

University of Kentucky

Institutional Biosafety Committee (IBC) Meeting

Date: 04FEB2026
Time: 12:03PM – 1:28PM
Location: Virtual Meeting via Zoom - <https://uky.zoom.us/j/82304184575?jst=1>

Minutes

Call to Order

The meeting was called to order by Douglas Harrison at 12:03PM EST.

Attendance

IBC Members Present

Maria Landron (Local, Non-Affiliated Member)	Brandy Nelson (Institutional Member)
Thomas Chambers (Local, Non-Affiliated Member)	Amelia Pinto (Institutional Member)
Doug Harrison (Chairperson)	Carol Pickett (Local, Non-Affiliated Member)
Cheryl Haughton (Animal Containment Expert)	Arthur Hunt (Plant Containment Expert)
Carrie Shaffer (Institutional Member)	Delphine Malherbe (Laboratory Staff Representative)
Delena Mazzetti (Biological Safety Officer)	Jan Smalle (Plant Containment Expert)
Mike Mendenhall (Local, Non-Affiliated Member)	Yadi Wu (Institutional Member)

Regrets

None

Guests

Elizabeth Brooks (Administrative Support Associate I)	Jeff Howell (IBC Administrative Professional II)
Robert Hayman (Assistant Biological Safety Officer)	Audra Strahl (IBC Administrative Professional II)

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Melissa Hollifield (Animal Compliance
Manager)

Kathryn Childress (Temporary STEPS
Office and Clerical)

Quorum

Per the University of Kentucky Institutional Biosafety Committee By-Laws, at least 6 voting members shall constitute a quorum.

Approval of Previous Month's Meeting Minutes

[2026.01.14 IBC Meeting Minutes DRAFT.pdf](#)

The previous month's minutes were approved. Doug Harrison initiated the motion. Thomas Chambers seconded the motion. All members present (14) voted in favor.

Old Business

None

Protocol Review

Amendments

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PI: Rina Plattner

IBC Protocol Number: IBC-24-474

Protocol Title: Role of Abl Family Kinases in Solid Tumors

Protocol Type: Amendment

Amendment To: Cells or tissues used in research, Genetic constructs, Manipulations planned, Personnel

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-F, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.9, NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-7, UK Administrative Regulation 6.3

Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)

Primary Reviewers: C. Haughton, A. Pinto, M. Landron

Brief Project Overview:

Our laboratory studies the Abl family of non-receptor tyrosine kinases (c-Abl, Arg) and their involvement in the development and progression of solid tumors such as melanoma and breast cancer. We are

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currently focusing on how these proteins are activated in these diseases; identifying biological processes that they mediate (e.g. invasion, migration, proliferation, survival, tumor growth, metastasis); and defining downstream signaling pathways that are activated by Abl kinases to promote the above biological processes. The biological safety issues associated with our research include the use of established human cancer cell lines, ecotropic retrovirus, lentivirus, recombinant DNA, use of radioactivity in conjunction with biohazardous materials, injection of human cancer cell lines into animals, and breeding of transgenic animals.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Immunohistochemistry, PCR/qRT-PCR, Transformation, Transfection, Use of viral vectors, Creation of viral vectors, Use of Human Source Material(s), Imaging/Microscopy

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: ABL1, ABL2/ mouse, human/ proto-oncogene-enzyme, proliferation/ expression in cell culture, silencing in cell culture, also utilizing constitutively active (PP) and kinase-dead forms (K>R)/ mammalian cell culture/ MIGR1, PK1, pcDNA3; BRAF/ human/ proto-oncogene-enzyme/ expression in cell culture/ mammalian cell culture/ pBabePuro; I κ B kinase/ human/ enzyme/ expression in cell culture/ mammalian cell culture/ pcDNA3; EGFP, EYFP, dsRed, mCherry/ jellyfish/ tag, cell tracking/ expression in cell culture/ mammalian cell culture/ various; STAT3/ human/ cell growth, survival/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed; luciferase/ firefly/ cell tracking/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed; beclin-1/ human/ autophagy protein/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed; Src/ human/ cell growth and survival/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed; cathepsins-B, L, D/ human/ protein degradation, autophagy/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed; NF- κ B, Sp1, Ets1/ human/ transcription factors/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed; ATG proteins/ human/ autophagy/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed; Rab5, Rab7/ human/ vesicular trafficking/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed; NM23/ human/ metastasis suppressor/ silencing in cell culture/ mammalian

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cell culture/ lentivirus;Fus1/Tusc2/ human/ tumor suppressor/ expression in cell culture/ mammalian cell culture/ pGEX2T, pcDNA3, MIGR1, pBabepuro, pIRES-DsRed;ERK2/ human/ proliferation/ expression in cell culture/ mammalian cell culture/ pBabepuro;beta-catenin/ human/ proliferation/ expression in cell culture/ mammalian cell culture/ pcDNA3, EGFP-tag;MYC/ human/ proliferation/ expression in cell culture/ mammalian cell culture/ MSCV-IRES-GFP;ETS1/ human/ proliferation/ expression in cell culture/ mammalian cell culture/ pCMV-IRES-GFP;CCL2, CCL5, CXCL1, CXCL5, CXCL8, IL6/ human or mouse/ cytokine/ expression into mouse or human melanoma cells/ mammalian cell culture/ LV-vectors from GeneCopoeia;ZEB1/ mouse or human/ transcription factor/ Make stable cell lines in human or mouse melanoma cells/ mammalian cell culture/ LV-230 from Genecopoeia.;ZEB1 shRNA/ human or mouse/ transcription factor/ Silence ZEB1 in human or mouse melanoma cells/ human or mouse/ PLK0.1 or PLK0.5 from Sigma;N-cadherin/ human or mouse/ E3 ligase/ over expression/ mammalian cell culture/ PLK0.5 from Sigma;N-cadherin/ Homo sapiens/ transmembrane protein/ shRNA for silencing the gene/ mammalian cell culture/ PLK0.5-viral vector from Sigma;NEDD4L/ Homo sapiens/ E3 ligase/ silencing in cell culture with shRNA/ mammalian cell culture/ PLK0.1 or PLK0.5 from Sigma;shNT (non-targeting shRNA)/ none/ none/ non-targeting shRNA/ mammalian cell culture/ PLK0.1 or PLK0.5 from Sigma;Div-DDR1 (constitutively active)/ homo sapiens/ receptor tyrosine kinase/ create stable melanoma cell lines/ mammalian cell culture/ pCDH from Dr. Ge

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pcDNA3/; Plasmid/Migr1/; Plasmid/pBabe Puro/; Plasmid/pEGFP-N1/; Plasmid/pGEX2T/; Plasmid/pIRES-dsRed2/; Plasmid/pKLO-IPTG-3XLacO/; Plasmid/Piggybac-cumate/; Plasmid/Piggybac-cum-shRNA/; Plasmid/pWZLblast/; Lentivirus/pLKO.1, PLK0.5/; Plasmid/PK1/; Plasmid/pCMV-mCherry/; Plasmid/pCMV-IRES-GFP/; Plasmid/MSCV-IRES-GFP/ Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/293T HEK/; Animal/NIH3T3/; Human/MDA-MB-435s/M14, M14-BR, M14-BMR/; Human/BT-549/; Human/MDA-MB-231/; Human/MDA-MB-468/; Human/WM3248/; Human/MCF-7/; Human/A375/; Human/WM239,WM278,SBCL2, WM35, Mel1617, 451-Lu,WM164,UACC-903,WM9, WM1232,WM3211,WM793, 12050-Lu. WM3248 and WM164 expressing constitutively active ABL1 and ABL2 (WM164-ABL1/2-PP, WM3248-ABL1/2-PP)/; Human/melanocytes/; Human/mammary epithelial cells/; Human/BT-474/; Human/SUM1315/; Animal/melan-a/; Human/Hermes-1/; Human/LOX IVMI/; Human/SK-Mel-2, SK-Mel-5, SK-Mel-28, UACC-62, UACC-257, malme-3M, and SK-MEL-2 resistant to MEK inhibitor (SK-MEL-2MR)/; Human/SK-Mel-30, SK-Mel-147 and their BRAF and MEK inhibitor resistant counterparts (-BMR)/; Animal/OSUMMER.12, OSUMMER.13 and their MEK inhibitor resistant counterparts (-MR)/; Animal/Ma-NRAS1-1007, Ma-NRAS2-1014 and their MEK inhibitor resistant counterparts (MR)/; Animal/YUMM5.2, YUMM1.7, YUMM3.3, B16F10, and YUMM-.BMR (cell lines resistant to BRAF and MEK inhibitors)/

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of

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Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Cells - Human, non-modified/subcutaneous injection/isoflurane/ABSL2/Gown, eye protection, gloves, mask or BSC/ABSL2/No/Excess cells will be autoclaved. Sharps use minimized. Cages only opened in changing station, BSC or chemical fume hood./; Mouse/Cells - Animal, genetically modified/subcutaneous injection/isofluorane/ABSL1/Gown, gloves, eye protection/ABSL1/No/Excess material will be autoclaved. Sharps will be minimized./; Mouse/Cells - Human, genetically modified/subcutaneous injection/isoflurane/ABSL2/Gown, eye protection, gloves/ABSL2/No/Excess material will be autoclaved. Sharps will be minimized. Cages will only be opened in changing stations, BSC, or chemical fume hood./; Mouse/Tissue - Human (ex. PDX tumor tissue)/subcutaneous/isoflurane anesthesia/ABSL2/Gown, eye protection, gloves, mask or BSL2 hood/ABSL2/No/Excess human tissue will be autoclaved. Use of sharp objects will be minimized. Cages will only be open in a cage changing station, BSC, or chemical fume hood.

Risk Assessment/Discussion:

Dr. Plattner has submitted an amendment to her current IBC protocol entitled *Role of Abl Family Kinases in Solid Tumors*. In this amendment, Dr. Plattner has updated lab personnel, added new lentiviral vectors and gene targets, added new human cell lines, and updated planned manipulations. The new lentiviral vector constructs will express NF-1, a tumor suppressor, and knockdown RTK (EGFR, PDGRF), known oncogenes. These new lentiviral constructs will be produced in Dr. Plattner's laboratory as previously described and approved using BSL2+ containment and will only be used in cell culture. None of the cells transduced with these new lentiviral vectors will be used in conjunction with animals. Dr. Plattner has also added a project to be completed in collaboration with Dr. Olivier Thibault's laboratory utilizing PDOX models obtained from St. Jude's. These fluorescently labeled PDOX models will be implanted into the brains of immune-compromised mice via stereotaxic injection. Growth of gliomas in mice will be assessed via IVIS imaging as previously described and approved. This work will be completed using ABSL2 containment and housing and is similar to previously approved work in Dr. Plattner's laboratory. The addition of these new projects and vector constructs does not significantly alter the biohazardous risks associated with Dr. Plattner's current IBC protocol.

IBC Discussion & Vote:

The amendment to IBC-24-474 (version 36.0) was approved pending minor modifications as listed below:

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ANIMAL RESEARCH – Animals with Biohazards Table:

Please update the route of administration to reflect new stereotaxic administration of PDOX models in collaboration with Dr. Thibault.

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There are entries for PPE Required in the table that state “mask or BSC”; however, masks are not indicated elsewhere in the protocol as a type of PPE used. Please clarify and update accordingly to state whether you will wear masks or utilize the BSC.

INFECTIOUS AGENTS – Please verify that all personnel have a signed Exposure Control Plan (ECP) personnel statement on file.

LOCATIONS - Biological Safety Equipment: Please select “Use of radioactive materials” in the list of procedures done in a BSC as described in the Scientific Summary.

SCIENTIFIC SUMMARY – There are references to “future studies” in the Scientific Summary that should be removed. Similarly, the statement “In the future, we may be interested in further delving...” is vague, and it is unclear if this work will ever take place. Please modify to remove verbiage that is vague or references future work. Projects that are not actively planned should be added as an amendment when preparing to initiate that work.

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Amelia Pinto initiated the motion. Maria Landron seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Yosra Helmy

IBC Protocol Number: IBC-25-12

Protocol Title: Evaluation of the efficacy of different antibiotic alternatives against infectious pathogens

Protocol Type: Amendment

Amendment To: Administrative information, Laboratory location(s), Manipulations planned, Organisms used in research, Personnel, Project Title

Applicable Guidelines & Regulations: UK Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA 29 CFR 1910.1030, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7

Maximum Containment Level: Biological Safety Level 2 (BSL2), Animal Biological Safety Level 2 (ABSL2)

Primary Reviewers: C. Haughton, T. Chambers, C. Shaffer

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Brief Project Overview:

This project will focus on the treatment of different bacterial pathogens that cause diarrhea in neonatal foals as well as foodborne pathogens in humans using different strains of probiotics and small molecules (SMs). It also focuses on testing these drug candidates in chickens infected with the bacteria. We will test the efficacy of different strains of probiotics (Lactobacillus spp., Bacillus spp, non-pathogenic E. coli spp., Bifidobacterium spp., Saccharomyces spp., Streptococcus spp, Enterococcus spp) and SMs on the growth of antibiotic-resistant pathogens causing neonatal foal's diarrhea (NFD). These probiotic strains are nonpathogenic and have a beneficial effect when administered adequately. In this project, I plan to use Enterococcus faecalis as a non-pathogenic probiotic strain (doi.org/10.1007/s12602-021-09840-1). The SMs are a set of natural products that have less potential to develop drug resistance. Each probiotic strain and the SM will be screened for their efficacy on each pathogenic bacteria. The best/most effective probiotic strain and/or SM will be also further evaluated in vitro. We are planning to test the efficacy of the small molecules that showed best efficacy in vitro in chickens infected with Salmonella Typhimurium. We also plan to test the efficacy of the best probiotic candidates on Salmonella and Campylobacter-infected chickens. In a separate study we are going to test the efficacy of vaccine against S. equi in foals, this vaccine has already been commercialized in Europe.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, PCR/qRT-PCR, Propagation of infectious agents, Use of Human Source Material(s), Use of infectious agents, Immunohistochemistry

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Bacillus subtilis (RG1-bacteria)/Humans, animals/; Campylobacter jejuni (RG2-bacteria)/Humans, animals/; Clostridium difficile (RG2-bacteria)/Humans, animals/; Enterococcus faecalis (RG2-bacteria)/Humans, animals/; Escherichia coli (RG2-bacteria)/Humans, animals/; Escherichia coli (entero-hemorrhagic) RG2 Bacteria/Humans, animals/; Listeria monocytogenes (RG2-bacteria)/Humans, animals/; Rhodococcus equi (RG2-bacteria)/Humans, animals/; Staphylococcus aureus (RG2-bacteria)/Humans, animals/; Streptococcus equi subsp. equi (RG2-bacteria)/Humans, animals/; Salmonella enterica (RG2-bacteria)/Humans, animals, farms/; Clostridiales spp. (RG2-bacteria)/Humans, animals/; Bifidobacterium bifidum (RG1-bacteria)/Humans, Mammals/; Bifidobacterium adolescentis (RG1-bacteria)/Humans, Mammals/; Bifidobacterium lactis BB-12 (RG1-bacteria)/Humans, Mammals/;

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Bifidobacterium longum (RG1-bacteria)/Humans, Mammals/; *Escherichia coli* Nissle 1917 (RG1-bacteria)/Humans, Mammals/; *Escherichia coli* G58-1 (RG1-bacteria)/Humans, Mammals/;

Lactobacillus rhamnosus (RG1-bacteria)/Humans, Mammals/; *Lactobacillus acidophilus* (RG1-bacteria)/Humans, Mammals/; *Lactobacillus reuteri* (RG1-bacteria)/Humans, Mammals/;

Lactobacillus leichmannii (RG1-bacteria)/Humans, Mammals/; *Streptococcus thermophilus* (RG1-bacteria)/Humans, Mammals/; *Bacteroides thetaiotaomicron* (RG1-bacteria)/Humans, Mammals/; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Humans/; *Parabacteroides merdae* (RG2-bacteria)/Humans/; *Klebsiella pneumoniae* (RG2-bacteria)/Humans, animals

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: N/A

Vector(s) [Vector Category/Vector Technical Name]: N/A

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Caco-2; Human/Ht-29; Animal/HD-11; Animal/equine embryonic lung

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Chicken, Agricultural/*Salmonella enterica* (RG2-bacteria)/Oral/in cages/ABSL2/Gloves, Coverall, masks, Eye protection, face shield/ABSL2/Yes/The chickens will be infected with the bacteria *S. enterica* serovar Typhimurium and then treated with the selected drug candidate. After a few days, the animals will be euthanized and the internal organs will be collected to determine the effect on the drugs on the bacterial colonization in the internal organs. Chickens will be maintained/ kept in metal cages provided by the DLAR.; Chicken, Agricultural/*Campylobacter jejuni* (RG2-bacteria)/oral/in cages/ABSL2/Gloves, Coverall, masks, Eye protection, face shield/ABSL2/Yes/The chickens will be infected with the bacteria and then treated with the selected drug candidate. After a few days, the animals will be euthanized and the internal organs will be collected to determine the effect on the drugs on the bacterial colonization in the internal organs. Chickens will be maintained/ kept in metal cages provided by the DLAR.; Chicken, Agricultural/*Bacteroides thetaiotaomicron* (RG1-bacteria)/Oral/Hand hold/ABSL1/lab coat, gloves, eye protection/ABSL2/Yes/Probiotic; Chicken, Agricultural/*Lactobacillus leichmannii* (RG1-bacteria)/Oral/Hand hold/ABSL1/lab coat, gloves, eye protection/ABSL2/Yes/probiotic; Chicken, Agricultural/*Parabacteroides merdae* (RG2-bacteria)/Oral/hand hold/ABSL1/lab coat, gloves, eye protection/ABSL2/Yes/probiotic; Equine, Agricultural/*Streptococcus equi* subsp. *equi* (RG2-bacteria)/Intranasal/Hand Hold, sedation if

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necessary/ABSL2/Gloves, Coveralls, masks, Eye protection, face shield/ABSL2/Yes/BSL2 Barn practices & procedures

Risk Assessment/Discussion:

Dr. Helmy has submitted an amendment to her current IBC protocol entitled *Evaluation of the efficacy of different antibiotic alternatives against infectious pathogens*. In this amendment, Dr. Helmy has updated the title of her project, added personnel, and added an infection study utilizing *Streptococcus equi* subsp. *equi* (causative agent of the equine disease commonly known as Strangles) in horses. Strangles is a contagious upper respiratory disease of horses transmitted via direct contact with infected horses or indirectly via contaminated equipment, environments, or via asymptomatic carrier animals. The pathogen is considered largely non-zoonotic, however rare human infections have been reported in individuals with underlying health conditions. The newly added project is broken up into three distinct Aims. Aim 1 is a field safety study that will evaluate the safety of Strangvac, a multicomponent recombinant protein vaccine against equine Strangles that is currently licensed for use in the European Union and the United Kingdom. This aim does not involve the administration of any biohazardous material to animals. The second and third aim will involve the administration of *S. equi* subsp. *equi* to horses. Aim 2 seeks to establish the lowest dose that reliably produces mild, self-limiting infection within 21 days. 9 horses will be used for this study. Horses in Aim 2 are 2 years old. With the established dose from Aim 2, Aim 3 will examine the efficacy of Strangvac in placebo-controlled, vaccinated, and control horses challenged intranasally with *S. equi* subsp. *equi*. 24 horses, approximately 5-6 months of age, will be used for Aim 3. The biosafety and biosecurity measures for both Aims 2 and 3 are identical. Horses will be housed at ABSL2 containment in the BSL2 Isolation Barn at the UK North Farm. Inoculum will be prepared in Dr. Helmy's laboratory in Gluck and transported to the North Farm. Personnel will change into farm dedicated clothing (coveralls or equivalent) in the trailer adjacent to the BSL2 barn. Upon entry, personnel will don the following PPE: disposable Tyvek suit, disposable gloves, shoes covers OR BSL2-barn dedicated boots, and face shield OR wrap around eye protection and fluid resistant surgical mask. One individual will restrain the horses via halter with lead rope attached while a second individual administers the inoculum intranasally. All bedding, manure, muck, and other contaminated disposable materials will be placed in hard-sided plastic bins lined with a red biohazard bag to be picked up by SteriCycle for autoclaving and final disposal. At the conclusion of Aims 2 and 3, and any time a horse shows signs of disease, they will be euthanized. Horse carcasses will be loaded and transported to the UKVDL for necropsy according to the UK BSL2 Isolation Barn Biosafety Manual. After necropsy, pickup of carcasses will be arranged with a third-party vendor for incineration. The BSL2 Isolation Barn will undergo terminal cleaning and disinfection according to the BSL2 Barn Biosafety Manual. For the duration of study, farm personnel will be dedicated exclusively to this project and will not have any contact with non-study horses. There is an IBC hold on the corresponding IACUC protocol, 2025-4703.

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IBC Discussion & Vote:

The amendment to IBC-25-12 (version 30.0) was approved pending minor modifications as listed below:

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DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Disinfectants: Please add RESCUE to the list of Disinfectants in Use.

DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Biohazardous Materials Transport Description: Please include the more specific language of biohazard transport from the Scientific Summary to the description in the transport section.

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Thomas Chambers initiated the motion. Carrie Shaffer seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Michael C. Tackenberg

IBC Protocol Number: IBC-25-130

Protocol Title: Genetic, molecular, and environmental determinants of circadian period length and output phase.

Protocol Type: Amendment

Amendment To: Administrative Information, Genetic constructs, Personnel, Manipulations planned

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-1, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, OSHA 29 CFR 1910.1030, NIH Guidelines Section III-D-2, NIH Guidelines Section III-F-3

Maximum Containment Level: Biological Safety Level 2 (BSL2), Animal Biological Safety Level 1 (ABSL1)

Primary Reviewers: C. Haughton, A. Hunt, M. Mendenhall

Brief Project Overview:

Biological rhythms (like the sleep/wake cycle) are controlled by biological clocks inside of the body. These biological clocks can be influenced by internal factors (like genetics) and external factors (like

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diet, exercise, and light exposure). This project looks to investigate how internal and external factors, together and separately, impact the timing of biological clocks.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, Creation of viral vectors, DNA/RNA isolation/purification, Genetics, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Transfection, Transformation, Use of viral vectors, Viral culture, Use of infectious agents

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

Infectious Agent(s)/Natural Host(s):

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Npas2/ Mouse/ Transcription factor/ Overexpression/ Mouse/ AAV(PHP.eB), AAV8;Clock/ Mouse/ Transcription factor/ Overexpression/ Mouse/ AAV(PHP.eB), AAV8;Bmal1/ Mouse/ Transcription factor/ Overexpression/ Mouse/ AAV(PHP.eB), AAV8; Per1/ Mouse/ Transcription repressor/ Overexpression/ Mouse/ AAV(PHP.eB), AAV8; Per2/ Mouse/ Transcription repressor/ Overexpression/ Mouse/ AAV(PHP.eB), AAV8; ASCL1/ Mouse/ Transcription repressor/ Overexpression/ Mouse/ AAV(PHP.eB), AAV8; Cre/ P1 bacteriophage/ Recombinase/ Recombination/ Mouse/ AAV(PHP.eB), AAV8;Gfp/ multiple sources/ Reporter/ Reporter/ Mouse/ AAV(PHP.eB), AAV8;GCaMP/ synthetic/ Reporter/ Reporter/ Mouse/ AAV(PHP.eB), AAV8;dCas9/ Streptococcus pyogenes or Staphylococcus aureus / RNA-guided (dead) endonuclease/ Transcription factor delivery/ Mouse/ AAV(PHP.eB), AAV8;pMag/nMag/ synthetic/ light-sensitive protein/ light-inducible elements/ Mouse/ AAV(PHP.eB), AAV8;Npas2 shRNA/ synthetic/ shRNA/ Gene knockdown/ Mouse/ pAAV(PHP.eB)/AAV8;Clock shRNA/ synthetic/ shRNA/ Gene knockdown/ Mouse/ pAAV(PHP.eB)/AAV8;Clock shRNA/ synthetic/ shRNA/ gene knockdown/ Mouse/ pAAV(PHP.eB)/AAV8;Nr1d1 shRNA/ synthetic/ shRNA/ gene knockdown/ mouse/ AAV(PHP.eB)/AAV8;Nr1d2 shRNA/ synthetic/ shRNA/ gene knockdown/ mouse/ AAV(PHP.eB)/AAV8 Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/pAAV (AAV2 ITR); Plasmid/pcDNA3

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/U2OS; Human/HEK293

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Viral Vector - Adeno-Associated Virus (AAV)/i.v. (tail vein injection)/Rotating tail injector platform/ABSL1/Gloves, gown, eye protection/ABSL1/No/Performed in BSC/

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Risk Assessment/Discussion:

Dr. Tackenberg has submitted an amendment to his current IBC protocol entitled *Genetic, molecular, and environmental determinants of circadian period length and output phase*. In this amendment, Dr. Tackenberg has updated personnel, IACUC approval information, gene targets, and DLAR locations. Utilizing an AAV8 backbone (previously approved), Dr. Tackenberg has added overexpression of Per1 and knockdown of Per2 and ASCL1. New AAV constructs will be produced and utilized as previously described and approved. Work with AAV will take place using BSL1/ABSL1 containment. The addition of these new AAV constructs does not significantly alter the biohazardous risks associated with Dr. Tackenberg's approved IBC protocol.

IBC Discussion & Vote:

The amendment to IBC-25-130 (version 22.0) was approved pending minor modifications as listed below:

SCIENTIFIC SUMMARY:

1. Please provide details on the function of the transgenes and potential risk of exposure to the new gene constructs.
2. Please clarify the expression of ASCL1 and the host. It is indicated elsewhere in the protocol that HEK293 cells will be used, but this is not explained in the Scientific Summary.

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Michael Mendenhall initiated the motion. Arthur Hunt seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Yekaterina Zaytseva

IBC Protocol Number: IBC-25-156

Protocol Title: The role of fatty acid metabolism in colorectal cancer

Protocol Type: Amendment

Amendment To: Cells or tissues used in research, Manipulations planned

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, UK Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA 29 CFR 1910.1030, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7, NIH Guidelines Section III-F

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Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)

Primary Reviewers: C. Haughton, D. Harrison, C. Pickett

Brief Project Overview:

Metabolic reprogramming is a hallmark of cancer, controlling various aspects of malignant development and progression. Activation of de novo lipogenesis in cancer cells, which is increasingly recognized as one of the characteristics of aggressive cancers, correlates with a poorer prognosis and shorter disease-free survival in many tumor types including colorectal cancer (CRC). Fatty Acid Synthase (FASN), a key enzyme of de novo lipid synthesis, is upregulated in many cancers including CRC; increased FASN activity is associated with decreased survival and increased disease recurrence. Recently, a first-in-class, oral FASN inhibitor (TVB-2640) entered a Phase I clinical trial (3V2640-CLIN-002) in solid tumor patients demonstrating a favorable tolerability profile with no significant adverse events; however, tumor characteristics that would indicate responsiveness to FASN inhibition are not fully understood. The purpose of our studies is: (i) to determine the effect of novel, selective and reversible FASN inhibitors on tumor growth as a monotherapy and in combination with other therapeutic agents in CRC xenografts and CRC patient-derived xenografts (PDXs); (ii) to identify potential biomarkers associated with CRC responsiveness to FASN inhibition; (iii) investigate the effect of sphingolipid metabolism on metastasis in CRC; (iv) delineate the role of FASN in initiation of CRC using transgenic mouse models and established from them organoid cultures, and (v) evaluate the effect of FASN-mediated exosomes on CRC metastasis

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Cell culture, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Use of infectious agents, Use of viral vectors

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: FASN (Fatty Acid Synthase)/ Human/ metabolic enzyme/ shRNA knockdown in cell culture/ Human cells/ lentivirus;SPHK1 (Sphingosine Kinase 1)/ Human/ metabolic enzyme/ shRNA knockdown in cell culture; expression in cell culture/ Human cells/ lentivirus;SPHK2 (Sphingosine Kinase 2)/ Human/ metabolic enzyme/ shRNA knockdown in cell culture; expression in cell culture/ Human cells/ lentivirus;CD36/ Human/ cell adhesion/ shRNA knockdown in cell culture;

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expression in cell culture/ Human cells/ lentivirus;GFP/ Jellyfish/ tracking/ expression/ human cells, mouse cells/ lentivirus;Luciferase/ Firefly/ tracking/ expression/ human cells, mouse cells/ lentivirus;GFPT-1/ Human/ Metabolic enzyme/ shRNA knockdown in cell culture/ Human cells/ lentivirus;OGT/ Human/ Metabolic enzyme/ shRNA knockdown in cell culture/ Human cells/ lentivirus Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/pLKO.1/; Lentivirus/pLKO.1 FASNshRNA/; Lentivirus/shRNA plasmid-SPHK1shRNA/; Lentivirus/shRNA plasmid- SPHK2shRNA/; Lentivirus/shRNA plasmid control/; Lentivirus/shRNA plasmid - CD36/; Lentivirus/pLX_TRC317 SPHK1 & SPHK2/; Lentivirus/pLenti-C-Myc-DDK-P2A-Puro - CD36 & control/; Lentivirus/Mission shRNA lentivirus - Human GFPT-1 & OGT/; Lentivirus/Mission shRNA lentivirus - Human CD166

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HCT116/; Human/HT29/; Human/pt 93/; Human/pt 130/; Human/pt 2387/; Human/pt 2377 PT/; Human/pt 2377LM/; Human/HCT116 NTC/; Human/HT29 NTC/; Human/HCT116 FASN shRNA/; Human/HT29 FASN shRNA/; Human/HCT116 CD36 shRNA/; Human/HT29 CD36 shRNA/; Animal/CT26/; Animal/MC38/; Human/HCT116 CD166sh RNA/; Animal/CT26 FASN shRNA/; Animal/CT26 CD36 shRNA/; Animal/CT26 SPHK1 shRNA/; Animal/CT26 SPHK2 shRNA/; Animal/MC38 FASN shRNA/; Animal/MC38 CD36 shRNA/; Animal/MC38 SPHK1 shRNA/; Animal/MC38 SPHK2 shRNA/; Human/HCT116 CD36 over expression

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Tissue - Human (ex. PDX tumor tissue)/xenografts/anesthesia/ABSL2/gloves, cover, eye protection/ABSL2/No/Tissues are transferred in a sealed secondary container to the animal facility and implanted subcutaneously into NSG mice which are anesthetized as described in IACUC protocol. Subcutaneous implantation of tumor tissue will be done in a designated BSC in DLAR ABSL2 procedure rooms as described in IACUC protocol.//; Mouse/Cells - Human, genetically modified/xenografts /anesthesia/ABSL2/gloves, cover, eye protection/ABSL2/No/Cells are transferred in a sealed secondary container to the animal facility and implanted subcutaneously into Nu/Nu mice which are anesthetized as described in IACUC protocol. Cell injections will be done in a designated BSC in DLAR ABSL2 procedure room as described in IACUC protocol./; Mouse/Cells - Human, genetically modified/tail vein injections/anesthesia/ABSL2/gloves, cover, eye protection/ABSL2/No/Cells are transferred in a sealed secondary container to the animal facility and injected of tail vein of Nu/Nu mice which are anesthetized as described in IACUC protocol. Cell injections will be done in a designated BSC in DLAR ABSL2 procedure room as described in IACUC protocol./; Mouse/Cells - Human, genetically modified/splenic injections/anesthesia/ABSL2/gloves, cover, eye protection/ABSL2/No/Cells are transferred in a sealed secondary container to the animal facility and injected of tail vein of Nu/Nu mice which are anesthetized as described in IACUC protocol. Cell injections will be done in a designated BSC in DLAR ABSL2 procedure room as described in IACUC protocol./; Mouse/Cells - Animal, non-

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modified/xenografts/anesthesia/ABSL2/gloves, cover, eye protection/ABSL2/No/Mouse cells are transferred in a sealed secondary container to the animal facility and injected subcutaneously into BALB/c wild type mice which are anesthetized as described in IACUC protocol. Injections will be done in a designated BSC in DLAR ABSL2 procedure rooms described in IACUC protocol./; Mouse/Cells - Human, genetically modified/cecum injections/anesthesia/ABSL2/gloves, cover, eye protection/ABSL2/No/Cells are transferred in a sealed secondary container to the animal facility and injected in cecum of Nu/Nu mice which are anesthetized as described in IACUC protocol. Cell injections will be done in a designated BSC in DLAR ABSL2 procedure room as described in IACUC protocol./; Mouse/Cells - Animal, genetically modified/xenografts/anesthesia/ABSL2/gloves, cover, eye protection/ABSL2/No/Mouse cells are transferred in a sealed secondary container to the animal facility and injected subcutaneously into BALB/c wild type mice which are anesthetized as described in IACUC protocol. We will inject Balb/c mice with mouse cell lines CT26 and MC38 with Luciferase reporter and altered expression of FASN, CD36, SPHK1 and SPHK2 genes. Injections will be done in a designated BSC in DLAR ABSL2 procedure rooms described in IACUC protocol.

Risk Assessment/Discussion:

Dr. Kate Zaytseva has submitted an amendment to her current IBC protocol entitled *The role of fatty acid metabolism in colorectal cancer*. In this amendment, she has added new human cells, added a description of animal transport for IVIS imaging, and added two projects in animals. Her laboratory will be creating new stably transduced cells that express luciferase and/or GFP reports with altered expression of FASN, CD36, SPHK1, and SPHK2 genes. These stably transduced cells will be produced using an already approved lentivirus vector system. These particle cells differ with the addition of tracking genes luciferase and GFP. These stably transduced cells will be administered to mice as previously approved and described. These mice will undergo IVIS imaging. In a second new project, Dr. Zaytseva's laboratory will isolate exosomes via ultracentrifugation from HT29, HCT116, SW480, and CT26 cells with altered expression of FASN. Mice will be pretreated with isolated exosomes administered to anesthetized mice via tail vein injections. After 4 weeks of exosome exposure, mice will be injected with HT29 LM3-GFP-Luc cells to assess whether pretreatment influences the formation of liver metastasis. Both new animal projects will utilize ABSL2 experimental procedures. Mice will be housed using ABSL1 containment. This new work is very similar to previously approved work in Dr. Zaytseva's laboratory and does not significantly alter the biohazardous risks associated with Dr. Zaytseva's IBC protocol. There is an IBC hold on the corresponding IACUC protocol, 2019-3236.

IBC Discussion & Vote:

The amendment to IBC-25-156 (version 23.0) was approved pending minor modifications as listed below:

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DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Animal Transport Description:

Please clearly describe how animals are transported. Are DLAR-provided closed carts utilized?

SCIENTIFIC SUMMARY:

1. Please provide more detail regarding centrifugation of biohazardous materials during exosome isolation. For instance, are tubes filled within the BSC? Are tubes sealed? If so, how? Are tubes wiped with disinfectant prior to loading into centrifuge buckets/cups? Are centrifuge buckets/cups fitted with aerosol-tight safety lids? How are centrifuge buckets/cups disinfected prior to removal from BSC and transport to centrifuge? Additional details regarding centrifugation of biohazardous materials is online here -> <https://researchsafety.uky.edu/biological-safety/laboratory-equipment/centrifuges>
2. How is material removed from centrifuge tubes afterwards? Does this step involve the use of sharps? What other steps are taken to minimize the potential of exposure during these steps?
3. Are exosomes prepared fresh for administration to animals? Are they frozen ahead of animal work? Please specify.

*

Doug Harrison initiated the motion. Carol Pickett seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

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New Protocols

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N/A

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Renewals

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PI: Cassandra Gipson-Reichardt

IBC Protocol Number: IBC-25-181

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Protocol Title: Glutamate, Neuroinflammation, Acetylcholine, HIV and Addiction

Protocol Type: Renewal

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-D-2, NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-3, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9

Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: C. Haughton, B. Nelson, D. Malherbe

Brief Project Overview:

The goals of the proposed studies are to understand neurobehavioral mechanisms of different patterns of drug use. The use of viral vectors allows for specific manipulation of neuronal populations or signaling pathways which allow a more thorough understanding of the neural circuitry of addiction. Viruses that allow for control of specific neurons or knockdown of specific proteins will be used in alternate experiments.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Cell culture, Creation of viral vectors, DNA/RNA isolation/purification, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Proteomics, Use of viral vectors, Viral culture, Use of infectious agents, Bacterial culture, Propagation of infectious agents

Transport: Yes

Materials Transported: Animals

Infectious Agent(s)/Natural Host(s): EcoHIV (RG2-virus)/Rats; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: GFP/Aequorea victoria /Reporter/Expression/Rat/AAV/; calcium/calmodulindependent kinase II alpha (CaMKII α) /Rattus norvegicus /Regulatory /Expression/Rat/AAV/; hM4(Gi) : Derived from human muscarinic receptor M4, with two point mutations (Y113C, A203G) /Human /Regulatory /Expression/Rat/AAV/; mCherry /Discosoma sea anemone /Reporter/Expression/Rat/AAV/; CaMKII α -hM3D(Gq)- mCherry /calcium/calmodulindependent kinase II alpha (CaMKII α) promotor: Rattus norvegicus hM2D : Derived from human muscarinic receptor M4, with

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two point mutations (Y113C, A203G) •mCherry: derived from GFP from *Discosoma* sea anemone/Regulatory /Expression/Rat/AAV/; PR (progesterone receptor) /*Rattus norvegicus* /Regulatory /Expression/Rat/AAV/; GFAP /*Rattus norvegicus* /Regulatory /Expression/Rat/AAV/; Cre Recombinase/P1 bacteriophage/Regulatory/Expression/Rat/AAV/; Estrogen Receptor Beta (ER-B)/*Rattus norvegicus*/Regulatory/shRNA knockdown/Rat/AVV/; elongation factor 1 (ef1a)/*Rattus norvegicus*/Regulatory/Expression/Rat/AVV/; Dopamine Receptor 1 (D1R)/*Rattus norvegicus*/Regulatory/Expression/Rat/AVV/; The LCK (lymphocyte-specific protein tyrosine kinase)/*Rattus norvegicus*/Regulatory/Expression/Rat/AVV/; Channelrhodopsin 2-mCherry /*Chlamydomonas reinhardtii* /Regulatory /Expression/Rat/AAV/; EYFP (Enhanced Yellow Fluorescent Protein)/*Aequorea victoria*/Tracker/Expression/Rat/AAV/; Tyrosine Hydroxylase (TH)/*Rattus norvegicus*/Regulatory/Expression/Rat/AAV

Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/pAAV-EF1a-DIO-hM4D(Gi)-mCherry/; Adeno-Associated Virus (AAV)/pAAV-EF1a-DIO-hM3D(Gq)-mCherry/; Adeno-Associated Virus (AAV)/AAV5-EF1a-DIO-mCherry/; Adeno-Associated Virus (AAV)/pAAV-5 Ef1a-DIO EYFP/; Adeno-Associated Virus (AAV)/pAAV-CaMKIIa-hM3D(Gq)-mCherry/; Adeno-Associated Virus (AAV)/pAAV-CaMKIIa-hM4D(Gi)-mCherry/; Adeno-Associated Virus (AAV)/AAV-CaMKIIa-eGFP/; Adeno-Associated Virus (AAV)/pENN.AAV.hSyn.HI.eGFP-Cre.WPRE.SV40/; Adeno-Associated Virus (AAV)/AAV-FLEX-rev-ChR2(H134R)-mCherry/; Adeno-Associated Virus (AAV)/pAAV.GfaABC1D.PI.Lck-GFP.SV40/; Naked nucleic acid/Morpholino oligos/; Naked nucleic acid/Morpholino oligos

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/293FT

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Rat/Viral Vector - Adeno-Associated Virus (AAV)/Intracranial cannulae/anesthesia/ABSL1/Gloves, Lab Coat, eye protection/ABSL1/No/Delivered through intracranial cannulae into the prelimbic area. Isoflurane is used as an anesthetic using a Isoflurane Vaporizer; Rat/EcoHIV (RG2-virus)/IV/ Manual/ABSL2/Gloves, Lab Coat, eye protection/ABSL2/No/The animals are manually restrained. A PNP3M injector is attached to a syringe, and we can deliver the virus through the catheter in a saline solution.; Rat/Naked Nucleic Acid-r/sDNA/Injection intracranial anesthesia/ABSL1 Gloves, eye protection, lab coat/ABSL1/No

Risk Assessment/Discussion:

Dr. Gipson-Reichardt has submitted a renewal of her IBC protocol entitled *Glutamate, Neuroinflammation, Acetylcholine, HIV and Addiction*. In Dr. Gipson-Reichardt's laboratory, multiple

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AAV vector constructs and Eco-HIV are utilized in rats. AAVs expressing DREADDs, reporters, CRE, or shRNA are obtained from Addgene and administered to anesthetized rats via intracranial injection. Some rats will also be administered antisense or control sequence morpholinos. Rats will then undergo a number of downstream behavioral assessments and ultimately peptide/cellular analysis following sacrifice. AAVs will be handled using BSL1/ABSL1 containment. A second project utilizes EcoHIV, a rodent-specific HIV in which the gp120 coding region has been replaced with gp80 from ecotropic murine leukemia virus. EcoHIV cannot infect human cells in culture and poses minimal risk to personnel. Chimeric EcoHIV will be produced and utilized using BSL2 containment. This includes work within a BSC. Rats will be administered EcoHIV through intravenous administration through a catheter port to establish infection. Because administration of EcoHIV via catheter port does not require the use of needles, rats are manually restrained. EcoHIV infected rats will undergo a number of downstream assays and/or manipulations, including cART therapy and behavioral testing prior to euthanasia and analysis of spleen and brain tissues. This project will be completed using ABSL2 procedures and containment. Dr. Gipson-Reichardt's current IBC protocol will expire on March 22, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-25-181 (version 5.0) was returned for modifications listed below:

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DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Disinfectants in Use: Please include an entry for the disinfectant utilized in the Rodent Behavior Core facility.

LOCATIONS – Research Locations: Please remove reference to HSV in the Procedures column for “DLAR 079 B, C, and D.”

SCIENTIFIC SUMMARY:

1. Please reorganize the Scientific Summary to clearly outline what manipulations are being performed such that reviewers can envision how each procedure is done. Clearly identify the biohazardous materials utilized in each manipulation, recognize the associated risks to personnel and specify how these risks will be mitigated. Please remove references, objectives, and background materials that are irrelevant to the biohazardous materials in use.
2. Please clarify if rats infected with EcoHIV are expected to shed virus.

*

Delphine Malherbe initiated the motion. Brandy Nelson seconded the motion. All IBC members present (14) voted in favor of the motion.

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Request for extension of IBC protocol approval for IBC-25-181, PI Dr. Gipson-Reichardt, due to approaching expiration date of original protocol. IBC-25-181 will expire on March 18, 2026.

The motion for a 60-day extension of IBC protocol IBC-25-181 approval period was approved.

Doug Harrison initiated the motion. Brandy Nelson seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Anel Jaramillo

IBC Protocol Number: IBC-25-185

Protocol Title: Neurobiology of anxiety and alcohol-use disorder

Protocol Type: Renewal

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-3, NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-3, NIH Guidelines Section III-F-6, OSHA Act of 1970 Clause 5(a)(1), UK Administrative Regulation 6.3, NIH Guidelines Section IV-B-7, UK Administrative Regulation 6.9, NIH Guidelines Section III-E-1

Maximum Containment Level: Biological Safety Level 1 (BSL1), Animal Biological Safety Level 1 (ABSL1)

Primary Reviewers: C. Haughton, Y. Wu, M. Mendenhall

Brief Project Overview:

The scope of this research is to understand the neurobiology of anxiety and alcohol-use disorder.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Use of viral vectors

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

Infectious Agent(s)/Natural Host(s): N/A

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Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: GCaMP/rat, chicken, jellyfish/tracking gene/expression/mouse/AAV1, AAV9, AAVrg/; ChR2/tiarina fusus/membrane protein/activating/mouse/AAV8/; hM3D(Gq)-mCherry/human/membrane protein/activating/mouse/AAV5/; hM4D(Gi)-mCherry/human/membrane protein/inhibiting/mouse/AAV5/; KORD/human/membrane protein/inhibiting/mouse/AAV8/; CRE/bacteriophage/tracking gene/expression/mouse/AAV1, AAVrg/; EGFP/jellyfish/tracking gene/expression/mouse/AAV1, AAV5, AAVrg

Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/AAV5; Adeno-Associated Virus (AAV)/AAV9; Adeno-Associated Virus (AAV)/AAV1; Adeno-Associated Virus (AAV)/AAV8

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: N/A

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Viral Vector - Adeno-Associated Virus (AAV)/intracranial injections/Angle Two mice stereotaxic/ABSL1/Lab coat, gloves, eye protection/ABSL1/No/animals anesthetized and placed into a stereotaxic frame outfitted with a fitted nosecone delivering constant flow of isoflurane and on top of a heating pad. Animal will be covered with a sterile drape.

Risk Assessment/Discussion:

Dr. Jaramillo has submitted a renewal of her IBC protocol entitled *Neurobiology of anxiety and alcohol-use disorder*. AAV vectors obtained from Addgene will be used to express DREADDs, tracking genes, and CRE in mice. AAV is not associated with disease in healthy adults and can safely be handled using BSL1/ABSL1 containment. AAVs will be administered to anesthetized mice via intracranial injection. After 4-6 weeks, mice will be sacrificed and brain tissue removed for imaging. All waste is described as handled according to UK Research Safety guidance. Dr. Jaramillo's current IBC protocol will expire on March 3, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-25-185 (version 8.0) was approved pending minor modifications as listed below:

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GENERAL INFORMATION – Manipulations planned: While “PCR/qRT-PCR,” is checked, there is no description of PCR/DNA work in the Scientific Summary. Please modify the Scientific Summary to include a description of this work or UNCHECK the “PCR/qRT-PCR” selection from this section.

SCIENTIFIC SUMMARY:

1. Please clarify whether any AAVs are being created/produced in the laboratory or if they are being purchased from a vendor. If they are being produced in the laboratory, please include a description of how they are produced.
2. Modify language regarding the AAV vectors and gene constructs to state potential hazards and risks to personnel. Be sure to acknowledge that there is some level of risk involved, rather than “no potential hazards.”
3. Please include a description of the PCR/q-RT-PCR work alluded to in the General Information – Manipulations Planned section of the IBC protocol.

*

Michael Mendenhall initiated the motion. Yadi Wu seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Andrew Stewart

IBC Protocol Number: IBC-26-04

Protocol Title: Gene Therapy Approaches to Induce and Control Neuronal Growth in Rodents With Spinal Cord Injuries

Protocol Type: Renewal

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-D-2, NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-1, NIH Guidelines Section III-F, NIH Guidelines Section III-F-1, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9

Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)

Primary Reviewers: C. Haughton, D. Harrison, Y. Wu

Brief Project Overview:

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Work in my lab focuses on developing gene therapies to modulate neurons after spinal cord injury. We have developed specific approaches to target neurons using constitutively active or inducible promoters to produce proteins that will hopefully produce functional improvements after spinal cord injury via either regenerative or regeneration-independent mechanisms. Collectively we have identified many molecular targets that can be manipulated to produce functional improvements and/or axon growth after SCI. We have generated infrastructure for the cloning and assembly of novel viral vectors as well as the production and packaging of AAVs. Our work described below highlights the current approaches being used by our lab to repair the damaged spinal cord.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, Creation of Viral Vectors, DNA/RNA isolation/purification, Genetics, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Transfection, Transformation, Use of Viral Vectors, Use of Infectious Agents

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: AKT3/Human/Oncogene and cell growth/induce regeneration in a neuron specific manner/bacteria, human cell line, mouse and rat spinal cord/AAV retro/;

rTA/Bacteria/Regulatory/Make inducible expression of AKT3/bacteria, human cell line, mouse and rat spinal cord/AAV retro/; rtTA and TET-ON 3G/Bacteria/Regulatory/Make inducible expression of AKT3/bacteria, human cell line, mouse and rat spinal cord/AAV retro/;

eGFP/dTomato/mCherry/tdTomato/Jelly Fish/Tracking/Label Neurons/bacteria, human cell line, mouse and rat spinal cord/AAV retro / Lentivirus/; Cre/Bacteria/Regulatory/KO PTEN from transgenic mouse line. Make a stable cell line of neuronal stem cells that express cre recombinase to test Syn1-DIO constructs in vitro./Mouse and Rat Spinal Cord, human neuronal stem cells and packaging cells/AAV retro/; Flp/Bacteria/Regulatory/expression of genes in a Flp dependent manner/Mouse and Rat Spinal Cord/AAV retro/; Cre/Bacteria/Regulatory/create stable cell line producing cre recombinase to test other cre-dependent constructs./Neural Stem cell line/Lenti-Cre-IRES-PuroR/; miR30-shRNA(Kv1.2 murine)/shRNA/Translation/knockdown the expression of the potassium channel Kv1.2./Neural Stem Cell Line/AAV-Retro/; Crispr Cas9 and Guide arms against REST/NRSF/Bacteria/Translation/Knockout of the DNA binding domain of gene REST/NRSF/HEK293/Expression Plasmid/; ApoA1/Human/Lipid Trafficking/Study of HDL and Dysfunctional HDL/HEK293/AAV plasmid/; ApoA1(milano)/Human/Lipid Trafficking/Study of HDL and Dysfunctional HDL/HEK293/AAV Plasmid/; PKA

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(PRKACA)/Human/Regulatory/Catalytic domain of PKA to study effects on neural excitability./Hek293, Mouse and Rat Spinal Cord/AAV-Retro/; PKA (L 206 -> R) (PRKACA)/Mouse/Regulatory/Mutant catalytic domain of PKA to confer constitutive activity via interference with regulatory domain./Hek293, Mouse and Rat Spinal Cord/AAV-Retro/; 3xHA-eGFP-OMP25(C' 170-206)/Mouse/Mitochondria Reporter/Mitochondrial targeted reporter with HA tag for neuron-specific pull down studies./Hek293, Mouse and Rat Spinal Cord/AAV Retro/; DDR2/Human/Receptor/Study of collagen receptor for axon growth over collagen/Hek293, hNPC/AAV Plasmid/; EPAC1 (VLVLE to AAAAA)/Mouse/Regulatory/Expression of constitutively active EPAC1 in vitro and in spinal-projecting neurons in vivo to study regeneration/Mouse and Rat Spinal Cord, HEK293s, Mouse Primary Neuron Culture/AAV retro/; miR30(CXCL12/shRNA/Translation/Knockdown of Mouse CXCL12 in vitro and in vivo/Mouse and Rat Spinal Cord and Hek293/AAV2/; miR30(CXCR4)/shRNA/Translation/Knockdown of Mouse CXCR4 in vitro and in vivo/Mouse and Rat spinal cord, Hek293/AAV retro/; BFP (Blue Fluorescent Protein)/Aequorea victoria/Reporter/Reporter Gene/Mouse and Rat Spinal Cord, HEK293/AAV2/; L1CAM/RAT/Cell Adhesion Molecule/Express in Stem Cells for Transplantation/Mesenchymal Stem Cells/pLenti-EF1a-L1CAM-CMV-BFP/PuroR/; NCAM1/Rat/Cell Adhesion Molecule/Expression Stem Cells for Transplantation/Mesenchymal Stem Cells/pLenti-EF1a-NCAM1-CMV-BFP/NeoR/; CNTN1/Rat/Cell Adhesion Molecule/Express in Stem Cells for Transplantation/Mesenchymal Stem Cells/pLenti-EF1a-CNTN1-CMV-BFP/HygroR/; miRFP670 Nano/Nostoc punctiforme/Reporter Gene/Reporter Gene for Vector Transduction/Rat and Mouse/pAAV-(Antisense) WPRE3-myrAKT3-TRE3G (SENSE) Syn1-TETON3G-2a-miRFP670 Nano WPRE3/; pAAV-(Antisense) WPRE3-dTomato-TRE3G (SENSE) Syn1-TETON3G-2a-miRFP670 Nano WPRE3/; pAAV-(Antisense) WPRE3-3' beta Actin-myrAKT3-TRE3G (SENSE) Syn1-TETON3G-2a-miRFP670 Nano WPRE3/; Kir2.1/synthetic/Ion Channel/Establish Stable Cell Line/HEK293/Lentivirus/; PGC1alpha/Mouse/Transcription Factor/Co-activator/Gene Expression in neurons in mice/Mouse, Rat, HEK293, mouse Neural Stem Cells /AAV retro/; HA_eGFP-MitoTag/Jelly Fish/Reporter Gene/Label Mitochondria and Pull Down/Mouse, Rat, HEK293, Mouse Neural Stem Cells/AAV Retro/; shRNA (Kv1.2)/shRNA/Regulatory/Knockdown Kv1.2/Mouse/AAV Retro/; HA-eGFP-MitoOMM/Jelly Fish/Tracking/Identification and isolation of neuron-specific mitochondria/Hek293, Mouse and Rat Spinal Cord/pAAV-CamKIIa-HA-eGFP-MitoOMM/; 3xFlag-BFP-MitoOMM/Jelly Fish/Tracking/Tracking and isolation of Astrocyte-specific mitochondria/Hek293, Rat and Mouse Spinal Cord/pAAV-GFAP-3xFlag-BFP-MitoOMM/; Myc-RFP-MitoOMM/Jelly Fish/Tracking/Tracking and Isolation of Oligodendrocyte-specific mitochondria/Hek293, Mouse and Rat spinal cords/pAAV-MAG2.2-Myc-RFP-MitoOMM/; Pink1/Mouse/Regulatory/Overexpression of Pink1 in spinal projecting neurons within mouse and Rat spinal cords after injury./Hek293, Mouse and Rat spinal cords/pAAV-Syn1-Pink1-p2a-HA-eGFP-MitoOMM/; Firefly Luciferase/Firefly/Reporter/In vivo bioluminescence reporter/Hek293, Mouse spinal cord/pcDNA-Axolotl Enhancer- Hsp68 min- eGFP-p2a-ffLuc/; pLenti-Axolotl Enhancer- Hsp68 min- eGFP-p2a-ffLuc/; pcDNA-Hsp68 min- eGFP-p2a-ffLuc/; pLenti-Hsp68 min- eGFP-p2a-ffLuc/;

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pLenti-Axolotl Enhancer- Hsp68 min- RunX1-p2a-ffLuc/; pLenti-Axolotl Enhancer- Hsp68 min- ffLuc/; pLenti-CMV-eGFP-p2a-ffLuc-wpre/; pLenti-CMV-Runx1-p2a-ffLuc-wpre/; Runx1/Mouse/Transcription Factor/Transcription Modification/Hek293, Mouse Spinal Cords/pLenti-Axolotl Enhancer- Hsp68 min- RunX1-p2a-ffLuc/; pLenti-CMV-Runx1-p2a-ffLuc-wpre/; CD44/Mouse/Cell Adhesion Molecule/Express CD44 to determine role in growth in spinal cord lesions/Hek293, mouse spinal cord/pAAV-Syn1-CD44-HA-WPRE3/; Pink1 reporter split-luciferase/Synthetic/Reporter/Measure Pink1 activity in vivo in a cell-specific manner/Hek293, mouse spinal cord/pAAV-(CMV/Syn1/GFAP/CamK2a)-Pink1 reporter split-Luciferase/; CRISPR-CAS9 U6-Guide Arms (Pink1)/Bacteria/Translation/Knockout of the Pink1 protein in Hek293 cells/Hek293/CMV-CRISPR-CAS9 U6-Guide Arms (Pink1)

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/PHR-EF1 alpha-TET-On 3G/; Adeno-Associated Virus (AAV)/pAAV-Thy1PS-rTA/; Lentivirus/PHRIG-AKT3-IRES-eGFP/; Adeno-Associated Virus (AAV)/pAAV-hSyn1-Cre-P2A-dTomato/; Adeno-Associated Virus (AAV)/pAAV-TRE3G-tdTomato/; Adeno-Associated Virus (AAV)/AAV-hSyn1-rtTAV16/; Adeno-Associated Virus (AAV)/AAV-hSyn1-TET-ON 3G/; Adeno-Associated Virus (AAV)/AAV-hSyn1-rTA/; Adeno-Associated Virus (AAV)/pAAV-TRE3G-AKT-IRES-eGFP/; Adeno-Associated Virus (AAV)/pAAV-TRE3G-eGFP/; Adeno-Associated Virus (AAV)/pAAV-ihSYN1-DIO-tTA/; Adeno-Associated Virus (AAV)/pAAV-ihSYN1-DIO-AKT-IRES-dTomato/; Adeno-Associated Virus (AAV)/pAAV-ihSYN1-DIO-dTomato/; Adeno-Associated Virus (AAV)/pAAV-EF1a-fDIO-Cre/; Adeno-Associated Virus (AAV)/AAV phSyn1(S)-FlpO-bGHpA/; Lentivirus/pMD2.G VSV-G/; Lentivirus/Lenti-Cre-IRES-Puro/; Adeno-Associated Virus (AAV)/pAAV-CAG-Flex-tdTomato/; Adeno-Associated Virus (AAV)/pAAV-CMV-eGFP-mir30-shRNA(Kv1.2 murine)/; Plasmid/CMV-CRISPR-CAS9 U6-Guide Arms (REST/NRSF)/; Adeno-Associated Virus (AAV)/pAAV-Ef1a-ApoA1-IRES-eGFP/; Adeno-Associated Virus (AAV)/pAAV-Ef1a-ApoA1(milano)-IRES-eGFP/; Plasmid/pcDNA-CMV-PKA/; Adeno-Associated Virus (AAV)/AAV-Syn1-eGFP-2a-mKv1.2/; Adeno-Associated Virus (AAV)/AAV-TRE-eGFP-2a-PKA/; Adeno-Associated Virus (AAV)/AAV-TRE--eGFP-2a-PKA(L 206-> R)/; Plasmid/pMXs-3xHA-eGFP-OMP25/; Plasmid/Syn1-3xHA-eGFP-OMP25/; Adeno-Associated Virus (AAV)/Syn1-3xHA-eGFP-OMP25(c'170-206)/; Plasmid/pAAV-CamK2a-RFP/; Plasmid/pCDNA-Hb9-eGFP/; Plasmid/pDONR223-DDR2/; Adeno-Associated Virus (AAV)/pAAV-hSyn1-DDR2-2a-eGFP/; Adeno-Associated Virus (AAV)/pAAV-Syn1-eGFP-2a-PKA (L 206 R)/; Adeno-Associated Virus (AAV)/pAAV-Syn1-HA-EPAC1 (VLVLE to AAAAA)/; Adeno-Associated Virus (AAV)/pAAV-CMV-BFP-mir30(CXCL12)/; Adeno-Associated Virus (AAV)/pAAV-Syn1-eGFP-mir30(CXCR4)/; Lentivirus/pLenti-EF1a-L1CAM-CMV-BFP/PuroR/; Lentivirus/pLenti-EF1a-NCAM1-CMV-BFP/NeoR/; Lentivirus/pLenti-EF1a-CNTN1-CMV-BFP/HygroR/; Adeno-Associated Virus (AAV)/pAAV-(Antisense) WPRE3-myrAKT3-TRE3G (SENSE) Syn1-TETON3G-2a-miRFP670 Nano WPRE3/; Adeno-Associated Virus (AAV)/pAAV-(Antisense) WPRE3-dTomato-TRE3G (SENSE) Syn1-TETON3G-2a-miRFP670 Nano WPRE3/; Adeno-Associated Virus (AAV)/pAAV-(Antisense) WPRE3-3' beta Actin-myrAKT3-TRE3G (SENSE) Syn1-TETON3G-2a-miRFP670 Nano WPRE3/; Adeno-Associated Virus (AAV)/pAAV-Syn1-EPAC1 (VLVLE to AAAAA) - 3' Beta Actin/; Lentivirus/pRSV-REV/;

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Lentivirus/pMDLg/pRRE/; Lentivirus/HK_13_BLA_Lenti_KIR2.1/; Adeno-Associated Virus (AAV)/pAAV-CMV-eGFP-U6-shRNA(Kv1.2)/; Adeno-Associated Virus (AAV)/pAAV-Syn1-PGC1alpha-HA_eGFP_MitoTag/; Adeno-Associated Virus (AAV)/pAAV-Syn1-HA_eGFP_MitoTag/; Adeno-Associated Virus (AAV)/pAAV-CamKIIa-HA-eGFP-MitoOMM/; Adeno-Associated Virus (AAV)/pAAV-GFAP-3xFlag-BFP-MitoOMM/; Adeno-Associated Virus (AAV)/pAAV-MAG2.2-Myc-RFP-MitoOMM/; Adeno-Associated Virus (AAV)/pAAV-Syn1-Pink1-p2a-HA-eGFP-MitoOMM/; Plasmid/pcDNA-Axolotl Enhancer- Hsp68 min- eGFP-p2a-ffLuc/; Lentivirus/pLenti-Axolotl Enhancer- Hsp68 min- eGFP-p2a-ffLuc/; Plasmid/pcDNA-Hsp68 min- eGFP-p2a-ffLuc/; Lentivirus/pLenti-Hsp68 min- eGFP-p2a-ffLuc/; Lentivirus/pLenti-Axolotl Enhancer- Hsp68 min- RunX1-p2a-ffLuc/; Lentivirus/pLenti-Axolotl Enhancer- Hsp68 min- ffLuc/; Lentivirus/pLenti-CMV-eGFP-p2a-ffLuc-wpre/; Lentivirus/pLenti-CMV-Runx1-p2a-ffLuc-wpre/; Adeno-Associated Virus (AAV)/pAAV-Syn1-CD44-HA-WPRE3/; Adeno-Associated Virus (AAV)/pAAV-(CMV/Syn1/GFAP/CamK2a)-Pink1 reporter split-Luciferase/; Plasmid/CMV-CRISPR-CAS9 U6-Guide Arms (Pink1)/; Plasmid/pCAGGS-FuG-B2

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Hek 293; Human/hNPC (Human Neural Progenitor Cell) ATC-5004; Human/hiPSC (Human Induced Pluripotent Stem Cells); Human/Hek 293-REST-KO; Animal/Mouse Neural Stem Cell; Human/Hek 293-Pink1-Knockout

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Viral Vector - Adeno-Associated Virus (AAV)/Spinal Cord Injection/Anesthesia/Vertebral Clips/ABSL1/Mask/Gloves/Eye Protection/Lab Coat/Hair Bonet/ABSL1/No/Sterile technique, We use a BSC for injection although not required. Mice will be housed in ABSL2 containment for 24 hours after treatment until the wound has closed. Some AAVs contain the use of antisense oligonucleotides/; Mouse/Viral Vector - Lentivirus/Spinal Cord Injection/Anesthesia/Vertebral Clips/ABSL2/Mask/Gloves/Eye Protection/Lab Coat/Hair ABonet/ABSL2/No/Sterile technique. Perform spinal cord injections in BSL2 certified biosafety cabinet. House mice in ABSL2 containment for 72 hours post-injection. Disinfect the area after use and dispose of all sharps into sharps containers.

Risk Assessment/Discussion:

Dr. Stewart has submitted a renewal of his IBC protocol entitled *Gene Therapy Approaches to Induce and Control Neuronal Growth in Rodents With Spinal Cord Injuries*. A number of different AAV constructs are administered to anesthetized mice and rats via spinal cord injection. Work with AAV in animals is completed using BSL1/ABSL1 procedures and housing. Dr. Stewart's laboratory uses 3rd party vendors (such as VectorBuilder) for AAV packaging, as well as packaging some AAV vectors in house. Packaging of AAV in Dr. Stewart's laboratory is completed using BSL2 containment. Lentivirus is also administered

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to anesthetized mice via spinal cord injection using ABSL2 containment and housing. Lentivirus will be packaged in house for in vitro work, but VectorBuilder will packaging lentivirus for animal work. When packaged in house, lentivirus is generated using a 3rd generation packaging plasmid mix using BSL2+ containment. Lentiviral work is restricted to the PI, Dr. Stewart. Animals administered lentivirus will be housed at ABSL2 containment for a minimum of 72 hours post-administration. Dr. Stewart's current IBC protocol will expire on February 17, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-04 (version 6.0) was approved pending minor modifications as listed below:

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SCIENTIFIC SUMMARY – It is unclear what is being done after the transduction of cells and injection of biohazardous materials into animals. Please expand upon and describe end-point assays and manipulations.

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Doug Harrison initiated the motion. Yadi Wu seconded the motion. All IBC members present (14) voted in favor of the motion.

*

Conflicts of Interest: None

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PI: Erin Garcia

IBC Protocol Number: IBC-26-05

Protocol Title: Mechanisms of competition and cooperation in Burkholderia species

Protocol Type: Renewal

Applicable Guidelines & Regulations: UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7

Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: D. Malherbe, C. Shaffer, C. Pickett

Brief Project Overview:

Our work focuses on understanding the ways that bacteria interact with each other through cooperation and competition. We use Burkholderia species, which are intrinsically antibiotic-resistant bacteria found in the soil that can cause lung infections in people with cystic fibrosis. These bacteria produce proteins

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on their surface that can kill other bacteria. Since these proteins are naturally antibacterial and could be useful for developing therapeutics to treat human infections, we would like to better understand how they work. We also investigate how Burkholderia bacteria use these and other proteins within biofilms, which are adherent communities of bacteria that can form in the environment, on medical devices, or during infections.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, DNA/RNA isolation/purification, Genetics, PCR/qRT-PCR, Cell culture, Use of Infectious Agents, Propagation of Infectious Agents, Imaging/Microscopy, Proteomics, Transformation, Animal work (breeding, surgeries, etc.)

Transport: Yes

Materials Transported: Biohazardous Materials, Animals

Infectious Agent(s)/Natural Host(s): Burkholderia cepacia (RG1-bacteria)/Human; Burkholderia multivorans (RG2-bacteria)/Human; Burkholderia dolosa (RG2-bacteria)/Humans; Burkholderia thailandensis (RG1-bacteria)/Human; Animal, Burkholderia cenocepacia (RG1-bacteria)/Human; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: mCherry/commercial/tracking/reporter/B. multivorans, B. dolosa, B. thailandensis/pUC18-miniTn7-kan/; gfp/commercial/tracking/reporter/B. thailandensis, B. multivorans, B. dolosa/pUC18-miniTn7-kan /; lacZ/E. coli/enzymatic protein/reporter/B. thailandensis, B. multivorans, B. dolosa/pUC18-miniTn7-kan, pEGZH3T /; cdiA (fragment, bcpA homolog)/E. coli, Photobacterium luminescens/membrane protein (antibacterial)/generate chimeric bcpA-cdiA/B. multivorans, B. dolosa, B. thailandensis/pEXKm5/; cdil (bcpl homolog)/E. coli, P. luminescens/cytoplasmic protein/expression/B. multivorans, B. dolosa, B. thailandensis/pUC18-miniTn7-kan/; bcpAIOB/B. thailandensis, B. multivorans, B. dolosa, B. cepacia, B. cenocepacia/structural, membrane proteins, enzymatic proteins/expression, complementation, knock-out/B. thailandensis, B. multivorans, B. dolosa/pUC18-miniT7-kan, pEXKm5, pSchraB2, pET28, pJET2.1/; gltIJKL/B. multivorans, Escherichia coli/enzymatic proteins, membrane proteins/expression, complementation, knock-out/B. multivorans/pUC18-miniTn7-kan, pEXKm5/; various lipopolysaccharide (LPS) biosynthesis genes/B. multivorans, B. dolosa/enzymatic proteins, membrane proteins/expression, complementation, knock-out/B. multivorans, B. dolosa/pUC18-miniTn7-kan, pEXKm5/; bacterial regulatory proteins (to be identified)/B. multivorans, B. dolosa/regulatory proteins/expression, complementation, knock-out/B. multivorans, B. dolosa/pUC18-miniTn7-kan, pEXKm5, pET28, pJET2.1 /; bacterial outer membrane proteins/B. multivorans, B. dolosa, B. thailandensis, E. coli/membrane proteins/expression, complementation, knock-out/B. multivorans, B. dolosa, B. thailandensis/pUC18-miniTn7-kan, pEXKm5/; bacterial metabolic proteins/B. multivorans, B. dolosa, B.

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thailandensis/enzymatic proteins/expression, complementation, knock-out/*B. multivorans*, *B. dolosa*, *B. thailandensis*/pUC18-miniTn7-kan, pEXKm5/; bacterial promoters/*B. multivorans*, *B. dolosa*/regulatory DNA elements/reporter, cloning, expression/*B. multivorans*, *B. dolosa*/pUC18-miniTn7-kan, pEGZH3T, pCR2.1-TOPO

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pEGZH3/; Plasmid/pET28/; Plasmid/pUC18T-miniTn7/; Plasmid/pScRhaB2/; Plasmid/pFlpe4/; Plasmid/pEXKm5/; Plasmid/pUT-miniTn5-kan/; Plasmid/pTNS3/; Plasmid/pJET1.2/; Plasmid/pCR2.1 - TOPO TA

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/CFBE41o-

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Invertebrate - Non-Insect/Non-Arthropod/*Burkholderia dolosa* (RG2-bacteria)/oral/N/A/ABSL2/gloves, lab coat, eye protection/ABSL2/No//; Invertebrate - Non-Insect/Non-Arthropod/*Burkholderia cepacia* (RG1-bacteria)/oral/N/A/ABSL2/gloves, lab coat, eye protection/ABSL2/No//; Invertebrate - Non-Insect/Non-Arthropod/*Burkholderia multivorans* (RG2-bacteria)/oral/N/A/ABSL2/gloves, lab coat, eye protection/ABSL2/No//; Invertebrate - Non-Insect/Non-Arthropod/*Burkholderia thailandensis* (RG1-bacteria)/oral/N/A/ABSL2/gloves, lab coat, eye protection/ABSL2/No

Risk Assessment/Discussion:

Dr. Garcia has submitted a renewal of her IBC protocol entitled *Mechanisms of competition and cooperation in Burkholderia species*. In her laboratory, Dr. Garcia uses different *Burkholderia* species to study the ways in which bacteria interact with one another. Specifically, they are focused on investigating the function of gene (bcpA_{IOD}) that encode contact-dependent growth inhibition (CDI) systems. Towards that goal, Dr. Garcia's laboratory uses pathogens that are part of the *Burkholderia cepacia* complex (Bcc). These bacteria are commonly found in the soil and are the cause of severe pulmonary infections in immunocompromised individuals and those with cystic fibrosis or chronic granulomatous disease. *B. multivorans* is considered a RG2 organism, whereas *B. cepacia*, *B. dolosa*, *B. thailandensis*, and *B. cenocepacia* are considered RG1 agents. Clinical isolates will be obtained from the UK Hospital Pathology Laboratory for co-culture with genetically modified *Burkholderia* strains. Dr. Garcia's laboratory constructs gene deletions, point mutations, and gene replacements in *Burkholderia* species. Reporter genes (GFP, RFP, lacZ) are introduced, as well as genes native to *Burkholderia* species and antibiotic-resistant cassettes for selection. These genes confer resistance to kanamycin, tetracycline, and chloramphenicol, which are not utilized to treat *Burkholderia* infections in the clinic. All work is completed using BSL2 containment with personnel wearing lab coat, gloves, and eye protection. Dr. Garcia describes a number of protocols utilizing *Burkholderia* species, including various biofilm

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assays, lysis via French Press, flow cytometry of fixed bacteria, phenotypic assays, infection of eukaryotic cell lines, and infection of *C. elegans*. Dr. Garcia's current IBC protocol will expire on February 20, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-05 (version 8.0) was approved pending minor modifications as listed below:

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SCIENTIFIC SUMMARY -

1. Please clarify where genetic manipulations of bacteria are done. Is this performed in a BSC or on the open bench?
2. In the description of Protocol #3, will spectrophotometer cuvettes be sealed to avoid potential spillage of bacterial samples during transport to and from the spectrophotometer? How are the cuvettes sealed/closed?
3. Please include the incubation time for fixative prior to flow cytometry.
4. Please clarify where the French Press described in Protocol #7 is located. Is it on the open benchtop or inside of a BSC? If this is done on the open benchtop, what additional precautions are taken to minimize risk of personnel exposure?

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Carrie Shaffer initiated the motion. Delphine Malherbe seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

*

PI: Chang-Guo Zhan

IBC Protocol Number: IBC-26-06

Protocol Title: Development of Cocaine-Metabolizing Enzymes

Protocol Type: Renewal

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-1, NIH Guidelines Section III-E-2, NIH Guidelines Section III-F, NIH Guidelines

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Section III-F-1, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9

Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+)

Primary Reviewers: C. Haughton, B. Nelson, D. Harrison

Brief Project Overview:

Cocaine addiction and overdose are a well-known public health problem. According to the 2008 National Survey on Drug Use and Health, 36.77 million Americans have used cocaine. There is no FDA-approved medication specific for cocaine abuse treatment. The disastrous medical and social consequences of cocaine abuse, such as lost lives, decreases in productivity, increases in crime, disruption of households, and the expense of cocaine interdiction efforts, have made the development of an anti-cocaine medication a high priority. Our recently designed and discovered cocaine hydrolases (CocHs), including high-activity mutants of human butyrylcholinesterase (BChE) against cocaine, have been recognized as promising candidates for anti-cocaine therapeutic agents that can quickly detoxify cocaine in the body. Our accomplished animal studies showed that administration of the purified CocH protected mice from the toxic effects of cocaine. The current studies will examine the in vivo effectiveness of multiple CocHs for cocaine degradation. The CocHs will be prepared and tested first for their in vivo activities in hydrolyzing a sub-lethal dose of cocaine. The most active CocHs, once identified, will be tested for their effectiveness in protecting against a lethal dose of cocaine and will be developed to increase their half-lives in the body. To study and compare the catalytic activity of CocHs against cocaine, large-scale preparation of CocHs is needed. Lentiviral expression system and adeno-associated viral (AAV)-helper free system which have the advantage of providing stable, high level and long-term expression of target proteins are designed to use in our lab. Both lentiviral and adeno-associated viral systems include a significant number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus. However, they still pose some biohazardous risk since they can transduce primary human cells. All the lentiviral construction work will be done under BSL2+ containment, including use of dedicated lab coat or disposable lab gown and work with lentivirus or lentivirus-containing materials will only take place in a dedicated BSC.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, Creation of viral vectors, DNA/RNA isolation/purification, PCR/qRT-PCR, Transfection, Transformation, Use of infectious agents, Use of viral vectors, Use of Human Source Material(s)

Transport: No

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Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Human butyrylcholinesterase/human cDNA/metabolic enzymes/expression in cell culture, insertion into animals/CHO-S cells, rats/Lentivirus, AAV

Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/pLenti/; Adeno-Associated Virus (AAV)/AAV

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/293F/293T/293FT/293AAV;

Animal/CHO-S

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Rat/Viral Vector – Lentivirus/IV/Physical restrainer or anesthesia/ABSL2/Gloves, lab coat, eye protection/ABSL2/Yes/Use exclusively in the BSC; lentivirus may be shed 72-hours post administration; Rat/Viral Vector - Adeno-Associated Virus (AAV)/IV/Physical restrainer or anesthesia/ABSL1/Eye protection, gloves, lab coat/ABSL1/No

Risk Assessment/Discussion:

Dr. Zhan has submitted a renewal of his IBC protocol entitled *Development of Cocaine-Metabolizing Enzymes*. Dr. Zhan's laboratory is interested in anti-cocaine therapeutic agents. Dr. Zhan's in vitro studies include the expression of Coch mutants in HEK293 or CHO cells for collection and purification. The catalytic activity of purified enzyme will be tested against cocaine. Dr. Zhan's in vivo studies will first test high activity mutants of human BChE in mice to identify the most promising Coch candidates for further testing in rats. Rats will be administered lentiviral or AAV vectors expressing genes for the most promising CochHs. AAV is packaged in Dr. Zhan's laboratory using the AAV helper-free system. AAV is manipulated using BSL1 containment. Lentivirus is produced using a 3rd generation packaging system using BSL2+ containment. All work with lentivirus is completed within a BSC. Viral vectors (AAV or lentivirus) will be injected via tail-vein injection into rats within a BSC in DLAR. Rats are restrained using a physical restraining chamber. Coch concentration will be determined by saphenous vein blood collection. Rats administered lentivirus will be housed at ABSL2 containment, whereas rats administered AAV will be housed at ABSL1 containment. Dr. Zhan's current IBC protocol will expire on February 14, 2026.

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IBC Discussion & Vote:

The protocol renewal IBC-26-06 (version 4.0) was approved pending minor modifications as listed below:

PERSONNEL: The personnel list is not congruent with the SciSure profile. Please ensure personnel are listed correctly in both the IBC protocol and the SciSure database.

SCIENTIFIC SUMMARY:

1. Please remove extraneous information unrelated to biological procedures from the Scientific Summary. Limit the discussion to the manipulations planned in conjunction with biohazardous materials and mitigation measures.
2. How is biohazardous material removed from sealed tubes after centrifugation? Does this step utilize sharps? Please expand this description.
3. Please clarify which steps are performed on the benchtop versus inside a biological safety cabinet.

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Doug Harrison initiated the motion. Brandy Nelson seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Michael Flythe

IBC Protocol Number: IBC-26-10

Protocol Title: B22-4062: The ecology and physiology of anaerobic bacteria in grazing animals

Protocol Type: Renewal

Applicable Guidelines & Regulations: NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), UK Administrative Regulation 6.3, UK Administrative Regulation 6.9

Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: J. Smalle, C. Shaffer, M. Landron

Brief Project Overview:

We study the bacteria found in the gastrointestinal tracts of cows, goats, and horses. Most of these bacteria are beneficial to the animals, and harmless to humans. However, some have been implicated in disease in rare cases. For instance, some strains of *Streptococcus bovis* can cause meningitis and

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endocarditis. The risk of contracting these diseases in the laboratory is low because oxygen prevents the growth *S. bovis*, so it will not survive on a bench top or a glove for very long. However, a needle stick could transmit *S. bovis*. The literature indicates that the animal strains of *S. bovis* are considerably different than those that infect humans (Kurtovic et al 2003). We always work with the animal strains.

Occasionally, we have to isolate and identify unknown microorganisms. There is always the chance that an unknown isolate will be pathogenic. However, we always: 1) identify isolates as rapidly as possible, and 2) treat an unknown as if it were a pathogen until it is identified.

There is not a specific IACUC protocol associated with this IBC protocol. During this three year period we will work exclusively with organisms we already isolated or from strains obtained from culture collections.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture

Transport: No

Materials Transported: N/A

Infectious Agent(s)/Natural Host(s): *Streptococcus bovis* (RG1-bacteria)/Bovine, Equine, zoonotic potential for human/; *Peptostreptococcus anaerobius* (RG2-bacteria)/Humans, animals/; *Enterococcus faecalis* (RG2-bacteria)/Humans, animals/; *Fusobacterium necrophorum* (RG2-bacteria)/Humans, animals (including horses, cattle, sheep, goats, pigs, and fowl)/; *Prevotella bryantii* (RG2-bacteria)/Humans, animals/; *Clostridium sticklandii* (RG1 bacteria)/bovine

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: N/A

Vector(s) [Vector Category/Vector Technical Name]: N/A

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: N/A

Animal Use: No

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: N/A

Risk Assessment/Discussion:

Dr. Flythe has submitted a renewal of his IBC protocol entitled *The ecology and physiology of anaerobic bacteria in grazing animals*. In this project, Dr. Flythe seeks to study the bacteria found in the GI tract of cows, goats, and horses. Whereas most of these bacteria are beneficial to their animal hosts and harmless to humans, some are capable of causing disease, particularly in individuals with a compromised immune system. Bacterial agents in use include *Streptococcus bovis* (RG1), *Peptostreptococcus anaerobius* (RG2), *Enterococcus faecalis* (RG2), *Fusobacterium necrophorum*

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(RG2), *Prevotella bryantii* (RG2), and *Clostridium sticklandii* (RG1). Bacteria will be handled/manipulated using BSL2 containment within a BSC (when possible) or an anaerobic chamber. Bacterial isolates are characterized via 16S homology, DNA extraction and PCR, optical density measurements, and HPLC of non-hazardous metabolites. Personnel wear lab coats, nitrile gloves, and eye protection for all procedures. Bacteria are not genetically modified in any way. Dr. Flythe's protocol was granted a 3-month extension of approval at the December 2025 IBC meeting due to the extended US Government shutdown last fall. His extended IBC protocol will expire March 6, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-10 (version 8.0) was approved pending minor modifications as listed below:

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SCIENTIFIC SUMMARY – Please remove the statement “Surgical masks and N95 masks are available, but not specifically required for any procedures.” if these PPE are not required for this work.

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Jan Smalle initiated the motion. Carrie Shaffer seconded the motion. All IBC members present (14) voted in favor of the motion.

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Conflicts of Interest: None

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Amendments

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PI: Amelia Pinto

IBC Protocol Number: IBC-24-83

Protocol Title: Risk group two virus protocol

Protocol Type: Amendment

Amendment To: Genetic constructs, Organisms used in research

Applicable Guidelines & Regulations: NIH Guidelines Section III-F-1, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, OSHA 29 CFR 1910.1030, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7, NIH Guidelines Section III-D-2

Maximum Containment Level: Biological Safety Level 2 (BSL2), Animal Biological Safety Level 2 (ABSL2)

Primary Reviewers: C. Haughton, T. Chambers, B. Nelson

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Brief Project Overview:

To grow BSL-2 virus strains and to carry out immunology virology and pathogenesis studies in small animal models using mice. Mouse models will be screened for the development of inflammatory disease, allowing investigation of viral and host factors that contribute to inflammation disease pathogenesis. Small animal models will be used for the application of system-biology approaches to identify host inflammatory networks and will be used to identify host genes/polymorphisms that contribute to acute and/or chronic disease. Small animal models will be used to determine factors involved in infection that contribute to severe disease. Finally, longer-term small animal models will be used to test the efficacy/safety of vaccines, antivirals and immune therapies.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Use of Viral Vectors, Use of Infectious Agents, Transformation, Transfection, Proteomics, Propagation of Infectious Agents, PCR/qRT-PCR, Immunohistochemistry, Imaging/Microscopy, Histology, Genetics, DNA/RNA isolation/purification, Cell culture, Bacterial culture, Animal work (breeding, surgeries, etc.)

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Chikungunya Virus (CHIKV) 181/25 vaccine strain (RG2-virus)/Vaccine-lab generated/; Cytomegalovirus (CMV) (RG2-virus)/Humans /; Dengue Virus (DENV) Serotype 1-4 (RG2-virus)/Biodefense and Emerging Infections Research Resources Repository (BEI Resources) /; Herpes Simplex Virus-1 (HSV-1) (RG2-virus)/Human/; Herpes Simplex Virus-2 (HSV-2) (RG2-virus)/Human/; Influenza A Virus (RG2-virus)/Humans, Animals, Birds/; Japanese Encephalitis Virus (JEV) SA 14-14-2 vaccine strain (RG2-virus)/Mosquito/Human/; La Crosse Virus (LACV) (RG2-virus)/Humans, Animals, and Insects/; Kunjin Virus (RG2-virus)/Mosquito/Human /; Langat Virus (LGTV) (RG2-virus)/Humans, Animals, and Insects /; Lone Star Virus (LSV) (RG2-virus)/Humans, Animals, and Insects /; Mayaro Virus (MAYV) (RG2-virus)/Humans, Animals, and Insects /; Modified Vaccinia Ankara Virus (MVA) (RG2-virus)/Vaccine-lab generated /; Murine Polyomavirus (MPyV) (RG1-virus)/Mice/; Murine Hepatitis Virus (MHV) (RG2-virus)/Mice/; Murine Cytomegalovirus (MCMV) (RG1-virus)/Mice/; Pichinde Virus (PICV) (RG2-virus)/Humans and Mice/; Rift Valley Fever -MP12 (RVFV-MP- 12) vaccine strain (RG2-virus)/Humans, Animals, and Insects/; Sendai Virus (RG1-virus)/Mice, Hamsters, Rats, Guinea Pigs/; St. Louis Encephalitis Virus (SLEV) (RG2-virus)/Humans, Animals, and Insects/; Tacaribe complex Virus (TCRV) (RG2-virus)/Humans, Animals, and Insects/; Usutu virus (USUV) (RG2-virus)/Humans, Animals, and Insects/; Varicella-Zoster Virus (VZV) (RG2-virus)/Humans /; Venezuelan Equine Encephalitis Virus (VEEV) TC83 vaccine strain (RG2-virus)/Vaccine-lab generated /; Vesicular Stomatitis Virus (VSV) (RG2-virus)/Humans, Animals, and Insects/; West Nile Virus (WNV) (RG2-virus)/Humans, Animals, Birds, and Insects/; Yellow Fever Virus (YFV) vaccine strain 17D (RG2-virus)/Vaccine-lab generated/; Zika Virus

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(RG2-virus)/Humans, Animals, and Insects;/ Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human;/ Gadgets Gully Virus (GGYV) (RG1-virus)/Seals and Insects;/ Lymphocytic choriomeningitis virus (LCMV) Clone 13 strain (RG2-virus)/House mice (*Mus musculus*) are natural hosts. LCMV can also infect humans other animals (hamsters, guinea pigs, NHPs, etc); Lymphocytic choriomeningitis virus (LCMV) Armstrong strain (RG2-virus)/House mice (*Mus musculus*) are natural hosts. LCMV can also infect humans other animals (hamsters, guinea pigs, NHPs, etc)

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Zika Envelope /Flavivirus /Structural /Expression/Bacteria/pcDNA3.1;/ GFP/Commercial /Expression/Expression/Bacteria/pcDNA3.1, pIF577.pTC83, pJB200;/ Zika virus like particle/Flavivirus/antigen/structural Protein/expression/mammalian cell/ mice/pJB200, pIF577.pTC83;/ West Nile virus like particle/Flavivirus/antigen-structural proteins/expression/mammalian cell/mice/pJB200, pIF577.pTC83;/ Dengue virus 1-4 virus like particle/Flavivirus/antigen-structural proteins/expression/antigen-structural proteins/mice/pJB200, pIF577.pTC83;/ Mayaro virus like particle/Alphavirus/antigen-structural proteins/expression/

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pBR322; Plasmid/pcDNA3.1; Plasmid/pJB200.1; Plasmid/pIF577.pTC83

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/Vero; Human/293T; Animal/MDCK; Animal/BALB/3T3; Animal/BHK-21; Animal/RAW264.7; Insect/C6/36

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse, Dengue Virus (DENV) Serotype 1-4 (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized, ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other

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contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Chikungunya Virus (CHIKV) 181/25 vaccine strain (RG2-bacteria), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Gadgets Gully Virus (GGYV) (RG1-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport.

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The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , , ,

Mouse, Herpes Simplex virus 1 (HSV-1) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , , ,

Mouse, Herpes Simplex Virus-2 (HSV-2) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport.

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The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Kunjin Virus (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Langat Virus (LGTV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

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Mouse, La Crosse Virus (LACV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized, ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail intraperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Mayaro Virus (MAYV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized, ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail intraperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, St. Louis Encephalitis Virus (SLEV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized, ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, Animal experiments will be carried out in DLAR according to DLAR approved protocols. All

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protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Usutu virus (USUV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Vesicular Stomatitis Virus (VSV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous,

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Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, West Nile Virus (WNV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Zika Virus (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are

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used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Japanese Encephalitis Virus (JEV) SA 14-14-2 vaccine strain (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Modified Vaccinia Ankara Virus (MVA) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging

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Mouse, Rift Valley Fever -MP12 (RVFV-MP- 12) vaccine strain (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Venezuelan Equine Encephalitis Virus (VEEV) TC83 vaccine strain (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried

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Mouse, Yellow Fever Virus (YFV) vaccine strain 17D (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Lone Star Virus (LSV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other

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material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Pichinde Virus (PICV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Tacaribe complex Virus (TCRV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved

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personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Varicella-Zoster Virus (VZV) (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Influenza A Virus (RG2-virus), Subcutaneous, Intravenous, Intramuscular, Intraperitoneal, and Intracranial, Anaesthetized , ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, , Animal experiments will be carried out in DLAR according to DLAR approved protocols. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol specific virus and the DLAR Facility plan. Animals that will be infected by the subcutaneous, Intramuscular, intravenous, intracranial using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration. Forceps are used to hold appendage, foot for subcutaneous, tail for intravenous, and head for intracranial injections. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material contaminated with virus that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to

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handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB., , , , ,

Mouse, Lymphocytic choriomeningitis virus (LCMV) Clone 13 strain (RG2-virus), intraperitoneal Intramuscular, , anesthetized, ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, Yes, These animal will only be infected and used in HSRB 464D. They will not be housed in DLAR facility. All protocols involving infected animals will be carried out in compliance with an IACUC approved protocol. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration via Intramuscular route. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and autoclaved using approved protocol and brought to DLAR for disposal. Inocula and all other material contaminated with virus that requires transportation between the laboratory space in HSRB 464C and the animal room HSRB 464D will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between the main laboratory space in HSRB 464C and the animal housing area located within HSRB 464D.

Mouse, Plasmid, intraperitoneal, intramuscular, anesthetized, ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, Yes, The plasmid generated mRNAs encapsulated into LNPs will be administered to animals IP or IM similar to mRNA vaccine administration. Animal experiments will be carried out in DLAR and HSRB 464D according to IACUC approved protocols. Animals that will be injected using a BD SafetyGlide 1 ml syringe equipped with a 25G needle when possible. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to mRNA LNP administration. Animals will be housed in a standard filtered caging system. All manipulations of animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and returned to the designated DLAR morgue location for disposal. Inocula and all other material that requires transportation outside of the approved areas will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other materials during transport. The materials will be hand-carried between HKRB 651 and DLAR via the service elevator in the HKRB. Materials prepared in HSRB 464C will be carried in leakproof puncture resistant container to HSRB464D .,

Mouse, Lymphocytic choriomeningitis virus (LCMV) Armstrong strain (RG2-virus), intraperitoneal Intramuscular, , anesthetized, ABSL2, Disposable Gloves, Eye Protection, Lab Coat, ABSL2, Yes, These animal will only be infected and used in HSRB 464D. They will not be housed in DLAR facility. All

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protocols involving infected animals will be carried out in compliance with an IACUC approved protocol. Animals are anesthetized with ketamine/xylazine cocktail interperitoneally prior to virus administration via Intramuscular route. Virus infected animals will be housed in a standard filtered caging system. All manipulations of infected animals or cages are carried out in a BSC. Animals found dead or sacrificed are disposed of in a red biohazard bag and autoclaved using approved protocol and brought to DLAR for disposal. Inocula and all other material contaminated with virus that requires transportation between the laboratory space in HSRB 464C and the animal room HSRB 464D will be transported in primary containment (cryotubes, centrifuge tubes or bottles) placed within a secondary containment which is sealable, and puncture resistant. Only approved personnel will be allowed to handle the inocula and all other contaminated materials during transport. The materials will be hand-carried between the main laboratory space in HSRB 464C and the animal housing area located within HSRB 464D., , , , ,

Mouse, Nanoparticle-r/sNA, IP, IM, Manual restraint, ABSL1, Lab coat, eye protection, Disposable gloves, ABSL1, No

Risk Assessment/Discussion:

Dr. Pinto has submitted an amendment to her current IBC protocol entitled *Risk group two virus protocol* to update genetic constructs and organisms used in research. Specifically, she has added two new viral agents – Lymphocytic choriomeningitis virus (LCMV) Armstrong strain and Lymphocytic choriomeningitis virus (LCMV) Clone 13 strain. While the parent virus is a RG3 agent, both LCMV-Arm and LCMV-C13 are laboratory adapted strains that are considered RG2 agents. These two new viral agents will be utilized as previously described and approved for in vitro and in vivo work exclusively in the HSRB 464 suite. Additionally, Dr. Pinto has added work with virus like particles (VLPs). mRNA lipid nanoparticles will be produced in Dr. Pinto's laboratory and added to cells in vitro to determine protein expression and via IP injection into mice to measure immune response (as previously described and approved). The mRNA lipid nanoparticles will express GFP, Zika virus (ZIKV) envelope, ZIKV VLP, WNV VLP, DENV VLP, or MAYV VLP. Dr. Pinto has also updated the route of administration for all viral agents in mice to include intramuscular administration. New work with LCMV-Arm and LCMV-C13 will be done using BSL2/ABSL2 containment with personnel wearing disposable gloves, eye protection, and lab coat. Mice are anesthetized for administration of viral pathogens. mRNA lipid nanoparticles will be administered to mice via IP or IM using ABSL1 containment. Work will take place within a BSC prior to inactivation of potentially infectious materials via established methods (already described). The addition of these new viral pathogens and mRNA lipid nanoparticles does not significantly alter the biohazardous risks associated with Dr. Pinto's current IBC protocol. There is an IBC hold on the corresponding IACUC protocol, 2023-4366.

IBC Discussion & Vote:

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Amelia Pinto left the meeting at 1:25pm during discussion of her IBC protocol

The amendment to IBC-24-83 (version 63.0) was approved.

*

Tom Chambers initiated the motion. Brandy Nelson seconded the motion. All IBC members present (13) voted in favor of the motion. Amelia Pinto was not present for vote.

*

Conflicts of Interest: None

*

Incident Review

None

Protocol Issued Registration Numbers

Protocols issued registration numbers, including minor amendments. These protocols are exempt from IBC review and are registered with the UK Biological Safety Officer (BSO).

Satin, Jonathan, IBC-24-423 (formerly B21-3780-M2): Long-term Regulation of Cardiac Ion Channels / Cardioprotection, Amendment, BSO, 02/03/26, IBC-24-423 (v.41.0)

Mishra, Ila, Small peptide hormones in metabolic disorders, Amendment, BSO, 02/02/26 IBC-24-89 (v.46.0)

Helsley, Robert, Macronutrient metabolism in Cardiometabolic Disease, Amendment, BSO, 02/02/26, IBC-25-78 (v.22.0)

Bauer, Bjoern, Targeting LOX/COX/mPGES-1 to Reduce Seizure Burden in Epilepsy, Renewal, BSO, 01/30/26, IBC-25-169 (v.10.0)

Campbell, Kenneth, Cellular level contractile function in human heart failure, Amendment, BSO, 01/30/26, IBC-24-482 (v.54.0)

Lucero, Diego, New Modulators of Lipoprotein Metabolism: From the liver to the vascular wall., Amendment, BSO, 01/30/26, IBC-25-97 (v.26.0)

Cai, Weikang, Understanding astrocytes and microglia functions in neurological diseases., Amendment, BSO, 01/30/26, IBC-24-408 (v.76.0)

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Kent-Dennis, Coral, Exploring health-protective effect of food-derived bioactive compounds, Amendment, BSO, 01/22/26, IBC-24-188 (v.21.0)

Brzozowski, Lauren, Accelerating the Development of FHB-Resistant Soft Red Winter Wheat Varieties, Amendment, BSO, 01/22/26, IBC-25-51 (v.16.0)

Lima, Florence, Metabolic Bone Disease Registry, Amendment, BSO, 01/21/26, IBC-24-87 (v.35.0)

Prisinzano, Thomas, B23-4139: Evaluation of GPCR Ligands, Renewal, BSO, 01/15/26, IBC-25-168 (v.8.0)

Gipson-Reichardt, Cassandra, Glutamate, Neuroinflammation, Acetylcholine, and Addiction, Amendment, BSO, 12/09/25, IBC-24-350 (v.76.0)

Protocols Meeting Registration Requirements

Protocols that have been approved by the IBC pending minor modifications that have met approval requirements.

Zaytseva, Yekaterina, The role of fatty acid metabolism in colorectal cancer, Amendment, IBC, 02/04/26, IBC-25-156 (v. 25.0)

Liu, Xiaoqi, Plk1 in epigenetics of prostate cancer development and progression, Amendment, IBC, 01/30/26, IBC-24-114 (v.48.0)

Pendergast, Julie, Misalignment of Circadian Rhythms, Renewal, IBC, 01/30/26, IBC-25-166 (v.14.0)

Mahuwala, Zabeen, RNAC-MG-002 (AURORA): A Randomized, Double-Blind, Placebo-Controlled Phase 3 Trial of Descartes-08 in Patients with Generalized Myasthenia Gravis (MG), New, IBC, 01/28/26, IBC-25-19 (version 8.0)

Smalle, Jan, B22-4131: Plant growth regulation and secondary metabolites, Renewal, IBC, 01/23/26, IBC-25-167 (v.11.0)

Yang, Eddy, Novel combination therapies to treat cancer, Amendment, IBC, 01/21/26, IBC-24-94 (v.57.0)

Schwarze, Steven, MLS Teaching Labs, New, IBC, 01/16/26, IBC-25-140

IBC Training

None. All current IBC members have completed training online via SciShield.

New Business

Delena Mazzetti provided a reminder that the Fifth Regional Listening Session on NIH Effort to Strengthen and Modernize Biosafety Oversight is scheduled to take place on February 12, 2026.

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Adjournment

Douglas Harrison moved to adjourn the meeting at 1:28PM. Tom Chambers seconded the motion. All members present (13) voted in favor.

APPROVED