THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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		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		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		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		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	

55064		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 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	
55325		Targeting Constitutively Active Gαq for the Treatment of Uveal Melanoma
APPROVED	**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	
55326		Stable transfected uveal melanoma cell lines for bioluminescent imaging in animals
APPROVED	**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	
55362		Lentivirus preparation and transduction
APPROVED WITH STIPULATIONS	**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	

55042		Analysis of virulence in Klebsiella pneumoniae, Yersinia enterocolitica, Yersinia pseudotuberculosis and Salmonella typhimurium
APPROVED WITH STIPULATIONS	**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	
54342	Richard Superfine	Use of Green/Red fluorescent or photoactivateable Markers for Evaluating Effects of Forces on Cells
APPROVED	**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	
54024		Novel approaches for GVHD prevention
APPROVED	**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	
54042		Use of luciferase containing human and murine cancer cell lines for animal studies.
APPROVED	**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	

54222	Benjamin Vincent	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: Jonathan Serody **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.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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 January 9, 2019 meeting.** Minutes approved.
2.

3. **Applications under review:**

ID	PI	Project Title
56022	Ralph Baric	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 Rocky Mountain Labs. **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 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	

56027		Generation of mouse-adapted MERS viruses with mutations in the PLpro domain of nsp3
APPROVED	**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	
55822		Recombinant Sindbis viruses expressing murine granzymes
APPROVED	**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	
56502		Optogenetics in Behaving Animals
APPROVED	**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	
56070		Studying biological function of Tau using recombinant AAV vectors – AAV GFP
APPROVED WITH STIPULATIONS	**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	

53302		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 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	
49584		Evaluation of the immune response in humanized BLT mouse model
APPROVED WITH STIPULATIONS	**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	
55522		AAV Reporter Vectors
APPROVED WITH STIPULATIONS	**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	

56104		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		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		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		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		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: Fulton Crews **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: **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.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

INSTITUTIONAL BIOSAFETY COMMITTEE

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

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

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

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

Guests:

Open Meeting

7. **Review minutes from the February 6, 2019 meeting.** Minutes approved.
8. **Clinical Trial Review** –
9. **Applications under review:**

ID	PI	Project Title
		A Phase 1/2 Dose Escalation Study to Evaluate the Safety and Efficacy of HMI-102 in Adult Subjects with PAH Deficiency
APPROVED	**Summary:** The aim of this clinical trial is to determine the safety and tolerability of a single dose of HMI-102 when administered to subjects with phenylalanine hydroxylase (PAH) deficiency. PAH deficiency, often called phenylketonuria is a recessive genetic disorder cause by a mutation in the PAH gene which results in the deficiency of PAH. If untreated PAH deficiency results in progressive, irreversible brain impairment during infancy and early childhood. In this trial, a novel gene therapy product, HMI-102 has been developed to delivery a normal copy of the human PAH gene to liver cells using a replication-incompetent recombinant AAV serotype . Patients enrolled in the trial will receive a single intravenous infusion of the study vector at doses ranging from $1x10^{13}$ to $1x10^{14}$ vector genomes per kilogram body weight. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-C, BSL-1, AAV	
57805		Use of Commercially Purchased A549-luc-c8 in In-Vitro and Mouse Studies
APPROVED	**Summary:** The aim of this experiment is to use a commercially-produced luciferase-expressing cell line (A549-luc-c8) for bioluminescent imaging in mice injected with the cells via lung parenchyma administration. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, mice	

57842		Hydrodynamic injection of vWF variants
APPROVED	**Summary:** The aim of this experiment is to express von Willebrand factor (normal or mutant) in mice. The gene for murine vWF will be cloned into a pLIVE plasmid which will be administered to mice via hydrodynamic tail vein injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-1, mice	
56763		Mechanisms of gene silencing by long noncoding RNAs
APPROVED	**Summary:** The aim of this experiment is to determine the mechanism by which long non-coding RNAs regulate gene expression in mammalian nuclei. Lentiviral vectors encoding the CRISPR/Cas9 machinery and a library of sgRNAs targeting genes driven by the U6 promoter will be constructed at a 3rd party institute. The resultant vectors will be used to infect mouse embryonic stem cells in vitro. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The containment condition needs to be updated to BSL-2 for lentiviral vectors. **Community Comments:** None III-D, BSL-2, lentivirus	
57302		Generation of a murine model of ACL aplasia through Cep5711 deletion
APPROVED	**Summary:** The aim of this experiment is to generate a mouse that mimics a human genetic mutation resulting in the absence of ligaments in the knee. Cas9 nucleoprotein and gRNA's will be injected into embryos to create a transgenic mouse with a Cep5711 27kb deletion. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The containment condition needs to be updated to BSL-2 for lentiviral vectors. **Community Comments:** None III-E, BSL-2, mice	
56966	Michael Emanuele	Identification and characterization of genes and proteins involved in cell cycle and cancer proliferation
APPROVED	**Summary:** The aim of this experiment is to identify and characterize genes involved in proliferation and cell cycle progression in human cells. Genes of interest will either be expressed in cells or knocked down using shRNAs or CRISPR/Cas9. Plasmid- and retroviral/lentiviral-based systems will be used. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, plasmids, lentivirus, retrovirus	

57542		All optical closed-loop studies for next generation neuroprostheses
APPROVED	**Summary:** The aim of this experiment is to design physiological tools to modulate the brain of living animals. AAV vectors encoding fluorescent proteins, optogenetic tools, or genetically encoded calcium indicators will be injected into mouse brains. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-1, AAV, mice	
57162		Fxr1 CRISPR: Functional impact of alternative slicing regulation of trafficking and membrane dynamics genes
APPROVED	**Summary:** The aim of this experiment is to create a Fxr1 transgenic mouse model system to assess the functional impact of alternative splicing regulation on trafficking and membrane dynamic genes. Synthetic guide RNA and Cas9 nuclease complex will be injected into single cell embryos to remove exon 15 of Fxr1. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-E, BSL-1, mice	
57042		Neuroanatomical role of TRM9
APPROVED	**Summary:** The aim of this experiment is to express GFP and GFP-tagged variants of human TRIM9 in mammalian cells. Cells will be transfected with plasmid DNA, or alternatively, plasmids will be electroporated in utero in C57Bl6 mice. Mice will be sacrificed post-natally for neuroanatomical inspection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-1, plasmids, mice	
57682	Matthew Hirsch	Recombinant adeno-associated virus gene delivery in human corneas ex vivo
APPROVED WITH STIPULATIONS	**Summary:** The aim of these experiments is to optimize dose and volume of AAV vectors for human cornea transduction following intrastromal injections. GFP or EF1a-hIDUA cDNA will be cloned into AAV vectors which will be used to transduce human corneas ex vivo. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee request information on the source of the human corneas. **Community Comments:** None III-D, BSL-2, AAV	

57683	Matthew Hirsch	Recombinant adeno-associated virus mobilization
APPROVED	**Summary:** The aim of this experiment is to determine if recombinant AAV vectors can be mobilized in the presence of the AAV cap and rep genes. A plasmid containing AAV rep and cap genes will be transfected into human cells solely or along with a recombinant AAV plasmid expressing GFP or EF1a-IDUA and a replication-deficient adenovirus helper plasmid. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, AAV	
57275	Matthew Hirsch	Corneal recombinant AAV gene therapy for lysosomal storage diseases
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to investigate rAAV gene addition for the treatment of corneal MPS1, MPSV1 or cystinosis disease. Plasmids or AAV vectors expressing human idua, ctns or arsB cDNA will be constructed and use to transfect/transduce mammalian cells in vitro. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the title be altered to remove “gene therapy” and the IACUC comment be removed since no animal work at UNC proposed. **Community Comments:** None III-D, BSL-2, AAV	
57276		AAV gene therapy for ocular immune suppression
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to investigate different immune suppressive molecules to prevent blindness. Experiments will be performed in vitro and in a murine corneal trauma model. cDNA’s for genes of interest will be cloned into plasmids for transfection of human cells in culture. Alternatively, AAV’s expressing cDNA’s will be constructed for administration to mice via corneal intrastromal injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the title be altered to remove “gene therapy” and the IACUC number be updated to 19-069. **Community Comments:** None III-D, BSL-2, AAV	
57277		Recombinant adeno-associated virus gene delivery in porcine corneas ex vivo
APPROVED	**Summary:** The aim of this experiment is to determine the administered volume to dose relationship for AAV treatment of human corneal diseases. Recombinant AAV expressing GFP will be administered to porcine corneas obtained postmortem. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, AAV	

56530		Cancer vaccine and treatment
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to introduce tumor antigens to immune cells and stimulate an immune response against established tumors in mouse models. Plasmid DNA encoding various tumor antigens or siRNA's to genes of interest will be encapsulated into nanoparticles used for delivery in mice as potential treatments for tumors in mouse cancer models. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested a list of siRNA targets and additional comments for Section III. **Community Comments:** None III-E, BSL-1, plasmids, mice	
57822		Immortalization and transformation of MEF
APPROVED	**Summary:** The aim of this experiment is to immortalize mouse and human embryo fibroblasts (MEF's and HEF's) using retroviral vectors carrying the SV40 large T. Vector particles will be generated using a three-plasmid system of transfection in HEK293T cells. Resultant vector particles will be employed on HEF's and MEF's in culture. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, retrovirus	
56902		Genetic recombination in hepatocytes using AAV8
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to utilize AAV8-delivered Cre recombinase to drive recombination of floxed alleles in hepatocytes of living mice. AAV8 viral particles expressing Cre recombinase will be purchase from a 3rd party and introduced into live mice by IV tail vein injection. Floxed alleles include Sox9EGFP and lox-stop-lox tdTomato. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the floxed alleles be indicated. **Community Comments:** None III-D, BSL-1, AAV	
56862		Juvenile hemophilia B dog study
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to administer gene therapy to juvenile dog to monitor gene expression . **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that BSL level be upgraded to BSL-2 due to use of human cell lines, and details on anesthesia be provided. **Community Comments:** None III-D, BSL-2, , dogs	

57043		Gene therapy of hemophilia A
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express canine dogs that are normal and deficient in FVIII . **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that BSL level be upgraded to BSL-2 due to use of human cell lines, and details on anesthesia be provided. **Community Comments:** None III-D, BSL-2, , dogs	
57802		AAV vectors for gene therapy of hemophilias and von Willebrand disease
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express coagulation factors VII, VIII, IX or vWF in dogs that do not express these proteins due to an inherited genetic defect. Canine VII, VIII, IX or vWF cDNA will be cloned into AAV vector which will be infused into dogs by intravenous injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that BSL level be upgraded to BSL-2 due to use of human cell lines, and details on anesthesia be provided. **Community Comments:** None III-D, BSL-2, AAV, dogs	
57803		Gene therapy canine hemophilia B with an improved AAV vector
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express coagulation factor IX in hemophilia B (FIX deficient) dogs. Canine IX cDNA will be cloned into AAV vector which will be infused into dogs by intravenous injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that BSL level be upgraded to BSL-2 due to use of human cell lines, and details on anesthesia be provided. **Community Comments:** None III-D, BSL-2, AAV, dogs	
57806		Gene therapy for factor VII deficiency or hemophilia in a dog model of disease
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express coagulation factor VII or VIIa in dogs deficient in FVII, FVIII or FIX. Canine FVII or FVIIa cDNA will be cloned into AAV vector which will be infused into dogs by intravenous injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that BSL level be upgraded to BSL-2 due to use of human cell lines, and details on anesthesia be provided. **Community Comments:** None III-D, BSL-2, AAV, dogs	

56789		Modulation of C1B1 expression
APPROVED	**Summary:** The aim of this experiment is to express inducible shRNA to knockdown the protein C1B1 in human cells for use in culture or for implantation into mice. These cells may also be engineered to express GFP. Control or C1B1 shRNA will be cloned into pLV lentiviral vector, as will GFP. Cells will be transduced with one, or both, of the viral vectors as the endpoint, or for injection 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, lentivirus, mice	
47942		Oncogenic Potential of EBV Latency and transforming genes
APPROVED	**Summary:** The aim of this experiment is to determine the oncogenic potential of certain EBV latent genes and non-coding RNA's. EBV latent membrane proteins 1 and 2 and BamA will be cloned into plasmid or retroviral/lentiviral vectors which will then be used to transfect or transduce a number of human cell lines. Cell lines will then by inoculated IP or subcutaneously into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, plasmids, lentivirus, retrovirus, mice	
57602	Jason Reed	Regulation of plant stomatal aperture by SAUR (small auxin up RNA) proteins
APPROVED	**Summary:** The aim of this experiment is to create fusion proteins between Arabidopsis promoters and proteins and reporter genes (such as E. coli GUS and GFP) in Arabidopsis plants to reveal tissue-specific gene expression patterns or intracellular protein localization. Genes or regulatory sequences will be cloned into E. coli plasmids, and then introduced into Agrobacterium tumefasciens and then introduced into Arabidopsis thaliana plants. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-E, BSL-1, plasmids, plants	
57663		GFP & engineered GPCRs (DREADDs)
APPROVED	**Summary:** The aim of this experiment is to express GFP and engineered GPCRs (DREADDs) in the brains of rodent models for Cre expression. AAV's expressing GFP, hM3D, hM4D and rM3d will be site directed to different brain regions of mice by stereotaxic injections. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-1, AAV, mice	

57782		Cre recombinase, channelrhodopsin-2, halorhodopsin, GFP, YFP, mCherry, DREADD
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express GFP, YFP, mCherry, Cre recombinase, channelrhodopsin02, halorhodopsin, and DREADDs in the brains of mice. AAV's expressing the genes of interest will be site directed to different brain regions of mice by stereotaxic injections. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-1, AAV, mice	
53202	Xinghua Zeng	Production of control AAV vectors for internal and external investigators
APPROVED	**Summary:** The aim of this experiment is to produce control AAV vectors for as a core service to research investigators. GFP will be cloned into AAV vector which will be used to transduce cells in vitro. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, AAV	

1.
2. **NIH Incident Report:** Garry Coulson present a summary of a recent incident in the laboratory involving rDNA material. A researcher was dissecting pig corneas that had been transduced with rAAV-GFP when the researcher accidentally received a cut to the hand with the blade used to cut the tissues.
3. **Introduction to Biosafety course** – Garry Coulson discussed a new online-course for researchers that introduced basic concepts and best practices regarding laboratory biosafety.
4. **Training –** The Committee reviewed some information material and SOP's for distribution to all PI's and safety supervisors regarding safe use and training requirements for autoclaves.
5. **Sub-committee Approvals of Schedule G**: 1
   PI: Joseph Calabrese **Title:** Mechanisms of gene silencing induced by long noncoding RNAs (ID 56762, III-F)
6. **Schedule H report:** 53
7. **Next IBC meeting date:** April 3, 2019. Location: Burnett-Womack 9001

Adjourn.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

INSTITUTIONAL BIOSAFETY COMMITTEE

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

Members Present: Doug Cyr, Sandra Bradshaw, Keith Porterfield, Barbara Savoldo, Tori Baxter, Garry Coulson, Eric Lewis

Members Absent: Amy Sims, Aravinda DeSilva, Xiao Xiao, Craig Fletcher, Mary Beth Koza, Jessica Poole

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

Guests:

Open Meeting

1. **Review minutes from the March 6, 2019 meeting.** Minutes approved.
2. **Clinical Trial:** present on LentiGlobin BB305 clinical trial.
3. **Applications under review:**

ID	PI	Project Title
		A Phase 1/2 study evaluating gene therapy by transplantation of autologous CD34+ stem cells transduced ex vivo with the lentiglobin BB305 lentiviral vector in subjects with severe sickle cell disease.
APPROVED	**Summary:** The aim of this clinical study HGB-206 is to evaluate the safety and efficacy of transplantation with LentiGlobin BB305 Drug Product for the treatment of severe sickle cell disease (SCD) in adults. The LentiGlobin BB305 lentiviral vector is a replication-defective, self-inactivating (SIN) 3rd generation lentiviral vector pseudotyped with VSV-G. The vector is used to transduce the patient's own hematopoietic stem cells (autologous CD34+) with a variant of the normal beta-globin gene that comprises a single nucleotide modification at codon which conserves the protein's function and has been shown to possess anti-sickling properties by inhibiting the polymerization of the hemoglobin chains. Given that subjects are treated with autologous CD34+ cells in Nagendran study, the need for a suitable donor is obviated. Moreover, there is no need for immunosuppression after transplantation. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-C, lentivirus	
58802		Developing exosome-based delivery of TPP1 for Batten disease therapy
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to study the biodistribution of luciferase-labeled extracellular vesicles (EVs) in mice. Macrophages will be transfected with a plasmid expressing luciferase ex vivo and EV's collected from the conditioned media. EV's will then be administered to mice and their distribution assessed. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that Section III be completed for this study given the possibility that plasmid DNA may be incorporated into the EVs.	

	Community Comments: None III-D, BSL-1, plasmid, mice	
58822		Underlying neurobiological effects of modulating cortical and subcortical brain regions on alcohol sensitivity and intake
APPROVED	**Summary:** The aim of this experiment is to modulate brain regions involved in sensitivity to alcohol and alcohol self-administration. AAV vectors expressing hM4D-mCherry will be obtained from the Vector Core and injected into rats to transduce hM4D. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-1, AAV, rats	
56042		Trafficking and function of ApoE
APPROVED	**Summary:** The aim of this experiment is to understand the trafficking and function of the ApoE2, ApoE3 and ApoE4 proteins in primary rodent astrocytes and neurons. Lentiviral vectors expressing the genes of interest, or shRNA's to the genes of interest, will be constructed by the Vector Core. Vector particles will be used to infect primary rodent cells, including astrocytes and neurons. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, lentivirus	
57885	Jeffrey Dangl	Principles of Organization of Natural and Beneficial Plant-Microbe Communities
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to identify genes in naturally-occurring plant-associated bacteria with a role in establishing sustainable communities and conferring physiological advantages to the associated plants. Genes of interest (e.g. fluorescent markers, resistance cassettes etc) or transposons (Tn5) will be introduced into microbes that have been isolated from natural soil associated with healthy plants. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested verification that no rDNA is anticipated to be transferred to the host plant. **Community Comments:** None III-F, BSL-1, plasmids	
58902		In vivo migration of Mesenchymal Stem Cells (MSC) from GFP (Green Fluorescent Protein) mice
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to determine the function and mechanism by which Thy-1 affects MSC migration. Mesenchymal stem cells (MSC's) from commercially-obtained transgenic mice expressing GFP will be transferred from the GFP mice to Thy-1 null experimental mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that volumes proposed for administration the mice be adjusted to be in line with current IACUC limits.	

	Community Comments: None III-D, BSL-1, mice	
57975		The nuclear receptor RORalpha plays critical roles in mitochondrial quality control (lentivirus)
APPROVED	**Summary:** The aim of this experiment is to understand the insight into the role of RORalpha in the heart. The RORalpha shRNA will be cloned into a lentiviral vector which will be used to transduce H9c2 cells (cardiomyocytes) or isolated ventricular myocytes from neonatal rats. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, lentivirus	
57978		The role of CHIP in health and disease (adenovirus)
APPROVED	**Summary:** The aim of this experiment is to use a series of dilution constructs and point mutations of the protein CHIP to study its regulatory role in cellular metabolism, stress response and cellular degradation. Various constructs of CHIP or Cre-recombinase will be expressed in adenoviral vectors for exogenous expression in mammalian cells (HEK293, SHSY5Y, primary mouse fibroblasts, neurons and cardiomyocytes. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, adenovirus	
56542	Gary Johnson	Analysis of Kinome Dynamics In Cancer
APPROVED	**Summary:** The aim of this experiment is to identify the role of the cell-state plasticity in adaptive resistance to human breast and melanoma cancer cell lines. Fluorescent markers (eGFP and tdTomato) will be expressed in lentiviral vectors which will be used to transduce 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	
54744		Insertion of human genes into mouse germline
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express human genes of interest into the mouse to define targets for treatment of inflammatory disorders. Target genes will be cloned into plasmids which will then be electroporated into ES cells in vitro to generate transgenic mice expressing the human genes. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested information on means of anesthetizing the mice. Protocol should be III-E due to construction of transgenic mice at BSL-1. **Community Comments:** None III-D, BSL-1, plasmids, mice	

58482		Targeted CD19-CAR Lentivirus for In Vivo T Cell Gene Therapy
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to evaluate a bispecific antibody-targeted lentivirus to specific cells of interest (ie. human T cells) in vitro and in vivo, and evaluate the efficacy of CAR+ T cells to kill tumors cells in vitro and in vivo. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the reference to gene therapy in the title be specifically indicated for in mice, and not humans. **Community Comments:** None III-D, BSL-2, lentivirus, mice	
58823		Engineering B cells to produce antibodies of interest
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to modify human B cells ex vivo to produce antibodies of interest as a platform for long-term antibody delivery in vivo. For proof of concept. GFP will be cloned into AAV6 which will be used to transduce human B cells. These cells will ultimately be injected into NSG mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee indicated that no IACUC number was provided, unclear whether there was an existing animal protocol or a pending protocol. **Community Comments:** None III-D, BSL-2, AAV, mice	
59287	Sam Lai	Study of the mobility of Chlamydia trachomatis and Neisseria gonorhhea in human CVM
APPROVED WITH STIPULATIONS	**Summary:** The aim of this study is to test if lab-created antibodies can inhibit the mobility of recombinant Chlamydia trachomatis and Neisseria gonorrhea (both expressing fluorescent proteins) in human cervicovaginal mucus. No new recombinant strains will be created, the lab will be using strains already created by collaborators. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that reference to “viruses” be replaced with “bacteria”, the source of the bacterial strains be noted, and the classification of the protocol be amended from III-E to III-D. **Community Comments:** None III-D, BSL-2	
58522		pAAV2-CH19F2/R2-AIAT-luciferase
APPROVED	**Summary:** The aim of this experiment is to quantify the increase in transduction by the enhancer element “CH19F/R” and also to determine a tissue specificity of the enhancer element and the “AIAT” promoter. The enhancer element CH19F2/R2 and promoter AIAT will be cloned into plasmid containing AAV2 ITRs, luciferase and the SV40 polyA. The resulting vector, AAV9-CH19F2/R2-AIAT-luciferase, will be used to transduce cells or injected into mice via retro-orbital injection. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, AAV, mice	

58602		CRISPR LoxP knock-in mouse PCk2
APPROVED	**Summary:** The aim of this experiment is to generate a Pck2 flox allele mouse line to knock out the Pck2 gene in the heart. LoxP sites will be inserted into the introns of Pck2 using a specific guide RNA and Cas9 protein which will be injected into the mouse zygotes. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-1, plasmid, mice	
58962		knockout of genes of interest identified from single cell RNA-seq in zebrafish using the CRISPR/Cas9 system and study of cardiac regeneration
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to knock out genes of interest involved in cardiac regeneration using CRISPR/Cas9. Early 1-cell stage zebrafish embryos will be co-injected with f3b-specific single guide RNA and Cas9 nuclease mRNA to knock out the gene of interest. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested more information be provided in Section III Q1. **Community Comments:** None III-D, BSL-1, zebrafish	
58963		The role of fibroblast and endothelial cells in cardiac regeneration
APPROVED	**Summary:** The aim of this experiment is to generate transgenic lines of zebrafish to facilitate the visualization and ablation of subsets of fibroblast and endothelial cells during cardiac regeneration. A DNA construct with GFP flanked by FloxP sites and a DTA chain gene for negative selection are cloned into a plasmid which is then injected into one cell stage zebrafish embryos. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-1, plasmid, zebrafish	
59042		lentiviral overexpression of sgRNA for Rpl3l, Pfkfb2, Pfkm and other glycolytic rate limiting enzymes
APPROVED	**Summary:** The aim of this experiment is to determine the efficiency of sgRNA's to genes of interest (glycolytic rate limiting enzymes) in mouse cell lines. sgRNA's will be cloned into lentiviral vector which will be used to infect mouse cells lines. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, lentivirus	

59062		Adeno-associated viral overexpression of sgRNA for Rpl3l, Pfkfb2, Pfkm and other glycolytic rate-limiting enzymes
APPROVED	**Summary:** The aim of this experiment is to knock-down glycolytic rate-limiting enzymes in mouse neonatal heart to determine their role in cardiac maturation. sgRNA's against genes of interest will be cloned into AAV which will be injected into anterior-dorsal subcutis of the neonatal mouse. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, AAV, mice	
59083		Lenti or Adeno-associated viral overexpression of Rbfox1, Celf1, Carm1, pck2 or Lin28a in mouse hearts
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to overexpress genes of interest in mouse hearts to determine their protein modification and function. Genes will be cloned into AAV or lentiviral vectors which will then be delivered into mouse hearts. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee noted that the intracardiac injections were not on the proposed IACUC protocol, and thus conditionally approved the protocol pending an amendment to include this procedure in their IACUC protocol. **Community Comments:** None III-D, BSL-2, AAV, lentivirus, mice	
58362		Novel function of Gbl in cancer
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to generate human breast cancer cell lines stably expressing human Gbl genes or fluorescent markers. Genes of interest will be cloned into lentiviral vectors which will be used to transduce cells in vitro. Cells may be injected into mice either subcutaneous or into mammary pad. As a secondary aim, the UNC Animal Core will help generate knockin mouse models using standard CRISPR technology. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested further information on the creation of the knock-in mice. **Community Comments:** None III-D, BSL-2, lentivirus, mice	
58702		Neural mechanisms of opioids in PTSD and opioid immune conditioning
APPROVED	**Summary:** The aim of this experiment is to use viral vectors to delivery DREADD receptors in rats to manipulate brain cells and circuits to look at function. Viral vectors will either be generated by the UNC Vector Core facility or purchased through a vendor. DREADDS for hM3D, hM4D will be cloned into AAV which will be used to transduce neurons and astrocytes in vitro. Additionally, the rAAV will be infused directly into rat brains during surgery. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-1, AAV, rats	

58046		Express fluorescent proteins in bacteria
APPROVED	**Summary:** The aim of this experiment is to clone fluorescent proteins GFP, Citrine, tdTomato, dsRed, YFP, CFP or their derivatives into plasmids which will be expressed in bacteria for visualization by immunofluorescent microscopy. Recombinant bacteria will be used to infect cells in vitro or to infect mice in vivo. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, plasmids, mice	
58583		Gene therapy of hemophilia A
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express (hemophilia A) dogs by a gene therapy approach. FVIII cDNA will be cloned into dogs. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, , dogs	
58443		All-optical closed-loop studies for next generation neuroprostheses
APPROVED	**Summary:** The aim of this experiment is to manipulate and monitor neural activity with multiphoton optogenetics. AAV vectors encoding fluorescent proteins, optogenetic tools or calcium indicators (purchased from a vendor) will be injected into mouse brains. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-1, AAV, mice	
58483	Darrel Stafford	Characterization of enzymes in the vitamin K cycle
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to study the structure and function relationship of enzymes in the vitamin K cycle. The vitamin K epoxide reductase and vitamin K-dependent carboxylase will be cloned into an expression plasmid which will be transfected into HEK293 cells in vitro. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee noted the use of LentiCRISPR, thus the category for the protocol should be changed from III-F to III-D. **Community Comments:** None III-D, BSL-2, plasmids, lentivirus	

4. **Sub-committee Approvals of Schedule G**: 6
   PI: Blossom Damania **Title:** Innate immunity and KSHV (CpG) (ID 59322, III-F)
   PI: Blossom Damania **Title:** Innate immunity and KSHV (vIRF) (ID 59382, III-D)
   PI: Blossom Damania **Title:** Innate immunity and KSHV (vPK) (ID 59383, III-D)
   PI: Brian Jensen **Title:** The nuclear receptor RORalpha plays critical roles in mitochondrial quality control (plasmid DNA) (ID 56942, III-F)
   PI: Brian Jensen **Title:** The role of CHIP in health and disease (plasmid DNA) (ID 57976, III-F)
   PI: Leaf Huang **Title:** Targeted nanoparticles for therapy and imaging (ID 58382, III-D)
5.
6. **Schedule H report:** 32
7. **Next IBC meeting date:** May 1, 2019. Location: Burnett-Womack 9001

Adjourn.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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. **LCCC 1907-ATL-COM: Compassionate Use Re-Infusion of ATL.CAR.CD30** – Garry Coulson presented a compassionate use request for a patient who had received ATL.CAR.CD30 cells on LCCC 1532-ATL, but based on disease progression, is no longer eligible for reinfusion under LCCC 1532-ATL.
3.
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	

60342		Expression of Norway rat hepacivirus core and envelope genes by Venezuelan Equine Encephalitis virus (VEEV) Replicon Particles
APPROVED	**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	
60343	Ralph Baric	Defining the role of Dengue virus genotypic variation on the host neutralizing antibody response.
APPROVED	**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	
60345		Development of a Comprehensive Dengue Virus Molecular Clone Library Platform to Evaluate the Antigenic or Functional Variability of Viral Proteins
APPROVED	**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	
60346	Ralph Baric	Plasmids for Cre recombinase usage and verification
APPROVED	**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	

60347	Ralph Baric	Gene knock out and overexpression approaches to gain insight into gene function during virus infection (2019 Update)
APPROVED	**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	
60348	Ralph Baric	Real-time plasmid constructs for calculating coronavirus replication
	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	
60349		Mutants Removing Tertiary RNA Interactions - 2019 Renewal
APPROVED	**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	
60350		Infectious clones of bat SARS-like coronaviruses WIV1-CoV and SHC-014 (including reporter-expressing variants) or expressing WIV1 or SHC014 Spike genes - 2019 Renewal
APPROVED	**Summary:** The aim of this study is to generate reverse genetic infectious clones of bat SARS-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.	

	Community Comments: None III-D, BSL-3, plasmids, mice	
60351	Ralph Baric	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		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		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		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		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		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		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		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 that are deficient in this protein. dogs. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, , dogs	

60685		Gene therapy for canine hemophilia B with an improved vector
APPROVED	**Summary:** The aim of this experiment is to express coagulation dogs that are deficient in this protein. Human FIX will be cloned into vectors that will be used to dogs. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, , dogs	
60686		Gene therapy for factor VII deficiency or hemophilia in a dog model of disease
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express dogs that are deficient in these proteins. Human coagulation proteins will be cloned into vectors that will be used 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, , dogs	
60687		Retroviral Gene Therapy of Blood Protein Deficiencies
APPROVED	**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 dogs. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, , dogs	
60689		vectors for gene therapy of hemophilias and von Willebrand disease-1
	Summary: The aim of this experiment is to express coagulation FVII, FVIIa, FVIII, FIX (and variants), von Willebrand factor dogs that are normal or deficient in these proteins. Target genes will be cloned into that will be used to transduce dogs. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, , dogs	

60690		vectors for gene therapy of hemophilias and von Willebrand disease
APPROVED	**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 vectors that will be used dogs. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design **Community Comments:** None III-D, BSL-2, , dogs	
59722	Aziz Sancar	structure/Function of DNA photolyase and cryptochromes;DNA damage checkpoint and DNA repair; knockout cells
APPROVED	**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	
59742	Aziz Sancar	structure/Function of DNA photolyase and cryptochromes;DNA damage checkpoint and DNA repair; Viral Vector
APPROVED	**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	
60622	Aziz Sancar	structure/Function of DNA photolyase and cryptochromes;DNA damage checkpoint and DNA repair; Plant Studies
TABLED	**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	

60793		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: **Title:** Injection of Cre recombinase into mouse pups (ID 60382, III-D)
   PI: **Title:** Injection of Cre-GFP into mouse pups (ID 60384, III-D)
   PI: **Title:** Injection of GFP into mouse pups (ID 60385, III-D)
   PI: Benjamin Philpot **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.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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
		A Phase 2 Study of BC-819 in Patients with Non-Muscle Invasive Bladder Cancer whose Disease is Unresponsive to Bacillus Calmette-Guerin
APPROVED	**Summary:** The aim of this clinical trial, in which UNC is a secondary site as part of a multicenter trial, is to evaluate BC-819 (inodiftagene vixteplasmid; IND 13588) for the treatment of invasive bladder cancer for individuals unresponsive to BCG treatment, or patients who have failed at least one induction course with BCG ad are still eligible for further BCG treatment (BC-819 in combination with BCG).The therapeutic used in this trial, BC-819 has completed six clinical trials in NMIBC, ovarian and pancreatic cancer, and therefore has an established safety profile. The therapeutic is a recombinant DNA plasmid containing regulatory sequences from the gene which restricts expression of to malignant cells only which show elevated levels of relative to normal, healthy cells.The gene therapy product has been engineered to express only the chain, and not the chain which mediates entry into cells, therefore restricting the activity of to the cells that are directly transfected by the plasmid. Patients receive the ready-to-use therapy by intravesical administration which allows the product to be placed in localized direct contact with the tumor cells while avoiding systemic exposure of the patient. Intravesical instillation of BCG is the standard treatment for successfully managed superficial bladder cancer. The administration regimen for BC-819 is intended to be once weekly for a 10-week induction course, followed by a maintenance regiment of every three weeks up to week 96. **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**.

Meeting Minutes
June 5, 2019 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Keith Porterfield. Barbara Savoldo, Tori Baxter, Garry Coulson, Jessica Poole, Eric Lewis

Members Absent: Sandra Bradshaw, Amy Sims, Aravinda DeSilva, Xiao Xiao, Craig Fletcher, Mary Beth Koza

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

Guests: None

Open Meeting

1. **Review minutes from the May 1 and May 9, 2019 meetings.** Minutes approved.

ID	PI	Project Title
	Ann Beavan	**LCCC 1904-ATL:** Phase II Study of the Administration of T Lymphocytes Expressing the CD30 Chimeric Antigen Receptor (CAR) for Relapsed/Refractory CD30+ peripheral T Cell Lymphoma
APPROVED	**Summary**: The aim of this clinical trial is to evaluate the efficacy of ATL.CAR.CD30 for treatment of patients with relapsed/refractory CD30+ peripheral T cell lymphoma. Peripheral T-cell lymphoma (PTCL) is a heterogenous group of T-cell lymphomas which represent approximately 10-15% of non-Hodgkin Lymphoma (NHL) and is characterized by aggressive clinical nature and poor outcomes. The CD30 antigen is commonly expressed in varying intensities across each separate PTCL entity and has been a target of novel therapy options, The study therapeutic, ATLCAR.CD30, is an autologous T-lymphocyte chimeric antigen receptor product targeting the CD30 antigen. ATLCAR.CD30 was determined to be safe at a recommended phase 2 dose (RP2D) of 2 x 10^8 cells/m^2 in a recent phase Ib/II study of ATLCAR.CD30 administered to patients with Hodgkin Lymphoma and CD30+ NHL, ATLCAR.CD30. This multicenter, open-label phase 2 study will determine the efficacy and safety of ATLCAR.CD30 administered in two sequential infusions in subjects with relapsed/refractory CD30+ PTCL. Up to 20 subjects will be enrolled and will receive 2 infusions of 2 × 10^8 cells/m^2 of ATL product expressing the CAR.CD30. Monitoring during and after ATLCAR.CD30 cell infusion will be undertaken according to institutional standards for administration of blood products. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-C	

62526	Paul Armistead	Generation of antigen-specific transgenic T cells
APPROVED	**Summary**: The aim of this experiment is to develop a protocol for transducing T cells with antigen-specific transgenic T cell receptros (TCR's) and testing the transgenic T cells for their ability to selectively kill cancer cells. Retroviral vectors will be used to introduced a TCR alpha and beta chain pair into human cell lines or primary human T cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, retrovirus	
61927		Mineralocorticoid receptor antisense
APPROVED	**Summary**: The aim of this experiment is to understand how knock down of the mineralocorticoid receptor in the brain of live rats will affect alcohol consumption and stress resilience. Rats will receive an infusion of synthetic oligonucleotides which will reduce the expression of the receptor **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, rats	
62003	Nicholas Brown	Baculovirus expression of proteins in ubiquitin and ubiquitin-like protein pathway
APPROVED	**Summary**: The aim of this experiment is to determine the structure and biochemical mechanisms of proteins in ubiquitin and ubiquitin-like protein pathways. Genes involved in ubiquitin and ubiquitin-like pathways (e.g. APC11, Cullin1-5, RBX1, RVX2) will expressed in insect cells. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, baculoviral vector	
62323		GPR182 Transgenesis in Zebrafish
APPROVED	**Summary**: The aim of this experiment is to alter expression of gpr182 in zebrafish. Zebrafish embryos will be injected with a Tol2 plasmids encoding zebrafish gpr182 under the control of fli1a promoter. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, plasmid, zebrafish	

62562		Morpholino Injections in Zebrafish
APPROVED	**Summary**: The aim of this experiment is to knockdown irf8 expression in zebrafish. An irf8 morpholino purchased from commercial vendor will be injected into zebrafish embryos. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, zebrafish	
62642		Use of a floxed Mertk mouse to study the role of Mertk in tumor progression
APPROVED	**Summary**: The aim of this experiment is to determine how MertK influences the immune response in tumor microenvironments. Wildtype tumor cell lines and genetically modified tumor cell (luciferase, GFP, RFP) will be injected into a floxed Mertk mouse model to examine how genetics of both the tumor and the mouse effect the immune response to cancer. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, plasmid, mice	
62142		Transplantation of transgenic murine total bone marrow or hematopoietic stem and progenitor cells (HSPCs).
APPROVED	**Summary**: The aim of this experiment is to determine the multi-lineage reconstitution potential of transgenic mouse HSPC's or perform lineage tracing of transgenic HSPC's. Whole bone marrow cells or purified HSPCs isolated from a variety of transgenic mice with insertions or modifications will be transplanted into sub-lethally irradiated congenic recipients by retro-orbital injection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, mice	
61702		Analysis of virulence in fully virulent
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to identify and characterize virulence determinants of , in particular how these determinants interact with the host to cause disease. Bacteria will be transformed with plasmids to either expressing genes of interest or for mutational analysis to knockout genes of interest. Recombinant bacteria will then be used to infect mammalian cells in vitro or inoculate mice in vivo. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the volumes for all routes of inoculation be provided as well as the method for anesthesia of the mice. **Community Comments**: None III-D, BSL-3, plasmids, mice	

62482		Generation of Ntn3 knockout mouse using CRISPR/Cas9
APPROVED	**Summary**: The aim of this experiment is to knockout the Ntn3 gene in mice to study the effect on kidney development. Guide RNAs targeting the Ntn3 gene and RNA for Cas9 will be introduced into mouse zygotes for creation of the Ntn3 transgenic mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-E, BSL-1, mice	
62362		PANK2 gene therapy for treatment of PKAN and elucidation of disease biology
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to determine if overexpression of human PANK2 protein in the murine brain is detrimental to animal health in a wild type mouse. The wild type or codon-optimized flag-tagged PANK2 gene will be cloned into an AAV vector for AAV production in HEK293 cells. Recombinant AAV will be injected intracranially into mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that PANK2 and PKAN be defined and an updated IACUC number be provided. **Community Comments**: None II-D, BSL-1, AAV, mice	
62303		Roles of inflammation-induced enteric bacterial genes in experimental colitis
APPROVED	**Summary**: The aim of this experiment is to determine how specific genes in resident intestinal bacteria impact the development of experimental colitis. Genetically-modified (with deletions of over-expression of genes of interest) commensal, non-pathogenic enteric bacterial strains will be used to inoculate the GI tract of germ-free mice in gnotobiotic isolators. Colon inflammation and immune responses will be measured at the time of sacrifice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, plasmids, mice	
62403	Koji Sode	Development of molecular recognition elements for biosensing technology
APPROVED	**Summary**: The aim of this experiment is to produce recombinant protein for the fabrication of molecular recognition elements for biosensing technology, which will be dedicated to the diagnosis of certain diseases such as metabolic disorders. Genes of interest will be cloned into plasmids which will be used to transform E. coli to produce recombinant proteins for use in vitro. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, plasmids	

61862		Ovarian cancer mouse model using Gaussian Luciferase (GLuc) and GFP as reporter genes
APPROVED	**Summary**: The aim of this experiment is to develop a mouse model for ovarian cancer and evaluate immunotherapies. Ovarian cancer cells will be transfected with lentiviral plasmids expressing GFP or luciferase. Lentiviral vectors will then be used to transduce mouse and human ovarian cancer cells which will ultimately be injected into mice by i.p. injection. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, lentivirus, mice	
60522		Innate Immunity - nucleotide agonists
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to test the activation of innate immunity against infectious or damaging agents through in vitro stimulation of isolated cells and in vivo stimulation by using the ligand as vaccine adjuvant. Synthetic short oligonucleotides will be administered to primary mouse cells or cell lines or will be injected into mice through a number of routes. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the classification be changed from III-F to III-D, the IACUC number be updated and the volume for each route provided. **Community Comments**: None III-D, BSL-1, mice	
61422		Influenza Viral Peptide
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to express portions of the influenza hemagglutinin (HA) protein in insect cells in vitro. Purified HA protein or peptides will be injected into mice to induce an immune response to flu. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the title be amended to be more descriptive of the work, and that the volumes for each route be provided. **Community Comments**: None III-D, BSL-1, plasmids, mice	
61459		Dengue Viral Peptide
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to express the ectodomain portions of the dengue E-protein in mammalian cells in vitro. Purified recombinant protein or peptides will be injected into mice to induce an immune response to the peptides. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the title be amended to be more descriptive of the work, and that the volumes for each route be provided. **Community Comments**: None III-D, BSL-1, plasmids, mice	

61460		Zika Viral Peptide
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to express the ectodomain portions of the Zika E-protein in mammalian cells in vitro. Purified recombinant protein or peptides will be injected into mice to induce an immune response to the peptides. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the title be amended to be more descriptive of the work, and that the volumes for each route be provided. **Community Comments**: None III-D, BSL-1, plasmids, mice	
62182		Plexin-A1 Regulation by CIITA and Immunologic Function
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to stimulate immune responses in primary mouse cells in vitro or mice using synthetic short DNA oligonucleotides. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the volumes for each route be provided and the method for anesthetization during intranasal inoculation be provided. **Community Comments**: None III-D, BSL-1, mice	
61063		Plasmid DNA (pDNA)- based Vaccination
APPROVED	**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 (e.g. cytokines and cell-derived antigens) will be cloned into plasmids which will be purified and injected into mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-1, plasmids, mice	
61762		Adoptive transfer of retrovirally transduced murine iNKT & T cells
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to assess the potency of murine iNKT cells and T cells that express mB7-H3-chimeric antigen receptor (CAR) or mB7-H3-CAR ires-IL12. The insert genes mB7-H3CAR andmB7-H3-IL12 have already been cloned into retroviral vector SFG by a collaborator. Murine T cells and iNKT cells will be transduced with the retroviral vector and transduced cells adoptively transferred to recipient mice. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee request that Section III be completed. **Community Comments**: None III-D, BSL-2, retrovirus, mice	

2. **Sub-committee Approvals of Schedule G**: 4
   PI: George Breese Jr. **TITLE:** Etiology of affective disorders and addiction (ID, 58882, III-D)
   PI: Eduardo Lazarowski **TITLE:** Ecto-ATPase inhibitors restore airway surface hydration in CF (ID 62264, III-F)
   PI: Uma Nagarajan **TITLE:** Characterizing the effects of Neisseria gonorrhoeae and Chlamydia trachomatis during single and co-infection of primary human fallopian tube epithelia (ID 55147, III-D)
   PI: Koji Sode. **TITLE** Development of molecular recognition elements for biomarker detection (ID 62402, III-F)
3. **Schedule H report:** 35
4. **Next IBC meeting date:** July 10, 2019 GMB 2007.

Adjourn.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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:** A Phase 2 Multi-Center, Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Safety of TOL-3021 in Patients with New Onset or Established Type 1 Diabetes Mellitus.
3.

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
		A Phase 2 Multi-Center, Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Safety of TOL-3021 in Patients with New Onset or Established Type 1 Diabetes Mellitus
APPROVED	**Summary:** Type 1a diabetes mellitus (T1D) is primarily a disease of children and young adults that results from immune-mediated destruction of the insulin-producing β-cells of the pancreas. Insulin is the only known β cell-specific autoantigen, and insulin autoantibodies are usually the first to appear in young children with T1D. Intensive management of exogenous insulin therapy to achieve strict control of hyperglycemia has been shown to slow the development of long-term complications. However, many patients inevitably and gradually develop complications, including damage to eyes, kidneys, and the cardiovascular system, which result in early disability and death. Such intensive management of insulin therapy also puts patients at higher risk for hypoglycemic events. The aim of this clinical trial is to evaluate the safety of TOL-3021 in patients with T1D. TOL-3021 is designed to modulate immune responses to insulin specifically to have no off-target immune-toxicity. This antigen-specific approach has the advantage of decreasing the autoimmune response while leaving other processes, such as immune surveillance against malignancy and immune responses against infectious agents, intact.	

	TOL-3021 is a plasmid expression vector containing the coding sequences for the . TOL-3021 is provided in a sterile single-use 2 mL vial. TOL-3021, or placebo, will be administered weekly via an intramuscular injection of 0.5 mL for 52 weeks. Subjects will be provided with all necessary supplies for administration of the injections including tuberculin syringes for drawing study drug up from vial, 23 gauge needles in the length(s) appropriate for subject age, weight, and injection site to be used (see Table 4 as a guide), alcohol wipes, gauze pads, band aids, and a sharps container. **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 UNC Animal Core facility. **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 Clones 7_O18 and 18_L6
APPROVED	**Summary:** The aim of this experiment is to produce a transgenic mouse strain expressing the and regions of the human genome. Bacterial artificial chromosome (BAC) harboring the unmodified 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	Mark Heise	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		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		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		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		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		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. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments**: None III-D, BSL-2, , dogs	
63482		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		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		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		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**:. William Kim **TITLE:** CRISPR-mediated knockout in A375 xenograft study (ID 62683; III-D)
   PI:. William Kim **TITLE:** Enhancing the therapeutic efficacy of the fatty acid synthase inhibitor, fasnall (ID 62684; III-D)
   PI:. William Kim **TITLE:** Pre-clinical evaluation of gene editing of TAK1 in breast cancer (ID 62702; III-D)
   PI:. William Kim **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.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

INSTITUTIONAL BIOSAFETY COMMITTEE

Meeting Minutes
August 7, 2019 3:30 PM
Burnett-Womack 9001

Members Present: Doug Cyr, Sandra Bradshaw, Keith Porterfield, Amy Sims, Aravinda DeSilva, Barbara Savoldo, Shawn Hingtgen, Tori Baxter, Garry Coulson, Eric Lewis

Members Absent: Xiao Xiao, Craig Fletcher, Mary Beth Koza, Jessica Poole

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

Guests:

Open Meeting

1. **Review minutes from the July 10, 2019 meeting.** Minutes approved.
2. **Clinical Trial:** LCCC1804-ATL Phase I Trial of Personalized and Adaptive Neoantigen Dose-Adjusted Vaccine (PANDA-VAC) Administered Concurrently with Nivolumab **PI:** Dr. Jared Weiss
3. **Applications under review:**

ID	PI	Project Title
	Jared Weiss	LCCC1804-ATL Phase I Trial of Personalized and Adaptive Neoantigen Dose-Adjusted Vaccine (PANDA-VAC) Administered Concurrently with Nivolumab
APPROVED	**Summary:** This is a single center, open-label phase I clinical trial designed to determine the safety of personalized and adjusted neoantigen peptide vaccine (PANDA-VAC) administered concurrently with nivolumab in subjects with advanced squamous non-small cell lung cancer (NSCLC) or squamous cell carcinoma of head and neck (SCCHN). In this trial, the primary peptide vaccine will consist of personal neoantigens identified by whole exome sequencing and single cell sequencing of matched archival tumor and normal cell DNA obtained from individual subjects at baseline. The initial therapeutic neoantigen vaccine product will be comprised of six peptides administered IV at a dose of 300 micrograms (µg) per peptide and Poly-ICLC at a dose of 500 µg, Poly-ICLC is a synthetic double-stranded ribonucleic acid (dsRNA) 'host-targeted' therapeutic viral-mimic and pathogen-associated molecular pattern (PAMP) with broad innate and adaptive immune enhancing, vaccine adjuvant, antiviral and antiproliferative effects. The peptide vaccine and Poly-ICLC will be given in five priming doses on Days 1 and 4 of Week 1, Day 1 of Week 2, Day 1 of Week 3 and Day 1 of Week 4, followed by two booster vaccinations on Day 1 of Week 11 and Day 1 of Week 21. Additionally, subjects with partial response, stable disease, mixed response or oligoprogressive state or non-threatening progressive disease, following the full series of five priming and two booster vaccinations concurrently with nivolumab administration, may receive adapted vaccine adjusted to address neoantigens emerging during combination treatment. The five priming and two booster doses of adapted vaccine will be administered at the same intervals as primary vaccine. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-C	

59482		Therapeutic applications of highly engineered delivery agents
APPROVED	**Summary:** The aim of this experiment is to study how nano- and micro-particles can be manipulated as therapeutics for the treatment of cancer or infectious diseases. Mouse models of cancer will be generated by injected mice with a variety of cells lines expressing firefly luciferase. Additionally, mice may be injected with immune-modulating synthetic CpG. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, plasmids, mice	
64145		In vivo function of alternative splice variants of HNRNPA1
APPROVED	**Summary:** The aim of this experiment is to generate mouse models with constitutive expression of two splice variants of the hNRNPA1 gene to determine the in vivo function of exon 8 of this gene. Mouse zygotes will be injected with picoliter amounts of gRNA, Cas9 mRNA and linearized vector containing desired gene variants and then subsequently implanted into pseudopregnant dams. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-E, BSL-1, plasmids, mice	
59984	Nigel Key	Contribution of red blood cell/endothelial interactions to bleeding and thrombosis in MPN
APPROVED	**Summary:** The aim of this experiment is to determine the contribution of endothelial cell expressed Jak2 V617F mutations on red cell, platelet and white cell adhesion in vitro. The Jak2 V617F gene and GFP reporter will be cloned into a plasmid which will be used to transfect human umbilical vein endothelial cells (HUVEC) in vitro. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-F, BSL-2, plasmids	
64402		In vivo CRISPR screens of liver for tumor suppressors
APPROVED	**Summary:** The aim of this experiment is to identify genes in the liver that promote tumor formation when combined with loss of SWI/SNF complex genes. A guide RNA library will be cloned into a plasmid and injected into mice through hydrodynamic injection to induce expression in hepatocytes thereby creating hepatocyte-specific knockout animals that will be monitored for tumor formation. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. Classification should be changed to III-D and IACUC number updated to 19-176. **Community Comments:** None III-D, BSL-1, plasmids, mice	

63782		Injection of GFP-positive, human breast cancer cells into the mammary fat pad of mice
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to test the anti-tumor efficacy of new drug delivery carriers in tumor mice. To induced cancers, mice will be injected into their mammary fat pads with breast cancer tumor cells lines (MDA-MB-231-GFP, LM2-4175 and BoM-1833) generated by collaborators. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the volume for injection be provided. **Community Comments:** None III-D, BSL-2, plasmid/retroviral vector, mice	
58322		Knockdown and overexpresson of TCF4 using lentivirus
APPROVED	**Summary:** The aim of this experiment is to overexpress wildtype or mutant TCF4 in cells (mouse cortical neurons, HEK293) in vitro, or to knockdown expression using siRNA to TCF4. Wildtype or mutant mTCF4 will be cloned into lentiviral vector which will be used to transduced 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	
64442	Kent Rossman	The role of oncogenes in cell proliferation and signaling
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to assess the ability of known and potential oncogenes (e.g. KRAS) to modulate signaling and proliferation in vitro. Genes of interest will be cloned into plasmids or lentiviral vectors while will be used to transfect/transduce mammalian cells in vitro. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. Classification of experiments should be III-D. **Community Comments:** None III-D, BSL-2, plasmid, lentivirus	
64642	Brian Strahl	Mechanisms of DNA methylation regulation in chromatin
APPROVED	**Summary:** The aim of this experiment is to produce stable cells lines expressing genes of interest (i.e. chromatin regulatory proteins), or short hairpins targeting those genes of interest, for research in chromatin regulation and DNA methylation in mammalian cells. For expression of genes of shRNAs, a variety of plasmid and viral vector-based systems will be used. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. **Community Comments:** None III-D, BSL-2, plasmids, lentivirus, retrovirus, adenovirus	

64542		HSV Virus H129 ΔTK-TT
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to perform site-directed infusion of HSV delta-TK-TT into the brains of mice. Replication of the virus is dependent upon Cre recombinase recombination of the lox-stop-lox sequence. **Committee Comments**: The proposed containment and safety procedures are adequate for the experimental design. Committee requested that dose and concentration of virus to be infused be provided. **Community Comments:** None III-D, BSL-2, HSV, mice	

4. **Sub-committee Approvals of Schedule G**: 0
5. **Discussion of Ft. Detrick lab shut-down after failed safety inspection**– Doug Cyr discussed the recent shutdown of USAMRIID laboratories in response to failed inspected and safety concerns by the CDC Federal Select Agent Program.
6. **Updated Appendix 10A for Clinical Trials –** Garry Coulson discussed the updated Appendix 10A form for submission of human gene therapy clinical trials to the IBC for review. Committee voted and approved the new form and official rollout in October 2019. Current form will be accepted during this two-month window.
7. **Schedule H report:** 31
8. **Next IBC meeting date:** September 4, 2019

Adjourn.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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:** VB-111-701/GOG-3018: The OVAL Study – A Randomized, Controlled, Double-Arm, Double-Blind, Multi-Center Study of Ofranergene Obadenovec (VB-111) Combined with Paclitaxel vs Paclitaxel Combined with Placebo for the Treatment of Recurrent Platinum-Resistant Ovarian Cancer – **PI:** Linda Van Le

ID	PI	Project Title
	Linda Van Le	VB-111-701/GOG-3018: The OVAL Study – A Randomized, Controlled, Double-Arm, Double-Blind, Multi-Center Study of Ofranergene Obadenovec (VB-111) Combined with Paclitaxel vs Paclitaxel Combined with Placebo for the Treatment of Recurrent Platinum-Resistant Ovarian Cancer
APPROVED	**Summary**: The aim of this multi-center trial is to evaluate the safety, tolerability and efficacy of VB-111-701 and paclitaxel in patients with platinum-resistant ovarian cancer. Platinum resistant, and recurrent ovarian cancer represents a significant unmet medical need. The study drug, VB-111 (Ofranergene obadenovec), is a non-replicating adenovector (Ad5, E1 deleted), which contains a proprietary modified). VB-111 has a dual mechanism of action in cancer therapy applications: vascular disruption of angiogenic blood vessels nourishing the tumor and induction of a tumor directed immune response through cytokine secretion of activated APCs in the tumor microenvironment. VB-111 is formulated as a sterile vector solution in single use, 10 ml glass vials. Each vial contains 5ml of vector at a viral titer of 10_{12} VP/ml and vehicle (10% glycerol in Phosphate Buffered Saline). Administration is through a single intravenous infusion of diluted sample every 2 months until study end. **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	Ralph Baric	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	

66125	Ralph Baric	Generation of CMV promoter-driven constructs for coronavirus nonstructural and accessory proteins - 2019 renewal
APPROVED	**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	
66126		Adenovirus vector-based transduced mouse model of MERS-CoV infection - 2019 renewal
APPROVED	**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	
66142	Ralph Baric	Design of Spike variant Porcine Epidemic Diarrhea Virus (PEDV) infectious clones - 2019 renewal
APPROVED	**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	
66143		Introduction of stop codons into coronavirus accessory open reading frame (ORF) genes to prevent expression of Coronavirus accessory proteins - 2019 renewal
APPROVED	**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	

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	

65842	Xian Chen	The role of methyltransferase EHMT2 in epigenetic regulation and others
APPROVED	**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	
65702		Ex326 Cytochrome P450 (CYP) Humanized Rat
APPROVED	**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	
65482		Generating mice with Cre dependent Cd276 expression
APPROVED	**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	
65122		Gene Targeting Retinoblastoma Using Nanoparticles
APPROVED WITH STIPULATIONS	**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	

65123		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		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		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		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	

66282		Injection of Luciferase expressing cells into the mammary fat pad of mice
APPROVED	**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	
65443		Infection of neurons in mice with viral vectors
APPROVED WITH STIPULATIONS	**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	
65462		Generation of novel knockin mouse lines
APPROVED	**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 UNC Animal Models Core 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	
64223		Circuitry study of adult neurogenesis regulation and function
APPROVED WITH STIPULATIONS	**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	

66042		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		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	

3. **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.
4. **Sub-committee Approvals of Schedule G**: 5
5. **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)
6. **Schedule H report:** 42
7. **Next IBC meeting date:** October 2, 2019 Burnett-Womack 9001

Adjourn.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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. **Baric Lab** – Proposal for research using EBOV ΔVP30 (Schedule G 67055) – Presented by Dr. Baric.
3. **Lab** – Proposal for research using inactivated -infected samples – Presented by Garry Coulson. The lab 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	Ralph Baric	Defining the role of tripartite motif gene (TRIM) family members in the host immune response to Ebola virus infection
APPROVED	**Summary**: The aim of this experiment is to use the biologically-contained clone of EBOV (deltaVP30) as a tool to understand the role of TRIM proteins in regulating immune responses to EBOV, specifically how these proteins contribute to EBOV replication and virus-induced apoptosis in vitro. Since the deltaVP30 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.	

	• 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	
66562	Craig Cameron	RNA-dependent RNA polymerase mechanism
APPROVED	**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	
66563	Craig Cameron	Single-cell analysis of viral infection dynamics
APPROVED	**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	
66662	Craig Cameron	Picornavirus Genome Replication
APPROVED	**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	

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	Kristina De Paris	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	Kristina De Paris	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		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		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		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 which will be used dogs. **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. **Community Comments:** None III-D, , , dogs	
67342		in normal, hemophilia A, hemophilia B, VWD and FVII deficient dogs
APPROVED WITH STIPULATIONS	**Summary**: The aim of this experiment is to determine dogs with inherited bleeding disorders. . **Committee Comments**: The proposed containment and safety procedures are adequate for the experiment design. The Committee requested details on the . **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	

67422		Role of the Rho-GAP GRAF3 in human hypertension
APPROVED WITH STIPULATIONS	**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	
66243		Recombinant Adeno-Associated Virus (rAAV) vector-based vaccination of mice_Updated
APPROVED WITH STIPULATIONS	**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	

5. **Sub-committee Approvals of Schedule G**: 2
   PI: **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.

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

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

Members Absent: Rachel Graham, 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 October 2, 2019 meetings.** Minutes approved
2. **Clinical Trial:** 9801-CL-0101 – A Phase I, Open-label Study of ASP9801, an Oncolytic Virus, Administered by Intratumoral Injection in Patients with Advanced/Metastatic Solid Tumors. **PI:** Juneko Grilley-Olson
3. **Clinical Trial:** An Open-label Phase I Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Preliminary Efficacy of MEDI5395 in Combination with Durvalamab in Subjects with Select Advanced Solid Tumors. **PI:** Siddarth Sheth
4. **Clinical Trial:** A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy using Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the BB305 Lentiviral Vector in Subjects with Sickle Cell Disease at Risk of Stroke. **PI:** Kim Kasow
5. **Clinical Trial Protocol Amendments: 2**
   Trial: LCCC1811-ATL: Phase 1 Study of the Administration of T lymphocytes Expressing the Kappa Chimeric Antigen Receptor (CAR) and CD28 Endodomain for Relapsed/Refractory Kappa+ Non-Hodgkin Lymphoma. **PI:** Natalie Grover
   Trial: rad-IFN-CS-003: A Phase III, Open Label Study to Evaluate the Safety and Efficacy of Instiladrin (rad-IFN/Syn3) Administered Intravesically to Patients with High Grade, BCG Unresponsive Non-Muscle Invasive Bladder Cancer (NMIBC). **PI:** Michael Woods
6. **Applications under review:**

ID	PI	Project Title
	Juneko Grilley-Olson	9801-CL-0101 – A Phase I, Open-label Study of ASP9801, an Oncolytic Virus, Administered by Intratumoral Injection in Patients with Advanced/Metastatic Solid Tumors
CONDITIONAL APPROVAL	**Summary:** The aim of this study is to evaluate the safety, tolerability, and efficacy of ASP9801, a genetically engineered oncolytic vaccinia virus, in patients with advanced/metastatic solid tumors. ASP9801 is an attenuated recombinant vaccinia virus expressing transgenes for human and human . . The study consists of 2 parts: Dose Escalation and Recommended Phase 2 Dose (RP2D) Expansion. Each part of the study will be divided into Group A (cutaneous/ subcutaneous lesions) and Group B (visceral lesions). Group A will include subjects with cutaneous or subcutaneous tumors accessible for intratumoral (IT) injection. Group B will include subjects with visceral lesions accessible for IT injection with ultrasound or computed tomography (CT) guidance. For all subjects, the study will consist of the following periods: Screening (up to 28 days); Initial Treatment Period (two 28-day cycles); Optional Extended Treatment Period (contin. 28-day cycles); and Follow-up Period (safety and survival follow-up)	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. The Committee requested an SOP of how the biohazards will be safely handled and properly disposed of. III-C	
	Siddarth Sheth	An Open-label Phase I Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Preliminary Efficacy of MEDI5395 in Combination with Durvalamab in Subjects with Select Advanced Solid Tumors
CONDITIONAL APPROVAL	**Summary:** This is a Phase 1, first-in-human, open-label, dose-escalation, and dose-expansion study to assess the safety, tolerability, pharmacokinetics (PK), pharmacodynamics and preliminary efficacy of MEDI5395 in combination with durvalumab in subjects with selected advanced solid tumors. MEDI5395 is a novel genetically modified recombinant Newcastle disease virus (rNDV) expressing that is designed to selectively infect, replicate, and kill human cancer cells. Durvalumab is a human immunoglobulin (Ig) G1 kappa (IgG1κ) monoclonal antibody (mAb) that blocks the interaction of programmed cell death ligand 1 (PD-L1) (but not programmed cell death ligand-2) with programmed cell death1 (PD-1) on T-lymphocyte (T-cells) and cluster of differentiation (CD)80 (B7.1) on immune cells (IC) and is engineered to reduce antibody-dependent cell-mediated cytotoxicity (ADCC). All doses of MEDI5395 and durvalumab will be administered by intravenous (IV) infusion. . Durvalumab treatment will be started either sequentially or concurrently with MEDI5395. . Up to approximately 164 subjects may be enrolled in the study across approximately 30 sites globally. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested an SOP of how the biohazards will be safely handled and properly disposed of. III-C	
	Kim Kasow	A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy using Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the BB305 Lentiviral Vector in Subjects with Sickle Cell Disease at Risk of Stroke
APPROVED	**Summary:** The study will evaluate the efficacy and safety of hematopoietic stem cell (HSC) transplantation (HSCT) using LentiGlobin for SCD BB305 Drug Product: an autologous CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced with BB305 Lentiviral Vector (LVV) [previously referred to as LentiGlobin BB305 LVV] encoding the gene, suspended in cryopreservation solution in the final immediate container for the intended medical use. Subjects will undergo stem cell collection via mobilization with plerixafor (0.24 mg/kg) and subsequent apheresis to collect HSCs for both LentiGlobin for SCD BB305 Drug Product manufacture/cryopreservation and for cryopreservation of back-up cells for rescue. This is a single-arm, open label, multi-site, single dose, Phase 3 study in approximately 18 subjects ≥2 and ≤14 years of age with SCD and at elevated risk of stroke despite chronic blood transfusions. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-C	

67624	Albert Baldwin, Jr	Role of IKK, RelA, and RelB in cancer
APPROVED	**Summary:** The aim of this experiment is to explore the roles of IKK, RelA and RelB in cell growth, proliferation and gene expression in cancer. Insert genes (e.g RelA) will be cloned into a lentiviral vector which will be used to transduce cells in vitro. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentivirus, no animals	
67922		Injection of DNA into Zebrafish embryos
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express a gene of interest (sFLT1 with double HIA tag), or control (fluorescent reporters such as GFP) in zebrafish. Constructs possesses the gene of interest and desired promoter will be cloned into a plasmid, which will be injected into single-cell stage zebrafish embryos. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided, as well as the volume and concentration of inoculum used for injecting into the zebrafish embryos. III-D, BSL-1, plasmids, zebrafish	
68462		Injection of Morpholinos into Zebrafish Embryos
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to reduce the expression of a protein of interest (putative SUN2) in zebrafish and monitor subsequent phenotypes. No recombinant DNA manipulations will be performed. Morpholinos (synthetic DNA) will be injected into single-cell stage zebrafish embryos. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided, as well as the volume and concentration of inoculum used for injecting into the zebrafish embryos. III-D, BSL-1, morpholinos (synthetic DNA), zebrafish	
68362	Edward Brown	Analysis of cells latently infected with lentiviruses
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to determine the cellular pathways that lead to latent HIV infection and reactivation. This experiment will use wildtype plasmids encoding HIV as well as modified variants with certain genes deleted and replaced with reporter genes (eg. GFP, Luciferase, and CD24). Human T cells will be transduced with lentiviruses designed to overexpress human genes of interest (eg. Groucho repressor gamily genes, PRDM1, FOXP1, TCF7, GATA3). Will also deliver Cas9/CRISPR or shRNA encoding lentiviruses into human cell lines and primary human T cells to inhibit those human genes of interest. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Requested verification that the work with HIV would be performed in a BSL-2+ laboratory. III-D, BSL-2, BSL-2+, HIV, lentivirus	

68742		Chemically catalyzed epigenetic gene regulation in prostate cancer.
APPROVED	**Summary:** The aim of this experiment is to study the role of proteins of interest (eg. Let7c, p53, and Androgen Receptor) in human prostate cancer. This work will focus on the epigenetics and tumorigenesis. A novel dCAS9-FKBP will be transduced with adenovirus or adeno-associated virus (AAV), depending on the cell-type. Both in vitro and in vivo experiments will be performed. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, Adenovirus, AAV, mice	
68282	Blossom Damania	Role of KSHV vIL6 in tumorigenesis
APPROVED	**Summary:** The aim of this experiment is to assess the role of KSHV viral interleukin 6 (KSHV vIL6) in tumorigenesis. KSHV vIL6 will be cloned into a pcDNA3 vector and introduced into mammalian cells in vitro. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, plasmids	
68506	Dirk Dittmer	Use of shRNA lentiviruses in tissue culture
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to inhibit protein expression of genes of interest using siRNA and shRNA. The genes of interest include p53, p21, MDM-2, CDKN2A, TP53BP1, and others. Either siRNA or shRNA 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. The Committee requested the names of the genes of interest of the experiment. III-D, BSL-2, lentivirus	
68082		Analysis of S. aureus strains with selected deletions in virulence factors in vitro and in vivo
APPROVED	**Summary:** The aim of this experiment is analyze different *S. aureus* strains in vitro and in vivo (in mice) when certain virulence factors have been selectively eliminated. Genes Clfa, coa, vWbp, or streptokinase have been inactivated by transposon insertion. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, *Staphylococcus aureus*, mice	
68242		Antibody Depletion for rAAV Gene Delivery
APPROVED	**Summary:** The aim of this experiment is to develop an effective approach for the depletion of pre-existing antibodies against AAV. Insert genes of interest will be cloned into rAAV viral vectors, which will then be injected into rabbits. The constructs will include rAAV-CBA-hNAGLU-op and scAAV-hSGSH. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, AAV, rabbits	

67643		All-optical closed loop studies for next generation neural prostheses
APPROVED	**Summary:** The long-term aim of this project is to develop neural protheses to recover lost motor functions. One objective of this project is to study brain circuits involved in sensory processing and motor learning. A second objective is to develop non-invasive interfaces with the brain via optical methods. The experiments that will be performed for this project include inject AAV viral vectors containing insert genes (eg. fluorescent proteins, optogenetic tools, etc.) into mouse and ferret brains. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, AAV, mice, ferrets	
68102		Production of a mouse adapted chikungunya virus
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to modify the chikungunya virus vaccine strain 181/25 infectious clone to test whether it alters the virus' ability to replicate in mice. Point mutations of interest will be introduced in the infectious clone via PCR based mutagenesis, then subsequent viral replication rates, compared to wildtype virus, will be tested in cell-based assays and in mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested a risk assessment for experiment, particularly for the differential replication rates among the viral strains that will be examined. The Committee also requested the dose and volume that will be injected in mice. III-D, BSL-3, chikungunya virus, plasmids, mice	
68103		Immune Evasion of Neurovirulent alphaviruses
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to examine if introducing a mutation in nsP1 in alphaviruses Ross River virus (RRV), Venezuelan equine encephalitis virus (VEEV), and chikungunya virus (CHIKV) can modify their virulence phenotypes. The nsP1 mutation was previously identified in another alphavirus, Sindbis virus strain AR86. Mutations of interests will be made in infectious clones of those alphavirus. Viral replication rates and other phenotypes, compared to wildtype virus, will be tested in cell-based assays and in mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested a risk assessment for experiment, particularly for the experiments that will examine the differential replication rates among the different viral strains. The Committee also requested information about the VEEV strain as well as the dose, volume, and injection route that will be injected in mice. III-D, BSL-3, alphaviruses, mice	
68122		Expression of novel viral ORFs and noncoding RNA in lentivira retroviral vectors
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to express novel viral open reading frames (ORFs) and noncoding RNA in lentiviral and retroviral vectors. These ORFs and noncoding RNA will come from influenza A virus, , MERS-CoV, , or Kaposi's Sarcoma Herpes virus. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. Committee requested clarity of the viral ORFs that will be examined during this experiment. III-D, BSL-2, lentivirus, retrovirus	

68743		UPDATE of 57822 Immortalization and transformation of MEF
APPROVED	**Summary:** The aim of this experiment is to immortalize mouse and human embryo fibroblast (MEFs and HEFs, respectively) using retroviral vectors carrying the SV40 large-T. The SV40 large-I-immortalized will be transformed further by using lentiviral vectors expressing the H-RAS V612 either with or without the BSD/GFP selection marker. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, lentivirus, retrovirus	
68422		Aldh1l1 and Aldh1l2, a novel tumor growth regulators
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to analyze if folate metabolizing enzymes ALDH1L1 and ALDH1La gene knockout is responsible for tumor progression or suppression and effects on metabolism and other cellular effects like proliferation, migration, etc. The insert gene will be cloned into a vector which will be utilized to transduce cells in vitro. The 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 anesthesia method that will be used for the mouse experiments. III-D, BSL-2, plasmids, mice	
67542		Engineering Bacteriophage particles for Sustained Secretion of Therapeutics or Immunogens by Mucosal Commensals
APPROVED	**Summary:** The aim of this experiment is to deliver plasmids or genetic material into commensal bacteria in vivo using bacteriophage-based particles, with the goal of secreting therapeutic proteins. These particles consist of a plasmids packed into the T7 bacteriophage capsid. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be provided, as well as the injection routes that will be used for injecting the mice. III-D, BSL-2, T7 bacteriophage, mice	
68642		Inducible Fibronectin Fragment (Fnf) Mouse
APPROVED	**Summary:** To produce a mouse model in which human Fnf is overexpressed in a cre-dependent manner. This mouse model will be used to examine the role of Fnf induced reactive oxygen species (ROS) generation in surgically induced and age-related osteoarthritis. Vector will be propagated in *E. coli* and ultimately injected into mouse embryos with Cas9 protein and guide RNA. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-E, BSL-1, plasmids, mice	

68342		Use of Adeno-Cre virus
APPROVED	**Summary:** The aim of this experiment will be to use a dual reporter system (a Cre-reporter system) in mice that responds to Cre-recombinase for the purpose of fate-mapping and lineage tracing cancer cells during the metastic process. An adeno-Cre virus containing an insert gene will be exposed to cells and injected in mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, adenovirus, mice	
68263		Dissecting the neural projections of hyperarousal states
APPROVED	**Summary:** The aim of this experiment is to examine the projection-specific, cell-type specific neural circuitry in mouse brain that is relevant to rapid arousal states and motivated behaviors. Mice will be injected with CAV-Cre, which encodes the Cre gene. This virally carried gene integrates with the mouse genome, after which infected mouse cells express the Cre protein. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be updated. III-D, BSL-2, canine adenovirus, mice	
69022		The Function of Mesenchymal Stem Cells During GvHD
APPROVED	**Summary:** The aim of this experiment is to examine the function of mesenchymal stem cells (MSCs) during Graft-versus-Host-Disease (GvHD). A CRISPR/Cas9 system will be used to edit the gene that codes for either TSLP, IL7 or CXCL12 in MSCs. The gene edited MSCs will then be transplanted into mice. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested that the IACUC number be updated. III-D, BSL-2, plasmids, mice	
67362		DREADD Detecting basal and pathological changes in neurocircuits under evoked fMRI
APPROVED	**Summary:** The aim of this experiment is to precisely excite, inhibit, or lesion neuronal populations during functional studies and determine their influence over evoked responses in the brain. This will be accomplished by injecting AAV vectors with specific opsins, DREADDS, or caspase into the brains of mice or rats using stereotactic methods. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, AAV, rodents	
68142		Intracerebroventricular (ICV) delivery of rAAV for gene therapy treatment
APPROVED WITH STIPULATIONS	**Summary:** The aim of this experiment is to perform safety testing and phenotypic scoring in neonatal mice after receiving intracerebroventricular (ICV) injections of rAAV vector day 1 or 2 of life. The different rAAV vectors may contain protein coding region expressing either marker genes or therapeutic genes. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. The Committee requested the names of the genes of interest of the experiment. III-D, BSL-1, AAV, mice	

68502		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 using mouse models. Murine stem cell virus or lentivirus vectors carrying transgenes of interest will be transduced in cells in vitro. Some of those cells will be chosen and injected into mice. Transgenes include Cre, EGFP, YFP, RFP, ovalbumin, and luciferase. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-2, murine stem cell virus, lentivirus, mice	

4. **Sub-committee Approvals of Schedule G**: 8
   PI: Gaorav Gupta **Title:** Cas9 Ribonucleoprotein modification of cell lines (**ID:** 68722, III-F)
   PI: Frederico Innocenti **Title:** Characterization of functional genetic variation in genes of the angiogenesis pathways (**ID:** 67731, III-F)
   PI: Frederico Innocenti **Title:** Creation of isogenic TIME cells (**ID:** 67732, III-F)
   PI: Tal Kafri **Title:** The effects of host genetic background on lentiviral vector gene therapy_2019 Renewal (**ID**: 68382, III-D)
   PI: Tal Kafri **Title:** Role of cis elements and chromatin-modifying drugs in lentiviral gene expression_2019 Renewal **(ID**: 68383, III-D)
   PI: Tal Kafri **Title:** HIV BSD_2019 Renewal (**ID:** 68384, III-D)
   PI: Tal Kafri **Title:** HIV-1 Cre_2019 Renewal (**ID:** 68385, III-D)
   PI: Patrick Sullivan **Title:** GWAS region deletion (**ID:** 67862, III-F)
5. **Schedule H report:** 26
6. **Next IBC meeting date:** December 4, 2019 Burnett-Womack 9001

Adjourn.

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:** RPL-002-18: A Randomized, Controlled, Open-Label, Phase 2 Study of Cemiplimab as Single Agent and in Combination with RP1 in Patients with Advanced Cutaneous Squamous Cell Carcinoma – Diana Wallack presented on behalf of Dr. Collichio. **Approved with stipulations** (see below)
3. **Managed Access Program (MAP):** CCTL019B2003I Managed Access Program (MAP) to provide access to CTL019, for acute lymphoblastic leukemia (ALL) or large B-cell lymphoma patients with out of specification leukapheresis product and/or manufactured tisagenlecleucel out of specification for commercial release – Garry Coulson presented this single-subject IND for the use of tisagenlecleucel that is out-of-specification for commercial release. Dr. Grover 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
	Frances Collichio	RPL-002-18: A Randomized, Controlled, Open-Label, Phase 2 Study of Cemiplimab as Single Agent and in Combination with RP1 in Patients with Advanced Cutaneous Squamous Cell Carcinoma
APPROVED WITH STIPULATIONS	**Summary:** The aim of this Phase 2 study is to evaluate the clinical benefit of cemiplimab monotherapy versus cemiplimab in combination with RP1, a selectively replication competent HSV-1, for patients with advanced cutaneous squamous cell carcinoma. The virus contains a codon-optimized sequence for hGM-CSF and a codon-optimized sequence for GALV-GP R-. GALV-GP R- expression leads to cell to cell fusion (syncytia) formation in infected tumor cells through binding to the constitutively expressed PiT-1 receptor for GALV. The objective of treating with RP1 is to have a direct oncolytic effect on tumors and to induce an immune response to tumor antigens, including tumor neoantigens, which is proven to be therapeutically beneficial in other tumors. As the objective of treating with cemiplimab is to enhance anti-tumor immune responses through PD-1 blockade, combination of RP1 with cemiplimab is expected to provide a synergistic effect. This is a phase 2, randomized, controlled, multicenter study of cemiplimab at a dose of 350 mg administered intravenously (IV) every 3 weeks or cemiplimab at a dose of 350 mg administered IV every 3 weeks in combination with RP1 administered intratumorally (IT) at a dose level of 1 x 10^6 particle forming units (PFU)/mL followed by a subsequent dose of RP1 at a dose level of 1 x 10^7 PFU/mL every 3 weeks for patients with	

	advanced CSCC (metastatic or locally advanced). In the combination group, dosing with cemiplimab will start at the second dose of RP1. Patients will visit the clinic at the timepoints outlined in the Study Schedule. Safety will be assessed by history and physical examination results. Standard safety evaluations will occur on the first day of each cycle. **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		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		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		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		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	

69623		Over expression and knockout of non-coding RNAs in breast cancer cell lines for mouse xenograft assays
APPROVED	**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	
70602		Analysis of genes in avirulent (BSL2 level)
APPROVED	**Summary:** The aim of this experiment is to identify and characterize virulence determinants of , 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 , 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	
69082	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	
69003		Analysis of virulence in fully virulent
APPROVED	**Summary:** The aim of this experiment is to identify and characterize virulence determinants of , including how these genes are transcriptionally regulated under various conditions. Genes of interest will either knocked out, or overexpressed, in fully-virulent strains of , 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	

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.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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.

3. **LCCC-1955-ATL-COM** – Compassionate Use of Autologous Activated T-Cells Expressing the 2nd Generation GD2 Chimeric Antigen Receptor, IL-15, and iCaspase9 Safety Switch for an Adult Patient with Relapsed or Refractory Neuroblastoma (Dr. George Hucks). Garry Coulson presented this single-patient compassionate use protocol using CAR-T cells modified with the iC9.GD2.CAR.IL15 cassette vector. Dr. Hucks 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		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		Vero E6 cell passaging of wildtype and ExoN(-)
APROVED	**Summary:** The aim of this experiment is to passage ExoN(-) in Vero E6 cells to increase fitness of the attenuated virus in vitro and in vivo. Once a more-fit 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 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		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		Ex337 -Cas9 Mouse
APROVED	**Summary:** The aim of this experiment is to produce mice with Cas9 and eGFP expression from the . 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 -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 . 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 -Cas9 Mouse
APROVED	**Summary:** The aim of this experiment is to produce mice with Cas9 and eGFP expression from the . 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		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:** . **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. mice	
71203		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	

70327	Wanda O'Neal	Knockdown and Overexpression of various genes relevant to Cystic Fibrosis
APROVED	**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	
70542		D3 domain mutations to explore Muc5b Biology
APROVED	**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	
71362	Philip Spanheimer	Aberrant transcriptional regulation by TFAP2C as a Novel mechanism of Hormone resistance in luminal breast cancer
APROVED	**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	
70422		Luciferase expressing tumor cells for intraoperative devices to prevent tumor re0ccurrence.
APROVED	**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	

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.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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:

Open Meeting

1. **Review minutes from the January 8, 2020 meetings.** Approved
2. **Proposal for research with 2019-nCoV at BSL-3:** presented a research proposal for working with the 2019-nCoV virus under BSL-3 containment in his lab. 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, 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, has 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 extensive expertise and history in working with pathogenic coronaviruses under BSL-3 high-containment, the IBC has approved research proposal with 2019-nCoV under strict BSL-3 containment.
3. **Applications under review:**

ID	PI	Project Title
72702	Ralph Baric	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		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 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 - 2020 Renewal
APPROVED	**Summary**: The aim of this experiment is to determine whether certain residues are important for viral replication and pathogenesis. Recombinant will be generated with attenuating mutations in the . 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 SARS-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 SARS-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	

72708		Generating mutants expressing
APPROVED	**Summary**: The aim of this experiment is to generate viral mutants of expressing the . will be ligated into the 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	
72722		Generating mutants expressing from the SARS-like Wuhan coronavirus
APPROVED	**Summary**: The aim of this experiment is to generate viral mutants of expressing the protein . Synthetically -produced protein from the novel coronavirus will be ligated into the 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	
72723		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
APPROVED	**Summary**: The aim of this experiment is to create vaccine candidates for the spike and nucleocapsid proteins of the novel SARS-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	
72822		Generation of expressing the , including reporter-expressing variants
APPROVED	**Summary**: The aim of this experiment is to generate viral mutants of in which the protein from . Synthetically -produced 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	

72304		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		Ex345 -Cas9-ERT2 Mouse Strain -
APPROVED	**Summary**: The aim of this experiment is to do a targeted insertion in the mouse 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		Ex346 -Cas9 Rat
APPROVED	**Summary**: The aim of this experiment is to generate a transgenic rat strain with Cas9 and eGFP expression from the . 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		Ex347 -Cas9 Rat
APPROVED	**Summary**: The aim of this experiment is to generate a transgenic rat strain with Cas9 and eGFP expression from the . 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 Ex1-2 Mouse
APPROVED	**Summary**: The aim of this experiment is to create a transgenic mouse strain with human 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 Mouse
APPROVED	**Summary**: The aim of this experiment is to create a transgenic mouse strain with human 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		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		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		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		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: Title: Viral infection of gene knockout mice (III-D, ID 72343 / 72386)
   PI: Yuliya Pylayeva-Gupta Title: Immunomodulatory mechanisms in Kras-driven pancreatic cancer_Lenti (III-D, 71182)
   PI: Yuliya Pylayeva-Gupta Title: Immunomodulatory mechanisms in Kras-driven pancreatic cancer_pLKO.1 (III-D, 71183)
   PI: Yuliya Pylayeva-Gupta 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: 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.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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:

Open Meeting

1. **Review minutes from the February 5, 2020 meetings: Approved**
2.

3.

4. **Applications under review:**

ID	PI	Project Title
73102		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		Generation of attenuated - 2020 renewal
APPROVED	**Summary**: The aim of this experiment is to generate and examine them in mice. Recombinant viruses will be generated containing combinations of these mutations: .These mutations are based on conserved residues that have been identified as important residues for	

	. The genome is maintained as six cDNA cassettes in plasmids that are propagated in *E. coli*. 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 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, , 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.	

	III-D, BSL-1, plasmids, mice	
68042		Generation of a zebrafish (Danio rerio) model of kidney development and disease
APPROVED	**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	
73722		Use of Venezuelan equine encephalitis virus strain ZPC738 as non-select agent challenge virus for mouse vaccination studies
APPROVED	**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	
73723		Fluorescent influenza viruses for in vivo tracking of infection
APPROVED	**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	
73724		Venezuelan equine encephalitis virus replicons expressing proteins from influenza A, and KSHV
APPROVED	**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	

	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	
73842		Nonintegrating lentiviral vectors towards clinical trials
APPROVED	**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	
73142		Functional analysis of human and chiroptera hepatovirus pX sequence
APPROVED	**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	

	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	

	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	
73928		Study of stabilized variants of Lipoprotein lipase- mRNA version
APPROVED	**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	
73642		Gene Therapy of Hemophilia and von Willebrand Disease
APPROVED	**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 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, , dogs	
73262		Sendai Virus Infection of airway epithelium in vitro or in vivo
APPROVED	**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	
72062		Meiotic Recombination in *D. melanogaster*
APPROVED	**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.	

	Committee Comments: The proposed containment and safety procedures are adequate for the experimental design. III-D, BSL-1, plasmids, fruit flies	
73942		Multimodality Therapy: B16
APPROVED	**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	
71762		Crosstalk between intestinal inflammation, bone and bone proteins
APPROVED WITH STIPULATIONS	**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	
73162		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 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	
73143		Lentiviral production of stable cell line of luciferase expression gene

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

5. **Sub-committee Approvals of Schedule G**: 2
   PI: **Title:** Ex351 -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)
6. **Schedule H report:** 40
7. **Next IBC meeting date:** April 1, 2020 Burnett-Womack 9001

Adjourn.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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. **LCCC1818-ATL:** A Phase 1 Study of Autologous Activated T-cells Targeting the B7-H3 Antigen in Subjects with Recurrent Epithelial Ovarian Cancer. **PI:** Paola Gehrig
3. **Applications under review:**

ID	PI	Project Title
	Paola Gehrig	LCCC1818-ATL A Phase 1 Study of Autologous Activated T-cells Targeting the B7-H3 Antigen in Subjects with Recurrent Epithelial Ovarian Cancer.
APPROVED	There is a critical need to identify new treatment options for recurrent ovarian cancer. Preclinical observations support B7-H3 as a viable target of CAR-T therapy. Based on these observations, a phase 1 study of CAR.B7-H3 T cells in subjects with recurrent epithelial ovarian cancer will be conducted to determine the safety and tolerability of this treatment modality and identify a recommended phase 2 dose for further study. The primary objective is to determine the safety and tolerability of autologous CAR.B7-H3 T cell product administered intraperitoneally after lymphodepletion with cyclophosphamide and fludarabine to subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer. This is single center, open-label phase 1 dose escalation trial that uses modified 3+3 design to identify a recommended phase 2 dose (RP2D) of CAR.B7-H3 T cell product. An expansion cohort will enroll additional subjects at the RP2D for a total enrollment of up to 21 subjects on the protocol. **Committee Comments:** The proposed containment and safety procedures are adequate for the experimental design. III-C	
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	
74883		Enhancement of the Bat Coronavirus HKU4 binding to the human DPP4 receptor - 2020 renewal
APPROVED	**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	
74942		Investigating potential ORFs on the negative strand of Flaviviruses
APPROVED	**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	
74962		Adaptation of the 2019-nCoV (SARS-CoV-2) infectious clone to mice expressing wild-type mouse ACE2
APPROVED WITH STIPULATIONS	**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	
73062		Kras-driven pancreatic cancer and metastasis_pLVTH-M-GFP
APPROVED WITH STIPULATIONS	**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	
73182		Kras-driven pancreatic cancer and metastasis_Lenti_LucS_LucOS_Cre
APPROVED	**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	
74142		Adjuvant GVI3A derived from Venezuelan equine encephalitis virus (VEE) replicon particles
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. **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	
74284		AAV-mediated Gene Therapy for the Treatment of Neurogenetic Diseases
APPROVED	**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	
74482	Nilu Goonetilleke	Generation of cell lines that stably express human leukocyte antigens (HLA) for use as antigen presenting cells to T cells
APPROVED	**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	
71726		Oncogenes include NeuT, EGFP, and ampicillin resistant protein
APPROVED	**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. 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	Gaorav Gupta	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 SARS-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 lab 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		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		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		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		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. **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	
74922		Study of stabilized variants of Lipoprotein lipase
APPROVED WITH STIPULATIONS	**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	
74862		Expression of ciliary proteins in airway epithelial cells in cultured cells and in vivo
APPROVED WITH STIPULATIONS	**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	
74102		Influenza Virus H1N1 inoculation of Hamster Airways
APPROVED WITH STIPULATIONS	**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 (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. III-D, BSL-2, recombinant influenza virus, hamsters	
73162		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		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: Wolfgang Bergmeier **Title:** Transfusion of transgenic platelets (III-E, ID 74262)

PI: **Title**: Ex352- -Cas9- -Mouse (III-E, ID 74302)

PI: **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:** 20
3. **Next IBC meeting date:** May 6, 2020 Web-Conference Call

Adjourn.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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:

Open Meeting

1. **Review minutes from the April 1, 2020 meetings: Approved**
2. **NIH Reportable Incident** – Garry Coulson discussed a recent incident in a high containment laboratory involving a potential exposure to a recombinant SARS-CoV-2 strain acquired through a mouse bite by an infected mouse.
3. **Applications under review:**

ID	PI	Project Title
76579		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 (, MERS-CoV, SARS-CoV-2, HKU3, HKU4, HKU5, and SARS-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		Combination of nsp14 and nsp16 attenuating mutations in the 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 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	

	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, , MERS-CoV, mammalian cell lines, mice	
76581		Viral pathogenesis of strains with mutations in the papain-like proteinase domain that prevents ubiquitination - 2020 renewal
APPROVED	**Summary**: The nsp3 region of 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 viruses and experiments therein will be executed within the BSL3 environment. Pathogenesis of these viruses will then be compared to wild-type . Mice will be infected intranasally with each viral strain, mice will be monitored/weighed daily for 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 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, , mammalian cell lines, mice	
76582		Recombinant monoclonal antibody fragment antigen-binding (Fab) expression - 2020 Renewal
APPROVED	**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	
76585		Coronavirus nsp14 ExoN determinants of fidelity - 2020 renewal
APPROVED WITH STIPULATIONS	**Summary**: To determine if fidelity-altering mutations will alter replication in vitro and replication and pathogenesis in vivo, fidelity-altering mutations identified in experiments performed in collaboration with at will be introduced individually and in pools into the infectious cDNA background, in the presence and absence of inactivated nsp14 ExoN activity. Viruses will be constructed as described above.	

		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
76587		for increase in transduction efficiency, production yield and evasion of pre-existing antibodies
APPROVED WITH STIPULATIONS		**Summary**: The aim of this experiment is to engineer a portfolio of . This may be done for the purpose of studying either the basic biology or utilities as . This work proposes to create and screen for multiple libraries that has distinct physical and biological profiles including but not limited to . The libraries will contain randomized deletion, insertion or substitution in the genome. The libraries will be tissue culture cells or injected in mice. The libraries will then be propagated in the presence of human or mouse . 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 vectors, which will then be used for 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, , mammalian cells, mice
76683		Mechanisms of Formation of Pseudoexfoliation Material on Human Surgical Lens Capsules (Ad.CLU)
APPROVED WITH STIPULATIONS		**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.

	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	
75662		Investigating the molecular functions underlying endometrial cancer
APPROVED	**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	
75362		EBV-infected human mononuclear cells
APPROVED WITH STIPULATIONS	**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	
75782	Daniel Dominguez	Modulation of Gene Expression Using Lentiviral Particles
APPROVED	**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	
75522		The role of androgen and cofactors in prostate cancer development
APPROVED	**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.	

	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 attenuated 3526 strain of Venezuelan Equine Encephalitis (VEE) expressing the Spike and Nucleocapsid proteins from the SARS 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		COVID-19 in vitro
APPROVED	**Summary**: **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 , NP and GP (1:1:1 ratio), with eGFP incorporated into the VP40 capsid construct. The VLPs are prepared using viral proteins from the 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 and NCI-H460, will be stably transfected with the	

	gene expressed from the SV40 promoter. The generated cells will express will ultimately be injected into mice. The 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	
75343		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		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		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: Sarah Linnstaedt **Title:** Injection of FKBP5 silencing siRNA (III- F, ID 76827)
   PI: **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.