate a recombinant NL63 coronaviruses that expresses either nano Luciferase or Cre recombinase in place of open reading frame 3 (ORF3).

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>112574</td><td></td><td>Transfection of Fetal Liver Cells</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to study CalDAG-GEFI's role in platelet activation. A retroviral vector will be used to transfect murine fetal liver cells and induce mutant CalDAG-GEFI expression. Successfully transfected cells will be used for further study in vivo.

<u>Committee Comments:</u> The committee granted conditional approval pending comments from one of the IBC's animal use experts.</td></tr>
</table>

111695	Frank Conlon	Lentiviral transduction in cells
Approved with Stipulations	Summary: The aim of this experiment is to use lentiviral vectors to induce reporter gene expression *in vitro.* Committee Comments: The committee granted conditional approval pending the PI's signature and amendment of the title to better represent the proposed work.	
112479	Doug Cyr	Study of Hsp70/Hsp40 function in ER Protein Quality Control
Approved	Summary: The aim of this experiment is to study how loss of Hsp70 and Hsp40 chaperone function impacts the folding of membrane proteins. A lentiviral CRISPR system will be used to inactivate the target genes *in vitro*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
112234	[redacted]	Luciferase (Lux)-expressing Bacille Calmette Guerin (BCG)
Approved with Stipulations	Summary: The aim of this experiment is to use a reporter strain of BCG to monitor replication and dissemination *in vivo* in humanized mice. Committee Comments: The committee granted conditional approval pending comments from one of the IBC's animal use experts and discussion with UEOHC regarding potential medical surveillance for work with BCG.	
112315	[redacted]	HIV-specific Chimeric Antigen Receptor (CAR) T-Cell Vectors for HIV Eradication
Approved	Summary: The aim of this experiment is to evaluate HIV-specific CAR T-cells in humanized mice. HIV-specific CAR constructs will be used to generate replication incompetent vectors *in vitro* for use *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>111754</td><td>Mark Heise</td><td>Identification and Characterization of Conserved Alphavirus Functional Domains</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to identify potential antiviral targets by locating conserved domains in alphavirus replicase proteins. Chikungunya virus will be used to evaluate conserved domains for functional importance, and functionally important domains will be tested in other alphaviruses.

<u>Committee Comments:</u> The committee granted conditional approval pending the addition of a risk assessment statement to Section II Question 2.</td></tr>
<tr><td>111755</td><td></td><td>Analysis of RNA structural determinants for their impact on Chikungunya virus replication and viral protein synthesis</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to disrupt RNA secondary structures in Chikungunya virus and assess the impact mutations have on viral fitness in vitro.

<u>Committee Comments:</u> The committee granted conditional approval pending the addition of a risk assessment statement to Section II Question 2.</td></tr>
<tr><td>112214</td><td>Tal Kafri</td><td>Lentiviral vector packaging cassette renewal of 42782</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to generate an improved packaging cassette for lentiviral vectors by substituting the current gag sequence in lentiviral constructs with the gag region from the NDK strain of HIV.

<u>Committee Comments:</u> The committee granted conditional approval pending amendment of the title to better represent the proposed work.</td></tr>
<tr><td>111534</td><td></td><td>Lentiviral vectors to study immune responses</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to identify key genes related to immune defense systems by manipulating T cells or hematopoietic stem cells (HSCs) with lentiviral vectors for study in vivo.

<u>Committee Comments:</u> The committee granted conditional approval pending comments from one of the IBC's animal use experts.</td></tr>
</table>

112654	[redacted]	Aggregation of mRNA nanoparticles for ImmornaBio studies
Approved with Stipulations	Summary: The aim of this experiment is to study mRNA nanoparticle formulations *in vivo*. Committee Comments: The committee granted conditional approval pending comments from one of the IBC's animal use experts and the amendment of Section III Question 1 to include route-specific dosing information.	
111794	[redacted]	SARS-CoV-2 VSVg Pseudovirus
Tabled	Summary: The aim of this experiment is to investigate the effect of peptide-based therapies on viral infection. Committee Comments: The committee voted to table this submission until the March 2023 meeting due to insufficient information about the construct, proposed manipulations, and anticipated risks associated with the vector.	
111834	[redacted]	rgRSV124
Tabled	Summary: The aim of this experiment is to investigate the effect of peptide-based therapies on enveloped viral infection. Committee Comments: The committee voted to table this submission until the March 2023 meeting due to insufficient information about the construct, proposed manipulations, and anticipated risks associated with the vector.	
111835	Robert Tarran	EV-D68 (US/MO/14-18947)
Tabled	Summary: The aim of this experiment is to investigate the effect of peptide-based therapies on non-enveloped viral infection. Committee Comments: The committee voted to table this submission until the March 2023 meeting due to insufficient information about the construct, proposed manipulations, and anticipated risks associated with the vector.	

3. Discussion:

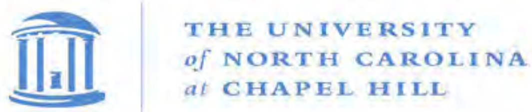
a. UNC SARS-CoV-2 Biosafety Guidance Review

4. **NIH Reportable Incidents:** 0
5. **WCG IBC Review:** 0
6. **Sub-Committee Review:** 1

   III-F

   i. **ID:** 112478 **PI:** Doug Cyr **Title:** Mechanism for degradation of membrane proteins by ER chaperones
7. **Schedule H Report:** 20
8. **Next IBC meeting:** March 1, 2023

Adjourn



Meeting Minutes
March 1, 2023 1:00 PM
Hybrid Conference

Members Present: Amanda Craigen, Barbara Savoldo, Catherine Brennan, Christopher Broberg, Doug Cyr, Ilana Galex, Keith Porterfield, Rachael Turner, Rachel Graham, Victoria Baxter, William Bucha

Members Absent: Craig Fletcher, Shawn Hingten, Wil Lawson

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from February 1, 2022 meeting**
2. **Clinical Trials**
   i. **Presentation**
      PI:
      Title:
      Status: Approved
3. **Applications under review:**

ID	PI	Title
112514	Ralph Baric	Generation of pseudotyped vesicular stomatitis viruses (VSV) expressing SARS-CoV-2 and BANAL-236 spike proteins
Approved	Summary: The aim of this experiment is to generate recombinant VSV pseudoviruses encoding the spike protein from either SARS-CoV-2 or BANAL-234, and to develop the pseudovirus for use as a BSL-2 alternative to SARS-CoV-2 and BANAL-236, viruses which typically require BSL-3 precautions. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

113218		CRISPR-modified tumor cells injected into mice to study cancer
Approved	Summary: The aim of this experiment is to study the role of target genes related to tumor development by using a lentiviral CRISPR system to create knockouts in human or mouse cancer cell lines. The recombinant cells will be used for *in vivo* studies. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
113134	Adriana Beltran Lopez	Generation of induced pluripotent stem cells
Approved with Stipulations	Summary: The aim of this experiment is to reprogram stem cells using a Sendai viral vector to induce endogenous expression of certain pluripotent genes. Committee Comments: The committee granted conditional approval pending verification that this proposal only covers *in vitro* experiments.	
113178		Implantation of TCR transductants CD8+ T cells into BLT (Bone Marrow, Liver, Thymus) mice
Approved	Summary: The aim of this experiment is to generate a pseudotyped lentiviral vector to transduce CD8+ T cells with different T cell receptor (TCR) genes, and to use the modified cells for *in vivo* studies. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
113054	Martina Gentzsch	Interactions of the epithelial sodium channel (ENaC) and the epithelial chloride channel (CFTR)
Approved	Summary: The aim of this experiment is to use a lentiviral vector to generate cells expressing the ENaC and/or CFTR for *in vitro* study of the regulation and interactions of these ion channels. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>112994</td><td>Erin Heinzen Cox</td><td>Generation of Ngn2 expressing human induced pluripotent stem cell lines.</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to generate induced pluripotent stem cells that express Ngn2 with lentiviral vectors.

Committee Comments: The committee granted conditional approval pending clarification on Section 2 Question 9.</td></tr>
<tr><td>112554</td><td></td><td>Expression of Cre recombinase in astrocytes</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to facilitate Cre-dependent manipulations of astrocytes in vivo by using AAV vectors to express Cre recombinase and mCherry.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>113274</td><td>Nancie Maciver</td><td>Metabolic reprogramming to improve EGFRvIII CAR T cell persistence</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to analyze the impact that Drp1 knockout has on CAR T cell metabolism. Retroviral vectors will be used to generate CAR T cells with Drp1 KO cells.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>113234</td><td></td><td>Molecular Mechanisms of Histoplasma pathogenesis</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to study the evolution of virulence factors in Histoplasma capsulatum by using plasmids to transfer genes of interest. Modified H. capsulatum will be used to assess test the role of target genes in its pathogenicity using model systems of infection.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

<table>
<tr><td>113056</td><td>Samantha Pattenden</td><td>Using lentivirus to transduce HEK293T mammalian cells with siRNAs targeting the WIZ zinc finger RNA and non-targeting siRNAs (control) for studying the role of the G9a/GLP/WIZ methyltransferase complex in epigenetic regulation</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to study the G9a/GLP/WIZ methyltransfrase complex by using siRNAs and lentiviral vectors to knock out the WIZ or G9 gene in vitro.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>113255</td><td></td><td>Sendai Virus Infection of airway epithelium in vitro and in vivo</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to monitor the extent and duration of infection of a recombinant Sendai Virus. The recombinant virus will express either reporter genes or CRE, and studies will be performed both in vitro and in vivo.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>113154</td><td>Scott Randell</td><td>Markers for cells engrafted in tissue culture experiments</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use lentiviral vectors to label cells and study their behavior after engraftment in vitro.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>113094</td><td>Ronald Swanstrom</td><td>General Protocol for CRISPR-based genetic knockouts to validate novel host restriction factors for HIV-1</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to identify novel host restriction factors for HIV-1 in myeloid cells by using a lentiviral CRISPR system to generate knockout cells lines.

<u>Committee Comments:</u> The committee granted conditional approval pending amendment of Section 2 Question 8 to include the specific target genes of interest.</td></tr>
</table>

111834		Using rgRSV124 in HEP2/HEK293T cell lines, primary normal human bronchial epithelial cells and in vivo mouse models to study the antiviral effects of peptides identified in the airway against RSV
Approved	Summary: The aim of this experiment is to investigate the efficacy of peptide-based therapies on non-enveloped viral infection. This proposal specifically covers *in vitro* and *in vivo* studies with rgRSV124. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
111835	Robert Tarran	Using EV-D68 in Rhabdomyosarcoma muscle cells, neuroblastoma cells SH-SY5Y and primary human bronchial epithelial cells to study the antiviral effects of peptides identified in the airway against a non-enveloped virus.
Approved with Stipulations	Summary: The aim of this experiment is to investigate the efficacy of peptide-based therapies on non-enveloped viral infection. This proposal specifically covers *in vitro* studies with the recombinant enterovirus EV-D68 (strain US/MO/14-18947). Committee Comments: The committee granted conditional approval pending amendment to the risk assessment in Section 2 Question 2 to include the specific metrics that will be monitored.	
112674		Systematic in vivo characterization of genes involved in neuropsychiatric disorders
Approved	Summary: The aim of this experiment is to elucidate cellular mechanisms underlying neuropsychiatric disorders by using a CRISPR guide library to induce targeted knockouts within the brain *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
112771		In utero electroporation of plasmids containing guide RNAs
Approved	Summary: The aim of this experiment is to target the antisense transcript of the paternal allele of UBE3A by introducing plasmids encoding guide RNAs *in utero*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

4. **Discussion:** n/a
5. **NIH Reportable Incidents:** 0
6. **WCG IBC Review:** 1
   i. **PI:** [redacted] **Title:** [redacted]
   Status: Application submitted; Revisions in-progress as of February 21, 2023
7. **Sub-Committee Review:** 10

III-D

i. **ID:** 112894 **PI**: [redacted] **Title:** Fiber Photometry - real-time measurement of population-level neuronal activity in the PrL during alcohol self-administration

ii. **ID:** 112894 **PI**: [redacted] **Title:** Using recombinant Vesicular stomatitis virus in which the glycoprotein is deleted, and SARS-CoV-2 Spike plasmid is added (rVSV-DG-Spiked pseudotyped virus) in mammalian cells to study the effects of our peptides in reducing viral entry and load in mammalian cells and rodents.

III-E

i. **ID:** 113354 **PI**: [redacted] **Title:** Ex450-KLKB1 Mouse

ii. **ID:** 113294 **PI**: [redacted] **Title:** Targeting hybrid PRTN3 transgene to humanize the mouse Prtn3 gene

iii. **ID:** 113157 **PI**: [redacted] **Title:** Generation of FOLR2 floxed mouse

iv. **ID:** 113295 **PI**: [redacted] **Title:** TARGETED Tacatd2 (TROP2) CRISPER/Cas-mediated Genome Engineering:

III-F

i. **ID:** 112701 **PI**: Maria Azcarate-Peril **Title:** Microbiome Core and Research Lab

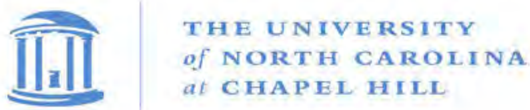
ii. **ID:** 113135 **PI**: Adriana Beltran Lopez **Title:** Generation of reporter lines

iii. **ID:** 112454 **PI**: Andrew Lee **Title:** Thymidylate Synthase

iv. **ID:** 113057 **PI**: Samantha Pattenden **Title:** Transient and stable transfections of plasmid DNA in HEK293T cells to study histone methyltransferase biology

7. **Schedule H Report:** 15
8. **Next IBC meeting:** April 5, 2023

Adjourn.



Meeting Minutes
April 20, 2023 1:00 PM
Web Conference

Members Present: Victoria Baxter, Catherine Brennan, Christopher Broberg, Amanda Craigen, Doug Cyr, Rachel Graham, Matthew Hirsch, Wil Lawson, Barbara Savoldo, Rachael Turner

Members Absent: William Bucha, Craig Fletcher, Shawn Hingten, Ilana Galex, Keith Porterfield

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from March 1, 2022 meeting**
2. **Applications under review:**

ID	PI	Title
113394	[redacted]	Evaluation of the SARS-CoV-2 Omicron BQ.1.1 subvariant
Approved with Stipulations	Summary: The aim of this experiment is to express the spike gene from the BQ.1.1 SARS-CoV-2 subvariant on the lab's mouse adapted (MA) strain. The resulting construct will be used to characterize the *in vivo* pathogenicity of BQ.1.1 and to evaluate the therapeutic efficacy of interventions *in vitro* (e.g., monoclonal antibodies, sera from vaccinated donors). Committee Comments: The committee granted approval pending re-labeling of the provided vector map with a key for the symbols.	
113395	[redacted]	Evaluation of the SARS-CoV-2 Omicron XBB.1 and XBB.1.5 subvariants
Approved with Stipulations	Summary: The aim of this experiment is to express the spike gene from the XBB.1 and XBB.1.5 SARS-CoV-2 subvariant on the lab's mouse adapted (MA) strain. The resulting construct will be used to characterize the *in vivo* pathogenicity of BQ.1.1 and to evaluate the therapeutic efficacy of interventions *in vitro* (e.g., monoclonal antibodies, sera from vaccinated donors). Committee Comments: The committee granted approval pending re-labeling of the provided vector map with a key for the symbols.	

113874	[redacted]	Generation of a cDNA infectious clone system for a bat MERS-like coronavirus (MjHKU4r-CoV-1)
Approved with Stipulations	Summary: The aim of this experiment is to further the labs effort to develop pan-coronavirus vaccines and antivirals with new data generated from *in vitro* experiments with an infectious clone or MjHKU4r-CoV-1. A published sequence by Chen et al. will be used to synthetically reconstruct MjHKU4r-CoV-1. Committee Comments: The committee granted approval pending an amendment to the risk mitigation statement in Section II Question 2 to include the anticipated baseline titer for the proposed construct based on data from *in vitro* MERS studies.	
113876	[redacted]	Generation of a full-length infectious clone of bat [redacted] like coronavirus WIV16-CoV, including reporter expressing variants: 2023 renewal
Approved	Summary: The aim of this experiment is to generate an infectious clone of the [redacted]-like coronavirus W1V16-CoV. This construct was successfully developed and will be maintained for potential use in future studies. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
113877	[redacted]	Coronavirus Transcription Regulatory Network Remodeling and Its Effects on Replication and Virulence: 2023 renewal
Approved	Summary: The aim of this experiment is to study how introducing novel transcription regulatory sequences (TRS) into transcription regulatory networks (TRN) impacts the replication and virulence of betacoronaviruses ([redacted] MERS-CoV, HKU3, HKU5, WIV-1, SHC-014, and WIV-16). Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
113434	[redacted]	Genetically modified macrophages and engineered Peripheral Blood Macrophages (PBM)
Approved	Summary: The aim of this experiment is to study human glial cell derived neurotrophic factor (GDNF) protein expression *in vivo* using modified macrophages. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>111695</td><td>Frank Conlon</td><td>Lentiviral transduction in cells</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to package lentiviral vectors encoding GFP, microRNA-871, microRNA-183, or Brachyury and infect human cell lines and primary murine cardiomyocytes in vitro.

Committee Comments: The committee granted approval pending amendment to the protocol’s title and clarification in Section II Question 5 regarding the target gene variants.</td></tr>
<tr><td>113774</td><td></td><td>Tau seeding in alcohol drinking mice</td></tr>
<tr><td>Approved with Stipulation</td><td colspan="2">Summary: The aim of this experiment is to study the impact of alcohol consumption on in vivo tau propagation and tau pathology by using reporter vectors on an adeno-associated virus 2/8 backbone.

Committee Comments: The committee granted approval pending verification from the lab that vector manipulations and injection preparations will be performed in a BSC.</td></tr>
<tr><td>91778</td><td>Hua Mei</td><td>Study the functions of a list of genes regulating corneal regeneration and wound healing</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to modulate the expression of genes related to corneal regeneration and wound healing in primary human corneal cells to study changes in cell proliferation, stemness, differentiation, and fibrosis.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>113521</td><td></td><td>Targeting Mechanisms of Cancer Metastasis</td></tr>
<tr><td>Approved with Stipulation</td><td colspan="2">Summary: The aim of this experiment is to investigate certain gene targets and their role in cancer metastasis. Target gene expression will be modulated with microRNA, small interfering RNA, small hairpin RNA, CRISPR/Cas9, and/or lentiviral constructs.

Committee Comments: The committee granted approval pending additional details regarding the role of the company mentioned in the protocol and any related Facility Use Agreements.</td></tr>
</table>

<table>
<tr><td>113518</td><td>Scott Randell</td><td>Cystic Fibrosis Research and Translational Core Center: Core C Cell Models Core</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to extend cell life of primary human airway cells by expressing specific proteins along with selection agent resistance and a fluorescent marker protein. Vesicular stomatitis virus (VSV) pseudotyped lentiviral vectors will be used to alter in vitro gene expression.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design. Duplicate protocols submitted under different titles to correspond with the PI’s grants.</td></tr>
<tr><td>113519</td><td>Scott Randell</td><td>Identification of Airway Epithelial Stem Cells</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to extend cell life of primary human airway cells by expressing specific proteins along with selection agent resistance and a fluorescent marker protein. Vesicular stomatitis virus (VSV) pseudotyped lentiviral vectors will be used to alter in vitro gene expression.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design. Duplicate protocols submitted under different titles to correspond with the PI’s grants.</td></tr>
<tr><td>113520</td><td>Scott Randell</td><td>Research and Development Program Component II: Epithelial Function in Cystic Fibrosis</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to characterize the phenotypic outcome in vitro that targeted gene knockouts or induced expression of target genes human airway epithelial cells.

<u>Committee Comments:</u> The committee granted approval pending the removal of “or another gene of interest yet to be specified” from the answer to Section II Question 8.</td></tr>
<tr><td>113554</td><td>David Williams Jr</td><td>Inhibition of MBD2-NuRD function</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to disrupt the recruitment and function of the MBD2-NuRD complex by using lentiviral vectors to express small peptides in vitro which blocking binding or induce degradation.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

3. **Discussion:** n/a

4. **NIH Reportable Incidents:** 0

5. **WCG IBC Review:** 1

   i. **PI:** [redacted] **Title:** [redacted]

   Status: Application submitted; Revisions in-progress as of February 21, 2023

6. **Sub-Committee Review:** 10

   III-D

   i. **ID:** 114094 **PI**: [redacted] **Title:** Non-replicating adenoviral vector induced knock out studies for the improvement of T cell immunotherapies for cancer

   III-E

   i. **ID:** 113875 **PI**: [redacted] Generation of C3 knockout mice via CRISPR

   ii. **ID:** 113754 **PI**: [redacted] **Title:** vPK/ORF36 mice

   iii. **ID:** 113334 **PI**: [redacted] **Title:** Developmental genetics in Steptocarpus

   iv. **ID:** 113679 **PI**: [redacted] **Title:** Tet2(H1803R) Knock-in Mouse

   i. **ID:** 113680 **PI**: [redacted] **Title:** Invivofectamine 3.0/ USP15 siRNA complex

   III-F

   i. **ID:** 113661 **PI**: Jill Dowen **Title:** Building cell lines for degradation of cohesin and labeling the cohesin interactome

   ii. **ID:** 113654 **PI**: Whitney Edwards **Title:** Elucidating the role of protein lipidation pathways in cardiac development and disease

   iii. **ID:** 113856 **PI**: Dale Ramsden **Title:** DNA Double Strand Break Repair

   iv. **ID:** 113555 **PI**: David Williams Jr **Title:** Structural and biophysical characterization of the NuRD complex

7. **Schedule H Report:** 15

8. **Next IBC meeting:** May 3, 2023

Adjourn.

Meeting Minutes
June 7, 2023 1:00 PM
Hybrid Conference

Members Present: Amanda Craigen, Barbara Savoldo, Catherine Brennan, Christopher Broberg, Doug Cyr, Ilana Galex, Keith Porterfield, Rachael Turner, Rachel Graham, Victoria Baxter, William Bucha

Members Absent: Craig Fletcher, Shawn Hingten, Wil Lawson

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from May 03, 2023 meeting**
2. **Applications under review:** 17

ID	PI	Title
116688	Ralph Baric	Recombinant vaccine strain of Chikungunya virus for use in RT-PCR assays
Approved	Summary: The aim of this experiment is to grow a recombinant vaccine strain of Chikungunya virus to use as a positive control in RT-PCR assays. This virus will not be manipulated beyond growth of stocks and as an experimental control. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
116710	[redacted]	Creation of full-length infectious clones of wild-type West Nile virus (WNV) and Usutu virus (USUV) expressing reporter genes (nanoluciferase)
Approved	Summary: The aim of this experiment is to facilitate measurement and mapping of antibody neutralization by create two recombinant flaviviruses that express reporter proteins. The constructs will be used for experiments *in vitro* and *in vivo*. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>116711</td><td>[redacted]</td><td>Recombinant Influenza viruses for antibody cross-reactivity studies</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to obtain reconstituted viral stocks of recombinant PR8 influenza viruses for use in vivo to test antibody cross-reactivity.

Committee Comments: The Committee granted approval pending amendment to Section 2 Question 10 to clarify the constructs replication competence, and Section 3 Question 5 to clarify DCM PPE will be worn.</td></tr>
<tr><td>116876</td><td>Ralph Baric</td><td>Expression of the Appalachian Ridge Coronavirus spike in HKU5: 2023 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to generate recombinant coronavirus constructs which express the Appalachian Ridge CoV spike in the HKU5 background.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>116877</td><td>[redacted]</td><td>Generation of replicons from the attenuated 3526 strain of Venezuelan Equine Encephalitis (VEE) expressing the Spike glycoproteins from either an HKU 2-related coronavirus or Appalachian Ridge coronavirus: 2023 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to produce VEE-3526 replicon particles expressing the spike glycoprotein from an HKU 2-related coronavirus or the Appalachian Ridge coronavirus. These replicon particles will be used in vitro and in vivo as vaccine candidates and/or for antibody production.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>116878</td><td>[redacted]</td><td>Reverse genetic clones for HKU 2 Bat Coronaviruses: 2023 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to use reverse genetic clones of HKU 2 bat coronaviruses to evaluate their capacity to infect available human, swine, and bat cells under different conditions. This proposal only covers work in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

<table>
<tr><td>115534</td><td></td><td>Kaposi Sarcoma-associated Herpesvirus (KSHV) deletion viruses – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to generate KSHV constructs with targeted deletions for use in vivo.

Committee Comments: The Committee granted approval pending amendment of Section 2 Question 2 to include a quantitative risk assessment statement, Section 2 Question 11 to add animal strains, Section 3 Question 1 to include dosing information, Section 3 Question 3 to clarify restraint procedure, Section 3 Question 5 to list required PPE, and Section 3 Question 6 with additional clarification about experiments.</td></tr>
<tr><td>116315</td><td></td><td>Luciferase expressing human cell lines – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to generate human lymphoma cell lines that express the luciferase gene for use in vivo.

Committee Comments: The Committee granted approval pending amendment of Section 3 Question 5 to list required PPE and confirmation that dosing information in Section 3 Question 1 is identical for both IP and IV exposures.</td></tr>
<tr><td>115347</td><td>Mark Heise</td><td>An intrinsically safe, biologically constrained system for identifying and testing antiviral drug resistance mutants.</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to develop a biologically constrained alphavirus replicon system to study drug resistance. The replicons will lack alphavirus structural proteins, rendering it incapable of producing infectious virus containing resistance mutations.

Committee Comments: The Committee granted approval pending amendment of the title to clarify alphavirus replicons are the agent of interest.</td></tr>
<tr><td>116294</td><td></td><td>Continuation of 46163 CNS and systemic delivery of safer 5th generation lentiviral vectors with reduced viral sequence including opposite orientation</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to generate safer lentiviral vectors with decreased potential to recombine or affect host gene expression. This proposal focuses on producing constructs with limited parental viral sequence in the vector genome or which use a vector cassette to generate RNA structures capable of silencing undesired protein expression.

Committee Comments: The Committee granted approval pending amendment of Section 2 Question 8 to clarify miRNA targets, Section 3 Question 3 to include anesthesia method, and Section 3 Question 5 to include eye protection and sleeves in the PPE list.</td></tr>
</table>

<table>
<tr><td>116295</td><td>[redacted]</td><td>Continuation of 47102 A safe Non-humanized mouse model for HIV-1 infection</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to continue using the approved constructs in vitro and in vivo to generate a safe non-humanized mouse model for HIV-1 infection.

<u>Committee Comments:</u> The Committee granted approval pending amendment of Section 3 Question 5 to include sleeves in the PPE list.</td></tr>
<tr><td>116326</td><td>[redacted]</td><td>Recombinant Sendai Viruses expressing additional genes from other viruses.</td></tr>
<tr><td>Tabled</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to generate recombinant Sendai Viruses for study in vivo.

<u>Committee Comments:</u> Form may have been re-submitted unintentionally. The committee voted to table this protocol due to unresolved safety concerns and comments that had been discussed previously at the 11/03/2021 meeting.</td></tr>
<tr><td>115255</td><td>Matthew Smith</td><td>Lentivirus of WT and R132H mutant of human IDH1 gene – 2023 Renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use lentiviral vectors based on HIV or SIV types 1 or 2 to transfect human cells for protein extraction and analysis.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>115256</td><td>Matthew Smith</td><td>Adenoviral (E1A deleted) and AAV Vectors Ad-GFP – 2023 Renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to clone a reporter gene into an AAV helper plasmid for co-infection with Adenoviral vectors in vitro. The constructs will be transfected into human cell lines for protein extraction and analysis.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

<table>
<tr><td>115257</td><td>Matthew Smith</td><td>Retroviral vectors of WT and R132H mutant of human IDH1 gene – 2023 Renewal</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use retroviral vectors to express variations of the IDHI gene in vitro for protein extraction and analysis.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>115855</td><td></td><td>Developing CAR-T cell therapy for medulloblastoma using endogenous mouse tumor models and single-cell transcriptomic analysis</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to study how CAR-T cells interact with brain tumors that develop in vivo.

<u>Committee Comments:</u> The Committee granted approval pending amendment of Section 3 Question 1 to clarify route of exposure and dosing information and Section 3 Question 5 to include booties and sleeves in the PPE list.</td></tr>
<tr><td>115294</td><td></td><td>NLRs in Gastrointestinal Inflammation and Cancer - NLRC3 rDNA – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to determine how varying levels of the NLRC3 gene affects immune cells or cancer cells. Lentiviral vectors will be used to modulate target gene expression.

<u>Committee Comments:</u> The Committee granted approval pending amendment of Section 2 Question 5 to clarify rsNA manipulations performed in-house, Section 2 Question 11 to add animal strains, Section 3 Question 1 to include dosing information for each exposure route, Section 3 Question 3 to correct a typo, Section 3 Question 5 to list required PPE, and Section 3 Question 6 with additional clarification about experiments.</td></tr>
</table>

3. **Discussion:** n/a
4. **NIH Reportable Incidents:** 0
5. **WCG IBC Review:** 5
    a. **PI:** **Title:**

       Status: Initial application submitted Tuesday, May 23, 2023.

    b. **PI:** **Title:**

       Status: Initial application submitted Monday, May 22, 2023.

c. **PI:** [redacted] **Title:** [redacted]

   Status: Initial application submitted Monday, May 22, 2023.

d. **PI:** [redacted] **Title:** [redacted]

   Status: Reviewed and approved by WCG IBC on Friday, May 19, 2023.

e. **PI:** [redacted] **Title:** [redacted]

   Status: Reviewed and approved by WCG IBC on Friday, May 05, 2023.

6. **Sub-Committee Review:** 3

   III-E

   i. **ID:** 115854 **PI**: [redacted] **Title:** Generating mice with Cre dependent Cd276 expression

   III-F

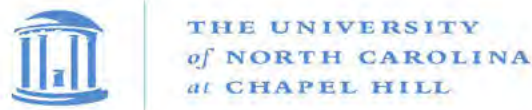
   i. **ID:** 115834 **PI**: Martina Gentzsch **Title:** Development and production of antibodies for CFTR detection

   ii. **ID:** 115894 **PI**: Matthew Lockett **Title:** 3D co-culture models to quantify estrogen responses in the presence of CAFS

7. **Schedule H Report:** 10

8. **Next IBC meeting:** July 12, 2023

Adjourn.



Meeting Minutes
August 02, 2023 9:00AM
Web Conference

Members Present: Amanda Craigen, Barbara Savoldo, Catherine Brennan, Christopher Broberg, Doug Cyr, Ilana Galex, Matthew Hirsch, Rachael Turner, Rachel Graham, Ronnie Weed, Victoria Baxter, William Bucha, Wil Lawson

Members Absent: Craig Fletcher, Shawn Hingtgen

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from July 12, 2023 meeting**
2. **Applications under review:** 22

ID	PI	Project Title
119054	[redacted]	Mutational analysis of SARS-CoV-2 replicase protein nsp13
Approved with Stipulations	Summary: The aim of this experiment is to use targeted mutagenesis and structural analysis to identify vital sites for nsp13 protein function. The resulting mutant libraries will better characterize nsp13, assist with development of small molecule antivirals, and help identify small molecule mechanisms of action. Committee Comments: The Committee granted approval pending amendment of Section II Question 2 to remove the information regarding animal work from the risk assessment statement since this application only covers *in vitro* experiments.	
119074	[redacted]	SARS-CoV-2 expressing an OVA CD4 epitope
Approved	Summary: The aim of this experiment is to express the ovalbumin CD4 epitope from a SARS-CoV-2 accessory ORF to investigate the protective role of CD4+ T-cell responses during infection/vaccination. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>118814</td><td></td><td>AAV and lentiviral vectors for measuring tumor xenograft size in mice</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use lentiviral vectors to label tumor cells with luciferase for xenograft mouse studies, and AAV vectors to deliver treatment in vivo.

Committee Comments: The Committee granted approval pending amendment of Section III Question 5 to include the full list of DCM PPE requirements</td></tr>
<tr><td>118359</td><td></td><td>Mouse models of chikungunya virus-induced arthritis (2023 Renewal)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to study how targeted mutations of an infectious clone of chikungunya virus impact replication in vivo. This is a 5-year renewal and only the IACUC protocol number was updated.

Committee Comments: The Committee granted approval pending clarification on whether human cells will be used in this project and amendment of Section II Question 2 to include in vivo morbidity monitoring in the risk assessment statement.</td></tr>
<tr><td>118360</td><td></td><td>Improved chikungunya virus vectors (2023 Renewal)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to study how targeted mutations of an infectious clone of chikungunya virus impact replication in vivo. This is a 5-year renewal and only the IACUC protocol number was updated.

Committee Comments: The Committee granted approval pending amendment of the vector map provided to include information regarding mutation targets/locations.</td></tr>
<tr><td>118361</td><td></td><td>fluorescent influenza viruses for in vivo tracking of infection (2023 Renewal)</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to use an influenza reporter virus to tag infected cells for study in vitro and in vivo. This is a 5-year renewal and there were no amendments to the protocol.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
</table>

<table>
<tr><td>118362</td><td></td><td>Venezuelan equine encephalitis virus replicons expressing proteins from influenza A, SARS CoV and KSHV (2023 Renewal)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to biochemically characterize proteins and/or produce antiserum against select proteins using VEE replicons to express genes of interest. This is a 5-year renewal and there were no amendments to the protocol.

Committee Comments: The Committee granted approval pending amendment of the vector map provided to include information regarding mutation targets.</td></tr>
<tr><td>118363</td><td>Mark Heise</td><td>Analysis of RNA structural determinants for their impact on VEE replication (2023 Renewal)</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to insert reporter genes or mutate key stem loop structures within the TC83 vaccine strain of VEE and study how the changes impact viral replication.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>118364</td><td></td><td>Recombinant H1N1 Influenza Virus Infectious Clone (2023 Renewal)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to elucidate the role of specific host genes in regulating virus-induced immunity and disease by comparing seasonal influenza infection in different mouse models.

Committee Comments: The Committee granted approval pending amendment of Section II Question 2 to include in vivo morbidity monitoring in the risk assessment statement.</td></tr>
<tr><td>118274</td><td></td><td>Injection of Pseudorabies virus (PRV) for neural circuit tracing (2023 Renewal)</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to map specific neural circuits by using PRV as a retrograde tracer in vivo.

Committee Comments: The Committee granted approval pending amendment of Section III Question 4 to state the work will take place in their assigned DCM BSL-2 space instead of</td></tr>
</table>

118275		Injection of adenoassociated virus (AAV) for neural circuit modulation (2023 Renewal)
Approved with Stipulations	Summary: The aim of this experiment is to modulate specific neural circuits by using AAV vectors to express chemogenetic, optogenetic, and/or control flurophores in target neuronal populations. Committee Comments: The Committee granted approval pending amendment of Section III Question 4 to state the work will take place in their assigned DCM BSL-2 space instead of	
117874		Developing and employing retroviral and lentiviral vectors for in vitro and in vivo gene delivery Renewal 2023
Approved with Stipulations	Summary: The aim of this experiment is to use transient transfection or stable packaging cell lines to optimize retroviral and lentiviral vectors for gene delivery. Committee Comments: The Committee granted approval pending clarification regarding the commercial gene libraries used by this core lab service.	
118254		Ad-Cre-GFP
Approved with Stipulations	Summary: The aim of this experiment is to transfect primary mouse bladder organoid cell lines with Ad-Cre-GFP. Committee Comments: The Committee granted approval pending amendment of the title and aim to better represent the proposed experiments and removal of Section III since this application only covers *in vitro* experiments.	
117914		Engineering Temperate Bacteriophage for Sustained Secretion of Protein Therapeutics or Immunogens by Mucosal Commensals - 2023 Renewal
Approved with Stipulations	Summary: The aim of this experiment is to develop genetically modified bacteriophage capable of transducing genes of interest into commensal bacterial populations. Committee Comments: The Committee granted approval pending clarification on the type of oral exposure route the *in vivo* work will use.	

118874		Engineering antigen-specific tolerance via recombinant plasmid - 2023 Renewal
Approved	Summary: The aim of this experiment is to create immunological tolerance in mice vaccinated against a model antigen, the ova protein Committee Comments: The committee granted approval pending amendment of Section III Question 1 to correct the IM injection volume from 100uL to the university standard volume of 50uL, or to obtain an IACUC-approved exception to exceed the standard volume.	
118554		Gene Therapy with AAV Vectors
Approved with Stipulations	Summary: The aim of this experiment is to optimize an AAV vector for alpha-1 gene therapy. An adenovirus will be used to enhance AAV replication and packaging *in vivo*. Committee Comments: The Committee granted approval pending amendment of the title to better represent the proposed experiments and amendment of Section III Question 1 to include the dose of inoculum.	
118074		Cancer cell lines engineered with replication-deficient lentiviral vectors to express luciferase and other neoantigens to study immune-mediated control of tumor growth in mice
Approved with Stipulations	Summary: The aim of this experiment is to monitor tumor growth *in vivo* via live imaging with luciferase and to induce tumor-specific immune responses with neoantigens. Committee Comments: The Committee granted approval pending receipt of vector maps, amendment of Section II Question 6 to clarify the neo-antigen genes of interest, amendment of Section III Question 1 to clarify the intended orthotopic injection site, and amendment of Section III Question 3 to remove extraneous information.	
118174		Generation of human or mouse CAR T cells for administration as anti-tumor therapy
Approved	Summary: The aim of this experiment is to modulate *in vivo* tumor growth using Chimeric Antigen Receptor (CAR) T cells modified to target specific tumor antigens. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

109023	Karen Mohlke	Susceptibility to Type 2 Diabetes and Related Quantitative Traits
Approved	Summary: The aim of this experiment is to identify regulatory elements and gene functions that influence susceptibility to type 2 diabetes or biomarkers of cardiovascular risk. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
118276	William Polacheck	Genetic knockout of Notch and adherens junction components
Approved with Stipulations	Summary: The aim of this experiment is to investigate the role of Notch signaling on the development and maintenance of the vascular transport barrier. Committee Comments: The Committee granted approval pending receipt of vector maps and amendment of Section II Question 6 to specify the target genes.	
118054		Targeting Fluid Stress-induced Chemoresistance in Ovarian Cancer Using Mechanism-based Photoimmunoconjugate Nanoparticles
Approved	Summary: The aim of this experiment is to use firefly luciferase for non-invasive bioluminescent imaging of tumor progression to monitor tumor progression and treatment response. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
118733		Postnatal ICV injection of LNPs containing Cre mRNA
Approved with Stipulations	Summary: The aim of this experiment is to establish a new, non-viral delivery system for therapeutic nucleic acids in the mammalian brain. The proposed experiments serve to (1) evaluate the mRNA distribution in the brain, (2) to test the efficiency of different Cre mRNA versions/concentrations and (3) to analyze the effect of different LNP formulations in vivo. Committee Comments: The Committee granted approval pending amendment of the title and aim to better represent the proposed experiments and removal of Section III since this application only covers *in vitro* experiments.	

117498	Cary Moody	Interplay between the DNA damage response (DDR) and the life cycle of DNA tumor viruses
Approved with Stipulations	Summary: The aim of this experiment is to elucidate how DNA tumor viruses induce the DDR and its role in viral replication. Committee Comments: The Committee granted approval pending amendment of the rDNA category from III-F to III-D and amendment of Section II Question 2 to specify the DNA tumor viruses.	

3. **Discussion:** 2

   i. [redacted]

   Status: **Approved**
   Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

   ii. [redacted]

   Status: **Approved**
   Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.

4. **NIH Reportable Incidents:** 0

5. **WCG IBC Review:** 4

WCG Review (III-C)		
PI	**PI**	**PI**
[redacted]	[redacted]	Initial application received Thurs. 06/29/2023
[redacted]	[redacted]	Reviewed and approved Fri. 07/21/2023
[redacted]	[redacted]	Reviewed and approved Fri. 07/21/2023
[redacted]	[redacted]	Reviewed and approved Wed. 07/26/2023

6. **Sub-Committee Review:** 3

III-E		
ID	**PI**	**Project Title**
118358	[redacted]	Role of ADR1 and NRG1 protein families in innate plant immunity

.

III-F		
ID	**PI**	**Project Title**
118974	Stephanie Gupton	Netrin glycosylation distinguishes chemotaxis and haptotaxis
118734	Koji Sode	Development of molecular recognition elements for biomarker detection

7. **Schedule H Report:** 21

8. **Next IBC meeting:** September 6, 2023

Adjourn.

Meeting Minutes
September 06, 2023 1:00PM
Web Conference

Members Present: Amanda Craigen, Barbara Savoldo, Christopher Broberg, Doug Cyr, Ilana Galex, Matthew Hirsch, Rachael Turner, Rachel Graham, Ronnie Weed, Shawn Hingtgen, Wil Lawson

Members Absent: Catherine Brennan, Craig Fletcher, Victoria Baxter, William Bucha

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from August 2, 2023 meeting**
2. **Applications under review:** 25

ID	PI	Project Title
120494		Propagation of the Bat Coronavirus HKU5 Infectious Clone: 2023 Renewal
Approved	Summary: The aim of this experiment is to continue using HKU5, a recombinant infectious clone related to and MERS-CoV, for replication and pathogenesis studies. This is a 5-year renewal with minor revisions (i.e., amended aim to include a risk assessment, updated IACUC protocol numbers). Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
120495		Propagation of the MERS-CoV Infectious Clone and Deletion of Accessory ORFs: 2023 Renewal
Approved	Summary: The aim of this experiment is to continue using an infectious cDNA of MERS-CoV to generate genomic RNA and isolate infectious progeny virus with deleted accessory ORFs. This a 5-year renewal with minor revisions (i.e., amended aim to reflect renewal, updated IACUC protocol numbers). Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

120496	[REDACTED]	[REDACTED] NSP16 Catalytic and Binding Domain Ablation in Infectious Clone: 2023 Renewal
Approved	Summary: The aim of this experiment is to continue using attenuated, infectious clones of [REDACTED] for replication and pathogenesis studies. This a 5-year renewal with minor revisions (i.e., amended aim to include a risk assessment, updated IACUC protocol numbers). Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
120514	Ralph Baric	An Infectious Clone for Human Coronavirus HKU1 (BL2): 2023 Renewal
Approved with Stipulations	Summary: The aim of this experiment is to generate a recombinant infectious clone of coronavirus HKU1 to use in replication and pathogenesis studies. This a 5-year renewal with minor revisions (i.e., amended aim to include a risk assessment). Committee Comments: The Committee granted conditional approval pending amendment of the aim to better reflect the purpose of these experiments.	
120515	[REDACTED]	Norway rat hepacivirus infection of mice
Approved	Summary: The aim of this experiment is to develop mouse models of hepacivirus infection, pathogenesis, and genetics. This project will utilize the Collaborative Cross mouse resource at UNC to identify mouse lines that support chronic infection and map loci associated with chronic infection. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
120534	[REDACTED]	Generation of a cDNA infectious clone system for a bat MERS-like coronavirus (MjHKU4r-CoV-1) and mouse model to study antiviral countermeasures
Approved with Stipulations	Summary: The aim of this project is to generate an infectious, reporter clone of MjHKU4r-CoV-1 and establish a pathogenic mouse model to evaluate replication, pathogenesis, and antiviral countermeasures *in vivo.* Committee Comments: The Committee granted conditional approval pending amendment of the risk assessment statement within the aim to include a “once established” clause.	

<table>
<tr><td>120535</td><td>Ralph Baric</td><td>Decreasing Influenza B pathogenicity through reassortment</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to identify genes associated with increased pathogenicity in recently isolated influenza B viruses by performing reassortments with previously characterized strains.

Committee Comments: The Committee granted conditional approval pending amendment of the aim to clarify this work will screen for decreased pathogenesis.</td></tr>
<tr><td>119914</td><td></td><td>Chimpanzee Adenovirus and Modified Vaccinia Virus Ankara viral vector prime/boost immunization using recombinant chlamydial antigens – 2023 renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to determine the efficacy of chlamydial antigen vaccination using viral vectors

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>119374</td><td></td><td>Ultrasound-assisted gene delivery</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use reporter genes and focused ultrasound contrast agents to evaluate the efficacy of non-invasive gene delivery methods to the heart.

Committee Comments: The Committee granted conditional approval pending amendment of the title to be more specific, amendment of the aim to clearly describe the constructs used for this work, and amendment of Section III Question 1 to clarify any administration differences between the proposed constructs.</td></tr>
<tr><td>119178</td><td>Marguerite Hood Pishchany</td><td>High throughput transposon mutagenesis of bacteria isolated from human vagina</td></tr>
<tr><td>Tabled</td><td colspan="2">Summary: The aim of this experiment is to develop methods for transposon mutagenesis (high throughput, random insertion) to study the gene functions of microbes isolated from the human vagina.

Committee Comments: The Committee voted to table this protocol due to the following concerns: the PI’s Schedule F did not include the biological agents mentioned in this protocol, the application did not have a risk assessment, the application did not clarify the source of the material (e.g., patient samples, purified bacterial cultures, etc.), and the description of the constructs was vague.</td></tr>
</table>

119854		Characterizing the effects of systemic energy balance on autophagy dynamics in murine models of TNBC
Approved with Stipulations	Summary: The aim of this experiment is to assess autophagic flux in different murine models of systemic energy balance to determine if autophagy is necessary for *in vivo* tumorigenesis of Triple negative breast cancer (TNBC). Committee Comments: The Committee granted conditional approval pending amendment of Section III Question 5 to include all required PPE for ABSL-2.	
120014	Tal Kafri	Regulation of vector-based gene expression via splicing manipulation. Renewal 2023
Approved	Summary: The aim of this experiment is to develop an inducible gene expression system using DNA oligos directed to the splice sites contained within expression cassettes in lentiviral vectors. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
120395		Engineering a highly specific viral targeting system for systemic gene therapy applications
Approved with Stipulations	Summary: The aim of this experiment is to evaluate how efficiently bispecific antibody-directed lentiviruses can target and transduce specific cells *in vitro* and *in vivo*. Committee Comments: The Committee granted conditional approval pending amendment of Section III Question 5 to include all required PPE for ABSL-2.	
119959	Stanley Lemon	Mechanism of replication pathogenesis and host immune responses to hepatitis responses to hepatitis A virus (HAV)
Approved with Stipulations	Summary: The aim of this experiment is to study host cell factors involved in hepatovirus infections, characterize immune responses to HAV infection, and investigate molecular mechanisms behind HAV replication and pathogenesis. Committee Comments: The Committee granted conditional approval pending amendment of the aim to include a risk assessment for this work.	

119994		Neutralizing Antibody and AAV FIX Gene Therapy – 2023 Renewal
Approved with Stipulations	Summary: The aim of this experiment is to mediate reporter gene expression in mouse tissues using AAV vectors. Committee Comments: The Committee granted conditional approval pending amendment of Section II Question 8 to include all target genes shown on the attached vector maps and amendment of Section III Question 5 to include all required PPE for ABSL-2.	
119354		Development of Intravenous AAV Vectors for Intractable Epilepsy – 2023 Renewal
Approved with Stipulations	Summary: The aim of this experiment is to develop novel AAV vectors capable of selectively crossing the blood-brain barrier, transducing neurons, and expressing antiseizure neuropeptides. Committee Comments: The Committee granted conditional approval pending amendment of Section III Question 5 to include all required PPE and Section III Question 7 to clarify BSC use. However, PI verified that construct is appropriate for ABSL-1 so these 2 items were ultimately not required.	
117498	Cary Moody	Interplay between the DNA damage response (DDR) and the life cycle of DNA tumor viruses
Approved	Summary: The aim of this experiment is to study the induction of DDR in Kaposi's sarcoma-associated herpesvirus (KSHV) and Human papillomavirus (HPV) and how this process influences viral replication. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
120214	Scott Randell	Molecular Therapy Core Center, Core F: Cell Culture Models
Approved	Summary: The aim of this experiment is to describe the services offered by Molecular Therapy Core Center, Core F. This group will assist different studies by creating modified human airway epithelial cells using lentiviral vectors. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>120216</td><td>Scott Randell</td><td>Identification of Airway Epithelia Stem Cells</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to extend the lifespan of primary human airway cells.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>119276</td><td></td><td>Genetic Manipulation of Clostridium difficile-Tn916 - 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to integrate target genes into C. difficile chromosome. Gene targets will either facilitate trans complementation of mutations or encode a reporter gene.

Committee Comments: The Committee granted conditional approval pending amendment of the aim to include a risk assessment for this work and amendment of Section III Question 5 to include all required PPE for ABSL-2.</td></tr>
<tr><td>119277</td><td>Rita Tamayo</td><td>Genetic Manipulation of Clostridium difficile-Expression Vectors – 2023 Renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to integrate target genes into C. difficile chromosome. Gene targets will either facilitate trans complementation of mutations or encode bacterial signaling molecules.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>119278</td><td></td><td>Genetic Manipulation of Clostridium difficile-Fluorescent reporters – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use recombinant reporter proteins to monitor gene expression in C. difficile during its lifecycle in vitro and in vivo.

Committee Comments: The Committee granted conditional approval pending amendment of Section III Question 5 to include all required PPE for ABSL-2.</td></tr>
</table>

119279	[redacted]	Genetic Manipulation of Clostridium difficile-Allelic Exchange pMTL – 2023 Renewal
Approved with Stipulations	Summary: The aim of this experiment is to study C. difficile pathogenesis using strains with mutations in known virulence gene candidates or their regulators. Committee Comments: The Committee granted conditional approval pending amendment of the aim to include a risk assessment for this work	
120134	[redacted]	Expression of NUT-BRD4 fusion protein in murine squamous cell carcinoma cell lines
Tabled	Summary: The aim of this experiment is to study how NUT-BRD4 expression in murine squamous cell carcinoma cell lines alters immune regulatory gene expression and MHC Class I antigen presentation. Committee Comments: The Committee voted to table this protocol due to the following concerns: the application did not include a Section III despite referring to animal work in Section II Question 5.	
120654	[redacted]	Use of luciferase containing human and murine cancer cell lines for animal studies.
Approved with Stipulations	Summary: The aim of this experiment is to monitor *in vivo* tumor growth using transgenic reporter cells. Committee Comments: The Committee granted conditional approval pending amendment of Section II Question 7 to include the current IACUC protocol information, amendment of Section III Question 3 to clarify the meaning of "intracardiac tail vein injections," and amendment of Section III Question 5 to include all required PPE for ABSL-2.	

3. **Discussion:** 2

   a. Summary of Recent Incidents

      i. **20230720**: Chris Broberg presented on a potential exposure to rsNA that occurred at BSL-2 on 07/20/2023 involving a finger puncture from a lancet that occurred during an attempt to perform a submandibular bleed on a mouse. The mouse was infected with LCMV, strain A22.

      ii. **20230717**: Chris Broberg presented on a potential exposure to rsNA that occurred at BSL-2 on 07/17/2023 involving a finger puncture from a needle that occurred during an attempt to perform a cardiac puncture on a mouse. The mouse was engrafted with human cord blood CD34+ cells and acute myeloid leukemia cells

transduced with GFP-FF*luc* fusion protein, and had also been treated with CAR-T cells that were transduced with retroviral vectors.

iii. **20230723:** Amanda Craigen presented on an incident that occurred at BSL-3 on 08/02/2023 involving a malfunction with the supply air controller. No work was being conducted within the lab at the time of the incident, and the lab was subsequently shut-down for further investigation.

iv. **20230811**: Amanda Craigen presented on an incident involving a failure to obtain IBC approval for a clinical trial. The PI sent notification of this error on 08/11/2023. The study was approved by the Institutional Review Board on 06/27/2022 and initiated participant enrollment and dosing on 11/09/2022.

b. Updates for Schedule G Form

4. **NIH Reportable Incidents:** 4

5. **WCG IBC Review:** 4

WCG Review (III-C)		
PI	**Protocol**	**Status**
		Application received Thurs. 08/24/2023
		Application received Wed. 08/23/2023
		Application undergoing pre-review
		Reviewed and approved on Wed. 08/23/2023
		Reviewed and approved on Thurs. 08/10/2023

6. **Sub-Committee Review:** 6

III-E		
ID	**PI**	**Project Title**
120436		Ex462-Galt Mouse

121234		Ex466-Lamp2-Q245X
120158		Creating a Calcrl CRISPR/Cas Mouse to Study the Lymphatics
120757		FcgRIIß Crisper knock-out
120758		KSHV orf50 CRISPR knock-out

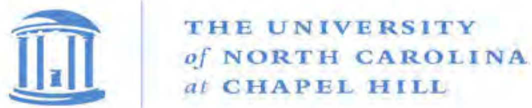
.

III-F		
ID	**PI**	**Project Title**
120334		The use of CpG in vaccination of mice or treatment of cancer
117498		Elucidating the Neural Circuits of Binge Drinking

7. **Schedule H Report:** 19

8. **Next IBC meeting:** October 4, 2023

Adjourn.



INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
October 04, 2023 1:00PM
Hybrid Conference

Members Present: Amanda Craigen, Catherine Brennan, Christopher Broberg, Doug Cyr, Matthew Hirsch, Rachael Turner, Rachel Graham, Ronnie Weed, Victoria Baxter, Wil Lawson

Members Absent: Barbara Savoldo, Craig Fletcher, Ilana Galex, Shawn Hingtgen, William Bucha

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from September 6, 2023 meeting**
2. **Applications under review:** 20

ID	PI	Project Title
121734		Pathogenicity and Immune Evasion of the SARS-CoV-2 BA.2.86 Variant Through Reverse Genetics Infectious Clone Based Studies
Approved	Summary: The aim of this experiment is to study the pathogenicity of the SARS-CoV-2 BA.2.86 variant using the lab's reverse genetics platform and mouse models. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
121540	Richard Boucher Jr.	IL1B Induces Mucin Hyperconcentration in Cystic Fibrosis Airways – 2023 Renewal
Approved	Summary: The aim of this experiment is to express target genes in primary human brionchiole epithelial cells using lentiviral vectors. This a 5-year renewal with no changes to the protocol. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>118754</td><td></td><td>Role of miR-29 in models of Alzheimer (2023 Renewal)</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to study the role of microRNA miR-29 in vitro using AAV-based viral vectors to overexpress the target gene. This a 5-year renewal where the only change was updating the IACUC protocol numbers related to sourcing the cells used in vitro.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>121259</td><td></td><td>Using caspase to elucidate the functional of anatomical circuits during goal-directed behavior</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use AAV-based viral vectors to investigate the neural networks associated with goal-directed behavior (e.g., reaching, walking) via caspase3-mediated cell death.

Committee Comments: The Committee granted conditional approval pending correction of the typo in the title (i.e., “functional” to “function”) and changing the containment level listed in Section II Question 6 to BSL-1.</td></tr>
<tr><td>120497</td><td>Marguerite Hood Pischany</td><td>High throughput transposon mutagenesis of bacteria isolated from human vagina</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to develop methods for transposon mutagenesis (high throughput, random insertion) to study the gene functions of microbes isolated from the human vagina.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>121574</td><td></td><td>Ad-Cre injection into mice</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use Ad-Cre viral vectors to modulate target genes in genetically engineered mouse models.

Committee Comments: The Committee granted conditional approval pending amendment of the title and aim to clarify this project, and Section II Question 11 to include the strains of mice used for the proposed experiments.</td></tr>
</table>

<table>
<tr><td>121575</td><td>[redacted]</td><td>In vitro Lentiviral CRISPR of genes of interest in cell lines</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use Ad-Cre viral vectors to modulate target genes in genetically engineered mouse models.

Committee Comments: The Committee granted conditional approval pending amendment of the title and aim to clarify this project, Section II Question 11 to include the strains of mice used for the proposed experiments, and Section III Question 1 to clarify the orthotopic target.</td></tr>
<tr><td>121175</td><td>[redacted]</td><td>Analysis of Recombinant Influenza Ya88 strain with reporter genes – 2023 Renewal</td></tr>
<tr><td>Approved</td><td colspan="2">Summary: The aim of this experiment is to develop in vivo models for influenza to test the efficacy of novel antivirals. This a 5-year renewal with no changes to the protocol.

Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>121614</td><td>[redacted]</td><td>Enabling repeated systemic gene therapy by eliminating antigen specific host adaptive immunity – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to evaluate the efficacy of CD22-targeting as a method to attenuate adaptive immune response to viral gene delivery systems. This is a 5-year renewal where the only change was updating the IACUC protocol numbers.

Committee Comments: The Committee granted conditional approval pending amendment of Section II Question 8 to clarify the packaged transgene(s) and Section III Question 1 to clarify the maximum tail vein injection volume.</td></tr>
<tr><td>121554</td><td>[redacted]</td><td>Polyploid Vectors 3, 7, and 8-GFP and luc – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use genetic material from different AAV serotypes to develop “polyploid” AAV-based vectors. This is a 5-year renewal where the only change was updating the IACUC protocol numbers.

Committee Comments: The Committee granted conditional approval pending amendment of the title to better clarify the project and correction of “retro-orbital vein” in Section III Question 1 to state “retro-orbital sinus.”</td></tr>
</table>

121555		CTL responses to AAV vectors-OVAepitopeSubstitution – 2023 Renewal
Approved with Stipulations	Summary: The aim of this experiment is to study the AAV vector induced immune response. This is a 5-year renewal where the only change was updating the IACUC protocol numbers. Committee Comments: The Committee granted conditional approval pending amendment of the title to clarify the CTL and OVA acronyms and correction of “retro-orbital vein” in Section III Question 1 to state “retro-orbital sinus.”	
120894		Cre-dependent knockout of interleukin-1 beta gene
Approved	Summary: The aim of this experiment is to use an AAV-based viral vector mediated Cas9 system to facilitate a Cre-dependent knockout of Il1b. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
121794		Investigating the forces shaping genetic variation across time, space, and species in Aedes
Approved with Stipulations	Summary: The aim of this experiment is to study how different polymorphic mutations impact the fitness of Aedes mosquitos. Committee Comments: The Committee granted conditional approval pending amendment of Section II Question 8 to clarify the target genes	
121674	Douglas Phanstiel	Optimization of lentivirus transduction conditions using a control GFP expressing lentivirus
Approved	Summary: The aim of this experiment is to optimize the method for transducing cells with lentiviral vectors to ensure high success rates and low toxicity. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

121034	Rita Tamayo	Genetic manipulation of C.difficile—CRISPRi
Approved with Stipulations	Summary: The aim of this experiment is to modulate gene expression in Clostridioides difficile using CRISPRi. The gene of interest will vary but will include targets involved with DNA replication, RNA regulation, and cell division. Committee Comments: The Committee granted conditional approval pending amendment of the aim to include a risk assessment for this work and amendment of Section II Question 8 to clarify the target genes.	
121654		Introducing luciferase into NUT carcinoma cell lines for in vitro and in vivo cytotoxicity assays
Approved with Stipulations	Summary: The aim of this experiment is to create NUT carcinoma reporter cells using lentiviral vectors. Committee Comments: The Committee granted conditional approval pending amendment of Section III Question 1 to clarify the volume for each route of administration.	
121655	Benjamin Vincent	Knockout of BRD4-NUTM1 in NUT Carcinoma cell lines using conditionally stabilized Cas9 ('DDCas9')
Approved	Summary: The aim of this experiment is to develop a conditionally stabilized Cas9 to facilitate assays with NUT carcinoma knockout cells that are difficult to culture. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design	
121057	Matthew Wolfgang	5044984 - Pseudomonas Virulence Gene Exp (2023 Renewal)
Approved with Stipulations	Summary: The aim of this experiment is to investigate the regulation of genes associated with virulence in P. aeruginosa. This is a 5 year renewal that added several target genes to Section II Question 8. Committee Comments: The Committee granted conditional approval pending amendment of the aim to include a risk assessment for this work.	

121634		Using recombinant DNA in vitro and in vivo to investigate basal and classical molecular subtype differences in pancreatic ductal adenocarcinoma - 2023 Renewal
Approved with Stipulations	Summary: The aim of this experiment is to elucidate gene-specific functions that impact the cell biology and drug response of classical and basal-like subtypes of pancreatic ductal adenocarcinoma and related cancers. Committee Comments: The Committee granted conditional approval pending amendment of the title to be more concise and Section III Question 1 to clarify the orthotopic target(s) and administration volume(s).	
120194	Xing-Hua Zeng	Production of control AAV vectors for internal and external investigators
Approved	Summary: The aim of this project is to provide vector core services to investigators. Specifically, this application describes AAV-based vector production. This is a 5-year renewal with no changes to the protocol. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

3. **Discussion:** 0
4. **NIH Reportable Incidents: 0**
5. **WCG IBC Review: 4**

WCG Review (III-C)		
PI	**PI**	**PI**
		Reviewed and approved 09/15/2023
		Reviewed and approved 09/15/2023
		Reviewed and approved 09/26/2023
		Reviewed and approved 10/03/2023

6. **Sub-Committee Review:** 6

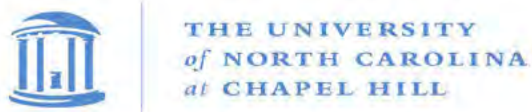
III-E

ID	PI	Project Title	Approved Date
121514		Ex467-Lamp2-L325fs	9/12/2023
121957		Cell biology of cell shape changes in cytokinesis	10/09/2023
121454	Angeliki Tsangaratou	Epigenetic and transcriptional regulation of T cell differentiation and function – renewal 2023	10/09/2023

III-F			
ID	PI	Project Title	Approved Date
121094	Dirk Dittmer	Exploration of gene functions and interactions	Pending
121494	Alain Laederach	Structure in noncoding RNA and Splicing	10/09/2023
117583	Rachel Noble	Production of or use of purchased plasmids or synthetic nucleic acids for use as positive controls for PCR, quantitative PCR, and digital droplet PCR – 2023 Renewal	n/a – deleted since 2023 renewal was submitted by mistake

7. **Schedule H Report:** 7
8. **Next IBC meeting:** November 1, 2023

Adjourn.



Meeting Minutes
December 06, 2023 1:00PM
Web Conference

Members Present: Amanda Craigen, Amanda Lytle, Barbara Savoldo, Catherine Brennan, Christopher Broberg, Doug Cyr, Ilana Galex, Matthew Hirsch, Rachael Turner, Rachel Graham, Ronnie Weed, Shawn Hingtgen, Wil Lawson

Members Absent: Craig Fletcher, Kelly Drayton, Victoria Baxter

Ad hoc Members (not requested to be present): Stanley Lemon and Ann Matthysse

Open Meeting

1. **Review minutes from November 1, 2023 meeting**
2. **Clinical Trials**
   i. **Study:** [redacted] **PI:** [redacted]
      Presenter: [redacted]
      Status: Approved
3. **Applications under review:** 18

ID	PI	Project Title
123774	[redacted]	Effect of metabolism on carcinogenesis of ovarian and endometrial cancer
Approved with Stipulations	Summary: The aim of this experiment is to induce ovarian and/or endometrial cancer *in vivo* using adenoviral vectors. This a 5-year renewal where the only change was updating IACUC protocol numbers. Committee Comments: The Committee granted conditional approval pending amendment of Section III Question 3 to replace buprenorphine with dexdomitor (dexmedetomidine).	
124254	[redacted]	Molnupiravir mutagenesis to map viral genomic plasticity of hepacivirus
Approved	Summary: The aim of this experiment is to chemically induce viral evolution *in vivo* to facilitate the identification of segments within the Norway rat hepacivirus (NrHV) genome vital to viral viability. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

124255	████	Adaptation of HKU4/5 Species Specificity – 2023 Renewal
Approved	Summary: The aim of this experiment is to generate a mouse-adapted version of the HKU4/5 bat coronaviruses to facilitate pathogenesis studies. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
123834	████	Generating genetic mutations and transgenes to study glial cell development and function
Approved with Stipulations	Summary: The aim of this experiment is to use a CRISPR/Cas9 system, binary expression Gal4/UAS system, and/or Tol2-based transposon system to deliver reporters and sensors *in vivo* to study the development and interactions of neural elements. Committee Comments: The Committee granted conditional approval pending amendment of Section III Question 4 to include clarification on BSC use. The following changes were made administratively: updated the IACUC protocol number and changed the rsNA category from III-E to III-D.	
124414	Dale Cowley	Adenovirus-Cre Fibroblast Infection
Approved	Summary: The aim of this experiment is to use adenoviral vectors in murine fibroblasts test Cre-mediated gene recombination systems *in vitro.* Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	
124014	Nathaniel Hathaway	Designing Chemically Modulated AAV Vectors for Transcriptional Regulation
Approved	Summary: The aim of this experiment is to generate AAV vectors that will allow transcriptional regulation via chemical modifiers. Committee Comments: The proposed containment and safety procedures are adequate for the experimental design.	

<table>
<tr><td>123814</td><td>Tal Kafri</td><td>Simple and lenti viral vectors expressing CRISPR/Cas9 gRNA Renewal 2023</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to generate simple retroviral or lentiviral vectors for use by other investigators.

<u>Committee Comments:</u> The Committee granted conditional approval pending amendment of Section II Question 2 to clarify what is meant by "simple" viral vectors.</td></tr>
<tr><td>123675</td><td></td><td>pINDUCER Lentiviral gene expression system for overexpression of genes of interest – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use a lentiviral system to overexpress certain genes of interest to study bladder and kidney cancer biology.

<u>Committee Comments:</u> The Committee granted conditional approval pending amendment of the title, Section II Question 2 to better clarify the planned experiments, Section III Question 1 to clarify orthotopic injection sites, Section III Question 3 to replace "averting" with ketamine and dexdomitor (dexmedetomidine) to clarify what is meant by "simple" viral vectors.</td></tr>
<tr><td>124003</td><td></td><td>Engineering human B cells to secrete broadly neutralizing antibodies in a proof of concept model.</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use a lentiviral system to engineer human B cells to secrete broadly neutralizing antibodies against HIV, influenza, RSV, or SARS-CoV-2.

<u>Committee Comments:</u> The Committee granted conditional approval pending addition of “booties, bonnets, and sleeves” to Section III Question 5 to reflect current DCM PPE requirements. The following changes were made administratively: updated the IACUC protocol number.</td></tr>
<tr><td>123758</td><td>Bo Li</td><td>Constructing Plasmids for Cloning and Expression of Natural Product Biosynthetic Genes</td></tr>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to amplify biosynthetic genes from Pseudomonas, Burkholderia, and Streptomyces species for cloning into E.coli or heterologous expression in one of the target species.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>123394</td><td></td><td>Lineage-Specific Gene Expression of coagulation proteins in platelets in dogs with inherited bleeding disorders – 2023 Renewal</td></tr>
</table>

<table>
<tr><td>Approved</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use a lentiviral vector system to correct inherited bleeding disorders via expression of canine coagulation factors in animal models. This is a 5-year renewal where the only change was updating the IACUC protocol number.

<u>Committee Comments:</u> The proposed containment and safety procedures are adequate for the experimental design.</td></tr>
<tr><td>123394</td><td></td><td>rAAV Gene Therapy for Hemophilia – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use an rAAV vector system to correct inherited bleeding disorders via expression of canine coagulation factors in animal models. This is a 5-year renewal where the only change was updating the IACUC protocol number.

<u>Committee Comments:</u> The Committee granted conditional approval pending amendment of Section III Question 1 to clarify dosing parameters for each route of exposure.</td></tr>
<tr><td>123395</td><td></td><td>Lentiviral Gene Therapy of Hemophilia A – 2023 Renewal</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use a lentiviral vector system to correct inherited bleeding disorders via expression of canine coagulation factors in animal models. This is a 5-year renewal where the only change was updating the IACUC protocol number.

<u>Committee Comments:</u> The Committee granted conditional approval pending amendment of Section III Question 1 to clarify dosing parameters for each route of exposure.</td></tr>
<tr><td>123396</td><td></td><td>Ultrasound-Mediated Gene Delivery (Plasmids) for Hemophilia A and B</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2"><u>Summary:</u> The aim of this experiment is to use a plasmid vector system to correct inherited bleeding disorders via expression of canine coagulation factors in animal models

<u>Committee Comments:</u> The Committee granted conditional approval pending amendment of Section III Question 1 to clarify dosing parameters for each route of exposure.</td></tr>
<tr><td>123894</td><td></td><td>Respiratory Virus Infections in vitro and in vivo</td></tr>
</table>

<table>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to use recombinant or chimeric constructs of RSV or parainfluenza viruses to investigate the functions of non-structural target proteins.

Committee Comments: The Committee granted conditional approval pending amendment of Section II Question 2 to include a risk assessment statement and additional details regarding the purpose behind these experiments.</td></tr>
<tr><td>123434</td><td>Kevin Pruitt</td><td>Biological activity of Dishevelled proteins and the impact of post-translational modifications on cellular functions and signaling pathways in cancer</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to elucidate the novel nuclear role of Dishevelled (DVL) protein paralogs and how post-translational modifications of DVL proteins impact function.

Committee Comments: The Committee granted conditional approval pending amendment of the rsNA category from III-D to III-F.</td></tr>
<tr><td>124078</td><td></td><td>Herpes Virus oncogenesis, latency, and reactivation: Transformation by Epstein-Barr Virus – 2023 Amendment</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to determine the oncogenic potential of EBV genes, miRs, and lncRNAs. The EBV latent genes of interest will be cloned into a plasmid or viral vector to transfect mammalian cells for use in vivo.

Committee Comments: The Committee granted conditional approval pending addition of “booties, bonnets, and sleeves” to Section III Question 5 to reflect current DCM PPE requirements.</td></tr>
<tr><td>124094</td><td></td><td>Herpes Virus oncogenesis, latency, and reactivation: Transformation by Epstein-Barr Virus– 2023 Amendment</td></tr>
<tr><td>Approved with Stipulations</td><td colspan="2">Summary: The aim of this experiment is to determine the oncogenic potential of EBV, select EBV latent genes, and/or non-coding RNAs when introduced into a human papilloma virus (HPV) infected cell line

Committee Comments: The Committee granted conditional approval pending addition of “booties, bonnets, and sleeves” to Section III Question 5 to reflect current DCM PPE requirements.</td></tr>
</table>

4. **Discussion:** 0
5. **NIH Reportable Incidents:** 0
6. **WCG IBC Review:** 0
7. **Sub-Committee Review:** 5

	III-E			
	ID	**PI**	**Project Title**	**Approved Date**
1	80608	[redacted]	Genetic editing using CRISPR-Cas technology in mice	12/07/2023
2	124215	[redacted]	Ex468 Human LAMP2 Mouse	12/01/2023
3	124216	[redacted]	EX474 Human ALB Ex1-Int1 Mouse	12/01/2023

	III-F			
	ID	**PI**	**Project Title**	**Approved Date**
1	124238	Douglas Phanstiel	Cell type specific proteomic profiling of cerebral organoids	12/13/2023
2	123914	Benjamin Philpot	Role of TCF4 in Pitt Hopkins syndrome	12/13/2023

7. **Schedule H Report:** 11
8. **Next IBC meeting:** January 10, 2023

Adjourn.