University of Kentucky (UK)
Institutional Biosafety Committee (IBC)

Date: 06AUG2025
Time: 12:02 PM – 2:07 PM
Location: Virtual Meeting via Zoom - https://uky.zoom.us/j/83005689329

Minutes

Call to Order

The meeting was called to order by Doug Harrison at 12:02PM.

Attendance

IBC Members Present

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

Regrets

None

Guests

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

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University of Kentucky (UK)
Institutional Biosafety Committee (IBC)

Approval of Previous Month's Meeting Minutes

2025.07.02 IBC Meeting Minutes - DRAFT.pdf

Cheryl Haughton initiated a motion to approve the July 2nd, 2025, IBC meeting minutes. Arthur Hunt seconded the motion. All IBC members present (14) voted in favor of the motion.

Old Business

None.

New Business

Introduction of New IBC Members – Doug Harrison introduced newly appointed IBC members Maria Landron, Amelia Pinto, and Carrie Shaffer.

Protocol Review

IBC approval is granted only when biosafety containment and procedures are reviewed and found to be adequate for the research being undertaken and when all biosafety laboratory inspection and training requirements are satisfactorily met. All biosafety laboratory inspection and training requirements are verified by the UK Biological Safety Officer (BSO) or designee prior to final approval. Current UK Biosafety training requirements are available online HERE. Current UK Biosafety Laboratory Inspection Program requirements are available online HERE.

Resubmissions

PI: William de Souza

IBC Protocol Number: IBC-25-05
Protocol Title: Transmission dynamics and virus-host interactions of arboviruses
Protocol Type: Resubmission
Amendment To: N/A
Applicable Guidelines & Regulations: UK Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7, OSHA 29 CFR 1910.1030
Maximum Containment Level: Biological Safety Level 2 (BSL2)
Primary Reviewers: D. Harrison, M. Mendenhall, B. Nelson

Brief Project Overview:

This project studies how viruses that are spread by blood-sucking invertebrates (like mosquitoes, midge, and ticks) infect people and how these infections spread. We use multiple scientific methods to understand these diseases, including studying how viruses work (virology), how our bodies fight off infections (immunology), how the viruses mutate, and how the virus evolves (genomics). We also use computers to analyze large amounts of data, which also helps us understand how these viruses spread; we study things like how often people get sick (epidemiology) and where these viruses live in nature (ecology). We also study how these viruses make people sick by doing experiments in the laboratory. Our research helps us learn how viruses and people interact, which is important for preventing and treating these diseases.

Summary of Biohazard Materials & Manipulations:

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Manipulations Planned: Cell culture, DNA/RNA isolation/purification, Imaging/Microscopy, PCR/qRT-PCR, Propagation of infectious agents, Viral culture, Use of Human Source Material(s), Use of infectious agents, Flow cytometry/Cell sorting

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Chikungunya Virus (CHIKV) 181/25 vaccine strain (RG2-virus)/Vaccine-lab generated/; Mayaro Virus (MAYV) (RG2-virus)/Humans, animals, and insects (mosquitoes)/; Venezuelan Equine Encephalitis Virus (VEEV) TC83 vaccine strain (RG2-virus)/Vaccine-lab generated/; Oropouche (OROV) (RG2-virus)/Humans, animals, and insects (midges)/; La Crosse Virus (LACV) (RG2-virus)/Humans, animals, and insects (mosquitoes)/; Dengue Virus (DENV) Serotype 1-4 (RG2-virus)/Humans, animals, and insects (mosquitoes)/; Zika Virus (RG2-virus)/Humans, animals, and insects (mosquitoes)/; Yellow Fever Virus (YFV) vaccine strain 17D (RG2-virus)/Vaccine-lab generated/; West Nile Virus (WNV) (RG2-virus)/Humans, animals, insects (mosquitoes)/; Japanese Encephalitis Virus (JEV) SA 14-14-2 vaccine strain (RG2-virus)/Humans, animals, and insects (mosquitoes)/; St. Louis Encephalitis Virus (SLEV) (RG2-virus)/Humans, animals, insects (mosquitoes)/; Bourbon Virus (BRBV) (RG2-virus)/Humans, animals, and ticks/; Rift Valley Fever -MP12 (RVFV-MP- 12) vaccine strain (RG2-virus)/Vaccine-lab generated /; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Humans/; Usutu virus (USUV) (RG2-virus)/Humans, animals, insects (mosquitoes)

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]: Animal/Vero clone E6/; Animal/Vero cells/; Human/HEK293/; Insect/Aedes albopictus clone C6/36/; Human/HuH-7 /; Animal/Immune Cells/; Human/Immune Cells/; Animal/Immune Cells/; Human/human primary astrocytes /; Human/Human Umbilical Vein Endothelial Cells

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:

This is a resubmission of Dr. De Souza's IBC protocol request, IBC-25-05, that was reviewed at the June 6, 2025, IBC meeting and returned to the PI for significant revisions.

The de Souza laboratory investigates virus-host interactions and transmission dynamics of arboviruses using virology, immunology, genomics, and computational biology. The lab works with a panel of RG2, non-select agent arboviruses, including attenuated vaccine strains such as CHIKV 181/25, VEEV TC83, and RVFV MP12. Exposure to the infectious arboviruses listed on this IBC protocol is the major biohazard risk identified, as well as handling of human and non-human primate samples which introduce the risk of exposure to bloodborne pathogens or zoonotic infectious agents including Herpes B virus. All work with infectious materials is conducted within a Class II BSC. Centrifugation steps are carried out utilizing sealed centrifuge buckets/safety cups that are loaded/unloaded in a BSC and wiped with disinfectant prior to removal. Dr. De Souza has clarified that only 1 virus will be handled at any time to prevent cross-contamination. Disposable gloves, lab coats, and eye protection are utilized for work with infectious materials. Liquid biohazardous waste is decontaminated utilizing fresh household

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bleach (10% bleach, final concentration, for a minimum of 20 minutes). Solid biohazardous waste is decontaminated via autoclave. Live materials are not removed from the BSC for open bench work until inactivation (via fixation, TRIzol, etc.). All work with infectious agents is restricted to HKRB 650K and 650J. RNA samples from RG3 agents obtained from collaborators will be received with a certificate of inactivation. Work with infectious agents described in this IBC protocol will be done at BSL2 containment.

IBC Discussion & Vote:

The protocol IBC-25-05 (version 15.0) was approved.
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Mike Mendenhall 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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Fevzi Yalniz

IBC Protocol Number: IBC-25-57
Protocol Title: Autolus-OOS-EAP: Expanded Access Program (EAP) for Obecabtagene Autoleucel (obe-cel) Out-of-specification (OOS) in Adult Patients with Acute Lymphoblastic Leukemia (AUTO1-OS1)
Protocol Type: Resubmission
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-C-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, NIH Guidelines Section III-D-1
Maximum Containment Level: Biological Safety Level 2 (BSL2)
Primary Reviewers: D. Harrison, B. Nelson, T. Chambers

Brief Project Overview:

Obe-cel, Aucatzyl® is used to treat B-cell Acute Lymphoblastic Leukimia (B-cell ALL). Obe-cel, Aucatzyl®, is an FDA-approved treatment when in specification. This EAP (Expanded Access Program) for Obecabtagene Autoleucel (obe-cel) that does not meet the commercial release specifications of Obe-Cel/Aucatzyl® in the United States.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Use of Human Source Material(s), Human Clinical Trial
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)]: anti-CD19/Human/Chimeric Antigen Receptor/Expression/Autologous Cells/LV18970; CD8 stalk & transmembrane domain/Human/transmembrane domain & structural/Expression/Autologous Cells/LV18970; CD137 cytoplasmic domain (4-1BB)/Human/Cytoplasmic Domain/Expression/Autologous Cells/LV18970; CD3 zeta cytoplasmic domain/Human/Cytoplasmic Domain/Expression/Autologous Cells/LV18970

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Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/LV18970
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:

This is a resubmission of Dr. Yalniz's IBC protocol request, IBC-25-57, that was reviewed at the July 2, 2025, IBC meeting and returned to the PI for significant revisions.

The purpose of this study is to provide access to out-of-specification (OOS) Obe-cel for patients with relapsed/refractory B-cell ALL when product meeting manufacture specifications is not available and remanufacturing is not feasible. Obe-cel is a lentiviral vector modified autologous T-cell product. Obe-cel was approved by the US Food and Drug Administration in November 2024. Obe-cel will be handled by UK Healthcare personnel as directed by the study sponsor and manufacturer. The study sponsor and treating physician evaluate the risks associated with using OOS product to ensure there is a positive benefit/risk assessment based on the nature of the product and the status of the patient. In this resubmission, the investigator has clarified that OOS product that contains detectable replication-competent lentivirus, mycoplasma, or that otherwise fails sterility testing will not be released for administration to study patients. It is unknown how many study patients will be enrolled at UK and dependent on the ability to manufacture Obe-cel from each patient. Obe-cel OOS product will be received by the UK Cell Therapy Laboratory for storage until administration. OOS product will be prepared according to manufacturer's instructions. Product will be thawed at patient bedside and administered to study patients by the administering physician. Infusion bags are inspected for leaks, cracks, or fractures throughout preparation and administration steps. Any spills are cleaned and decontaminated according to UK Healthcare SOP. The risk to UK healthcare workers is relatively low and no greater than what a healthcare worker may encounter in a hospital setting working with patients (ex. Exposure to bloodborne pathogens). All waste materials will be disposed of as regulated medical waste. UK Healthcare workers are trained to minimize risk in the healthcare setting. This training includes proper use of PPE, waste handling, spill response, etc. All work described in this IBC protocol is conducted at BSL2 containment in accordance with UK Healthcare policies and procedures.

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IBC Discussion & Vote:

The protocol IBC-25-57 (version 8.0) was approved.
*
Doug Harrison initiated the motion. Brandy Nelson seconded the motion. All IBC members present (14) voted in favor of the motion.
*
Conflicts of Interest: None
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Amendments

PI: Feng Li

IBC Protocol Number: IBC-24-32
Protocol Title: Influenza D Virus Entry and Tissue Tropism
Protocol Type: Amendment
Amendment To: Other, Biological Safety Level (BSL)

Applicable Guidelines & Regulations: OSHA 29 CFR 1910.1030, NIH Guidelines Section IV-B-7, NIH Guidelines Section III-D-3, NIH Guidelines Section III-F-2, OSHA Act of 1970 Clause 5(a)(1), UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, NIH Guidelines Section III-D-7
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+)
Primary Reviewers: D. Harrison, D. Malherbe, T. Chambers

Brief Project Overview:

This IBC protocol supports our research for better understanding how influenza viruses replicate and survive in cells and cause respiratory diseases in host species. Influenza viruses have four types, namely A, B, C, and D. We examine and compare these four influenza viruses with respect to their communication with host cells, entry process, and replication properties.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Cell culture, Immunohistochemistry, Propagation of Infectious Agents, Flow Cytometry/Cell Sorting, PCR/qRT-PCR, Proteomics, Transfection, Transformation, Use of Infectious Agents, Bacterial culture, Creation of Viral Vectors, Imaging/Microscopy, Viral culture
Transport: Yes
Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Influenza D Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Human/; Influenza D Virus (RG2-virus)/Bovine/; Influenza A Virus (RG2-virus)/Human/; Influenza B Virus (RG2-virus)/Human/; Influenza B Virus (RG2-virus)/Human/; Influenza C Virus (RG2-virus)/Human/; Influenza C Virus (RG2-virus)/Human/; Influenza C Virus (RG2-virus)/Bovine/; Influenza A Virus (RG2-virus)/Equine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human cell line/; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/human/; Influenza A Virus (RG2-virus)/Human/; Influenza A Virus (RG2-virus)/Humans/; Influenza A Virus (RG2-virus)/Humans/; Influenza A Virus (RG2-virus)/Humans/; Influenza A Virus (RG2-virus)/Humans/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine /; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine /; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (PR8) (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine/; Influenza A Virus (RG2-virus)/Swine
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: HEF/influenza D/bovine/Oklahoma/660/2013/viral glycoprotein/Reverse genetics/multiple mammalian cell lines/pHW2000/; PB1/influenza D/bovine/Oklahoma/660/2013/polymerase/Reverse genetics/multiple mammalian cell lines/pHW2000/; PB2/influenza D/bovine/Oklahoma/660/2013/polymerase/Reverse genetics/multiple mammalian cell lines/pHW2000/; P3/influenza D/bovine/Oklahoma/660/2013/polymerase/Reverse genetics/multiple mammalian cell lines/pHW2000/; NP/influenza D/bovine/Oklahoma/660/2013/nucleoprotein/Reverse genetics/multiple

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mammalian cell lines/pHW2000/; P42/influenza D/bovine/Oklahoma/660/2013/structural protein/Reverse genetics/multiple mammalian cell lines/pHW2000/; NS/influenza D/bovine/Oklahoma/660/2013/nonstructural protein/Reverse genetics/multiple mammalian cell lines/pHW2000/; siRNAs targeting endocytosis/Thermofisher /small RNA/Knockdown endocytosis pathway/Mammalian cell lines/Lentiviral vector/; mCherry/Addgene/fluorescent protein (Marker)/Viral entry/Mammalian cell lines/pCDNA3.1/; Glycoprotein (GP) of Ebola virus /provided by our collaborator Dr. Greg Melikian (Emory University)/Ebola virus envelop protein/Viral entry/Mammalian cell lines/pCDNA3.1/; VSV-G/Addgene/Vesicular stomatitis virus-glycoprotein/Viral entry/Mammalian cell lines/pCDNA3.1/; Renilla luciferase/Promega/reporter/Viral entry/Mammalian cell lines/pCDNA3.1/; CRISPR/Cas-9/IDT/Gene targeting sialic acid synthesis, viral receptor/Viral entry/Mammalian cell lines/Lentiviral vector/; Cytidine monophosphate N-acetylneuraminic acid (CMAH)/ST cells MDBK cells/Sialic acid synthesis pathway gene/viral entry/mammalian cell line (porcine and bovine)/Lentiviral vector/; Bifunctional UDP-N-acetyglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE)/A549 cells/Sialic acid synthesis pathway/viral entry/mammalian cell line (human, porcine and bovine)/Lentiviral vector/; Bovine ICV HEF/Genescript/Viral Glycoprotein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Bovine ICV PB1/Genescript/Polymerase/Reverse genetics/multiple mammalian cell lines/pHW2000/; Bovine ICV PB2/Genescript/Polymerasae/Reverse genetics/multiple mammalian cell lines/pHW2000/; Bovine ICV P3/Genescript/Polymerase/Reverse genetics/multiple mammalian cell lines/pHW2000/; Bovine ICV NP/Genescript/Nucleoprotein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Bovine ICV P42/Genescript/Structural Protein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Bovine ICV NS/Genescript/Nonstructural Protein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza PB1/Our lab/Polymerase/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza PB2/Our lab/Polymerase/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza PA/Our lab/Polymerase/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza HA/Our lab/Viral Glycoprotein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza NP/Our lab/Nucleoprotein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza NA/Our lab/Viral Glycoprotein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza M/Our lab/Structural protein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza M/Our lab/Structural protein/Reverse genetics/multiple mammalian cell lines/pHW2000/; Equine Influenza NS/Our lab/Nonstructural protein/Reverse genetics/multiple mammalian cell lines/pHW2000/; sgRNAs targeting GNE (UDP-GlcNAc 2-epimerase/ManNAc kinase)/Addgene/sgRNAs/Knockout UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE)/Multiple mammalian cell lines/LentiCRISPRV2/; HA (H5N1) (mutation in HA cleavage site)/A/American_wigeon/South_Carolina/AH0195145/2021_clade2.3.4.4b_vaccine (provided by Dr. David Ho, Columbia University)/Glycoprotein/Viral entry/multiple mammalian cell lines/pCDNA3.1/; HA (H5N1) (mutation in HA cleavage site)/A/Texas/37/2024_clade2.3.4.4b (provided by Dr. David Ho, Columbia University)/Glycoprotein/Viral entry/Multiple mammalian cell lines/pCDNA3.1/; N1 (H5N1)/A/Texas/37/2024_clade2.3.4.4b (provided by Dr. David Ho, Columbia University)/Glycoprotein/Viral entry/multiple mammalian cell lines/pCDNA3.1

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pHW2000; Plasmid/pcDNA3.1; Baculovirus/Baculovirus vector pAcAB3; Lentivirus/LentiCRISPRV2

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/MDCK; Human/A549/; Human/Calu-3/; Human/HRT-18G/; Human/HEK293T/; Animal/ST/; Animal/MDBK/; Human/NHBE/; Insect/SF9

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:

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Dr. Li has submitted an amendment to his approved IBC protocol, IBC-24-32, entitled Influenza D Virus Entry and Tissue Tropism. In this amendment, Dr. Li has updated administrative information, including training dates and location details, and added new strains of seasonal human influenza A from the International Reagent Resource (IRR), established by the CDC, and circulating swine influenza A viruses from collaborators at Iowa State University. All influenza A strains added in this amendment at RG2 and will be handled at BSL2 containment. Specifically, all virus replication experiments will be completed in a dedicated BSC with lab coats, disposable gloves, surgical mask, and eye protection. While the swine influenza A strains are not known to cause disease in humans, Dr. Li acknowledges the potential for spillover and as such will treat these materials as infectious. Dr. Li intends to study the replication fitness of these strains in MDCK and A549 cells. Liquid biohazardous waste generated will be decontaminated with fresh bleach (final concentration 10% bleach for a minimum of 20 minutes). Solid biohazardous waste will be decontaminated via autoclave. Dr. Li is currently approved to work with several RG2 influenza strains, and the addition of these new strains does not significantly alter the biohazardous risks associated with this IBC protocol.

IBC Discussion & Vote:

The amendment to IBC-24-32 (v.26.0) was approved pending minor modifications as listed below:
*
INFECTIOUS AGENTS – Infectious Agents Table: The Infectious Agents table indicates viral stock volumes of 1 mL. Is this accurate? Please review and revise, as necessary.

SCIENTIFIC SUMMARY: Please clarify the steps taken to mitigate potential cross-contamination between different strains.

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Doug Harrison 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
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PI: Samuel Awuah

IBC Protocol Number: IBC-24-90
Protocol Title: Understanding and developing novel small molecule anticancer agents
Protocol Type: Amendment
Amendment To: Administrative Information, Laboratory Location(s), Personnel
Applicable Guidelines & Regulations: UK Administrative Regulation 6.9, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7, NIH Guidelines Section III-D-1, NIH Guidelines Section III-A-1, OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+)
Primary Reviewers: C. Pickett, Y. Wu, M. Mendenhall

Brief Project Overview:

The Awuah research laboratory conducts research at the interface of chemistry and biology to understand human biology processes and disease states. Our primary interest is to develop novel small molecule compounds that shed light on biological pathways contributing to cancer. Compounds synthesized in our lab will be biologically evaluated in live cells to assess capacity to kill cancer selectively.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Cell culture, Bacterial culture, Creation of Viral Vectors, Flow Cytometry/Cell Sorting, PCR/qRT-PCR, Histology, Use of Infectious Agents, Transfection, Transformation, Use of Human Source Material(s), Use of viral vectors, Animal work (breeding, surgeries, etc.), Imaging/Microscopy, Immunohistochemistry
Transport: Yes
Materials Transported: Animals, 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)]: Brunello library (Addgene #73178)/H. sapiens/genome-wide/expression in human cells/Human cell lines/pLV-U6g-EPCG/; ribosomal DNA/H. sapiens/transcription/expression in yeast/S. cerevisiae/pHrP2, pHRD-IRES-Luc, pUC19, pRS314-URA3/; rDNA-IRES-Luciferase/H. sapiens/transcription/expression in yeast/S. cerevisiae, Human cell lines/pHrP2, pHRD-IRES-Luc, pUC19, pRS314-URA3/; URA3/S. cerevisiae/metabolic/expression in yeast/S. cerevisiae/pHrP2, pHRD-IRES-Luc, pUC19, pRS314-URA3/; HIS3/S. cerevisiae/metabolic/expression in yeast/S. cerevisiae/pBSIIKS_HIS3_I, pBCSK_HIS3_II, 720-2µ-maxHIS3/; ADE2/S. cerevisiae/metabolic/expression in yeast/S. cerevisiae/pICE ADE2/; MET25/Y. lipolytica/metabolic/expression in yeast/S. cerevisiae/pFa6a MET25/; LYS2/S. cerevisiae/metabolic/expression in yeast/S. cerevisiae/pICE LYS2/; TRP1/S. cerevisiae/metabolic/expression in yeast/S. cerevisiae/pFA6a-TRP1/; LEU2/S. cerevisiae/metabolic/expression in yeast/S. cerevisiae/pFA6a LEU2/; HK3/H. Sapiens/Genome/Gene deletion/Human cell line/pSpCas9(BB)-2A-GFP/; HK1/M. musculus/Genome/Gene deletion /Murine cell line/pSpCas9(BB)-2A-GFP/; HK2/M. musculus/Genome/Gene deletion/Murine cell lines/pSpCas9(BB)-2A-GFP/; HK3/M. musculus/Genome/Gene deletion/Murine cell lines/pSpCas9(BB)-2A-GFP/; ABCC1/H. sapiens/Genome/Gene deletion/Human cell lines/pSpCas9(BB)-2A-GFP/; SPRED2/H. sapiens/Genome/Gene deletion/Human cell lines/pSpCas9(BB)-2A-GFP/; ATP5l/H. sapiens/Genome/Gene deletion/Human cell lines/pSpCas9(BB)-2A-GFP/; Cas9/Streptococcus pyogenes M1/Genome/Gene deletion/Human and murine cell lines/pSpCas9(BB)-2A-GFP/; GFP/Organism Aequorea victoria (Water jellyfish) (Mesonema victoria)/Genome/Reporter gene/Human and murine cell lines/vectors listed within this protocol
Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/pLV-U6g-EPCG; Plasmid/pHrP2; Plasmid/pHRD-IRES-Luc; Plasmid/pUC19; Plasmid/pRS314-URA3; Plasmid/pICE ADE2; Plasmid/720-2µ-maxHIS3; Plasmid/pFa6a MET25; Plasmid/pICE LYS2; Plasmid/pFA6a-TRP1; Plasmid/pFA6a LEU2; Plasmid/pBSIIKS_HIS3_I; Plasmid/pBCSK_HIS3_II; Plasmid/pSpCas9(BB)-2A-GFP
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/SKOV3/; Human/HeLa/; Human/HT-29/; Human/OVCAR8/; Human/A2780/; Human/LS-174T/; Human/Caco-2/; Human/MCF-7/; Human/K562/; Animal/CT26/; Human/HEK293T/; Human/A549/; Human/MDA-MB-231/; Human/HCC1937/; Human/MDA-MB468/; Human/MDA-MB-175/; Human/PC3/; Human/H460/; Human/MRC5/; Human/MCF10A/; Human/OVCAR5/; Human/RPE-MYC/; Human/RPE-Neo/; Human/P-493/; Animal/4T1/; Animal/4T1_Luciferase/; Human/MDA_Luciferase/; Human/DLD1/; Human/HCT 116/; Human/HCC1937/; Human/BT 333 cells/; Human/SUM159/; Human/SKBR3/; Human/Sk-Mel-2 Parental /; Human/Sk-Mel-2 MR/; Human/Sk-Mel-30 Parental ; Human/Sk-Mel-30 MR/; Human/M14 Parental/; Human/M14-BMR/; Animal/YUMM5.2 Parental/; Human/YUMM5.2 BMR/; Human/MDA-MB-231-D3H2ln-luc/; Animal/4T1_iRFP
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/Cells - Human, non-modified/Injection mammary fat pads/anesthesia/ABSL2/Lab coat, gloves, eye protection/ABSL1/No; Mouse/Tissue - Human (ex. PDX tumor

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tissue)/subQ/anesthesia/ABSL2/gloves, lab coat, eye protection/ABSL1/No; Mouse/Cells - Human, genetically modified/SubQ/Injection mammary fat pads/anesthesia/ABSL2/Lab coat, gloves, eye protection/ABSL1/No

Risk Assessment/Discussion:

Dr. Awuah has submitted an IBC amendment to his approved IBC protocol, IBC-24-90, entitled Understanding and developing novel small molecule anticancer agents. In this amendment, Dr. Awuah updates administrative information, personnel, and includes additional plasmids for CRISPR/Cas9 knockout targeting HK1, HK2, and HK3 in mouse cancer cell lines and ABCC1, SPRED2, and ATP%I in human cells. Cells will be transfected using lipofection/electroporation, and GFP positive cells will be FACS-sorted for clonal expansion. This work will be completed at BSL2 containment utilizing lab coat, disposable gloves, and eye protection while working inside a BSC. Solid wastes will be decontaminated via autoclave, whereas liquid waste generated during cell culture will be treated with fresh bleach (final concentration 10%) for a minimum of 20 minutes prior to drain disposal. Once single-cell clones have been isolated, Dr. Awuah's lab intends to validate knockouts via sequencing followed by downstream analysis via Seahorse Analyzer. This new project does not significantly alter the biohazardous risks associated with this IBC protocol.

IBC Discussion & Vote:

The amendment to IBC-24-90 (version 20.0) was approved pending minor modifications as listed below:
*
SCIENTIFIC SUMMARY:

1. Please clearly state that xenografts are not created using lentivirus-transduced cells.
2. Please ensure that all genes listed in the Gene Information table in the Recombinant/Synthetic Nucleic Acid tab are referenced here in the Scientific Summary.

*
Mike Mendenhall initiated the motion. Yadi Wu seconded the motion. All IBC members present (14) voted in favor of the motion.
*
Conflicts of Interest: None
*

PI: Mautin Barry-Hundeyin

IBC Protocol Number: IBC-24-98
Protocol Title: Study of immune tumor microenvironment in gastrointestinal cancers
Protocol Type: Amendment
Amendment To: Personnel, Genetic constructs
Applicable Guidelines & Regulations: NIH Guidelines Section IV-B-7, NIH Guidelines Section III-D-1, NIH Guidelines Section III-F-1, UK Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA 29 CFR 1910.1030, OSHA Act of 1970 Clause 5(a)(1)
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 1 (ABSL1)
Primary Reviewers: C. Haughton, Y. Wu, B. Nelson

Brief Project Overview:

Traditionally, gastrointestinal cancers have been treated by three ways- surgery, chemotherapy and radiation. In the last few decades, immunotherapy has emerged as an exciting new treatment modality. It involves using the body's immune system to fight cancer. Our laboratory seeks to understand how to leverage the power of the

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immune system to treat cancers of the pancreas, stomach and liver. In summary, we will study the role of macrophages in these cancers.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Cell culture, Use of Viral Vectors, Use of Infectious Agents, Transformation, Transfection, Propagation of Infectious Agents, PCR/qRT-PCR, Use of Human Source Material(s), Imaging/Microscopy, Immunohistochemistry, Histology, Genetics, Flow Cytometry/Cell Sorting, DNA/RNA isolation/purification, Bacterial culture, Proteomics
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)]: Human/Regulatory gene/Expression or Knockdown/Cell Culture/Lentiviral Vector/; ICOSL/Mouse/Regulatory gene/Expression/Cell Culture/pcDNA3.1 expression plasmid vector/; ICOSL/Mouse/Regulatory gene/Knockdown/Cell Culture/Lentiviral Vector/; OPN(SPP1)/Mouse/Phosphoprotein/Knockout/Mouse tumor cell line/OPN Double Nickase Plasmid (m)/; Luciferase/Firefly luciferase/Tracking/Expression/Tumor cell lines/pCAG-luciferase/; Cas9n (D10A)/S. pyogenes/cleavage enzyme/expression to KO Spp1 (mouse)/mouse tumor cells/OPN Double Nickase Plasmid (m)/; GFP/Jellyfish/Tracking/Expression/Mouse tumor cell line/OPN Double Nickase Plasmid (m)
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pcDNA3.1/; Lentivirus/pLV[Exp]-EGFP:T2A:Puro-EF1A/; Lentivirus/pLV[shRNA]-EGFP:T2A:Puro-U6/; Plasmid/pmCherry-C1/; Plasmid/pEGFP-C1/;Plasmid/pCAG-luciferase/; Plasmid/OPN Double Nickase Plasmid (m)/;Plasmid/Control Double Nickase Plasmid
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/YTN Mouse Gastric Cancer Cell Lines /; Animal/KPC Pancreatic cancer cell lines /; Animal/RAW 247 Macrophages /; Animal/Panc01 Pancreatic cancer cell lines/; Animal/MC38 Colon cancer cell lines/; Animal/CT26 Colon cancer lines/;Human/THP-1 Human Monocytes/; Human/TCP-1008 Stomach (Gastric) Cancer Panel/; Human/HEK 293T
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/Cells - Animal, genetically modified/Subcutaneously or intraperitoneally/Anesthesia /ABSL1/Gown and gloves/ABSL1/No/Cells will be washed three times before implanting to the mice/; Mouse/Cells - Animal, non-modified/Subcutaneously, intraperitoneally, tail vein, or spleen injection/Anesthesia /ABSL1/Gown and gloves/ABSL1/No/IL-8/CXCL8 expression will be upregulated using lentiviral overexpression techniques in YTN mouse derived gastric cancer cell lines OR MC38/CT26 colon cancer cell lines. These will be injected into the spleen of mice aged 6-8 weeks to generate liver metastasis into C57BL/6 mice as described in the survival surgery section.

Risk Assessment/Discussion:

Dr. Barry-Hundeyin has submitted an IBC amendment to her current IBC protocol IBC-24-98, entitled Study of immune tumor microenvironment in gastrointestinal cancers. This amendment includes updates to administrative information, personnel, and genetic constructs. Specifically, Dr. Barry-Hundeyin has added several plasmid constructs for lipofectamine-mediated transfection into mouse tumor cells. The pCAG-Luciferase plasmid from Addgene will be transfected into mouse tumor cells to generate stable cell lines expressing Luciferase. These modified cells will be injected into anesthetized mice via subcutaneous or intraperitoneal route. Tumor progression will be monitored, and mice will undergo *in vivo* imaging. This work will be conducted at ABSL1 containment, and animals will be housed at ABSL1 housing. Dr. Barry-Hundeyin is already approved for similar

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work with lentivirus-transduced cells in animals. She has also added a project utilizing the OPN Double Nickase Plasmid (m) from Santa Cruz Biotechnology to knockdown expression of OPN(SPP1) in mouse cells. The OPN Double Nickase Plasmid (m) encodes a mutated Cas9 nuclease and gRNA targeting SPP1 (mouse) gene. These modified cells will be utilized for treatment of macrophages and animal work. Anesthetized mice will be injected subcutaneously or intraperitoneally with these modified cells to study the impact of SPP1 in tumor progression. A control CRISPR/Cas9 plasmid from Santa Cruz Biotechnology has also been added. This work will also be completed using ABSL1 containment and housing as previously described. These new plasmid constructs and project described in this amendment do not significantly alter the biohazardous risks associated with this IBC protocol. The methods of plasmid transfection present less risk to personnel than previously approved genetic modification methods (ex. Lentivirus transduction).

IBC Discussion & Vote:

The amendment to IBC-24-98 (version 30.0) was approved.

*

Yadi Wu 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: Andrew Stewart

IBC Protocol Number: IBC-24-333
Protocol Title: Gene Therapy Approaches to Induce and Control Neuronal Growth in Rodents With Spinal Cord Injuries
Protocol Type: Amendment
Amendment To: Cells or tissues used in research, Genetic constructs, Proteins produced
Applicable Guidelines & Regulations: NIH Guidelines Section III-F, NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-1, NIH Guidelines Section III-F-1, NIH Guidelines Section IV-B-7, UK Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section III-D-1, OSHA 29 CFR 1910.1030, NIH Guidelines Section III-D-2
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)
Primary Reviewers: C. Haughton, M. Mendenhall, D. Malherbe

Brief Project Overview:

I seek to regenerate the spinal cord after it has been damaged. My past work has deleted a gene (PTEN) from the mouse genome that inhibits regeneration of the spinal cord. Our results worked far better than anticipated. This was made possible due to mouse lines that are transgenic and interact with proteins (Cre) that are delivered using gene therapies. This approach can only work in these specific transgenic mice and will not work in any other organism. The aim of this project is to make a similar gene therapy strategy that will work in non-transgenic animals, or in other words, be able to work in any species or organism.

In order to accomplish this goal it is important to understand the pathways occuring in a cell. PTEN inhibits regeneration by stopping a signalling pathway going on inside of a cell. Specifically, two other proteins of interest are the PI3K and the AKT proteins. when PI3K interacts with AKT and turns it on, the damaged neurons in the spinal cord will regenerate. PTEN acts to stop this interaction and blocks PI3K from activating AKT. We have learned through research that a small mutation in AKT can render it always active and doesn't require activation by PI3K. Forcing this mutated AKT to be produced in damaged neurons can therefore bypass the PTEN block and cause

regeneration in a species-independent manner. Indeed, one recent previous study has performed this and found that it will work the same as deleting PTEN. My project, however, uses a different approach to deliver the gene that will be safer and more robust than the previously published paper. Specifically I am taking advantage of known genetic manipulations that will allow this gene to be only expressed in neurons, only when mice are delivered an antibiotic called doxycycline, and only in neurons that have been damaged during the spinal cord injury. This contrasts previous reports that would have effected only a single tract projecting into the spinal cord (1 out of 27 where I am affecting almost every spinal tract), in all cells including glia which can divide and form tumors, and in neurons unrelated to the damaged spinal cord. My project aims to enhance the safety and clinical relevance of gene therapy approaches to bring these approaches closer to being possible for paralyzed people.

Campion TJ 3rd, Sheikh IS, Smit RD, Iffland PH 2nd, Chen J, Junker IP, Krynska B, Crino PB, Smith GM. Viral expression of constitutively active AKT3 induces CST axonal sprouting and regeneration, but also promotes seizures. Exp Neurol. 2022 Mar;349:113961.

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 (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 constitutivley 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

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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/; 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-Luciferace/; 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-EF1alpha-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-

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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/; 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-Luciferace/; 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 an amendment to his approved IBC protocol IBC-24-333, entitled *Gene Therapy Approaches to Induce and Control Neuronal Growth in Rodents With Spinal Cord Injuries*. In this amendment, Dr. Stewart has updated administrative information, cells/tissues used in research, genetic constructs, and proteins produced. Specifically, Dr. Stewart has added a project that seeks to understand the genetic elements of axolotls that enable tissue regeneration in mice utilizing a number of new vectors, including new lentiviral vectors, a second project to generate a double-transgenic mouse line consisting of CD44 flox and CALCA (CGRP) Cre to get a CD44 knockout in CGRP neurons, and lastly a third project that aims to develop a novel biosensor to evaluate

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function of Pink1 in vivo. In the first new project, Dr. Stewart has added new lentiviral vectors with the HiRet envelope protein (based on VSV-g) to enable retrograde transport in neurons when administered to the spinal cord of anesthetized mice. The new lentiviral vectors will be purchased from VectorBuilder packaged and ready for use, which greatly reduces the risks associated with lentivirus production. Lentiviral vectors expressing Runx1 will be administered to anesthetized mice in a Class II BSC located in DLAR while wearing lab coat, disposable gloves, eye protection, surgical mask, and head cover. Transport of lentivirus from laboratory to DLAR location will be done in a shatter-proof secondary container transported on a cart. Animals will be housed at ABSL2 for a minimum of 72 hours post administration of lentivirus, after which they will be housed at ABSL1 containment. While Dr. Stewart was previously approved for in vitro work with lentivirus, this is the first project involving the administration of lentivirus to mice for this IBC protocol. The lentivirus utilized is a 3rd generation lentivirus. In the second new project, Dr. Stewart will deliver AAV expression CD44 glycoprotein to anesthetized mice in DLAR using ABSL1 containment. AAV will be obtained from VectorBuilder. This work will be completed in a Class II BSC for sterility while wearing lab coat, eye protection, disposable gloves, mask, and hair cover. Dr. Stewart is currently approved for numerous projects involving AAV administration to animals, and this additional AAV construct does not significantly alter the biohazardous risks associated with this IBC protocol. In the third new project, Dr. Stewart will utilize a new AAV vector developed with a collaborator at Central Michigan University that will express Pink1 reporter. The Pink1 reporter AAV will be delivered to the spinal cord of anesthetized mice as previously described for Project 2 above. All waste generated in these new projects will be handled as previously described and approved.

IBC Discussion & Vote:

The amendment to IBC-24-333 (version 73.0) was approved pending minor modifications as listed below:
*
SCIENTIFIC SUMMARY:

1. Please review the text added in this amendment for spelling and grammatical errors and update as necessary.
2. Describe the experimental methods that were added to this amendment in greater detail. If procedures will be completed “as previously described”, please point specifically to these procedures/details.
3. Expand on the procedures associated with lentiviral injections into mice. How will risk of needlestick be mitigated?
4. In the description of Runx1, please clearly note that this is an oncogene.

*
Cheryl Haughton initiated the motion. Mike Mendenhall seconded the motion. All IBC members present (14) voted in favor of the motion.
*
Conflicts of Interest: None
*

PI: Ka Wing Fong

IBC Protocol Number: IBC-24-385
Protocol Title: Understanding the mechanisms underlying advanced prostate cancer and finding novel therapies
Protocol Type: Amendment
Amendment To: Personnel, Genetic constructs, Manipulations planned
Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-F-3, 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, NIH Guidelines Section III-E-1, NIH Guidelines Section III-F-1

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Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)
Primary Reviewers: C. Haughton, C. Pickett, D. Harrison

Brief Project Overview:

Our research is important for delineating the fundamental oncogenic processes that drive prostate cancer progression in particular. Understanding the molecular mechanisms of tumorigenesis will facilitate the development of novel promising therapies for cancer patients who have developed currently untreatable terminal diseases. Scientific summary: Our laboratory focuses on understanding the genetic and epigenetic pathways to prostate cancer. A number of key transcription factors, including the Androgen Receptor (AR), USP21, TRIM28, SETDB1, E2F1 and CCDC83, have been related to epigenetic changes and implicated in prostate cancer. However, their downstream pathways remain largely unknown. Using 'omics' approaches including ChIP-Seq, RNA-Seq and proteomics, we have identified a number of target genes and molecular pathways regulated by these transcription factors. Also, we will investigate transcriptional, post-translational processes, together with cell signaling pathways that can be aberrantly modified in advance prostate cancer tumors and design therapeutic approaches to block oncogenic pathways. Findings from our study will contribute to the better understanding of the treatment-resistant prostate tumors and help to design the novel therapeutic approaches. Experimental Design: We utilize both in vitro and in vivo approaches in our research.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Genetics, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Proteomics, Transfection, Transformation, Use of viral vectors, Creation of viral vectors, Use of Human Source Material(s)
Transport: Yes
Materials Transported: Biohazardous Materials, Animals
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)]: TRIM28 (WT, S473A, S473D)/human/oncogenes, cell cycle/cell division/expression, knockdown, silencing/human cells/pLVX, pLenticrisprV2, HA-Flag, pLKO/; AR/human/oncogenes, cell cycle/cell division/overexpression, knockdown/human cells/pLVX, pLKO/; SETDB1/human/oncogenes, cell cycle/cell division/expression, knockdown, silencing/human cells/pLVX, pLKO.1, pLenticrisprV2/; E2F1-wildtype and mutants(E132 or ΔTA)/human/oncogenes, cell cycle/cell division/expression, knockdown, silencing/human cells/pLVX, pLKO.1, pLenticrisprV2/; CCDC83/human/oncogenes, cell cycle/cell division/expression, knockdown, silencing/human cells/pLVX, pLKO.1, pLenticrisprV2/; RB/human/tumor suppressor/knockdown, silencing/human cells/pLKO.1 and pLenticrisprV2/; LDHA/human/oncogenes, cell cycle/cell division/knockdown, silencing/human cells/pLenticrisprV2/; RSK/human/oncogenes, cell cycle/cell division/silencing/human cells/pLenticrisprV2/; luciferase/firefly/reporter/expression/human cells/pLVX/; SETDB1/Human/Oncogene/silencing gene expression/NA/It is purchased from IDT DNA technology as anti-sense DNA oligonucleotide ready to use/; TRIM28/Human/Oncogene/silencing gene expression/NA/It is purchased from IDT DNA technology as anti-sense DNA oligonucleotide ready to use/; RHOB/human/tumor suppressor/silencing/human cells/pLKO.1/; USP21/human/oncogenes, cell cycle/cell division/expression, knockdown silencing/human cells/pLVX, pLKO.1, pLenticrisprV2, HA-Flag
Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/pLVX/; Lentivirus/pLKO.1/; Lentivirus/pLenticrisprV2/; Lentivirus/HA-Flag

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Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HEK293T/; Human/22Rv1/; Human/C4-2B/; Human/DU145/; Human/LAPC4/; Human/LNCaP/; Human/PC-3/; Human/VCaP/; Human/LuCaP35CR/; Human/LuCaP96CR/; Human/NCI-H660/; Human/N2P1/; Human/LuCaP 145.1/; Human/LuCaP77CR/; Human/C4-2B EnzR/; Human/22Rv1 EnzR/; Human/C4-2R /; Human/MR49F
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/Cells - Human, genetically modified/Subcutaneously injection/Chemical Isoflurane (1-4% in O2, inhaled-nosecone)/ABSL2/Lab coat, glove, mask/ABSL2/Yes; Mouse/Patient-Derived Xenograft Materials (Human)/Subcutaneously injection/Chemical Isoflurane (1-4% O2, inhlaed nosecone)/ABSL2/Lab coat, glove, mask/ABSL2/Yes; Mouse/Cells - Human, non-modified/subcutaneously injection/chemical isoflurane (1-4% in O2, inhaled nosecone)/ABSL2/Lab coat, glove, mask/ABSL2/YesRisk Assessment/Discussion:

Dr. Fong has submitted an amendment to his existing IBC protocol IBC-24-385, entitled Understanding the mechanisms underlying advanced prostate cancer and finding novel therapies. In this amendment, Dr. Fong has updated personnel, administrative information, cells used in research, and new lentiviral constructs to express and knockdown USP21. Lentiviral particles will be packaged by Dr. Fong's laboratory utilizing 3rd generation packaging plasmids (pMD2.G, pRSV-Rev, and pMDLg/pRRE). Lentivirus will not be concentrated via centrifugation but will be clarified via filtration. All work with lentivirus will be conducted in a BSC utilizing lab coat, gloves, and eye protection at BSL2+ containment. Dr. Fong acknowledges the use of a lentivirus expressing a known oncogene, which does pose a greater risk to lab personnel should they be exposed. All personnel are advised of these risks, and signage will be posted to communicate ongoing work with oncogene-expressing lentivirus. Following lentivirus transduction of human prostate cancer cells, transduced cells will be administered to anesthetized mice subcutaneously within a BSC located in DLAR. At experiment endpoint, tumors will be excised for downstream nucleic acid extraction and immunoblotting. Another project added in this amendment will involve two human prostate cancer cells (not genetically modified) and PDX being administered subcutaneously to castrated mice. All subcutaneous injections will be done in anesthetized mice within a BSC. PPE will include lab coat, gloves, and eye protection. At experiment endpoint, tumors will be excised and snap frozen for down analysis including RNA and protein extraction for qPCR and others. These two new projects are very similar to previously approved work on Dr. Fong's protocol and do not significantly alter the biohazardous risks associated with this IBC protocol.

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IBC Discussion & Vote:

The amendment to IBC-24-385 (version 46.0) was approved.
*
Doug Harrison initiated the motion. Carol Pickett seconded the motion. All IBC members present (14) voted in favor of the motion.
*
Conflicts of Interest: None
*

PI: Feng Li

IBC Protocol Number: IBC-24-388
Protocol Title: Rotavirus Project
Protocol Type: Amendment
Amendment To: Other

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-2, 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 (BSL2), Animal Biological Safety Level 2 (ABSL2)
Primary Reviewers: C. Haughton, D. Harrison, D. Malherbe

Brief Project Overview:

This IBC is related to rotavirus research including cell culture-based experiments to determine viral replication fitness as well as animal experiments (in mice and horses) to measure viral replication and vaccine-mediated protection.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Genetics, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Propagation of infectious agents, Transfection, Transformation, Use of infectious agents, Use of viral vectors, Viral culture, Use of Human Source Material(s)
Transport: Yes
Materials Transported: Biohazardous Materials, Animals
Infectious Agent(s)/Natural Host(s): Equine Rotavirus A (RG1-virus)/Equine/; Porcine Rotavirus (RG2-virus)/Porcine/; Bovine Rotavirus (RG1-virus)/Bovine/; Simian rotavirus A (SA11) (RG2-virus)/Monkey/; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human/; Rotavirus A Odelia (RG2-virus)/Human/; Equine Rotavirus B (RG1-virus)/Equine
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: VP1/Simian Rotavirus A (SA11)/Structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; VP2/Simian Rotavirus A (SA11)/Structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; VP3/Simian Rotavirus A (SA11)/Structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; VP4/Simian Rotavirus A (SA11)/Structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; VP6/Simian Rotavirus A (SA11)/Structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; VP7/Simian Rotavirus A (SA11)/Structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; NSP1/Simian Rotavirus A (SA11)/Non-structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; NSP2/Simian Rotavirus A (SA11)/Non-structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; NSP3/Simian Rotavirus A (SA11)/Non-structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; NSP4/Simian Rotavirus A (SA11)/Non-structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; NSP5/Simian Rotavirus A (SA11)/Non-structural protein/Reverse Genetics/Multiple mammalian cell lines/pT7-SA11/; D1R/Vaccinia virus /Capping enzyme subunit/Reverse Genetics/Multiple mammalian cell lines/pCAGGS/; D12L/Vaccinia virus /Capping enzyme subunit/Reverse Genetics/Multiple mammalian cell lines/pCAGGS/; FAST/NelsonBay orthoreovirus/Membrane fusion/Reverse Genetics/Multiple mammalian cell lines/pCAGGS/; GFP/Addgene/Reporter/Viral replication/Multiple mammalian cell lines/pT7-SA11/; Renilla luciferase/Promega/Reporter/Viral replication/Multiple mammalian cell lines/pT7-SA11/; VP8/Simian Rotavirus A (SA11)/Structural protein/Expression/E. coli BL21 /pET-28/; VP8*/equine rotavirus B/Viral receptor core-binding domain/vaccine/E. coli BL21 /pET-28/; VP7/equine rotavirus B/Structural protein/vaccine/E. coli BL21 /pET-28
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pCAGGS; Plasmid/pT7-SA11; Plasmid/pBlueScript; Plasmid/pET-28a
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HRT-18G/; Animal/MA-104/; Animal/MA-104N*V/; Human/CaCo-2/; Animal/BIEC-c4/; Animal/OIEC/; Animal/MARC-145s/; Animal/Vero Cells/; Animal/BHK-T7/; Insect/Sf9

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Human/human neuroblastoma cells /; 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/Equine Rotavirus B (RG1-virus)/Oral inoculation/mouse pups physically restrained/ABSL2/Lab coat, disposable gloves, eye protection/ABSL2/Yes/Use of BSC; Equine, Agricultural/Equine Rotavirus B (RG1-virus)/Oral administration/Physical restraint/ABSL2/Tyvek suits, booties, scrubs or dedicated clothes, gloves, dedicated boots/ABSL2/Yes/Mare-foal pairs will be housed at UK Isolation Barn for duration of experiment; Mouse/Equine Rotavirus A (RG1-virus)/Oral inoculation/mouse pups physically restrained/ABSL2/Lab coat, disposable gloves, eye protection/ABSL2/Yes/Use of BSC

Risk Assessment/Discussion:

Dr. Li has submitted an amendment to his currently approved IBC protocol, IBC-24-388, entitled Rotavirus Project. In this amendment, Dr. Li has updated personnel, administrative information, laboratory locations, and added a new project that seeks to study the replication fitness and virulence of equine rotaviruses G3 and G14. The G3 and G14 equine rotaviruses were rescued via the reverse genetics system previously described and approved on Dr. Li's IBC protocol. These two strains are being utilized because they are the two dominant species of rotavirus currently circulating in horses. Mare-foal pairs will be utilized for this experiment with foals 5-10 days of age. 2 control pairs will be included as well. All work described here will be completed in the BSL-2 Barn (Isolation Barn), located at the North Farm. Foals will be challenged with equine rotaviruses via oral administration of 60mL of diluted infectious materials through nasal gastric tubing. Foals will be monitored for clinical signs of infection and samples (including rectal swabs) will be obtained for virus isolation and titration. Serum samples will be collected throughout. Rotavirus will be prepared in Dr. Li's laboratory inside a BSC wearing lab coats, gloves, and eye protection. Tubes containing viral inoculum will be placed in a leak-proof Ziplock bag within the BSC. The exterior of the bag will be wiped with disinfectant and placed inside a secondary container. The exterior of the secondary container will also be wiped with disinfectant prior to transport to North Farm. When inoculating foals and entering the BSL-2 Barn any time after inoculation, personnel will wear Tyvek coveralls, booties (or BSL-2 barn dedicated boots), farm-dedicated scrubs, gloves, eye protection, and surgical mask. All solid waste materials generated at the BSL-2 barn will be bagged for transport back to campus for autoclaving and final disposal. All personnel will shower in the adjacent trailer designated for this purpose upon leaving the BSL-2 barn. Dedicated scrubs will be laundered on site and never taken home. Farm personnel will not have any contact with non-study horses throughout the duration of this experiment. Mare-foal pairs will not be released back into the general population until virus isolation tests are negative for two days in a row. After which, mare-foal pairs will be kept in a dedicated pasture separate from the general population for another month prior to being released back into the general herd. All muck and other contaminated materials generated at the BSL-2 barn will be placed in hard-sided plastic bins lined with red biohazard bags and picked up for incineration via Stericycle. Closed bins will be stored in an adjacent shed prior to pick-up. Pickups are scheduled as often as needed to ensure timely disposal. Equine rotavirus a is a pathogen of horses, particularly foals less than 6 months of age. Adult horses are not likely to develop any clinical signs of infection. Although rotavirus can be zoonotic, there is no evidence to suggest that equine rotavirus A can cause disease in humans. This project is very similar to previously approved work utilizing equine rotavirus B. Although equine rotavirus A is not a known human pathogen, all work on this project will be conducted at BSL2/ABSL2 containment to prevent environmental spread or release.

IBC Discussion & Vote:

The amendment to IBC-24-388 (version 22.0) was approved pending minor modifications as listed below:
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ANIMAL RESEARCH – Animal IACUC Protocol Numbers: IACUC 2025-4634 needs to be added to the list of animal protocols.
SCIENTIFIC SUMMARY:

1. Please add a description to the Scientific Summary as to what steps are taken to identify and track foals administered rescued virus and ensure appropriate carcass disposal after the conclusion the experiment.
2. Please specify that tubes containing virus will be surface decontaminated prior to removal from the BSC for transport.
3. Regarding the PPE utilized in the BSL2 Isolation Barn, please describe how reusable PPE (ex. boots, protective eyewear, etc.) are decontaminated after each use.

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Doug Harrison 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
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PI: Mary Mohrin

IBC Protocol Number: IBC-24-412
Protocol Title: Molecular Mechanisms of Aging
Protocol Type: Amendment
Amendment To: Manipulations planned
Applicable Guidelines & Regulations: OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7, UK Administrative Regulation 6.9, NIH Guidelines Section III-E-3, NIH Guidelines Section III-D-1
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)
Primary Reviewers: C. Haughton, Y. Wu, D. Malherbe

Brief Project Overview:

As animals age, tissues lose function and disease incidence increases. A better understanding of why we age can help us identify processes to slow or stop aging and reduce disease. The best understood way to slow aging and extend lifespan is caloric restriction. When animals are calorically restricted, a key change is increased expression and activity of a family of proteins called Sirtuins. We use the hematopoietic (blood and immune) system as a model to study tissue aging and hematopoietic stem cells (HSCs) to study stem cell aging. We aim to study the role of Sirtuins in healthy aging of the hematopoietic system as well as in hematopoietic disease states like leukemia and clonal hematopoiesis.

For our studies we will use WT (C57/Bl6) mice as controls and use genetically modified mouse strains with changes in expression of Sirtuins or genes involved in hematopoietic diseases to study their effect on healthspan, lifespan, and disease development. We will also use human and mouse cells (cell lines as well as primary cells) to perform experiments in vitro to study biochemical and molecular changes that occur with age and interventions used to slow or stop aging.

To further investigate whether SIRT7 can be targeted to slow the progression of human leukemic diseases such as acute myeloid leukemia (AML), we will establish a mouse model by transplanting patient-derived bone marrow

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cells, with or without ex vivo SIRT7 overexpression. This experiment aims to investigate whether SIRT7 could be a potential target for treating human leukemia.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, Creation of viral vectors, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Genetics, Imaging/Microscopy, PCR/qRT-PCR, Immunohistochemistry, Histology, Proteomics, Transfection, Transformation, Use of Human Source Material(s), Use of viral vectors, Viral culture
Transport: Yes
Materials Transported: Animals, 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)]: GFP/jellyfish/reporter/tracker/overexpress GFP to track cells/primary mouse cells/Lentivirus/; Sirtuin 7/mouse/Deacetylase/overexpression/mouse primary cells/lentivirus
Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/pFUGw
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Human hematopoietic cells (blood or bone marrow origin); Human/293T
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/Cells - Animal, non-modified/retro orbital/anesthetized/ABSL2/lab coat, gloves, eye protection/ABSL2/No/control cells - uninfected/; Mouse/Cells - Animal, genetically modified/retro orbital/anesthetized/ABSL2/lab coat, gloves, eye protection/ABSL2/No/cells will be transduced with lenti or retro virus in vitro, then 1-3 days after they will be washed and transplanted into recipient mice/; Mouse/Cells - Human, non-modified/retro orbital/anesthetized/ABSL2/lab coat, gloves, eye protection/ABSL2/No/control cells - uninfected/; Mouse/Cells - Human, genetically modified/retro orbital/anesthetized/ABSL2/lab coat, gloves, eye protection/ABSL2/No/cells will be transduced with lentivirus in vitro or undergo electroporation, then 1-3 days after they will be washed and transplanted into recipient mice

Risk Assessment/Discussion:

Dr. Mohrin has submitted an IBC amendment to her approved IBC protocol IBC-24-412, entitled Molecular Mechanisms of Aging. In this amendment, Dr. Mohrin has updated administrative information and manipulations planned. Specifically, Dr. Mohrin has added a project that seeks to determine whether SIRT7 can be targeted in order to slow the progression of acute myeloid leukemia (AML) in humans. In this amendment, Dr. Mohrin's lab will receive human bone marrow samples from the UK Markey Cancer BPTP SRF to establish a xenotransplantation model in mice. CD34 positive cells will be isolated from bone marrow samples, and non-disease cells will be obtained from a vendor as a control. Cd34-positive human cells will be modified to overexpress SIRT7 via electroporation or lentivirus-mediated gene expression. Replication Competent Virus (RCV) testing will be completed for lentivirus-transduced cells prior to transplantation into mice via PCR for psi-gag and VSV-G sequences. Mice will be lethally irradiated prior to delivery of cells via retro-orbital transplantation. Mice are under anesthesia for transplantation of cells. Animals administered lentivirus-transduced cells will be housed at ABSL2 housing for 72 hours prior to moving to ABSL1 housing. Personnel will wear lab coat, disposable gloves, and eye protection for this work. This new project is very similar to previously approved work involving lentivirus transduction of HSPC cells to express SIRT7 obtained from donor mice for transplantation. This new project does not alter the biohazardous risks associated with this IBC protocol.

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IBC Discussion & Vote:

The amendment to IBC-24-412 (version 22.0) was approved pending minor modifications as listed below:
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SCIENTIFIC SUMMARY:

1. Please specify which procedures take place in a BSC vs the open bench.
2. Elaborate on the animal tissue harvesting procedures. Will sharps be used for the collection of bone marrow?
3. Clearly describe the restraint methods used when administering cells into animals.

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Delphine Malherbe 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
*

PI: Ilhem Messaoudi Powers
IBC Protocol Number: IBC-24-447
Protocol Title: Impact of microbial infections, age, and nutrition on the development and function of the immune system
Protocol Type: Amendment
Amendment To: Cells or tissues used in research, Manipulations planned, Personnel, Organisms used in research
Applicable Guidelines & Regulations: 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 (BSL2)
Primary Reviewers: Y. Wu, C. Pickett, A. Hunt

Brief Project Overview:

The immune system is a complex network of cells and mediators that work in concert to protect the host from microbial infections and cancer. Aberrant immune responses on the other hand can lead to auto-immune as well as chronic inflammatory diseases such as asthma and cardiovascular disease. The overall goal of the research projects in the Messaoudi laboratory is the investigate mechanisms that underlie dysregulation of the immune system. These goals can be broadly divided into 5 areas of emphasis:
- impact of age, and loss of sex hormones, on immune responses to vaccination;
- modulation of immune function by nutritional intake or substance use disorder;
- immune pathogenesis of filovirus infection using gene expression profiles (please note this work only uses RNA sent to us from our collaborators - there is NO filovirus work being carried out at UK);
- role of the microbiome in health and disease, notably with regards to diarrheal diseases in infants
- surveillance, propagation, and impact of age and pregnancy on the host immune response of SARS-CoV-2.
The rationale for these projects is that although it is well accepted that:
- immune fitness decreases as individuals age;
- obesity and alcohol intake lead to reduced ability to fight infection and capacity for wound healing;
- filoviruses interfere with host defenses; and
- early onset diarrhea delays the maturation of the infant immune system; the mechanisms underlying these defects are poorly understood.
To accomplish our research goals, we use tissues collected from human and nonhuman primate models and shipped to us from collaborators at Oregon Health and Science University (OHSU), Oregon National Primate Research Center (ONPRC), Rocky Mountain Laboratory (NIAID), university of Hawaii and others. By better

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understanding the mechanism of immune dysfunction in these vulnerable populations, we aim to help design interventions and treatments to improve human health.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, Cell culture, Flow cytometry/Cell sorting, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Propagation of infectious agents, Use of Human Source Material(s), Use of infectious agents, Proteomics, DNA/RNA isolation/purification, Viral culture
Transport: Yes
Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human/; Non-Human Primate (NHP) Materials (RG2)/Non-human primates/; Herpes Simplex Virus-1 (HSV-1) (RG2-virus)/Human/; Influenza A Virus (RG2-virus)/Human/; Respiratory Syncytial Virus (RSV) (RG2-virus)/Human/; Modified Vaccinia Ankara Virus (MVA) (RG2-virus)/Human/; Escherichia coli (RG2-bacteria)/Human/; Simian Varicella Virus (SVV) (RG2-virus)/Non-human primates/; Borrelia burgdoferi (RG2-bacteria)/Deer, wild rodents (mice), ticks/; Mycobacterium avium subsp. hominissuis (RG2-bacteria)/Soil and water/; Mycobacterium avium complex (MAC) (RG2-bacteria)/Soil and water/; Mycobacterium avium subsp. avium (RG2-bacteria)/Soil and water/; Mycobacterium chimaera (RG2-bacteria)/Soil and water/; Mycobacterium intraceullulare (RG2-bacteria)/Soil and water
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]: Animal/FRhl2/; Animal/4mBr5/; Animal/VERO/; Animal/VEROE6/; Animal/BSC-1/; Animal/Hep2/; Animal/A549 Wild Type and A549 deficient in: RL6, PKR, TLR3, MAVS/; Animal/HEK293/; Animal/THP-1/; Animal/MRC5/; Animal/Calu-3/; Animal/Immune Cells /; Animal/Immune Cells /; Animal/Immune Cells /; Animal/Immune Cells /; Animal/Immune Cells /; Human/Placental organoids/; Human/Lung organoids/; Animal/Lung organoids
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. Messaoudi has submitted an IBC amendment to her current IBC protocol IBC-24-447, entitled Impact of microbial infections, age, and nutrition on the development and function of the immune system. In this amendment, Dr. Messaoudi has updated lab personnel, added new human and animal primary clinical specimens, and added a new project with Mycobacterium Avium Complex (MAC) organisms. Human placenta tissue will be washed, digested, pelleted by centrifugation using sealed buckets, and resuspended in Matrigel. Work will be conducted within a BSC using previously approved PPE and waste disposal using BSL2 containment. Dr. Messaoudi's laboratory is approved to work with several human tissues and samples obtained from patients for a variety of projects. This new addition does not alter the biohazardous risks already approved on Dr. Messaoudi's IBC protocol. A new project involving the culture and in vitro infection of MAC has also been added. MAC cultures will be obtained from collaborators at Oregon State University or from BEI resources. MAC is considered non-tuberculous mycobacteria, or atypical mycobacteria, and is mainly associated with infections in immunocompromised individuals. PBMCs and lung organoids obtained from collaborators will be infected with MAC, followed by several downstream assays/manipulations including fixation for flow cytometry, RNA extraction, and single cell RNA sequencing. All biohazardous materials are inactivated (via fixation, Trizol, etc.) prior to being

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removed from the BSC for open bench work. MAC and other non-tuberculous mycobacteria are considered RG2 organisms and are ubiquitous in nature (found in soil, water, etc.). The primary hazards of working with MAC are direct contact of skin or mucous membranes, accidental parenteral inoculation, and ingestion. Sharps will not be utilized in this project, greatly minimizing the risk of potential exposure. A BSC will be utilized, and PPE and waste disposal will be conducted as previously approved using BSL2 containment. Dr. Messaoudi's laboratory is approved to work with several bacterial and viral RG2 organisms. This new project does not significantly alter the biohazardous risks associated with this IBC protocol.

IBC Discussion & Vote:

The amendment to IBC-24-447 (version 34.0) was approved.
*
Carol Pickett initiated the motion. Arthur Hunt seconded the motion. All IBC members present (13) voted in favor of the motion.
*
Conflicts of Interest: Delphine Malherbe abstained from the vote.
*

New Protocols

PI: Joshua Konkol

IBC Protocol Number: IBC-25-91
Protocol Title: Managing forest pathogens with RNA interference.
Protocol Type: New
Applicable Guidelines & Regulations: NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-2, NIH Guidelines Section III-F-3, NIH Guidelines Section III-F-5, NIH Guidelines Section III-F-8, 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 1 (BSL1)
Primary Reviewers: A. Hunt, J. Smalle

Brief Project Overview:

We will use double stranded ribonucleic acids (dsRNA) to prevent diseases in trees from several plant pathogens. Specifically, we will generate dsRNA that has the same sequence as a ribonucleic acid (RNA) sequence present in each of the plant pathogens Harringtonia lauricola, Bretziella fagacearum and Phytophthora cinnamomi. We will introduce the dsRNA solution into host trees (Sassafras albidum and Quercus alba) of these pathogens by either injecting it into the stem or allowing it to absorb through its roots. Afterwards, we will inoculate plants with the pathogens and assess if the use of dsRNA prevents or reduces symptoms of disease caused by the pathogens. Samples of the plants will be tested for the presence of dsRNA, the presence of the pathogen, and for microscopic observations of the effects of the dsRNA on the pathogen in the plant.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: DNA/RNA isolation/purification, Histology, Imaging/Microscopy, PCR/qRT-PCR, Plant Work, Propagation of infectious agents, Use of infectious agents
Transport: Yes
Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Harringtonia lauricola (RG1-fungus)/Sassafras albidum, Lindera benzoin; Phytophthora cinnamomi (RG1-fungus)/Quercus spp.; Bretziella fagacearum (RG1-fungus)/Quercus spp.
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of

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Construct/Host(s)/Vector(s)]: Beta-tubulin/Harringtonia lauricola, Bretziella fagacearum and Phytophthora cinnamomi respectively/Essential gene for cellular cytoskeleton/RNA interference/Harringtonia lauricola, Bretziella fagacearum and Phytophthora cinnamomi respectively/dsRNA/; Elongation factor 1 alpha/Harringtonia lauricola, Bretziella fagacearum and Phytophthora cinnamomi respectively/Essential gene for protein synthesis/RNA interference/Harringtonia lauricola, Bretziella fagacearum and Phytophthora cinnamomi respectively/dsRNA/; Green fluorescent protein/Aequorea victoria/Exhibits green fluorescence when exposed to UV light/RNA interference (negative control)/Aequorea victoria/dsRNA
Vector(s) [Vector Category/Vector Technical Name]: Naked nucleic acid/Naked dsRNA
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. Konkol has submitted a new IBC protocol entitled Managing forest pathogens with RNA interference. In this IBC protocol, Dr. Konkol will propagate and study three plant pathogens - Harringtonia lauricola, Bretziella fagacearum, and Phytophthora cinnamomi—all of which are native to Kentucky. These pathogens are cultured in petri dishes and liquid media within a biosafety cabinet (BSC) . All materials that contact pathogens are either autoclaved (solid media) or treated with 10% bleach (final concentration). The BSC is disinfected with 70% ethanol after use. Nitrile gloves, lab coats, and eye protection are worn throughout all procedures.

The protocol also includes the production of double-stranded RNA (dsRNA) targeting specific plant pathogen genes. These nucleic acids are non-toxic, non-replicative, and environmentally safe, designed to trigger gene silencing in plants and fungi. All disposables in contact with nucleic acids are autoclaved, and dsRNA products are stored at -80°C. Fluorophore-tagged dsRNA is used for microscopic observation. In vitro and in planta efficacy of dsRNA is tested by incubating pathogens with dsRNA and observing growth inhibition. Trees (Sassafras albidum and Quercus alba) are inoculated with dsRNA and later with pathogens to assess disease resistance. Inoculated trees are monitored for symptoms, and any plant material is autoclaved post-experiment. Risk of environmental spread is minimal due to the pathogens' reliance on specific insect vectors and their inability to survive outside host organisms. Additional precautions include monitoring for fungal mat formation and immediate destruction of any symptomatic or dead trees. None of the agents in use are pathogenic to humans. All work is conducted at BSL1/BSL1-P containment.

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IBC Discussion & Vote:

The protocol IBC-25-91 (version 5.0) was approved pending minor modifications as listed below:
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SCIENTIFIC SUMMARY: Please describe how pathogens will be transported from the laboratory to the site of tree inoculation.
*
Arthur Hunt initiated the motion. Jan Smalle seconded the motion. All IBC members present (14) voted in favor of the motion.
*
Conflicts of Interest: None
*

Renewals

*

PI: Luke Moe
IBC Protocol Number: IBC-25-66
Protocol Title: Functional metagenomics of soil-dwelling bacteria
Protocol Type: Renewal
Amendment To: N/A
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, NIH Guidelines Section III-F-2, NIH Guidelines Section III-F-3
Maximum Containment Level: Biological Safety Level 2 (BSL2)
Primary Reviewers: A. Hunt, J. Smalle, C. Pickett

Brief Project Overview:

We will be conducting research on the following areas of research related to environmental microbes: interactions between plants and bacteria at the plant root-soil interface (the rhizosphere), understanding the role of microbes in seed germination, and metabolism of amino acids by plant-associated bacteria.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, DNA/RNA isolation/purification, Genetics, PCR/qRT-PCR, Plant work, Use of infectious agents
Transport: No
Materials Transported: N/A
Infectious Agent(s)/Natural Host(s): Pseudomonas fluorescens (RG2-bacteria)/Humans, animals, plants; Saccharomyces cerevisiae (RG1-fungus)/Humans; Pediococcus spp. (RG1-bacteria)/Humans; Lactobacillus fermentum (RG1-bacteria)/Humans; Enterococcus faecalis (RG2-bacteria)/Humans
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: xylE/Pseudomonas putida/enzyme/expression/E. coli BL21(DE3)/pET28b/; 16s rRNA genes/DNA extracted from soil/ribosomal RNA gene/cloning for DNA sequencing/E. coli K12 derivative/pUC18/; Rhizosphere Metagenomic Library Genes/DNA extracted from soil/many different types of genes (unknown function)/screening DNA libraries for functional properties in a recombinant host/E. coli K12 derivative/pGEM, pCC1fos/; D-amino acid dehydrogenase/Pseudomonas putida/enzyme/expression/E. coli BL21(DE3)/pET28b/; ornithine cyclodeaminase/Pseudomonas putida/enzyme/expression/E. coli BL21(DE3)/pET28b/; lysine aminotransferase/Pseudomonas putida/enzyme/expression/E. coli BL21(DE3)/pET28b
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pET28b; Plasmid/pUC18; Plasmid/pGEM; Plasmid/pCC1fos
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. Moe has submitted a renewal of his IBC protocol IBC-25-66, entitled Functional metagenomics of soil-dwelling bacteria. Dr. Moe's current IBC protocol will expire on August 26, 2025. Dr. Moe's laboratory using culture-based and molecular biology techniques to study microbial community dynamics and the functional traits of individual organisms. The laboratory does focus on plant-associated bacteria but does not focus specifically on organisms

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that are plant pathogens. Culture-dependent work includes the use of commercial Escherichia coli K12 strains for cloning, as well as live cultures of model soil and plant-associated bacteria such as Pseudomonas putida (KT2440 and mt-2). These cultures are grown in small volumes (25 mL or less) and are used for genetic and transcriptomic studies. Additionally, the lab isolates bacteria from plant and grain samples to assess colony-forming units and identify strains with plant-beneficial traits, such as hormone production and phosphate solubilization. These cultures are handled in sealed containers within enclosed incubators to minimize exposure. In yeast-based fermentation studies, the lab uses a commercial strain of Saccharomyces cerevisiae and co-inoculates it with bacteria originally isolated from fermentation environments. These include strains from the families Lactobacillaceae and Acetobacteraceae, such as Pediococcus pentosaceus, Pediococcus acidilactici, Lactobacillus fermentum, Acetobacter aceti, and Enterococcus faecalis. While most of these are low-risk, RG1 organisms, E. faecalis is a Risk Group 2 organism due to its potential to cause opportunistic infections. Culture-independent work involves macerating plant tissues in sterile conditions to extract DNA using commercial kits. These kits contain lysis buffers that eliminate live material, and the extracted DNA is used for PCR and metagenomic sequencing. PCR procedures are conducted in a biosafety cabinet to prevent cross-contamination. The lab also uses E. coli BL21 to overexpress recombinant proteins from Pseudomonas putida for enzyme activity studies. The lab works primarily with Risk Group 1 organisms, including E. coli K12, Pseudomonas putida, Saccharomyces cerevisiae, and various lactic and acetic acid bacteria. These pose minimal risk under standard laboratory conditions. However, the use of Enterococcus faecalis, a Risk Group 2 organism, introduces a potential biosafety concern due to its ability to cause gastrointestinal illness in rare cases. Additionally, environmental isolates from plant samples are unknown the time of culturing, which warrants cautious handling until further characterization. To mitigate risks, all bacterial cultures are grown in small volumes using sterile technique to minimize aerosol generation. Cultures are maintained in sealed containers and are not transported outside the laboratory. Disinfection protocols include the use of 10% bleach or Lysol, and biological waste is regularly autoclaved. Personnel are equipped with lab coats, gloves, and protective eyewear, and utilize a BSC for work with RG2 organisms using BSL2 containment. The IBC protocol details emergency procedures for spills and monitoring of personnel for symptoms of exposure.

IBC Discussion & Vote:

The protocol IBC-25-66 (version 9.0) was approved.
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Jan Smalle 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: Ashley Seifert

IBC Protocol Number: IBC-25-67
Protocol Title: Understanding mechanisms regulating regeneration and scarring in vertebrates
Protocol Type: Renewal
Amendment To: N/A
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-3, NIH Guidelines Section III-F, UK Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 1 (ABSL1)
Primary Reviewers: C. Haughton, Y. Wu, D. Harrison

Brief Project Overview:

The concept of controlling fibrosis and inducing organ regeneration to repair damaged tissue is an awe-inspiring one, and one that would impinge beneficially on nearly every branch of medicine. In the studies briefly outlined here we wish to utilize a novel model of mammalian regeneration, African spiny mice (genus=Acomys) to compare appendage regeneration in Acomys, to wound repair and scarring in outbred laboratory mice (genus=Mus). We will also investigate neurodegeneration, aging and the response of Acomys to neurotoxins related to Parkinson's symptomology. In addition, we will study skin and limb regeneration in axolotls as an additional means of trying to understand the differences between scarring and regeneration and of trying to understand the molecular basis of limb regeneration.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Genetics, Histology, Immunohistochemistry, PCR/qRT-PCR, Transformation, Use of viral vectors, Imaging/Microscopy, Transfection
Transport: Yes
Materials Transported: Biohazardous Materials, Animals
Infectious Agent(s)/Natural Host(s): N/A
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: GFP/Jellyfish/Tracking /Expression/Mouse Cells/Lentivirus; AAV/; Sonic Hedgehog (Shh)/Axolotl/Embryonic development /Knockout/Axolotl/naked nucleic acid/; Fibroblast growth factor (Fgf) /Axolotl/tissue regeneration /knockout/Axolotl/naked nucleic acid/; GLI family zinc finger 3 (GLI3) /Axolotl/tissue patterning, development /knockout/Axolotl/naked nucleic acid/; Bone morphogenic protein (BMP) /Axolotl/growth factor /knockout/Axolotl/naked nucleic acid/; Gremlin1 /Axolotl/growth factor /knockout/Axolotl/naked nucleic acid/; MSX2 /Axolotl/growth factor /knockout/Axolotl embryo/naked nucleic acid
Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/CMV-GFP; Adeno-Associated Virus (AAV)/CAG-GFP; Naked nucleic acid/gRNA (Shh, FgF GLI3, BMP, GREMLIN1, MSX2)
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/Primary spiny mouse; Animal/Primary mouse; Animal/AL-1; Animal/Primary rat; Animal/Primary rabbit; Animal/Mongolian Gerbil
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/Cells - Animal, genetically modified/subQ to ear/anesthesia/ABSL1/lab coat, gloves, eye protection/ABSL1/No; Mouse/Viral Vector - Adeno-Associated Virus (AAV)/retro-orbital, intra-cranial/anesthesia/ABSL1/lab coat, gloves, eye protection/ABSL1/No

Risk Assessment/Discussion:

Dr. Seifert has submitted a renewal of his IBC protocol entitled Understanding mechanisms regulating regeneration and scarring in vertebrates. Dr. Seifert's current IBC protocol will expire on August 23, 2025. Dr. Seifert's laboratory investigates tissue regeneration in Acomys (spiny mice) compared to scarring in Mus (laboratory mice), using a combination of cell culture, gene editing, viral vector delivery, and transgenic animal generation. The study employs primary cell lines from various species, CRISPR/Cas9 gene editing in axolotls, single-cell RNA sequencing of T cells, and the development of embryonic stem cells and transgenic spiny mice. Recombinant lentiviruses and adeno-associated viruses (AAVs) expressing GFP are used to track cellular behavior and gene expression. Additionally, diphtheria toxin (DT) is used to selectively deplete TREG cells in transgenic mice to assess their role in regeneration. Lentivirus expressing GFP is produced by the UNC Viral Core utilizing 3rd generation packaging plasmids, which significantly reduces risk to Dr. Seifert's laboratory associated with

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lentivirus production. The gene insert, GFP, is not harmful to lab personnel should they be exposed. Rather, the risk of lentivirus work in this case is limited to insertional mutagenesis, potentially leading to cancer. All work with lentivirus will be done in a BSC following BSL2+ containment. PPE will include disposal gloves, dedicate lab coat or smock, and eye protection. All biohazardous waste will be decontaminated according to UK Research Safety guidance (liquid waste – 10% bleach final concentration for 20-minute contact time, solid waste via autoclave). Lentivirus will be used to transduce cells obtained from spiny mouse and laboratory mice derived from the ear pinna. Transduced cells will be cultured for a minimum of 3 days and washed to remove any residual lentivirus prior to transplantation into mice. Mice are anesthetized during injection of transduced cells, which greatly minimizes the risk of accidental needle stick during injection. Dr. Seifert's laboratory also utilizes CRISPR/Cas9 gene editing to inactivate genes of interest (Sonic hedgehog (Shh), Fibroblast growth factor (Fgf) family ligands and receptors, Gli3, Bmp family ligands and receptors, Gremlin1 and Msx2) in axolotl embryos. Axolotl larvae will be grown to 3 weeks of age, anesthetized, and utilized to study cell proliferation, cell death, skeletal staining, and other downstream assays and manipulations. They will also create transgenic spiny mice. To generate transgenic spiny mice, modified embryonic stem cells (ESCs) are injected into electro-transfused two-cell embryos. Offspring are genotyped to confirm successful knock-in of a construct containing a self-cleaving peptide (T2A), mClover3 fluorescent protein, and diphtheria toxin receptor (DTR) at the Foxp3 locus. This work will be done at BSL1/ABSL1 containment wearing lab coat, disposable gloves, and eye protection. To assess the role of regulatory T cells (TREGs) in tissue regeneration, both transgenic and wild-type mice are treated with diphtheria toxin (DT-B), which selectively induces apoptosis in DTR-expressing cells. TREG depletion is confirmed via flow cytometry and immunocytochemistry. Regenerative capacity is evaluated using an ear hole punch assay, with measurements taken over 85 days and histological analysis performed at key time points. Diphtheria toxin (DT-B) is not a select toxin subject to Federal Select Agent and Toxin regulations, however steps are taken to avoid exposure, including dissolving DT-B directly in sealed vials to avoid aerosolization, secure storage and labeling, and PPE including lab coat, gloves, and eye protection. Dr. Seifert's laboratory also utilizes an adeno-associated virus (AAV) produced by the UNC Viral Core expressing GFP. AAV-GFP will be administered to mice via retro-orbital and/or intracranial injections to study the pathways and mechanisms involved in Parkinson's disease. These are preliminary studies to determine the best AAV9 variant for alpha synuclein expression, and Dr. Seifert's laboratory does not seek to induce Parkinson's disease in mice currently. Once the pilot study described here has been completed, an IBC Amendment will be submitted with the specific AAV9 constructs that will be used to deliver alpha synuclein. Syringes containing AAV-GFP will be loaded in a BSC located in Dr. Seifert's laboratory, enclosed in a leak-proof, lock-tight secondary container, and transported to the animal facility for injections. Animals will be anesthetized for injections, which greatly minimizes the risk of accidental needle stick. Animal work and housing will be done at ABSL1 wearing lab coat, disposable gloves, and eye protection. Animal carcasses that have been administered AAV or lentivirus-transduced cells will be placed in a red bag and returned to DLAR for final disposal. Axolotl carcasses are picked up by UK EQM for final disposal.

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IBC Discussion & Vote:

The protocol IBC-25-67 (version 8.0) was approved pending minor modifications as listed below:
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SCIENTIFIC SUMMARY:

1. Please add a description of axolotl husbandry procedures and handling, and address the steps taken to prevent accidental escape/release.
2. Please expand the description of restraint for embryonic stem cell (ESC) injections.

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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.
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Conflicts of Interest: None
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PI: Florin Despa

IBC Protocol Number: IBC-25-72
Protocol Title: Cardiovascular consequences of diabetes; electrical remodeling in heart disease
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-E-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 6.3, UK Administrative Regulation 6.9
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 1 (ABSL1)
Primary Reviewers: Y. Wu, B. Nelson, C. Pickett

Brief Project Overview:

Research in our labs focuses on 1) cardiovascular and cerebrovascular consequences of obesity and type 2 diabetes, and 2) understanding how electrical activity of the heart is altered in heart disease. We combine fluorescence imaging, electrophysiology, biochemistry and molecular biology techniques in isolated cardiac myocytes and vascular smooth muscle cells, whole hearts and in vivo using animal models. Key results are validated by experiments in cells/tissue from humans.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Cell culture, DNA/RNA isolation/purification, Immunohistochemistry, PCR/qRT-PCR, Use of Human Source Material(s), Use of viral vectors, Imaging/Microscopy, Transfection
Transport: Yes
Materials Transported: Animals, Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human (Tissues/Blood, Cells)
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: solute carrier family 5 (sodium/glucose cotransporter 1, SGLT1), member 1 (slc5a1) /human /membrane protein /expression in primary cell culture /bacteria/cell culture /pshuttleCMV vector;/pAd/CMV/V5-DEST//; GCaMP2.2 (genetically-encoded calcium sensor; consists of circularly permuted GFP that is flanked by calmodulin at the C-terminus and by the calmodulin- binding peptide myosin light-chain kinase M13 at the N-terminus) /synthetic /tracking gene/expression in primary cell culture /bacteria/cell culture /pshuttleCMV vector; pAd/CMV/V5-DEST/; target-specific 19-25 nt (plus hairpin) shRNA designed to knockdown SGLT1 (slc5a1 gene) expression in rat /synthetic /gene expression (post-transcription) /expression in primary cell culture /cell culture /lentiviral vector (see attachment)/; Human Islet amyloid polypeptide (IAPP)/Human/Peptide hormone /expression in cell line/Bacterial/mammalian cells /pAdeno/; Low density lipoprotein receptor-related protein 1 (LRP1)/Human/Rat/Membrane Protein /expression in primary cells /Bacterial/mammalian cells /pAdeno/; Human Apolipoprotein E4 (ApoE4)/Human/Lipoprotein /expression in primary cells /Bacterial/mammalian cells /pAdeno/pCMV4/; Human Apolipoprotein E3 (ApoE3) /Human/Lipoprotein /expression in primary cells /Bacterial/mammalian cells /pAdeno/pCMV4/; Human Apolipoprotein E2 (ApoE2)

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/Human/Lipoprotein /expression in primary cells /Bacterial/mammalian cells /pAdeno/pCMV4/; Human Presenilin 1 (PSEN-1) /Human/Enzymatic Protein /expression in primary cells/ cell line/Bacterial/mammalian cells /pAdeno/pCMV3/; Human Amyloid Precursor Protein (APP) /Human/Amyloid Protein /expression in primary cells/ cell line/Bacterial/mammalian cells /pAdeno/pCAX/; Green Fluorescent Protein (GFP)/Synthetic /Tracking Gene/expression in primary cells/ cell line/Bacterial/mammalian cells /pAdeno
Vector(s) [Vector Category/Vector Technical Name]: Adenovirus/pshuttleCMV vector /; Adenovirus/pAd/CMV/V5-DEST /; Lentivirus/lentiviral vector (see attachment)/; Plasmid/pCMV4 /; Adenovirus/pAdeno/; Plasmid/pCAX/; Plasmid/pCMV3
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Cardiac myocytes /; Human/Smooth Muscle Cells /; Human/Smooth Muscle Cells /; Animal/Cardiac Myocytes /; Animal/Smooth Muscle Cells (A7R5)/; Animal/Insulinoma (Isolated clonal INS-1E cells from the parental INS-1 cells) /; Animal/Astrocytes /; Animal/Mouse Embryonic Fibroblast (MEF) /; Animal/Rat Brain Vascular endothelial cells(RBVEC)
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]: N/A

Risk Assessment/Discussion:

Dr. Despa has submitted a renewal of his IBC protocol entitled Cardiovascular consequences of diabetes; electrical remodeling in heart disease. Dr. Despa's current IBC protocol will expire on August 18, 2025. Dr. Despa's laboratory investigates molecular mechanisms underlying cardiac and metabolic diseases using human tissues, viral vectors, and genetically modified cell cultures and animals. Human tissues (e.g., heart, brain, kidney) are processed to analyze disease-related molecular markers. Tissue homogenization is performed using liquid nitrogen and mechanical disruption in designated areas and biosafety cabinets (BSCs), followed by protein analysis techniques such as western blot and ELISA. The project also involves the use of replication-deficient adenoviral and lentiviral vectors to overexpress or knock down genes in primary cardiac myocytes, INS-1E cells, and rat astrocytes. Transgenic animal models, including diabetic and cardiac-specific SGLT-modified rodents, are used to study gene function in vivo. The primary biohazardous risks associated with Dr. Despa's IBC protocol include the use of human tissues (risk of exposure to bloodborne pathogens such as HIV, Hepatitis B, or other unknown pathogens) and viral vectors (including replication-incompetent adenovirus and lentivirus). Adenovirus vectors will be used to overexpress SGLT1 and GCaMP2.2 in cardiac myocytes obtained from rats and mice, and Low density lipoprotein receptor-related protein 1 (LRP1), Human Apolipoprotein E4 (ApoE4), Human Apolipoprotein E3 (ApoE3), Human Apolipoprotein E2 (ApoE2), Human Amyloid Precursor Protein (APP), Human Presenilin 1 (PSEN-1), Green fluorescent protein (GFP) in mammalian cells. Lentivirus will be used to knockdown SGLT1 via shRNA in primary myocytes. All viral vectors are obtained from commercial sources (Vector Biolabs or Santa Cruz Biotechnology) and are not packaged/produced in Dr. Despa's laboratory, significantly reducing risk. None of the viral vector constructs are designed to express an oncogene, knockout a tumor suppressor, or other hazardous gene construct. All work with adenovirus and lentivirus vectors and transduced cells will be done at BSL2+ containment within a BSC. PPE will include lab coat, disposable gloves, and eye protection. Transduced cells are lysed for Western Blot or utilized for fluorescent microscopy or electrophysiology experiments. Liquid waste is decontaminated by adding fresh household bleach such that the final concentration of bleach is 10%. Contact time is a minimum of 20 minutes prior to drain disposal. Solid biohazardous waste is decontaminated via autoclave. Dr. Despa's animal work does not involve any use of biohazardous materials requiring IBC registration.

IBC Discussion & Vote:

The protocol IBC-25-72 (version 10.0) was approved.

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Yadi Wu 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: Allen Page

IBC Protocol Number: IBC-25-80
Protocol Title: Immunology of horses
Protocol Type: Renewal
Amendment To: N/A
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: Animal Biological Safety Level 2 (ABSL2), Biological Safety Level 2 (BSL2)
Primary Reviewers: C. Haughton, T. Chambers, D. Harrison

Brief Project Overview:

My laboratory has two major areas of research involving the immune response of horses. The first involves the characterization of the inflammatory response of horses to various stimuli, including exercise and injury. The other area is focused on the effect of corticosteroids on susceptibility to infectious disease, which will rely on experimental challenge with equine influenza virus.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), PCR/qRT-PCR, Use of infectious agents
Transport: Yes
Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Equine Herpesvirus (RG1-virus)/Horse; Equine Influenza Virus (EIV) (RG1-virus)/Horses
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]: Animal/Bronchoalveolar Lavage-Derived Leukocytes
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]: Equine, Agricultural/Equine Influenza Virus (EIV) (RG1-virus)/Inhaled/Halter and leadrope/ABSL2/Tyvek, gloves, boots, disinfectant, shower out, BSL-2 barn/ABSL2/Yes/Horses will be held in BSL-2 barn for 14 days, then moved to quarantine paddocks for additional 14 days post-challenge.

Risk Assessment/Discussion:

Dr. Page has submitted a renewal of his IBC protocol entitled Immunology of horses. Dr. Page's current IBC protocol will expire on September 1, 2025. Dr. Page's laboratory has two primary areas of research – the first involves characterizing the inflammatory response of horses to various stimuli, and the second area is focused on the effect of corticosteroids on susceptibility to infectious disease, specifically equine influenza virus. The infectious agents that Dr. Page's laboratory works with are both pathogens of horses – equine herpesvirus and equine influenza virus. Neither of these viral agents are known to cause disease in humans and are considered

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RG1 viruses. Dr. Page's laboratory will receive various horse samples, including whole blood, serum, plasma, joint fluid, etc. Samples will be sourced from the UK Veterinary Science department herd as well as from collaborators inside and outside the US. These samples will be utilized to isolate RNA for PCR, as well as ELISA analysis. Whole blood samples will be stimulated with equine herpes virus-1 within a BSC. Personnel will wear disposable gloves, lab coat, and eye protection. No active propagation of EHV-1 is planned at this time as virus has been grown and stored for future use. In a separate project, Dr. Page will challenge horses treated with corticosteroids with equine influenza virus grown and prepared by Dr. Feng Li. This project will be completed in the BSL2 Barn (Isolation Barn) located at the North Farm. PPE required for horse infection and handling will include disposable Tyvek suits, shoe overs or dedicated boots, and disposable gloves. Equine influenza virus is an infection of horses and is not known to cause disease in humans. All disposable PPE will be bagged for decontamination via autoclave. Personnel will also be required to shower in the adjacent trailer upon exit, further reducing the risk of environmental release. Horses will be administered equine influenza virus using a Flexi-Neb II nebulizer device that is cleaned and decontaminated according to the manufacturer's instructions. Following challenge, samples (blood, nasopharyngeal swabs) will be collected and transported back to the PIs primary laboratory for further processing. Samples will be double bagged for transport. Blood will be used for serology, cell-mediated immunity assays, or cytokine RNA analyses. Nasopharyngeal swabs will be used to assess viral shedding by injection into embryonated eggs or RT-PCR. Eggs will be inoculated with samples, sealed with paraffin wax, and placed into a dedicated "dirty" incubator. Following incubation, allantoic fluid is removed for testing by hemagglutination assay. Equine influenza may be shed by infected horses but typically stops at Day 7 after infection. Infected horses are kept in the BSL2 barn until day 14 but may be released sooner if there are no signs of infection. Horses will be moved to an isolated outdoor paddock for further observation prior to be released to the general population. Stall waste will be made to sit for 14 days prior to being hauled in a covered vehicle for commercial pickup. At the end of experiment, the BSL2 barn will be cleaned and decontaminated according to the BSL2 Barn Biosafety Manual. This protocol does not involve any agents known or suspected to be infectious to humans, but work will be conducted at BSL2/ABSL2 containment to prevent potential spread/release of equine pathogens.

IBC Discussion & Vote:

The protocol IBC-25-80 (version 10.0) was approved pending minor modifications as listed below:
*
LOCATIONS – Research Locations Table:

1. Please add the procedure room 402B and the biological safety equipment in use from the Feng Li lab to the Research Locations table and Biological Safety Equipment table.
2. Remove reference to “bacterial culture techniques,” from the Procedures column in the Research Locations listing for Gluck 416.

SCIENTIFIC SUMMARY:

1. Please specify the use of face shield OR wrap-around eye protection and fluid resistant surgical masks in the personnel PPE requirements for the inoculation of horses.
2. Please clarify the benchmarks utilized for release of horses after conclusion of experiment. Is this based on clinical signs of infection or diagnostic testing?

*
Doug Harrison initiated the motion. Tom Chambers seconded the motion. All IBC members present (14) voted in favor of the motion.
*
Conflicts of Interest: None

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*

PI: Linda Van Eldik
IBC Protocol Number: IBC-25-83
Protocol Title: Microglia responses to CNS injury: targeting p38 MAPK signaling
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section IV-B-7, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-1, NIH Guidelines Section III-F, NIH Guidelines Section III-F-1, OSHA 29 CFR 1910.1030
Maximum Containment Level: Biological Safety Level 2 (BSL2), Animal Biological Safety Level 1 (ABSL1)
Primary Reviewers: C. Haughton, M. Mendenhall, B. Nelson

Brief Project Overview:

A key mechanism that drives disease progression in many CNS disorders, including both acute and chronic brain injuries, involves inflammatory/ immune responses in the brain. A well-established mechanism underlying injury-induced brain inflammation is the p38a mitogen-activated protein kinase (MAPK) pathway. This research is testing the idea that suppression of p38a signaling at appropriate times and in appropriate cell types after CNS injury can lead to rescue of neurological function in mice after acute brain injury. We are using both pharmacological and genetic approaches. Pharmacological studies are testing the activity of a highly specific small molecule p38a inhibitor. Genetic studies are using mouse model approaches that allow us to delete p38a in specific cell types in the brain. This research will allow us to determine the cellular source of inflammation in the brain in the context of p38a-regulated signaling.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Cell culture, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Use of Human Source Material(s), Use of viral vectors
Transport: Yes
Materials Transported: Animals, Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human (CSF, Plasma, Fixed Brain Tissue)
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: GFP/jellyfish/tracking/expression in animals/mouse/AAV/; Cre/P1 bacteriophage/site specific recombination/target gene deletion in animals/mouse/AAV/; Luciferase/Photinus pyralis/tracking/expression in animals/mouse/AAV/; SYFP2/Aequorea victoria/tracking/expression in animals/mouse/AAV
Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/AAV-CaMKII(0.4)-iCre/; Adeno-Associated Virus (AAV)/AAV-GFa104-Luciferase/; Adeno-Associated Virus (AAV)/AAV-AiEO397m-SYFP2 (modified to include Cre sequence)/; Adeno-Associated Virus (AAV)/AAV-Gfa2-104-Cre-4x6T
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/BV2; Animal/Bend.3; Human/THP-1; Human/A-172; Human/SH-SY5Y
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 injection, anesthesia,

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ABSL1, lab coat, gloves, eye protection, ABSL1, NoMouse, Viral Vector - Adeno-Associated Virus (AAV), retro-orbital, anesthesia, ABSL1, lab coat, gloves, eye protection, ABSL1, No

Risk Assessment/Discussion:

Dr. Van Eldik has submitted a renewal of her IBC protocol entitled Microglia responses to CNS injury: targeting p38 MAPK signaling. Her current IBC protocol will expire on August 18, 2025. Dr. Van Eldik's laboratory investigates p38α-mediated neuroinflammation using conditional knockout mouse models and adeno-associated viral (AAV) vectors. Tissue-specific deletion of the p38α gene is achieved via Cre recombinase expressed in microglia, astrocytes, endothelial cells, and neurons, using both transgenic mouse lines and AAV constructs. AAV vectors, although replication-deficient and non-pathogenic, pose potential risks such as insertional mutagenesis. The transgenes do not pose significant risk and include GFP, luciferase, SYFP2 (all tracking genes) and Cre recombinase. To mitigate these risks, all AAV work is conducted under BSL1/ABSL1 conditions, with stereotaxic or intravenous administration in anesthetized adult mice, followed by proper containment and waste disposal. Human biospecimens (serum, CSF, autopsy tissue) and cultured human cell lines (e.g., THP-1 microglial cells) are handled under BSL-2 containment in a biosafety cabinet, with PPE including gloves, lab coat, and eye protection. Waste is autoclaved or disinfected with 10% bleach and 70% ethanol. Exposure risks include bloodborne pathogens and other unknown infectious agents in human tissue samples. Lab members are trained accordingly, and exposure incidents are managed through immediate decontamination and reporting to Employee Health.

IBC Discussion & Vote:

The protocol IBC-25-83 (version 9.0) was approved pending minor modifications as listed below:
*
INFECTIOUS AGENTS – Infectious Agent(s) table: Please indicate how the brain tissue is fixed.
SCIENTIFIC SUMMARY:
1. Include a more robust description of the processing of human sourced material for assays. Specifically, please clarify if human brain tissue is fixed upon receipt and if so, how this tissue was fixed. Also note what assays/manipulations are planned for human blood.

2. Please expand on the animal work. For example, there is no description of what happens to the animal after AAV injections, how animal tissue is harvested, or the downstream assays/manipulations planned for harvested tissues.

*
Mike Mendenhall 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
*

APPROVED

PI: Christine Brainson

IBC Protocol Number: IBC-25-85
Protocol Title: B22-4022-M2: Defining epigenetic vulnerabilities of lung cancer and lung disease
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-F, 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, B. Nelson, D. Malherbe

Brief Project Overview:

Lung cancer remains the leading cause of cancer related deaths world-wide and lung disease, including pulmonary fibrosis, is also a major cause of human morbidity and mortality. Precision medicine options, where the treatment is matched to the disease, are likely to yield much better treatment outcomes. However, finding precision medicine options for lung cancers and lung disease are complicated by the variety of cell types and genetic mutations that can contribute to an individual's lung cancer. Our main goal is to use both mouse models and human cells to examine how differing genetic mutations can transform varied lung cells, to test how lung cells interact with other cells in their environment, and to find precision medicine options for different types of lung cancer and lung diseases.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Cell culture, DNA/RNA isolation/purification, Immunohistochemistry, PCR/qRT-PCR, Use of Human Source Material(s), Use of viral vectors, Imaging/Microscopy, Transfection
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)]: GFP/Jellyfish/Tracking/Expression/K-12 E.coli/pUC19/; Green Fluorescent Protein/Aequorea victoria/tracking gene/to track cells/mammalian cells/pHage or pHage2, pAd5-CMV, pLXV, pLenti7/; Cre Recombinase/P1 bacteriophage/enzyme/to modify DNA with LoxP sites/mice, mammalian cells/Ad5-CMV,pSECC, PGK-Cre-shRNA/; FlpO Recombinase/Saccharomyces cerevisiae/enzyme/to modify DNA with Fret sites/mice, mammalian cells/Ad5-CMV/; Cas9/Streptococcus pyogenes/enzyme/to cause double strand breaks in DNA in a sequence guided manner/mammalian cells/pLenti-CRISPR, pSECC/; rtTA (reversible tetracycline transactivator)/fusion from E. coli and Herpes Simplex Virus/co-activator/inducible control of genes under tet operon promoter/mammalian cells/pHage, pHage2/; KrasG12V/Homo sapiens/oncogene/transformation/mammalian cells/pLenti7/; p53R175H/Homo sapiens/oncogene/transformation/mammalian cells/pLenti6/; EZH2/Homo sapiens/enzyme/epigenetic modulation; knockdown/mammalian cells/pDNR221, pLenti7.3, pHage, pCMV, pLKO.1/; BRG1 (aka SMARCA4)/Homo sapiens/enzyme/epigenetic modulation; knockdown/mammalian cells/pDNR221, pQCXIH, pHage, pLKO.1/; EGFR /Homo sapiens/Signaling protein/expression; knockdown/mammalian cells/pBabe-Puro, pDNR223, pHage, pLKO.1/; EGFRdel19/Homo sapiens/oncogene/transformation/mammalian cells/pBabe-Puro/; SV40 large and small T antigens/Simian virus 40/viral gene/transformation/mammalian cells/pLenti/; PIK3CA E545K/Homo sapiens/oncogene/transformation/mammalian cells/pBabe-Puro/; PIK3CA /Homo sapiens/Signaling protein/expression/mammalian cells/pBabe-Puro/; PIK3CA H1047R/Homo sapiens/oncogene/transformation/mammalian cells/pBabe-Puro/; CDK4/Homo sapiens/cell cycle regulator/immortalization/mammalian cells/pDNR223/; SOX2/Homo sapiens/transcription factor/lineage switching/mammalian cells/P2A-Hygro-Barcode, pHage2, pLKO.1/; Sox2/Mus musculus/transcription factor/lineage switching/mammalian cells/pHage2, pLKO.1/; TERT/Homo sapiens/telomerase/immortalization/mammalian cells/pBabe-Puro/; CBS (cystathionine beta synthase)/Homo sapiens and Mus musculus/metabolic enzyme/expression; knockdown/mammalian cells/pDNR221, pLenti6.3, pLKO.1/; FOXP2/Homo sapiens/transcription factor/expression; knockdown/mammalian cells/pDNR221, pLenti6.3, pLKO.1/; Ezh2 F677I methyltransferase dead/Mus musculus/epigenetic protein/expression/mammalian cells/MSCV-Hygro/; Ezh2/Mus musculus/epigenetic protein/expression/mammalian cells/MSCV-Hygro/;

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TGFb/Mus musculus/Signaling protein/expression/mice, mammalian cells/AAV6/; LKB1 (STK11)/Homo sapiens/metabolic regulator/expression; knockdown/mammalian cells/pBabe-Puro, pLKO.1/; MYC/Homo sapiens/transcription factor/expression; knockdown/mammalian cells/pLXV, pLKO.1/; KEAP1/Homo sapiens/metabolic regulator/expression/mammalian cells/pDNR223, pLenti6.3/; Gag/Pol/Tat/Rev/HIV/viral packaging/viral packaging/293T or PlatE cells/pCMV-VPR-delta8.2/; VSVg/vesicular stomatitis virus/viral packaging/viral packaging/293T or PlatE cells/pCMV-VSVg
Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/AAV6-TGFb/; Adenovirus/Ad5-CMV-GFP/; Adenovirus/Ad5-CMV-Cre/; Adenovirus/Ad5-CMV-FlpO/; Plasmid/pCMV/; Plasmid/pCMV-VSVg/; Plasmid/pCMV-VPR-delta8.2/; Plasmid/pDNR221, pDNR223 (aka pENTR)/; Retrovirus/U6-shRNA pgkCre/; Retrovirus/pBabe/; Retrovirus/MCSV-hygro/; Retrovirus/pQCXIH/; Lentivirus/pLKO.1/; Lentivirus/pSECC/; Lentivirus/pLenti6 and pLenti7/; Lentivirus/pHage and pHage2/; Lentivirus/P2A-Hygro-Barcode/; Lentivirus/pLXV
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/Primary mouse lung or esophageal cells/; Animal/Primary mouse endothelial cells/; Human/Patient-Derived Tumoroids/; Human/Diseased or normal human lung tissue/; Human/HBEC14/; Human/HBEC3KT/; Human/HBEC2/; Human/BEAS2B/; Human/A549/; Human/Calu1/; Human/Calu3/; Human/Calu6/; Human/NCI-H23/; Human/HCI-H460/; Human/NCI-H522/; Human/NCI-H520/; Human/NCI-H157/; Human/NCI-1975/; Human/NCI-H2122/; Human/NCI-H2009/; Human/NCI-H2009/; Human/NCI-H1819/; Human/NCI-H322/; Human/NCI-H1781/; Human/NCI-H1395/; Human/NCI-H2087/; Human/NCI-H441/; Human/NCI-H661/; Human/NCI-H2030/; Human/NCI-H1650/; Human/NCI-H1299/; Human/NCI-H3255/; Human/HCC4006/; Human/HCC2450/; Human/HCC95/; Human/HCC15/; Human/HCC827/; Human/HEK293T/; Human/PC9/; Human/PC9 GR4/; Human/PlatE
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]: Incomplete

Risk Assessment/Discussion:

Dr. Brainson has submitted a renewal of her IBC protocol entitled Defining epigenetic vulnerabilities of lung cancer and lung disease. Her current IBC protocol will expire on August 17, 2025. Dr. Brainson's research involves the transformation and culture of murine and human lung cells using advanced 3D organotypic systems, viral genetic manipulation, and in vivo tumor modeling in immunocompromised mice. Biohazardous risks include exposure to replication-deficient adenoviruses, lentiviruses, retroviruses, and adeno-associated viruses (AAV), as well as handling of human-derived tissues potentially harboring infectious agents (e.g., HBV, EBV). Adenovirus is obtained from the University of Iowa Viral Vector core and not packaged by Dr. Brainson's lab. AAVs are obtained from Vector Biolabs. AAV, adenovirus, and lentivirus are administered to mice via inhalation within a BSC in DLAR at ABSL2 containment. Lentivirus and retrovirus vectors are produced by Dr. Brainson's laboratory utilizing BSL2+ containment. All work will be conducted in the BSC with dedicated lab coat, gloves, and eye protection. Sharps are not utilized in conjunction with retrovirus or lentivirus vectors, which significantly reduces risk of accidental exposure. Mitigation strategies include strict adherence to BSL2 containment (BSL2+ for adenovirus, lentivirus, and retrovirus work) including use of biosafety cabinets, PPE (gloves, lab coats, eye protection), and decontamination protocols using 10% bleach and autoclaving of solid biohazardous waste. Viral vectors are handled in micro-liter volumes, with centrifugation performed only in biocontainment-equipped microcentrifuges. Human tissues and organoids are tested for pathogens by IDEXX laboratories, and all viral work is conducted with replication-incompetent systems, with additional testing for replication-competent virus (RCV) prior to animal use. Mice are housed under ABSL2 conditions post-inoculation (adenovirus and lentivirus; ABSL1 for AAV) and transitioned to ABSL1 after shedding risk subsides. Carcasses and waste are disposed of as regulated medical

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waste per institutional SOPs. These comprehensive biosafety measures ensure containment of biohazards and protection of personnel and the environment.

IBC Discussion & Vote:

The protocol IBC-25-85 (version 8.0) was approved pending minor modifications as listed below:
*
ANIMAL RESEARCH – Animals with Biohazards Table: Please complete the Animals with Biohazards table.
SCIENTIFIC SUMMARY:

1. Please revise the statement "All adenovirus contaminated surfaces…" to state that all work surfaces will be decontaminated. Ensure the BSC work surface is disinfected with 10% bleach after all work is complete.
2. When describing transport of animals, the term "long distance" is ambiguous. Please clarify the specific locations of transport.
3. It is unclear whether mouse necropsies are performed on the open bench or in a BSC. Please clarify.
4. Provide a description of how animal carcasses will be disposed of after exposure to AAV vectors.
5. Please state which procedures are associated with oncogenes.
6. Specify whether each batch of lentivirus is tested for RCV and what happens to batches that do not pass RCV testing.

*
Delphine Malherbe initiated the motion. Cheryl Haughton 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: Misung Jo

IBC Protocol Number: IBC-25-86
Protocol Title: Mechanisms of Periovulatory Processes
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-D-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 1 (ABSL1)
Primary Reviewers: C. Pickett, M. Mendenhall, A. Hunt

Brief Project Overview:

The purpose of our study is to understand ovarian physiology, especially focused on the periovulatory process. The periovulatory process includes cumulus oocyte complex expansion, ovulation, luteinization, and fertilization. The studies will use primary culture cells from mouse and human granulosa cells obtained from IVF patients. No infectious agents or materials will be used on live animals. Biohazard materials include LB plates inoculated with E coli. In addition, we will clone portions of target genes of interest into commercially available plasmids (pCR2.1) using standard techniques and use them for generating complementary strands of RNA or DNA as "probes" to

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detect mRNA expressed in the tissue or cells. Infectious agents include human granulosa cell samples and recombinant viruses (e.g., adenovirus). Adenoviruses previously generated or commercially purchased will be used to over-express or knock down the expression of genes of interest to study the effect of the genes on cell function by infecting mouse or human granulosa cells.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Transfection, Use of Human Source Material(s), 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)]: Runx2/Mouse/Transcription Factor /Generation of RNA Probe /E. coli (TOPO10)/pCR2.1/; Fos/Mouse/Transcription Factor /Generation of RNA Probe /E. coli (TOPO10)/pCR2.1/; Cbfb/Mouse/Transcription Factor /Generation of RNA Probe /E. coli (TOPO10)/pCR2.1/; SOX9/human/transcription factor/silencing/human cells/Adenovirus/; RUNX2/Human/Transcription factor/expression/Human cells/AdEasy/; GFP/Aequorea victoria/Reporter/tracking/human cells/Adenovirus
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pCR2.1; Plasmid/pShuttle; Adenovirus/SOX9-shRNA; Adenovirus/Ad-RunX2; Adenovirus/Ad-GFP-U6-scrambled-shRNA
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Human Granulosa Cells; Animal/Rat Granulosa Cells; Animal/Mouse Granulosa Cells; Human/Cell Line (AD-293)
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]: N/A

Risk Assessment/Discussion:

Dr. Jo has submitted a renewal of her IBC protocol entitled Mechanisms of Periovulatory Processes. Her current IBC protocol will expire on August 16, 2025. Dr. Jo's laboratory studies periovulatory gene function using rodent models and human granulosa cells. Biohazardous risks include exposure to recombinant adenoviruses and human-derived materials. Adenoviral vectors (e.g., Ad-RUNX2, Ad-SOX9-shRNA) are replication-deficient and used under BSL2+ conditions, including exclusive BSC work and dedicated lab coat, disposable gloves, and eye protection. Cells transduced with adenovirus will be utilized for RNA and protein isolation. Human granulosa cells, which may carry bloodborne pathogens (e.g., HIV, HBV, HCV), are handled exclusively in biosafety cabinets (BSCs), with sealed rotors used for centrifugation to prevent aerosol exposure. Personnel wear lab coats, disposable gloves, and eye protection, and follow universal precautions. All solid biohazardous waste is autoclaved before disposal, and workspaces are disinfected with 10% bleach post-procedure. Spill response protocols and incident reporting are in place. Adenovirus production was completed in 2019; current work uses existing stocks or commercially acquired virus.

IBC Discussion & Vote:

The protocol IBC-25-86 (version 10.0) was approved pending minor modifications as listed below:
*
SCIENTIFIC SUMMARY:

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1. Please acknowledge that RUNX2 can act as an oncogene in various cancers.
2. Include a description of how human granulosa cells are withdrawn and handled after gradient centrifugation.
3. Add a statement clarifying that cultured cells are not being administered to live animals.
4. If adenovirus will no longer be produced by the laboratory, please remove the description of adenovirus production and include a statement that adenovirus is not being produced by the laboratory.
5. Please add a more detailed description of adenovirus handling including the safety concerns and mitigation steps in place.

Mike Mendenhall initiated the motion. Carol Pickett seconded the motion. All IBC members present (14) voted in favor of the motion.
*
Conflicts of Interest: None
*

PI: Kathleen O'Connor

IBC Protocol Number: IBC-25-90
Protocol Title: Integrin regulation of carcinoma invasion
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-D-2, NIH Guidelines Section III-E-1, NIH Guidelines Section III-F-3, NIH Guidelines Section III-F-5, 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: Animal Biological Safety Level 2 (ABSL2), Biological Safety Level 2 - Enhanced (BSL2+)
Primary Reviewers: C. Haughton, Y. Wu, D. Malherbe

Brief Project Overview:

The O'Connor lab is interested in the how carcinomas invade and metastasize with the goal of eventually developing more effective ways of targeting these processes for therapeutic intervention. Toward this goal, the lab is focused on the pro-invasive integrin α6β4 signaling. These studies over the last decade have concentrated the ability of integrin α6β4 to alter what genes are expressed that produce an invasive phenotype and to regulate the small GTPase RhoA. The current focus in the lab is how the integrin α6β4 regulates epigenetics and DNA repair in triple negative breast cancer, with some further exploratory experiments in lung, colon, pancreatic, and other types of cancers. Of note, integrin α6β4 is a protein generated from ITGA6 (α6 subunit) and ITGB4 (β4 subunit) where we only change ITGB4 to alter integrin α6β4 expression. These studies are broken down into three main projects (funded or funding sought for each area) as they relate to animal studies and are listed below:

1. Integrin α6β4 Regulation of Cancer Epigenetics (R01 funded through 2024, submitted again in June 2025, pending)
2. Integrin α6β4 Regulation of DNA repair in Triple-Negative Breast Cancer (R01 submitted March 2025)
3. Chemotherapeutic response in triple negative breast cancer (TNBC; previously funded, seeking new funding)
4. Targeting S100A4(a key prometastatic protein downstream of integrin alpha6beta4) in non-small cell lung cancer (lung cancer, previously funded)

Summary of Biohazard Materials & Manipulations:

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Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Creation of viral vectors, Flow cytometry/Cell sorting, Genetics, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Proteomics, Transfection, Transformation, Use of Human Source Material(s), 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)]: RhoA, RhoB, RhoC/Human/enzymatic/signaling proteins/expression and shRNA knockdown/mammalian cells/pRK5, lentivirus/; Integrin beta4/Human/Structural, signaling protein/expression and shRNA knockdown/mammalian cells/pCDNA, lentivirus shRNA, retrovirus/; Rac1 and cdc42/Human/enzymatic/signaling protein/expression/mammalian cells/pCDNA/; Rho Kinase/Human/enzymatic/signaling protein/expression/mammlian cells/pCDNA/; Beta-gal/bacterial/tracking/expression/mammalian cells/pCMV/; Rho binding domain (RBD) of various Rho effectors, including Thotekin, Citron, ROCKII, mDIA1, PKN/Human/structural/expression/mammalian cells/pGEX/; Rac binding domain of Pak (PBD)/Human/Structural (for activity assessment)/expression/mammalian cells/pGEX/; Fragment of Protein Kinase Inhibitor (inhibits PKA)/Human/structural (for activity assessment)/expression/mammalian cells/pGEX/; AKAP13/Human/structural, signaling/expression/mammalian cells/pcDNA/; Autotaxin/human/enzymatic/signaling protein/expression/mammalian cells/pcDNA/; NFAT1, NFAT5/human/transcription factors, regulatory genes/expression and shRNA knockdown/mammalian cells/pcDNA, lentivirus/; MARCKS/human/enzymatic/signaling protein/expression/mammalian cells/pcDNA/; LPA1, LPA2 receptor/human/membrane protein/expression/mammalian cells/pcDNA/; XPLN/human/signaling/expression/mammalian cells/pGEX, pcDNA/; S100A2/human/structural signaling/expression/mammalian cells/pcDNA/; SFRP1/human/signaling/expression/mammalian cells/pGEX, pcDNA/; Non-muscle myosin IIA/human/structural protein/expression/mammalian cells/pGEX/; S100A4/human/structural signaling/expression and shRNA knockdown/mammalian cells/pcDNA, lentivirus/; Vimentin/human/structural protein/expression and shRNA knockdown/mammalian cells/pcDNA, lentivirus/; MST4/human/kinase/expression and shRNA knockdown/mammalian cells/lentivirus/; NDUFS2/human/structural protein/expression/mammalian cells/pcDNA/; GFP/jellyfish/tracking gene/Expression/mammalian cells/pcDNA/; Luciferase/firefly/tracking gene/Expression/mammalian cells/pcDNA/; PTPRZ1/Human/Structural signaling/Expression and shRNA knockdown/mammalian cells/pcDNA/; IDO1/Human/Enzymatic protein, signaling/Expression and shRNA knockdown/mammalian cells/pcDNA/; Tetraspanin 4/mouse/membrane/signal protein/expression/mammalian/pB-CAG-BGH
Vector(s) [Vector Category/Vector Technical Name]: Retrovirus/pCLXSN/; Retrovirus/PBabe/; Plasmid/GFP/; Plasmid/pCDNA/; Lentivirus/pKL0.1/; Plasmid/pGEX/; Lentivirus/pCDH-CMV/; Plasmid/pGL3-luciferase/; Plasmid/PRK5/; Plasmid/PX459 (pSpCas9(BB)-2A-Puro)/; Plasmid/pB-CAG-BGH
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/MDA-MB-231; Human/MDA-MB-435/; Human/MDA-MB-453/; Human/MDA-MB-468/; Human/MDA-MB-175/; Human/T47D/; Human/BT20/; Human/HS578T/; Human/Aspc-1/; Human/Panc-1/; Human/Bxpc-3/; Human/Suit2/; Human/Miapaca-2/; Human/MCF7/; Human/DLD-1/; Human/Clone A /; Human/Caco2/; Human/HT29/; Human/HT116/; Human/SW480/; Human/SW620/; Human/293T/; Human/Primary cell cultures/; Human/A549/; Human/EKVX/; Human/H358/; Human/Hop62/; Human/H322 M/; Human/H522/; Human/H838/; Human/H23/; Human/H460/; Human/Hop 92/; Human/H1299/; Human/H810/; Human/H82/; Human/H157/; Human/BT549/; Human/H2030/; Human/PC-9/; Human/HeLa/; Human/MCF10A/; Human/HMLE/; Human/LS174T/; Animal/4T1/; Human/HMT-

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3522 T4/; Human/SUM159PT/; Human/SUM149/; Animal/MLE-12/; Animal/KP7B/; Animal/KP/; Animal/KPP/; Animal/EMT6/;
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/Cells - Human, non-modified/implant or subcutaneous/Anesthesia/ABSL2/Lab coat, gloves, eye protection, mask/ABSL2/No; Mouse/Cells - Human, genetically modified/mammary fat pad injection/anesthesia/ABSL2/Lab coat, gloves, eye protection, mask/ABSL2/No; Mouse/Cells - Human, non-modified/Intravenous tail vein/scruffing and restraint device/ABSL2/Lab coat, gloves, eye protection, mask/ABSL2/No; Mouse/Cells - Human, genetically modified/Intravenous tail vein/scruffing and restraint device/ABSL2/Lab coat, gloves, eye protection, mask/ABSL2/No

Risk Assessment/Discussion:

Dr. O'Connor has submitted a renewal of her IBC protocol entitled Integrin regulation of carcinoma invasion. Her existing IBC protocol will expire on September 14, 2025. Dr. O'Connor's laboratory utilizes established and primary human cell lines, viral vectors (lentiviral, retroviral, and adenoviral), CRISPR/Cas9 gene editing, and animal models, all of which present potential biohazardous risks including exposure to human-derived materials, replication-competent viruses, and recombinant DNA. Lentivirus expressing shRNA are obtained from commercial vendors. They will also utilize pXLSN retrovirus and pBabe adenovirus to overexpress genes including integrin beta 4 that were obtained from a collaborator. All viral vectors will be handled using BSL2+ conditions, with all manipulations involving live cells or viral vectors performed in a certified biosafety cabinet (BSC). PPE includes gloves, lab coats, and eye/face protection (e.g., face shields or safety glasses with masks) especially during procedures involving human tissues or animal injections. Viral vector work is limited to low-titer preparations ($<10^5$/ml), and no concentrated virus is used. Cells are tested for replication-competent virus prior to animal injection. Animal work is conducted in ABSL-2 procedures and housing. Non-modified and modified cells will be administered via subcutaneous injection, mammary fat pad injection, or intravenous injection in anesthetized mice within a BSC in DLAR. Primary human breast cancer tissues will be utilized to establish PDX models by implantation into the mammary fat pad of SCID mice. Other human tissues are obtained fixed, paraffin embedded. Biohazardous spill response and accidental exposure response are clearly identified.

IBC Discussion & Vote:

The protocol IBC-25-90 (version 10.0) was approved pending minor modifications as listed below:
*
RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information Table: Please add p53 to the Gene Information Table.

LOCATIONS – Research Locations Table: Please clarify if the PPE listed for the DLAR entry is all in use at the same time. Please clarify. For instance, should the PPE required list “Lab coat OR surgical gown OR disposable coveralls”?

SCIENTIFIC SUMMARY: Please review the Scientific Summary and amend it to accurately reflect the procedures in the laboratory. For instance, there is reference to a “freestanding benchtop shield” in use. Is this accurate?
*
Delphine Malherbe 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
*

PI: Elizabeth Duncan
IBC Protocol Number: IBC-25-96
Protocol Title: Cloning planarian genes for RNA interference screening
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-D-4, NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-3, 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 1 (BSL1), Animal Biological Safety Level 1 (ABSL1)
Primary Reviewers: J. Smalle, T. Chambers, A. Hunt

Brief Project Overview:

Planarians are flatworms with very unique biological features: they can replace old cells indefinitely, effectively making them immortal, and they can regenerate entirely new worms from a small piece of excised tissue. Their regenerative capacity includes the ability to create new complex organs, including brain-like structures, intestines, skin, and primitive kidneys. In our lab, we are very interested in understanding how planarians can accomplish this remarkable feat. We are particularly interested in how they regulate the expression of specific genes at specific times during this process. We are also interested in how the worms sense dynamic changes to their body and/or environment, such as the loss of a major chunk of tissue, and then respond to them appropriately.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Genetics, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Proteomics, Transformation, Animal work (breeding, surgeries, etc.)
Transport: Yes
Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): N/A
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: unc-22/C. elegans/enzyme/dsRNA & riboprobe/E. coli/T4P/; mll1/2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; set1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; dpy-30/S. mediterranea/subunit of enzyme complex/dsRNA & riboprobe/E. coli/T4P/; cfap53/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; rootletin/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; rsph6A/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Ap1b1/S. mediterranea/subunit of structural complex/dsRNA & riboprobe/E. coli/T4P/; Armc4-1/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Armc4-2/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Bicc1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Cc2d2a/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Ccdc151/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Ccdc39/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Cep110/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Cep290/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Daw1/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dctn5/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dnaaf3/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dnah5/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dnai1/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dnm2-1/S. mediterranea/structural/dsRNA &

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riboprobe/E. coli/T4P/; Dnm2-2/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dnm2-3/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dnm2-4/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dnm2-5/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Drc1/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dync2h1/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Dyx1c1/S. mediterranea/unknown/dsRNA & riboprobe/E. coli/T4P/; Foxj1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Hectd1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Ift140/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Ift74/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Kif7/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Lox-1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Lox-2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Lox-3/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Lrp1-1/S. mediterranea/metabolic/dsRNA & riboprobe/E. coli/T4P/; Lrp1-2/S. mediterranea/metabolic/dsRNA & riboprobe/E. coli/T4P/; Lrp1-3/S. mediterranea/metabolic/dsRNA & riboprobe/E. coli/T4P/; Mmp21/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Myh10/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Ndst1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Nek8-1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Nek8-2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Pcsk5/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Pde2a-1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Pde2a-2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Pde2a-3/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Pde2a-4/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Pkd1l1-1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Pkd1l1-2/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Plxnd1/S. mediterranea/Plexin-D1/dsRNA & riboprobe/E. coli/T4P/; Prdm1-1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Prdm1-2/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Prickle1-1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Prickle1-2/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Prickle1-3/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Ptk7/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Robo1/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Smarca4-1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Smarca4-2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Snx17/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Sufu/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Tab1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Tbc1d32/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; Tmem67/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; HDAC 2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; HDAC 3/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; HDAC 4/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; HDAC 6/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; HDAC 8/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; HDAC 11/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Piwi1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Prog-1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Agat1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; HAT1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; p300/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; ACTR/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; CLOCK/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Gcn5/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; HBO1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; MOZ/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; SRC1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Tip60/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; KDM2A/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; KDM4A/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; LSD2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; ATM/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; ATR/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; SMAD4/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; BMP4/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; NOTCH2/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; SMAD4/G.

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guanajuatiensis/signaling/dsRNA & riboprobe/E. coli/T4P/; BMP4/G. guanajuatiensis/signaling/dsRNA & riboprobe/E. coli/T4P/; NOTCH2/G. guanajuatiensis/signaling/dsRNA & riboprobe/E. coli/T4P/; NOTUM/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; SLIT/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; LSD1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; UTX/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; JARID1A/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; JARID1C/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; TRR/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; LPT/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; MENIN/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; EZH2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; NSD1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; NSD2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; SETD2/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; BPTF/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; H3.3/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; H3.2/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; HIRA/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; ATRX/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; H2A.X/G. guanajuatiensis/regulatory/dsRNA & riboprobe/E. coli/T4P/; H2A.X/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; WSTF/G. guanajuatiensis/enzyme/dsRNA & riboprobe/E. coli/T4P/; WSFT/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; MYOD/G. guanajuatiensis/regulatory/dsRNA & riboprobe/E. coli/T4P/; MYOD/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; MYOGENIN/G. guanajuatiensis/regulatory/dsRNA & riboprobe/E. coli/T4P/; MYOGENIN/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; LIPASE/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; ARF1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; PLAC8/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; TRAF5/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; SHOC2/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; Rapunzel-6/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Dimethylglycine-dehydrogenase,-mitochondrial/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Wax-ester/triacylglycerol-synthase-family-O-acyltransferase/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Bestrophin-homolog/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Slc18a-1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Synaptotagmin-11/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Cytochrome-P450/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; carboxypeptidase-B-like/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Transposon-Ty3-I-Gag-Pol-polyprotein/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Trypsin-like-serine-protease/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Chymotrypsin-like-elastase-family-member-1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Secreted-frizzled-protein-like-protein/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; Frizzled-5/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; Sodium/potassium-transporting-ATPase-subunit-beta-2/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Armadillo-repeat-containing-4/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; Ryanodine receptor-1/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; Ryanodine receptor-3/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; NFX1-type zinc finger-containing protein 1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; DExD-box helicase 50/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; aldehyde dehydrogenase 5 family member A1 /S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; L-2-hydroxyglutarate dehydrogenase/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Mediator of RNA polymerase II transcription subunit 6/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Pre-mRNA-processing-splicing factor 8/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Zinc finger CCCH domain-containing protein 4/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; Vigilin/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; EGF-like domain-containing protein/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; histidine ammonia-lyase/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Collagenase 3/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Carbon catabolite-derepressing protein kinase/S.

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mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; TNF receptor-associated factor 2/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; E3 ubiquitin-protein ligase RNF217/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Piezo-type mechanosensitive ion channel component 2/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; ribosomal protein lateral stalk subunit P0/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; DNA-directed DNA polymerase/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; Tubulin alpha-1C chain/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; aminoacylase 1/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P/; chromosome 11 open reading frame 54/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; rfx1/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; rfx2/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; fam50/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; rfx5/S. mediterranea/regulatory/dsRNA & riboprobe/E. coli/T4P/; erk1/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; erk2/S. mediterranea/signaling/dsRNA & riboprobe/E. coli/T4P/; asic3/S. mediterranea/structural/dsRNA & riboprobe/E. coli/T4P/; cilk/S. mediterranea/enzyme/dsRNA & riboprobe/E. coli/T4P
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/T4P
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]: Planarian worm/dsDNA/feed/N/A/N/A/gloves/N/A/Yes/will feed in vitro transcribed double-stranded RNA mixed with commercial beef liver to planarian worms

Risk Assessment/Discussion:

Dr. Duncan has submitted a renewal of her IBC protocol entitled Cloning planarian genes for RNA interference screening. Her current protocol will expire on August 17, 2025. Dr. Duncan's laboratory utilizes recombinant DNA technology to produce double-stranded RNA (dsRNA) for gene knockdown studies in planarians, utilizing E. coli strain HT115, which lacks RNase III activity. The primary biohazardous risk stems from the use of genetically modified E. coli and potential exposure to dsRNA and planarian biological material. Risk mitigation includes the use of BSL-1 containment practices with enhanced precautions during bacterial culture, induction, and feeding procedures. After RNAi targeting, planarian worms are assayed for 1) behavior and morphology changes, 2) changes to gene expression (via RNA extraction), 3) changes to markers of normal internal structures using in situ hybridization (worms are fixed via 4% paraformaldehyde), and 4) chromatin modifications. Personal protective equipment (PPE) includes lab coats, gloves, and eye protection to prevent exposure to bacterial cultures, Trizol reagent (a phenol-based chemical), and paraformaldehyde (PFA), both of which are hazardous chemicals used in downstream analyses but are known to inactivate biohazardous materials present. Waste containing live bacteria or worms is decontaminated using 10% bleach for at least 20 minutes before disposal. Additional precautions are taken for water changes post-feeding to ensure decontamination of excreted bacterial products. Flow cytometry and in situ hybridization procedures are performed on fixed or chemically treated samples, further reducing biohazard risks.

IBC Discussion & Vote:

The protocol IBC-25-96 (version 8.0) was approved pending minor modifications as listed below:
*
SCIENTIFIC SUMMARY: The last section describes work with HeLa cells. If this work is done in Dr. Galperin's laboratory, this should be reflected in her IBC protocol. If this work will be done in your laboratory, it should be described here. Please clarify who is doing this work.

APPROVED

*

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

*

Conflicts of Interest: None

*

PI: Chase Kempinski

IBC Protocol Number: IBC-25-101
Protocol Title: Yeast engineering for terpene production
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-3, NIH Guidelines Section III-F-5, 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 1 (BSL1)
Primary Reviewers: A. Hunt, J. Smalle, M. Mendenhall

Brief Project Overview:

Enepret is a biotechnology company focused on providing a class of natural oils utilized across multiple markets, but essential to the pharmaceutical (where they are adjuvants/immune boosters in vaccines) and personal care (topical emollients) industries. However, the traditional source of these oils, deep sea shark liver oil, has been severely restricted. Contemporary alternatives like extraction from olive oil have not solved the supply and purity demands, leading to increased pressure on finding alternatives. Thus, there remains an impetus in the fine chemical industry to be able to produce and offer triterpene oils from other natural, non-animal, sources. Enepret produces these oils using engineered yeast. We have selected mutant baker's yeast that allow manipulation of their natural triterpene production pathway. We have taken these mutant yeasts and using the tools of genetic engineering knocked out (and inserted) genes which increase the production of triterpene oils. The inserted genes are derived from other organisms which are known to make large amounts of these triterpene oils (e.g. a species of green algae, Botryococcus). We plan to continue to manipulate the genome of our engineered yeast lines, further knocking out genes which repress the triterpene oil pathway and inserting genes that increase triterpene oil production. These genes that are to be inserted will mostly derive from the yeast's genome, but under new control, while other genes may be copies of highly active enzymes from other species. Because the yeast triterpene oil production platform is amendable to produce other terpenes it will be further used to make other valuable compounds, such as carotenoids and cyclized triterpenes.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, DNA/RNA isolation/purification, Genetics, PCR/qRT-PCR, Transformation
Transport: Yes
Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): N/A
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Squalene synthase (SQS)/Botryococcus braunii/Enzyme/Expression/donor DNA for genome integration to produce squalene/Saccharomyces cerevisiae/pXLEU-SQSfull/; Botryococcene synthase (BS)/Botryococcus braunii/Enzyme/Expression/donor DNA for genome integration to produce botryococcene/Saccharomyces cerevisiae/pXLEU-BSm/; Triterpene methyltransferase 1 (TMT1)/Botryococcus

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braunii/Enzyme/Expression/donor DNA for genome integration to produce methylated triterpene/Saccharomyces cerevisiae/yEP352-TMT1/; Triterpene methyltransferase 2 (TMT2)/Botryococcus braunii/Enzyme/Expression/donor DNA for genome integration to produce methylated triterpene/Saccharomyces cerevisiae/yEP352-TMT2/; Triterpene methyltransferase 3 (TMT3)/Botryococcus braunii/Enzyme/Expression/donor DNA for genome integration to produce methylated triterpene/Saccharomyces cerevisiae/yEP352-TMT3/; AUS1/Saccharomyces cerevisiae/Transporter/Expression/donor DNA for genome integration to produce sterol uptake/Saccharomyces cerevisiae/pXLEU-AUS1/; 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR)/Chinese hamster ovary cells/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pXHIS-HMGR/; Farnesyl diphosphate synthase (FPS)/Gallus gallus/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pXLEU-FPS/; Oxidosqualene cyclase 8 (BdOSC8)/Brachypodium distachyon/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pXHIS-BdOSC8/; Squalene epoxidase (BdSQE1)/Brachypodium distachyon/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pXHIS-BdSQE1/; INO2/Saccharomyces cerevisiae/Transcription factor/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT-EXC2/; ERBV-1 skipping sequence/Equine rhinitis B virus /2A ribosomal skipping sequence/A short nucleotide used between open reading frames to generate a polycistronic gene cassette/Saccharomyces cerevisiae/pT/; Hygromycin phosphotransferase (HPH)/Escherichia coli/Enzyme/Expression/donor DNA for genome integration to allow for selection/Saccharomyces cerevisiae/pT, pXHIS, pXURA/; Squalene epoxidase (ERG1)/Saccharomyces cerevisiae/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT/; Dual phytoene synthase and lycopene cyclase (crtYB)/Xanthophyllomces dendrorhous/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT/; Phytoene desaturase (crtI)/Xanthophyllomces dendrorhous/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT/; Astaxanthin synthase (Asy)/Xanthophyllomces dendrorhous/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT/; HO endonuclease (HO)/Saccharomyces cerevisiae/Enzyme/Switch yeast mating type/Saccharomyces cerevisiae/pT/; Cytochrome P450 reductase (crtR)/Xanthophyllomces dendrorhous/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT/; NADH kinase (POS5)/Saccharomyces cerevisiae/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pXHIS, pXURA/; Glucose-6-phosphate dehydrogenase (ZWF1)/Saccharomyces cerevisiae/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pXHIS, pXURA/; Cre recombinase (CRE)/Bacteriophage P1/Enzyme/Expression DNA to remove integrated genomic elements/Saccharomyces cerevisiae/pXURA/; Butryaldehyde dehydrogenase (BLD)/Clostridium saccharoperbutylacetonium/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT/;Acetoaceto-CoA reductase (phaB)/Ralstonia eutropha/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT/; NADPH-dependent aldehyde reductase (YqhD)/Escherichia coli/Enzyme/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pT/; Lipid droplet protein (FIT2)/Mus musculus/Structural protein/Expression/donor DNA for genome integration to increase terpene production/Saccharomyces cerevisiae/pXURA
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pET28a/; Plasmid/pXLEU/; Plasmid/pXHIS/;Plasmid/pXURA/; Plasmid/pGEM-T easy vector/; Plasmid/pFA6/; Plasmid/pD1218/; Plasmid/pXLEU-SQSfull/; Plasmid/pXLEU-BSm/; Plasmid/yEP352/; Plasmid/yEP352-TMT1/; Plasmid/yEP352-TMT2/; Plasmid/yEP352-TMT3/; Plasmid/pTwist-Amp/; Plasmid/pESC-Leu/; Plasmid/ΔERG9 (long-arms) -

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SeGAL2p: (ScOpt) SSL1-3:VPS13t -LoxP: HPH/; Plasmid/ΔERG9 (long-arms)- LoxP:HPH-MCS/; Plasmid/ΔERG9 (long-arms) - SeGAL2p: (ScOpt) SSL1-3-hinge-m:VPS13t -LoxP: HPH/; Plasmid/ΔERG9 (long-arms) - SeGAL2p: (ScOpt) SSL1-3-hinge:VPS13t -LoxP: HPH/; Plasmid/pxLEU:: FIT2-DGAT2/; Plasmid/ΔERG9 (long-arms) - SeGAL2p: (ScOpt) SSL1-3m:VPS13t -LoxP: HPH/; Plasmid/pUC57 LPP1-gRNA-GPDp:Cas9:ADH1t/; Plasmid/pD-BTS1gRNA-CAS9/; Plasmid/pESC-LEU-NOUR-SeGAL2p::SSL1-3m:VPS13t/; Plasmid/pD-ERG9gRNA-CAS9/; Plasmid/pxLEU - pGDP: mmFIT2: ADH1t/; Plasmid/pT-GAL80-MCS-HPH-GAL80 (delivered from Twist)/; Plasmid/pXURA-TEFp::HO:CYC1t/; Plasmid/10018 - pUC57-SeGAL2p-tHMGR-ADH1t/; Plasmid/pESC-Leu2-NrsR-SeGAl2p:BbSSfull:VPS13t/; Plasmid/10024 - pESC-LEU-NOUR-SuperMCS/; Plasmid/pESC-LEU-NOUR-TEFp::HO:CYC1t/; Plasmid/pxURA: SSL1_ScOpt/; Plasmid/10017 - pUC57-SeGAL2p-BSSfull-VPS13t/; Plasmid/pxLEU: BbSS trunc+Yeast hinge_ScOPT/; Plasmid/pUC57-LPP1gRNA/; Plasmid/pxUra: SSL3_ScOpt/; Plasmid/pD-ERG1gRNA-CAS9/; Plasmid/pxLEU: BbSS trunc_Yeast hinge+MSD_ScOpt
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. Kempinski has submitted a renewal of his IBC protocol entitled Yeast engineering for terpene production. Dr. Kempinski's current IBC protocol will expire on August 17, 2025. Enepret Inc. is engineering triterpene metabolism in Saccharomyces cerevisiae using genetically modified yeast strains to produce high-value compounds such as squalene, botryococcene, and astaxanthin. The protocol involves CRISPR/Cas9-mediated genome editing, integration of foreign genes from Botryococcus braunii and Xanthophyllomyces dendrorhous, and manipulation of the mevalonate pathway to enhance precursor flux. Although the yeast strains used are non-pathogenic and classified as RG1 organisms suitable for BSL1 containment, biohazardous risks include potential exposure to genetically modified organisms and recombinant DNA. Standard PPE—gloves, lab coats, and safety glasses—is required during handling to minimize exposure. Solid biohazardous waste is decontaminated via autoclave. Liquid biohazardous waste is decontaminated by adding fresh bleach (10% bleach v/v) for a minimum of 20 minutes prior to drain disposal. Work surfaces are decontaminated routinely with 10% bleach and/or 70% ethanol.

IBC Discussion & Vote:

The protocol IBC-25-101 (version 5.0) was approved.
*
Mike Mendenhall initiated the motion. Arthur Hunt seconded the motion. All IBC members present (14) voted in favor of the motion.
*
Conflicts of Interest: None
*

PI: Yadi Wu

IBC Protocol Number: IBC-25-88
Protocol Title: Characterize the role of EBF1 and Hivep2 in breast cancer progression
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section IV-B-7, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section III-D-1, NIH Guidelines Section III-F, NIH Guidelines Section III-F-8, OSHA 29 CFR 1910.1030

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Maximum Containment Level: Biological Safety Level 2 (BSL2), Animal Biological Safety Level 2 (ABSL2), Biological Safety Level 2 - Enhanced (BSL2+)
Primary Reviewers: C. Haughton, M. Mendenhall, J. Smalle

Brief Project Overview:

Breast cancer is the most common cancer in women and a major health problem. The clinical symptoms and outcome of breast cancer depend largely on whether it is confined to the breast or has spread to adjacent or distant parts of the body. In fact, approximately 90% of breast cancer deaths are caused by local invasion and distant metastasis of tumor cells, and the median survival time of patients with metastatic breast cancer is approximately 2 years. Thus, novel treatment strategies based on the biology of how breast cancer metastasizes are urgently needed to combat this life-threatening disease. EBF1 is a transcriptional factor of the EBF family. Despite both epidemiologic and global genetic evidence, EBF1 and Hivep2 play a crucial role in breast cancer progression. The function of EBF1 and Hivep2 in breast cancer has not been thoroughly explored.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Cell culture, Animal work (breeding, surgeries, etc.), Bacterial culture, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Genetics, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Transfection, Transformation, Use of infectious agents, Use of viral vectors, Viral culture, Creation 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)]: EBF1/EBF1 shRNA/human/transcription factor/expression/knockdown/human/pCMV; Lenti-LKO.1/; HIF1a/human/transcription factor/expression/human/pCMV/; GFP/jellyfish/Tracking/Tracking/human/pCMV-tag/; CDK6/human/kinase/expression/knockdown/human/pCMV-Tag foe expression;/; /Lenti-LKO.1 for knockdown/; p62/human/E3 ligase/knockdown/human/Lenti-LKO.1/; LSD1/human/enzyme/expression/knockdown/human/pCMV-Tag for expression;/; Lenti-LKO for knockdown/; Hivep2/human/transcription factor/knockdown/human/Lenti-LKO.1/; OGT/human/enzyme/knockdown/human/Lenti-LKO.1
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pCMV-Taq; Lenti-LKO.1
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/MCF7 /; Human/T47D/; Human/HEK293/; Human/MDA-Mb157/; Human/MDA-Mb435/; Human/MDA-MB231 /; Human/SUM159 /; Human/BT549 /; Animal/4T1 cell/; Human/293T cells/; Human/MDA-MB436 cells/; Human/MDA-MB468 cells/; Animal/EMT6/; Animal/MMTV-PyMT
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/Cells - Animal, genetically modified/mammary gland injection or lateral tail vein injection/Isoflurane Anesthesia /ABSL2/glove, mask and lab coat/ABSL1/No/The cells will be resuspended into PBS and cells will be injected into mammary gland fat pad. Injections are done in ABSL2 procedure room and housing in ABSL1 thereafter. /; Mouse/Cells - Human, genetically modified/mammary fat pad injection or lateral tail vein injection/Isoflurane Anesthesia /ABSL2/glove, mask and lab coat/ABSL1/No/The cells will be resuspended into PBS and injected into mammary fat pad or injected via the lateral tail vein. Injections are done in ABSL2

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procedure room and housing in ABSL1 thereafter.

Risk Assessment/Discussion:

Dr. Wu has submitted a renewal of her IBC protocol entitled Characterize the role of EBF1 and Hivep2 in breast cancer progression. Her current protocol will expire on October 3, 2025. Dr. Wu's laboratory investigates the molecular mechanisms underlying breast cancer metastasis, metabolism, and immune evasion, focusing on the roles of EBF1, Hivep2, CDK6, LSD1, and p62. The research involves molecular cloning, lentiviral transduction, human and murine cell culture, and animal models. Biohazardous risks include the use of recombinant DNA, lentiviral vectors, and human cell lines. All molecular cloning is conducted under BSL-1 using non-pathogenic DH5alpha E. coli strains, while cell culture and viral transductions are performed under BSL-2+ conditions in biosafety cabinets with lab coats, gloves, and eye protection. Lentivirus will be packaged in Dr. Wu's laboratory using 3rd generation packaging plasmids. Lentivirus will not be concentrated prior to use for cell transduction. Lentivirus-transduced cells are washed thoroughly before animal injection, and no live virus is administered to animals. Lentivirus transduced human and animal cells will be injected into mice via tail vein injection or mammary gland using ABSL2 containment. Mice are anesthetized for injections, which greatly minimizes risk of accidental needle stick. Animals will be housed at ABSL1. Potential exposure to lentiviral vectors is mitigated through use of BSC, appropriate PPE use, and proper waste decontamination (e.g., bleach and autoclaving) in accordance with UK Research Safety guidance.

IBC Discussion & Vote:

The protocol IBC-25-88 (version 9.0) was approved pending minor modifications as listed below:
*
SCIENTIFIC SUMMARY: Please clarify that the ultraviolet light will be turned off prior to wiping down the BSC with disinfectant.
*
Mike Mendenhall initiated the motion. Jan Smalle seconded the motion. All IBC members present (13) voted in favor of the motion.
*
Conflicts of Interest: Yadi Wu left the meeting early at 1:59PM and was not present for vote.
*

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UK Biosafety Manual Chapter Review

Biosafety Manual Working Draft.docx

The IBC had the following comments on submitted chapters:

1. Please make reference to the need for a double door entry from public corridors and other items addressed in the UK IBC Tissue Culture Facility policy.

*

Biosafety Manual text will be updated by BSO to reflect this information. The 2025 Biosafety Manual was approved with the changes described here. Doug Harrison initiated the motion. Tom Chambers seconded the motion. All IBC members present (13) voted in favor of the motion.

Incident Review

Nothing to report.

University of Kentucky (UK)
Institutional Biosafety Committee (IBC)

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).

Starr, Marlene, Obesity-mediated protection in sepsis and related studies of adipose tissue in health and disease, Amendment, BSO, IBC-24-507 (v.18.0), 8/7/2025
Campbell, Kenneth, Cellular level contractile function in human heart failure, Amendment, BSO, IBC-24-482 (v.34.0), 8/7/2025
Dutch, Rebecca, Purification of Viral Vectors with Electrodialysis Using Ultrafiltration Membranes, Amendment, BSO, IBC-24-398 (v.25.0), 8/7/2025
Helmy, Yosra, Evaluation of the efficacy of different probiotic strains and small molecules against diarrhea-causing pathogens, Amendment, BSO, IBC-25-12 (v.22.0), 8/6/2025
van Horne, Craig, Use of cell-based therapies for neurological conditions, Renewal, BSO, IBC-25-81 (v.10.0), 8/5/2025
Kolodziej, Andrew, BICARD: Biopsy Collection and Repository Database, Amendment, BSO, IBC-24-486 (v.18.0), 8/5/2025
Vaillancourt, Lisa, Genetic and Genomic Studies of Associations of Pathogenic Fungi (Colletotrichum, Fusarium, Stenocarpella) with Plants, Amendment, BSO, IBC-24-442 (v.21.0), 8/5/2025
Rao, Madhumathi, Metabolic Bone Disease Registry, Amendment, BSO, IBC-24-87 (v.29.0), 8/5/2025
Tong, Sheng, Development of magnetic-responsive nanomaterials for disease treatment, Amendment, BSO, IBC-25-47 (v.19.0), 8/5/2025
Stumpf, Elizabeth, Stumpf Lab Protocol, Renewal, BSO, IBC-25-92 (v.10.0), 7/29/2025
Palli, Reddy, Insect Physiology, Biochemistry and Molecular Biology, Amendment, BSO, IBC-24-82 (v.20.0), 7/29/2025
Yang, Xiuwei, The critical role of integrin-associated CD151 in progression of ER-negative mammary tumors, Amendment, BSO, IBC-25-28 (v.27.0), 7/25/2025
Pinto, Amelia, Risk group two virus protocol, Amendment, BSO, IBC-24-83 (v.37.0), 7/25/2025
Hersh, Louis, Peptidases in Human Disease, Amendment, BSO, IBC-24-390 (v.22.0), 7/24/2025
Shoemaker, Robin, Biosafety protocol for Shoemaker lab, Amendment, BSO, IBC-24-434 (v.16.0), 7/24/2025
Satin, Jonathan, IBC-24-423 (formerly B21-3780-M2): Long-term Regulation of Cardiac Ion Channels / Cardioprotection, Amendment, BSO, IBC-24-423 (v.27.0), 7/23/2025
Fu, Jian, Molecular mechanisms of lung and vascular inflammation, injury and repair, Renewal, BSO, IBC-25-82 (v.12.0), 7/23/2025
D'Orazio, Sarah, Role of cell wall enzymes in the virulence of Streptococcus species, Amendment, BSO, IBC-24-281 (v.23.0), 7/22/2025
Sullivan, Patrick, Platelet Bioenergetics after Traumatic Brain Injury, Renewal, BSO, IBC-25-68 (v.10.0), 7/21/2025
Xu, Ren, Microenvironment network in breast cancer development and progression, Amendment, BSO, IBC-24-40 (v.23.0), 7/16/2025
Morris, Ann, Molecular genetics of ocular development, differentiation, and regeneration in zebrafish, Amendment, BSO, IBC-24-432 (v.17.0), 7/16/2025
Piranavan, Paramarajan, A Phase 2, adaptive, randomized, open-label, assessorblinded active-controlled study to evaluate the efficacy and safety of rapcabtagene autoleucel versus Standard of Care in patients suffering from systemic lupus erythematosus (SLE) with active, refractory lupus nephritis (LN), Amendment, BSO, IBC-24-422 (v.28.0), 7/9/2025
Stumpf, Elizabeth, Stumpf Lab Protocol, Amendment, BSO, IBC-24-286 (v.31.0), 7/9/2025

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Park, Jonghyuck, Reprogramming Neuroimmune Responses for Functional Regeneration after Spinal Cord Injury; Developing immunotherapeutic nanoparticles for spinal cord injury; Enhancing the Therapeutic Potential of Nanoparticle-Mediated Immunotherapeutics for Spinal Trauma, Amendment, BSO, IBC-24-270 (v.33.0), 7/8/2025
Yamasaki, Tritia, Synuclein seeding activity as a biomarker in Parkinson Disease, Amendment, BSO, IBC-24-477 (v.29.0), 7/8/2025
Li, Feng, Rotavirus Project, Amendment, BSO, IBC-24-388 (v.14.0), 7/7/2025
Zuidema, Jonathan, Synthetic nucleic acid molecules delivery and incorporation into biomaterials, New, BSO, IBC-25-56 (v.12.0), 7/3/2025
Dreaden, Tyler, B22-4004-M: Fungal and bacterial plant pathogen ID from culture, tissue and soil, Renewal, BSO, IBC-25-63 (v.10.0), 7/3/2025
Sompol, Pradoldej, Oxidative Stress and Related Neurovascular Dysfunction in Alzheimer's Disease and Related Dementias, Amendment, BSO, IBC-25-42 (v.19.0), 7/3/2025
Papazoglou, Ioannis, Neuronal response to hypoglycemia, Amendment, BSO, IBC-24-35 (v.30.0), 7/2/2025
Kern, Philip, Inflammation in & Regulation of Obesity, Amendment, BSO, IBC-24-325 (v.17.0), 7/2/2025

Protocols Meeting Registration Requirements

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

Gipson-Reichardt, Cassandra, Glutamate, Neuroinflammation, Acetylcholine, HIV and Addiction, Amendment, IBC, IBC-24-350 (v.68.0), 8/5/2025
Morganti, Josh, Aging and Disorders of the CNS, Renewal, IBC, IBC-25-49 (v.13.0), 7/30/2025
Spry, Malinda, Thomas Hunt Morgan Biology Department Teaching Labs, New, IBC, IBC-25-65, 7/25/2025
Gonzalez Duque, Octavio, Effect of oral bacteria on innate epithelial responses, Renewal, IBC, IBC-25-70 (v.18.0), 7/24/2025
Murphy, M. Paul, Adeno Associated Viruses (AAV) for ADRD Models, New, IBC, IBC-25-54 (v.13.0), 7/23/2025
Fry, Christopher, Cellular regulators of skeletal muscle plasticity, Renewal, IBC, IBC-25-74 (v.14.0), 7/21/2025
Galperin, Emilia, Regulation of MAPK activity by EGF receptor endocytosis, Amendment, IBC, IBC-24-154 (v.35.0), 7/18/2025
Shridas, Preetha, Inflammation, lipoproteins and chronic disease., Renewal, IBC, IBC-25-59 (v.14.0), 7/18/2025
Lee, Young-Sam, B22-3975: cellular metabolites regulating cancer cell adaptation, Renewal, IBC, IBC-25-64 (v.12.0), 7/14/2025
Helsley, Robert, Macronutrient metabolism in Cardiometabolic Disease, Renewal, IBC, IBC-25-78 (v.10.0), 7/10/2025
Lu, Hong, Mechanisms of Aortic Aneurysm and Atherosclerosis, Renewal, IBC, IBC-25-79 (v.18.0), 7/10/2025
D'Orazio, Sarah, Host-pathogen Interactions during Listeria monocytogenes infection, Amendment, IBC, IBC-24-42 (v.33.0), 7/9/2025
Liu, Xiaoqi, Enhancing the efficacy of prostate cancer therapy, Amendment, IBC, IBC-24-355 (v.33.0), 7/3/2025

IBC Training

Delena Mazzetti reminded IBC members that IBC Member training is available in the SciShield Course Directory (https://uky.scishield.com/raft/training/courses) and that all IBC members must complete IBC member training annually.

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Adjournment

Doug Harrison initiated a motion to adjourn the meeting at 2:07pm. Tom Chambers seconded the motion. All IBC members present (13) voted in favor of the motion.

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