

University of Kentucky

Institutional Biosafety Committee (IBC) Meeting

Date: 01JULY2026

Time: 12:02PM – 1:44PM

Location: Virtual Meeting via Teams -

<https://teams.microsoft.com/meet/228287260728865?p=4Q1RK0guL8LLoUMS7e>

Minutes

Call to Order

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

Attendance

IBC Members Present

Doug Harrison (Chairperson)

Cheryl Haughton (Animal Containment Expert)

Maria Landron (Local, Non-Affiliated Member)

Brandy Nelson (Institutional Member)

Delena Mazzetti (Biological Safety Officer)

Mike Mendenhall (Local, Non-Affiliated Member)

Carol Pickett (Local, Non-Affiliated Member)

Arthur Hunt (Plant Containment Expert)

Delphine Malherbe (Laboratory Staff Representative)

Jan Smalle (Plant Containment Expert)

Amelia Pinto (Institutional Member)

Regrets

Carrie Shaffer (Institutional Member)

Thomas Chambers (Institutional Member)

Yadi Wu (Institutional Member)

Elizabeth Brooks (Administrative Support Associate I)

Guests

Marisa McGrath (Associate Biological Safety Officer)

Robert Hayman (Assistant Biological Safety Officer)

Jeff Howell (IBC Administrative Professional II)

Audra Strahl (IBC Administrative Professional II)

Melissa Hollifield (Animal Compliance Manager)

Quorum

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

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Approval of Previous Month's Meeting Minutes

[2026.06.03 IBC Meeting Minutes DRAFT.pdf](#)

The previous month's minutes were approved. Doug Harrison initiated the motion. Amelia Pinto seconded the motion. All members present (11) voted in favor.

Old Business

None.

New Business

Protocol Review

Resubmissions

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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: Organisms used in research, Manipulations planned

Applicable Guidelines & Regulations: OSHA 29 CFR 1910.1030, 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-D-7, NIH Guidelines Section III-D-1, NIH Guidelines Section III-D-2, NIH Guidelines Section III-E-1, NIH Guidelines Section III-F-3

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

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

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, Use of Human Source Material(s), Use of viral vectors

Transport: Yes

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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/ ; 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)/ 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/ ; Influenza C Virus (RG2-virus)/ Humans/ ; Influenza A Virus (RG2-virus)/ Birds

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 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

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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-acetylglucosamine 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/ Polymerase/ 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

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Risk Assessment/Discussion:

Dr. Li has submitted an amendment to his IBC protocol entitled Influenza D Virus Entry and Tissue Tropism. In this amendment, Dr. Li has updated administrative information, personnel and locations, added a new strain of Influenza C virus from CDC and an inactivated H2N3 Influenza A virus from the University of Missouri, and added two projects. The first new project will involve the collection of fecal and environmental samples from wildlife and poultry farms for the surveillance of H5N1. Dr. Li lab personnel will collect fecal and environmental samples from local ponds and rivers to collect feces of wild geese and birds. Samples are immediately placed in PrimeStore MTM, which will denature proteins and inactivate any pathogens present. RNA will be extracted for RT-PCR and cDNA library construction. Air samples will be collected from poultry farms, and filters soaked in PrimeStore MTM for RNA extraction as described above. This work will be completed using BSL2 practices and procedures. The second project will utilize an inactivated influenza A H2N3 prepared by colleagues at the University of Missouri. H2N3 is a low pathogenic strain of avian influenza virus and will be inactivated prior to shipment via BPL. The addition of these new projects does not significantly alter the biohazardous risks associated with the work in Dr. Li's laboratory.

IBC Discussion & Vote:

The amendment to IBC-24-32 (version 42.0) was approved pending minor modifications as listed below:

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SCIENTIFIC SUMMARY:

1. Please include a more detailed description of hand- and fecal sample container-disinfection. Will hand sanitizer be used after collection? Will disinfectants be available in case of spills? Will the containers be disinfected before transportation?
2. Please include information about the signs and symptoms of exposure to H5N1 including proper response and awareness of field work hazards, <https://www.cdc.gov/niosh/outdoor-workers/about/index.html>.

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Doug Harrison initiated the motion. Brandy Nelson seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Feng Li

IBC Protocol Number: IBC-24-388

Protocol Title: Rotavirus Project

Protocol Type: Amendment

Amendment To: Organisms used in research, Manipulations planned

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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: B. Nelson, D. Harrison, C. Pickett

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; Equine Arteritis Virus (EAV) (RG1-virus)/Equine; Equine Norovirus (RG1-virus)/Equine; Equine Picornavirus (hepatovirus) (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/NSP6/ 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/

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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 / ; 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 current IBC protocol entitled Rotavirus Project to add locations and three new equine infectious agents - Equine Arteritis Virus (EAV), Equine Norovirus, and Equine Picornavirus (hepatovirus). Dr. Li's laboratory has recently discovered a new norovirus and new picornavirus while deep sequencing foal diarrhea samples that may be responsible for foal diarrhea cases in 2026. They will receive foal diarrhea samples from collaborators for RNA extraction. Positive samples will be sequenced. Isolated virus will be cultured in animal and human cells. Although neither of these viruses are known to be pathogenic in humans, work with these materials will take place using BSL2 practices and procedures. The second new project involves the resumption of EAV surveillance as one of the EAV reference laboratories for the World Organization for Animal Health. Equine samples will be received for RT-qPCR and serum neutralization assays. EAV is not known to be pathogenic in humans; however, care will still be taken to minimize potential exposure by wearing lab coat, gloves, and eye protection, and working within a BSC. The addition of these new equine pathogens does not significantly alter the biohazardous risks associated with the work described in this IBC protocol.

IBC Discussion & Vote:

The amendment to IBC-24-388 (version 36.0) was approved.

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Doug Harrison initiated the motion. Brandy Nelson seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Christopher Fry

IBC Protocol Number: IBC-25-74

Protocol Title: Cellular regulators of skeletal muscle plasticity

Protocol Type: Amendment

Amendment To: Genetic constructs, Laboratory or Greenhouse procedures, Manipulations planned, Personnel, Administrative Information

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), OSHA 29 CFR 1910.1030, NIH Guidelines Section III-D-1, NIH Guidelines Section III-F, NIH Guidelines Section III-F-1, NIH Guidelines Section III-E-1

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

Primary Reviewers: C. Haughton, M. Landron, M. Mendenhall

Brief Project Overview:

The goal of our research is to understand the cellular and molecular mechanisms that allow striated skeletal muscle to adapt to external stimuli (i.e. atrophy and hypertrophy). We study cells, animals and human subjects to better understand how muscle adapts when challenged to develop interventions to improve muscle health and function in conditions where it is compromised, like weakness and frailty associated with illness and aging.

Summary of Biohazard Materials & Manipulations:

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

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)]: Follistatin/ mouse / cell growth/housekeeping/ expression/ Mouse/ plasmid/ ; Cre recombinase/ bacteriophage P1/ recombinase/ expression/ mouse cells/ Ad5CMV/ ; GFP/ jellyfish/ tracking/ expression/ mouse / Ad5CMV/ ; GDF8 (myostatin)/ mouse/ skeletal muscle cell regulation/ silencing/ mouse cells/ siRNA/miRNA/ ; Histone deacetylase 4 (HDAC4)/ mouse/ skeletal

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muscle cell regulation/ skeletal muscle cell regulation/ skeletal muscle cell regulation/ plasmid, AAV/ ; RRAD/ Homo sapiens/ small GTPase superfamily/ Expression/ mouse, human (primary cells)/ AAV/ ; tdTomato/ disc corals of the genus Discosoma/ reporter/ expression/ mouse/ AAV/ ; green fluorescent protein (GFP)/ jellyfish/ tracking/ expression/ Mouse/ plasmid

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/ Fst (NM_008046) Mouse Tagged ORF Clone/ ; Adenovirus/ Ad5CMVCre-eGFP/ ; Plasmid/ HDAC4 (NM_207225) Mouse tagged ORF Clone/ ; Plasmid/ GFP Mouse tagged ORF clone/ ; Naked nucleic acid/ Mstn Mouse siRNA Oligo Duplex (Locus ID 17700)/ ; Naked nucleic acid/ siRNA Related Product, Trilencer-27 Universal scrambled negative control siRNA duplex, SKU SR30004/ ; Adeno-Associated Virus (AAV)/ pscAAV[Exp]-{CMV-T7 from pCMV6-Entry}>{hRRAD[NM_001128850.2]-tdTomato}/ ; Adeno-Associated Virus (AAV)/ pAAV[Exp]-CMV>mHdac4[NM_207225.2]:WPRES3/ ; Adeno-Associated Virus (AAV)/ pAAV[Exp]-TRE>mHdac4[NM_207225.2]:WPRES3/ ; Adeno-Associated Virus (AAV)/ pscAAV[Exp]-{CMV-T7 from pCMV6-Entry}>{hRRAD[NM_001128850.2]-tdTomato}/ ; Adeno-Associated Virus (AAV)/ pscAAV[Exp]-{CMV-T7 from pCMV6-Entry}>{tdTomato}/ ; Adeno-Associated Virus (AAV)/ pscAAV[Exp]-{CMV-T7 from pCMV6-Entry}>{tdTomato}

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/ Myoblast/ ; Animal/ Fibroblast/ ; Human/ Myoblast/ ; Human/ Fibroblast/ ; Human/ MRC-5 fibroblast/ ; Animal/ C2C12

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/ Plasmid/ electroporation/ isoflurane anesthesia/ ABSL1/ gloves, labcoat, eye protection/ ABSL1/ No/ N/A/ ; Mouse/ Viral Vector - Adeno-Associated Virus (AAV)/ Intramuscular/ anesthesia/ ABSL1/ Lab coat, gloves, eye protection/ ABSL1/ No/ N/A

Risk Assessment/Discussion:

Dr. Fry has submitted an amendment to his current IBC protocol entitled Cellular regulators of skeletal muscle plasticity to update personnel, administrative details, and add a project utilizing adeno-associated virus (AAV) vectors in mice. AAVs obtained from VectorBuilder will be administered intramuscularly to anesthetized mice using ABSL1 procedures. The AAV vectors are engineered to express HDAC4 (histone deacetylase 4 responsible for skeletal muscle cell regulation), RRAD (small GTPase superfamily), and tdTomato (a reporter gene). Mice will be returned to ABSL1 housing, and 4-6 weeks after AAV transduction, mice will undergo quadriceps muscle contractile function testing. Once testing is complete, mice are euthanized and hind limb muscles extracted for Western blotting and RNA sequencing using BSL1 procedures. In addition to the in vitro work, they will also use the same AAVs to transduce primary human cells. After AAV transduction, cells will undergo calcium imaging and protein collection for Western blot. AAV work in cell culture will be completed in a certified BSC wearing lab coat, disposable gloves, eye protection, and surgical mask at BSL2 containment. Dr. Fry is currently approved for other viral vector work using adenovirus at BSL2+ containment. The addition of this new project does not significantly alter the biohazardous risks associated with this IBC protocol.

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IBC Discussion & Vote:

The amendment to IBC-25-74 (version 36.0) was approved pending minor modifications as listed below:

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ANIMAL RESEARCH – Animal IACUC: Please add the new IACUC protocol number 2026-4817

LOCATIONS – Research Locations Table: Please update this table to include entries for DLAR locations.

LOCATIONS – Biological Safety Equipment: Please mark, “Exposure of animals to biohazardous materials,” as one of the Biological Safety Cabinet (BSC) Procedures.

LOCATIONS – Biological Safety Cabinet Table: Please include entries for BSCs used in DLAR.

SCIENTIFIC SUMMARY: Please remove “etc,” from the description of work as the summary should contain a list of all assays being performed.

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Michael Mendenhall initiated the motion. Cheryl Haughton seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Xiaoqi Liu

IBC Protocol Number: IBC-24-114

Protocol Title: Plk1 in epigenetics of prostate cancer development and progression

Protocol Type: Amendment

Amendment To: Personnel, Genetic constructs, Manipulations planned

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section IV-B-7, UK Administrative Regulation 6.3, OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.9, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section III-E, NIH Guidelines Section III-E-3, NIH Guidelines Section III-F, NIH Guidelines Section III-F-8, NIH Guidelines Section III-F-1, NIH Guidelines Section III-D-4

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

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

Brief Project Overview:

We aim to understand the underlying mechanisms of cancer development and therapy resistance. Based on this, we will eventually develop new approaches to treat cancer. The approaches of our study cover standard cell culture and mouse work. Our work focuses on a gene called Polo-like kinase 1 (Plk1), a critical cell cycle regulator, whose expression is elevated in many cancers. To study the role of Plk1 in cancer progression and therapy resistance, we need to use various approaches (standard plasmid-

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based transfection, lentivirus-based infection to express or deplete Plk1 or other genes in human cancer cell lines). The personnel in the protocol has sufficient experience to use these agents and well aware the potential danger-associated with these agents. We will work with your committee closely to ensure we strictly follow the rule.

Plk1 is well known as a protein kinase playing critical roles in cell cycle regulation. The lab studies how Plk1-associated kinase activity regulates functions of different substrates, including EZH2 and BRD4. EZH2 is a component of PRC2 complex, responsible for H3K27 trimethylation. BRD4 recognizes acetylated histones. In addition, our lab also aim to identify whether PLK1 can phosphorylates two transcription factors, ASCL1 and Neurod1. which are critical drivers to promote neuroendocrine prostate cancer development. PLK1 can also phosphorylate two key factors-Nanog and HOXB13, which contribute to stemness and prostate cancer progression. In addition, three transcription factors, TTF1(NKX2.1), NMYC and Oct4 also been identified as PLK1 phosphorylation substrates, and these phosphorylation events can promote stemness and neuroendocrine development in prostate cancer.

Prostate cancer reconstitution model is well used to study on the development and differentiation of prostate cancer. Briefly, we will implant the new mouse cell line RB1/P53-DKO/myc-KI, which induces phenotype of neuroendocrine prostate cancer, into the kidney of NSG mice. The personnel in the protocol has sufficient experience to handle this procedure. We will work with your committee closely to ensure we strictly follow the rule.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, Histology, Genetics, PCR/qRT-PCR, Transformation, Propagation of Infectious Agents, Transfection, DNA/RNA isolation/purification, Creation of Viral Vectors, Use of Viral Vectors, Use of Human Source Material(s), 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)]: Plk1 / Mouse and Human / Cell Cycle/Division/ Expression or Knockdown / human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPRE, N2P1, N2P2, N2P3, NCI-H660, LAPSCPC-1, DU145/ pLKO.1 and pLenti-GIIICMV-C-term-HA, FuCRW, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; EZH2/ Mouse and Human / Oncogene / Expression or Knockdown / human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPRE, N2P1, N2P2, N2P3, NCI-H660, LAPSCPC-1, DU145 E.Coli/ pLKO.1 and pLenti-GIIICMV-C-term-HA, pET-GST, pCDNA3.1, pLV-puro or pLV-EF1a-IRES-Hygromycin, FuCRW/ ; BRD4/ Human / Oncogene / Expression or Knockdown / human PCa cells: LNCaP, C4-2, 22Rv1, DU145, PC3, NHPRE. Mouse prostate cancer cell: TRAMP-C2. E.Coli/ pLKO.1 and pLenti-GIIICMV-C-term-HA,pCDNA3.1, tetpLKO-puro, pCW57-RFP-P2A-MCS, FuCRW, pET-GST, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; SPOP/ Human / Tumor Suppressor / Expression or Knockdown / human PCa cells: LNCaP, C42, C4-2B, 22Rv1, DU 145,

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and PC3/ pCDNA3.1, pLenti-CMVpuro, tet-pLKO-puro, pCW57-RFP-P2A-MCS, pLKO.1, FuCRW/ ; FGF10/ mouse/ Oncogene / Expression / mouse UGSM / FuCGW/ ; GFP/ Jellyfish/ Tag/ Expression / human PCa cells: LNCaP, C42, 22Rv1, DU 145, and PC3/ pGFP/ ; Luciferase/ Firefly/ Tag/ Expression / human PCa cells: LNCaP, C42, 22Rv1, DU 145, and PC3/ pLV-Luc/ ; Rb1/ Mouse and Human/ Regulatory Gene/ Knockdown / human PCa cells: LNCaP, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW/ ; P53/ Mouse and Human/ Regulatory Gene/ Knockdown / human PCa cells: LNCaP, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW/ ; ASCL1/ Mouse and Human/ Oncogene / Knockdown or Expression / human PCa cells: LNCaP, LNCaP/AR/tet-sh-P53&Rb1, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; Neurod1/ Mouse and Human/ Oncogene / Knockdown or Expression / human PCa cells: LNCaP, LNCaP/AR/tet-sh-P53&Rb1, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; HOXB13/ Mouse and Human/ Oncogene/ Knockdown or Expression / human PCa cells: LNCaP, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; Nanog/ Mouse and Human/ Oncogene/ Knockdown or Expression / human PCa cells: LNCaP, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; NMYC/ HUMAN/ ONCOGENE/ Expression or Knockdown/ Human PCa cells: LNCaP, C4-2, 22Rv1, PC3, NHPRE, N2P1, N2P2, N2P3, NCI-H660, LAPSCPC-1, DU145/ pLKO.1 and pLenti-GIIICMV-C-term-HA, pET-GST, pCDNA3.1, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; TTF1/ Mouse and Human/ Oncogene/ Expression or Knockdown/ Human PCa cells: LNCaP, C4-2, 22Rv1, PC3, NHPRE, N2P1, N2P2, N2P3, NCI-H660, LAPSCPC-1, DU145/ pLKO.1 and pLenti-GIIICMV-C-term-HA, pET-GST, pCDNA3.1, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; OCT4/ Mouse and Human/ oncogene/ Expression or Knockdown/ Human PCa cells: LNCaP, 16D, C4-2, 22Rv1, PC3, NHPRE, N2P1, N2P2, N2P3, NCI-H660, LAPSCPC-1, DU145; mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1 and pLenti-GIIICMV-C-term-HA, pET-GST, pCDNA3.1, pLV-puro or pLV-EF1a-IRES-Hygromycin, FuCRW/ ; Stat6/ Mouse and Human/ Oncogene/ Knockdown or Expression / human PCa cells: LNCaP, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; ZEB1/ Mouse and Human/ Oncogene/ Knockdown or Expression / human PCa cells: LNCaP, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ; ZEB2/ Mouse and Human/ Oncogene/ Knockdown or Expression / human PCa cells: LNCaP, mouse prostate epithelia cells (RB1/P53-DKO/myc-KI)/ pLKO.1, FuCRW, pLV-puro or pLV-EF1a-IRES-Hygromycin/ ;

Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/ pLKO.1 puro / ; Plasmid/ pEGFP-N1 / ; Lentivirus/ pLV-puro/ ; Plasmid/ pCDNA3.1/ ; Lentivirus/ tet-pLKO-puro/ ; Lentivirus/ pCW57-RFP-P2A-MCS/ ; Lentivirus/ pLV-EF1a-IRES-Hygromycin/ ; Plasmid/ pCDNA6-GFP/ ; Lentivirus/ FU-CGW/ ; Lentivirus/ FU-CRW/ ; Lentivirus/ pLenti-GIII-CMV-Cterm-HA_1/ ; Plasmid/ pCMV-VSV-G/ ; Plasmid/ pET-GST/ ; Lentivirus/ pLV-EF1a-P2A-GFP-Cre-hygro

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/ LNCaP/ ; Human/ PC3/ ; Human/ 22Rv1/ ; Human/ Du145/ ; Human/ VCaP/ ; Human/ C4-2B/ ; Human/ C4-2R/ ; Human/ MR49F/ ; Human/ 22Rv1-R/ ; Animal/ TRAMP-C2/ ; Human/ LNCaP-Plk1/ ; Human/ C4-2-BRD4/ ; Human/ C4-2-BRD4-S24A/S1100A/ ; Human/ C4-2-BRD4-S24D/S1100D/ ; Human/ 22Rv1-BRD4/ ; Human/ 22Rv1-

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BRD4-S24A/S1100A/ ; Human/ 22Rv1-BRD4-S24D/S1100D/ ; Human/ C4-2B/SPOP-F133V/ ; Human/ NHPRE/ ; Animal/ UGSM/ ; Human/ LNCaP-EZH2-WT/ ; Human/ LNCaP-EZH2-T144A/ ; Human/ LNCaP-EZH2-T144D / ; Animal/ TRAMP-BRD4-S24A/S1100A/ ; Animal/ TRAMP-BRD4-S24D/S1100D/ ; Human/ NCI-H660 / ; Human/ N2P1/ ; Human/ LNCaP/AR/tet-sh-P53&Rb1/ASCL1-T233A/ ; Human/ LNCaP/AR/tet-sh-P53&Rb1/ASCL1-T233D/ ; Human/ LNCaP/AR/tet-sh-P53&Rb1/ASCL1-WT/ ; Human/ LNCaP/AR/tet-sh-P53&Rb1/Neurod1-WT/ ; Human/ LNCaP/AR/tet-sh-P53&Rb1/Neurod1-S52A/ ; Human/ LNCaP/AR/tet-sh-P53&Rb1/Neurod1-S52D/ ; Human/ N2P2/ ; Human/ 22Rv1-EZH2-WT / ; Human/ N2P1-EZH2-T144A/ ; Animal/ RB1/P53-DKO/myc-KI/ ; Human/ N2P1-NMYC-S400A/ ; Human/ N2P1-NMYC-S400D/ ; Human/ 22RV1-NMYC-S400A/ ; Human/ 22RV1-NMYC-S400D/ ; Human/ N2P1-TTF1-S23A/ ; Human/ N2P1-TTF1-S23D/ ; Human/ 16D/ ; Human/ N2P1-OCT4-3A/ ; Human/ N2P1-Oct4-3D/ ; Human/ 16D-Oct4-3A/ ; Human/ 16D-Oct4-3D/ ; Human/ N2P3/ ; Human/ LAPSCPC-1/ ; Human/ 22Rv1-TTF1-S23A/ ; Human/ 22Rv1-TTF1-S23D / ; Animal/ PCa PT/ ; Animal/ PLKPT

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/ SQ, IP, IV, orthotopic injection/ restrainer/gas anesthesia such as isoflurane/ ABSL2/ double-layered gloves, lab coats and eye protection/face mask/ ABSL2/ No/ castration/ ; Mouse/ Cells - Human, genetically modified/ SQ, IP, IV, orthotopic injection/ restrainer/gas anesthesia such as isoflurane/ ABSL2/ double-layered gloves, lab coats and eye protection/face mask/ ABSL2/ No/ castration; sub-renal capsule implantation/ ; Mouse/ Tissue - Human (ex. PDX tumor tissue)/ SQ tumor implantation/ gas anesthesia such as isoflurane/ ABSL2/ double-layered gloves, lab coats and eye protection/face mask/ ABSL2/ No/ human tumor inoculation/ ; Mouse/ Cells - Animal, genetically modified/ IV injection/ gas anesthesia such as isoflurane/ ABSL2/ double-layered gloves, lab coats and eye protection/face mask/ ABSL2/ No/ Tail vein injection

Risk Assessment/Discussion:

Dr. Liu has submitted an amendment to his current IBC protocol entitled Plk1 in epigenetics of prostate cancer development and progression. In this amendment, Dr. Liu includes an update to lab personnel and lentiviral constructs for prostate cell line transduction. This new project is very similar to currently approved work in Dr. Liu's laboratory and involves the lentivirus transduction of human and mouse prostate cancer cell lines for administration to mice via tail vein injection or seeded onto the kidney. The new lentivirus constructs will be produced as previously described and approved and will be delivered to cells to knock down expression of Stat6, ZEB1, and ZEB2, or overexpress Stat 6 2A, Stat 6 2D, ZEB1, and ZEB2. The new gene targets are known oncogenes, and Dr. Liu's laboratory has extensive experience working with oncogenic lentivirus constructs. Work with lentivirus will be done at BSL2+ containment, as previously described and approved. The addition of this new experiment does not significantly alter the biohazardous risks associated with Dr. Liu's currently approved IBC protocol. There is an IBC hold on the corresponding IACUC protocol, 2020-3678.

IBC Discussion & Vote:

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The amendment to IBC-24-114 (version 57.0) was approved pending minor modifications as listed below:

*

ANIMAL RESEARCH – Animals with Biohazards table: ABSL Housing for all entries should be ABSL1. ABSL Experimental Procedures for genetically modified animal cells should be ABSL1. Please update the table accordingly.

RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information table: Please add an entry in this table for CRE.

RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Vector Information table: Please update this table to accurately reflect gene editing work as described in the updated Scientific Summary text, if applicable.

SCIENTIFIC SUMMARY:

1. For the work added in this amendment, please describe the downstream assays and manipulations planned for xenograft mice and their tissues. If these assays and manipulations are described elsewhere in the Scientific Summary text, please reference those sections specifically.
2. Please clarify “gene editing” in the new work added in this amendment and clarify how this is done, if applicable.

*

Doug Harrison initiated the motion. Delphine Matherbe seconded the motion. All IBC members present (11) voted in favor of the motion.

*

Conflicts of Interest: None

*

PI: Kiersten Wise

IBC Protocol Number: IBC-24-129

Protocol Title: Management of Corn, Sorghum and Forage Diseases

Protocol Type: Amendment

Amendment To: Laboratory Location(s), Personnel, Laboratory or Greenhouse procedures

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

Maximum Containment Level: Biological Safety Level 2 - Plants (BSL2-P)

Primary Reviewers: A. Hunt, J. Smalle, D. Harrison

Brief Project Overview:

Research conducted in the Wise lab encompasses the most common diseases of Kentucky field crops, including corn, sorghum, and forages. The pathogens we research are plant pathogens only, endemic to Kentucky, often with a very specific host range. They are not known to infect healthy humans, animals or

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wildlife, including non-host plants. Exposure to these pathogens should not have adverse effects on personnel, especially when using defined research procedures. My research studies the biology of these pathogens (primarily in the laboratory) and evaluates management options for these pathogens (primarily in the field).

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Imaging/Microscopy, DNA/RNA isolation/purification, PCR/qRT-PCR, Plant work, Use of Infectious Agents, Field trial(s), Propagation of infectious agents

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Exserohilum turcicum (RG1-fungus) / Zea mays/ ; Cercospora zeae-maydis (RG1-fungus)/ Zea mays/ ; Stenocarpella maydis (RG1-fungus)/ Zea mays, bamboo/ ; Stenocarpella macrospora (RG1-fungus)/ Zea mays/ ; Pythium ultimum (RG1-fungus)/ Broad host range including corn, soybean, potato, wheat, fir, and many ornamental species./ ; Fusarium graminearum (RG1-fungus)/ Zea mays,wheat and barley/ ; Fusarium verticillioides (RG1-fungus)/ Zea mays, sorghum/ ; Rhizoctonia solani (RG1-fungus)/ Broad host range including turfgrass, soybean, potatoes, sugar beet, cucumber and rice/ ; Curvularia lunata (RG1-fungus)/ Plants and soil in tropical and temperate countries

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

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

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

Animal Use: No

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

Risk Assessment/Discussion:

Dr. Wise has submitted an amendment to her currently approved IBC protocol entitled Management of Corn, Sorghum and Forage Diseases. In this amendment, Dr. Wise includes updates to laboratory personnel, laboratory locations, and procedures in the laboratory and field work. She has also updated the source of fungal isolates used in the laboratory and growth chambers to include isolates originating from other states within the US. She has included an updated PPQ 526 permit for the receipt and laboratory/growth chamber use of these out-of-state isolates. Importantly, the field trials planned will only utilized isolates obtained from Kentucky, most often from Uk research farms. The fungal pathogens in use are not known to infect healthy humans or animals. Work will be completed using BSL2-P containment. The updates in this amendment do not significantly alter the biohazardous risks associated with Dr. Wise's IBC protocol.

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IBC Discussion & Vote:

The amendment to IBC-24-129 (version 15.0) was approved.

★

Doug Harrison initiated the motion. Arthur Hunt seconded the motion. All IBC members present (11) voted in favor of the motion.

★

Conflicts of Interest: None

★

PI: Rebecca Dutch

IBC Protocol Number: IBC-24-134

Protocol Title: Paramyxovirus and pneumovirus protein function

Protocol Type: Amendment

Amendment To: Personnel, Genetic constructs, Manipulations Planned

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

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

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

Brief Project Overview:

The paramyxovirus and pneumovirus families contain established major human pathogens, including measles virus, respiratory syncytial virus, human metapneumovirus, and human parainfluenza virus 3 (HPIV3). The paramyxoviruses also include the newly emerged human pathogens Hendra virus and Nipah virus. Our laboratory focuses on understanding the molecular details of infection by these viruses, from the starting point of viral entry to the ending steps of viral spread. The fusion (F) protein of each virus promotes fusion of viral and host cellular membranes, an event that is both an essential step mediating entry of enveloped viruses into the host cell and an excellent model system for investigating the basic molecular events in any membrane fusion process. The long-term objectives of our research are:

- To understand the precise mechanism(s) of the paramyxovirus F proteins in promotion of membrane fusion, to delineate the events involved in cellular entry of these important pathogens, and to aid in developing new antiviral treatment
- To understand how paramyxovirus glycoproteins work in tandem with other viral and host proteins to allow spread of the virus to new target cells;
- To decipher the molecular details of replication of these viruses

To achieve these goals, we study both the synthesis and function of paramyxoviral glycoproteins in transient expression systems, and determine specific residues or regions of the proteins involved in

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critical processes. We utilize pseudotype particle systems, in which the viral glycoproteins are incorporated into non-infectious particles, to study entry promoted by the Hendra and Nipah virus glycoproteins, allowing us to determine regions of the proteins and cellular components that play important role in initial events in infection. We also study virus-like particle formation as part of a large grant to improve vaccine approaches. Utilizing infection with human metapneumovirus, a recently identified, ubiquitous human pathogen, to analyze trafficking events involved in paramyxovirus entry, and to study the replication, assembly and spread of the virus. Finally, our laboratory will utilize the non-pathogenic henipavirus, Cedar virus, to analyze the critical process of virus entry and replication, providing critical new information for a viral family that contains several members of pandemic potential. Our research protocols involve recombinant DNA techniques, the use of human and animal cell lines, and the use of BSL-2 (or 2+) infectious pathogens, and the appropriate procedures will be followed to minimize risk and maintain a safe working environment.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, DNA/RNA isolation/purification, Flow Cytometry/Cell Sorting, Cell culture, Immunohistochemistry, PCR/qRT-PCR, Propagation of Infectious Agents, Genetics, Use of Infectious Agents, Proteomics, Transformation, Use of Viral Vectors, Transfection, Viral culture, Use of Human Source Material(s)

Transport: No

Materials Transported: N/A

Infectious Agent(s)/Natural Host(s): Cedar Virus (RG1-virus)/Australian Flying Fox/; Vesicular Stomatitis Virus (VSV) (RG2-virus)/None/; Respiratory Syncytial Virus (RSV) (RG2-virus)/Human/; Simian Varicella Virus (SVV) (RG2-virus)/Canine/Simian /; Human metapneumovirus (hMPV) (RG2-virus)/Human/; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human/; Human Parainfluenza Virus (HPIV) (RG2-virus)/Human/; Human Parainfluenza Virus (HPIV) (RG2-virus)/Human/; Non-Human Primate (NHP) Materials (RG2)/Monkey

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: HMPV F, G, SH and mutants (glycoprotein genes) /HMPV /glycoprotein /expression in cell culture /Mammalian cells /pCAGGS/; SV5 F and HN mutants (glycoprotein genes) /SV5 /glycoprotein /expression in cell culture /Mammalian cells /pCAGGS/; HMPV L, P, M, and N proteins /HMPV/Structural and RNA replication /expression in cell culture /Mammalian cells /pCAGGS/; Hendra virus F, G and mutants /Hendra Virus /glycoprotein /expression in cell culture /Mammalian cells /pCAGGS, lentivirus/; Nipah virus F, G and mutants /Nipah Virus /glycoprotein /expression in cell culture /Mammalian cells /pCAGGS/; RSV F and G /RSV/glycoprotein /expression in cell culture /Mammalian cells /pCAGGS/; Ebola Gp, transmembrane domain only /Synthetic /Portion of glycoprotein /express as part of chimeric protein in E. coli /E. coli /pET11/; SARS S, transmembrane domain only /Synthetic /Portion of glycoprotein /express as part of chimeric protein in E. coli /E. coli /pET11/; Influenza HA, transmembrane domain only /Synthetic /Portion of glycoprotein /express as part of chimeric protein in E. coli /E. coli /pET11/; rabies G, transmembrane domain only /Synthetic /Portion of glycoprotein /express as part of chimeric protein in E. coli /E. coli /pET11/; Nipah M /Nipah Virus /Structural Protein /expression in cell culture /Mammalian cells /pseudotype virus particles/; mcherry-tagged actin, tubulin, Rab

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GTPases /Human/Cytoskeletal Proteins /expression in cell culture /Mammalian cells /Adenovirus /; Hendra M /Hendra Virus /Structural Protein /expression in cell culture /Mammalian cells /pseudotype virus particles /; RSV P, M, L and N proteins /RSV/Structural and RNA replication /expression in cell culture /Mammalian cells /pCAGGS/; GTP evaluator proteins /Bacterial protein w/ yellow fluorescent protein added/GTP sensor /expression in cell culture /Mammalian cells /lentivirus /; SARS-CoV-2 Spike /SARS-CoV-2 (Dr. Gaya Amarasinghe) /viral fusion protein /expression in cell culture /Mammalian cells /pTwist and pCAGGS/; ACE2 /Human/Human receptor protein /expression in cell culture /Mammalian cells /pCAGGS/; TMPRSS2 /Human/Human serine protease /expression in cell culture /Mammalian cells /pCAGGS/; Cedar virus N, P and L /Cedar Virus /viral replication protein genes /expression in cell culture /Mammalian cells /pCMV/; Cedar virus F, M and G /Cedar Virus /Viral attachment and fusion protein genes/expression in cell culture /Mammalian cells /pCAGGS/; HPIV P, NP, and L/Human Parainfluenza Virus/glycoprotein/protein expression in cell culture/Mammalian cells/PCAGGS/; HPIV L tagged ECFP or EYFP/viral/viral polymerase /FRET/human/pCMV

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pGEM4A/; Plasmid/pET111/; Plasmid/pCAGGS/; Plasmid/VSV-deltaG/; Plasmid/NL43R-E-Luc3/; Adenovirus/Adenovirus /; Plasmid/p(+))JPS07E2/; Plasmid/pTwist/; Plasmid/CedPV pOLTV5 /; Lentivirus/lentivirus- pLVX-M-Puro_HeVF-P2AeGFP-T2A-HeV M /; Lentivirus/lentivirus-pMD2.G /; Lentivirus/lentivirus-psPAX2 /; Plasmid/complete HPIV3 cDNA /; Plasmid/HPIV3 L-ECFP/; Plasmid/HPIV3 L-EYFP/; Plasmid/pCMV/

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HeLa T4/; Animal/Vero /; Animal/BHK/; Animal/MDBK/; Animal/CHO/; Animal/BSR/; Human/293T/; Human/A549/; Animal/Pt kid/; Animal/Pt brain/; Animal/RO5T/; Animal/RO6E/; Human/HAE (human airway epithelial) cultures /; Human/BEAS-2B/; Human/16 HBE/; Human/CV-1/

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. Dutch has submitted an amendment to her currently approved IBC protocol entitled Paramyxovirus and pneumovirus protein function. In this amendment, Dr. Dutch has included updates to personnel, lentivirus production, and added 2 new recombinant HPIV3 strains that express fluorescent tags (CFP, YFP, or GFP). Previously, 2nd generation lentivirus was produced by the UK Viral Vector Core. Since this service is no longer available, Dr. Dutch will produce the same lentivirus vectors in her laboratory. Lentivirus vectors will be produced via transfection of gene of interest plasmid + helper plasmids, and the supernatant used directly to transduce cells. Lentivirus will not be concentrated. This work will be completed using BSL2+ containment, as previously described and approved. Work will be restricted to a Class II BSC and personnel will wear disposable gloves, goggles, and a dedicated lab coat. The new fluorescent tagged HPIV3s will be used for ampFISH testing and optimization. The new recombinant strains of HPIV3 do not introduce risk beyond that of their originally approved work with HPIV3. This work

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will also be conducted using BSL2+ containment, as previously described and approved. These changes do not significantly alter the biohazardous risks associated with Dr. Dutch's IBC protocol.

IBC Discussion & Vote:

The amendment to IBC-24-134 (version 30.0) was approved pending minor modifications as listed below:

*

INFECTIOUS AGENTS – Infectious Agent(s) table: Please update the “Non-Human Primate (NHP) Materials (RG2)” entry in the table to state Vero Cells are used in the Source column. Please also update Symptoms of Exposure and Route(s) of Exposure as “none” is not accurate. Note that exposure to NHP materials may result in exposure to Hepatitis A & B, Macacine Herpesvirus 1, Herpes B, SIV and others through routes of exposure including parenteral inoculation, ingestion, inhalation, splash to eyes or mucous membranes.

SCIENTIFIC SUMMARY:

1. The use of NHP materials are not described in the Scientific Summary. Please briefly describe procedures associated with Vero Cells and indicate potential risk of exposure. Note that NHP materials present many of the same risks as working with human sourced materials and present the additional consideration of exposure to Herpes B virus.
2. The statement “We do no concentrate of centrifuge lentivirus...” is grammatically incorrect and should be revised and clarified. Please review the summary for additional grammatical errors (i.e. goggles is misspelled “googles” under the risk minimization paragraph.
3. Please clearly state all PPE used for each procedure.

*

Amelia Pinto initiated the motion. Maria Landron seconded the motion. All IBC members present (11) voted in favor of the motion.

*

Conflicts of Interest: None

*

PI: Yosra Helmy

IBC Protocol Number: IBC-25-12

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

Protocol Type: Amendment

Amendment To: Personnel, Organisms used in research, Administrative Information

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

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

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

Brief Project Overview:

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

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): *Bacillus subtilis* (RG1-bacteria)/ Humans, animals/; *Campylobacter jejuni* (RG2-bacteria)/ Humans, animals/; *Clostridium difficile* (RG2-bacteria)/ Humans, animals/; *Enterococcus faecalis* (RG2-bacteria)/ Humans, animals/; *Escherichia coli* (RG2-bacteria)/ Humans, animals/; *Escherichia coli* (entero-hemorrhagic) RG2 Bacteria/ Humans, animals/; *Listeria monocytogenes* (RG2-bacteria)/ Humans, animals/; *Rhodococcus equi* (RG2-bacteria)/ Humans, animals/; *Staphylococcus aureus* (RG2-bacteria)/ Humans, animals/; *Streptococcus equi* subsp. *equi* (RG2-bacteria)/ Humans, animals/; *Salmonella enterica* (RG2-bacteria)/ Humans, animals, farms/; *Clostridiales* spp. (RG2-bacteria)/ Humans, animals/; *Bifidobacterium bifidum* (RG1-bacteria)/ Humans, Mammals/; *Bifidobacterium adolescentis* (RG1-bacteria)/ Humans, Mammals/; *Bifidobacterium lactis* BB-12 (RG1-bacteria)/ Humans, Mammals/; *Bifidobacterium longum* (RG1-bacteria)/ Humans, Mammals/; *Escherichia coli* Nissle 1917 (RG1-bacteria)/ Humans, Mammals/; *Escherichia coli* G58-1 (RG1-bacteria)/ Humans, Mammals/; *Lactobacillus acidophilus* (RG1-bacteria)/ Humans, Mammals/; *Lactobacillus reuteri* (RG1-bacteria)/ Humans, Mammals/; *Lactobacillus leichmannii* (RG1-bacteria)/ Humans, Mammals/; *Streptococcus thermophilus* (RG1-bacteria)/ Humans, Mammals/; *Bacteroides thetaiotaomicron* (RG1-bacteria)/ Humans, Mammals/; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/ Humans/; *Parabacteroides merdae* (RG2-bacteria)/ Humans/; *Klebsiella*

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pneumoniae (RG2-bacteria)/ Humans, animals/ ; *Shigella flexneri* (RG2-bacteria)/ Human, animal/ ; *Helicobacter pylori* (RG2-bacteria)/ Human/ ; *Aeromonas* spp. (RG2-bacteria)/ aquatic animals/ ; *Vibrio* spp. (RG2-bacteria)/ aquatic animals

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

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

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

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Chicken, Agricultural/ *Salmonella enterica* (RG2-bacteria)/ Oral/ in cages/ ABSL2/ Gloves, Coverall, masks, Eye protection, face shield/ ABSL2/ Yes/ The chickens will be infected with the bacteria *S. enterica* serovar Typhimurium and then treated with the selected drug candidate. After a few days, the animals will be euthanized and the internal organs will be collected to determine the effect on the drugs on the bacterial colonization in the internal organs. Chickens will be maintained/ kept in metal cages provided by the DLAR. / ; Chicken, Agricultural/ *Campylobacter jejuni* (RG2-bacteria)/ oral/ in cages/ ABSL2/ Gloves, Coverall, masks, Eye protection, face shield/ ABSL2/ Yes/ The chickens will be infected with the bacteria and then treated with the selected drug candidate. After a few days, the animals will be euthanized and the internal organs will be collected to determine the effect on the drugs on the bacterial colonization in the internal organs. Chickens will be maintained/ kept in metal cages provided by the DLAR. / ; Chicken, Agricultural/ *Bacteroides thetaiotaomicron* (RG1-bacteria)/ Oral/ Hand hold/ ABSL1/ lab coat, gloves, eye protection/ ABSL2/ Yes/ Probiotic/ ; Chicken, Agricultural/ *Lactobacillus leichmannii* (RG1-bacteria)/ Oral/ Hand hold/ ABSL1/ lab coat, gloves, eye protection/ ABSL2/ Yes/ probiotic/ ; Chicken, Agricultural/ *Parabacteroides merdae* (RG2-bacteria)/ Oral/ hand hold/ ABSL1/ lab coat, gloves, eye protection/ ABSL2/ Yes/ probiotic/ ; Equine, Agricultural/ *Streptococcus equi* subsp. *equi* (RG2-bacteria)/ Intranasal/ Hand Hold, sedation if necessary/ ABSL2/ Gloves, Coverall, masks, Eye protection, face shield/ ABSL2/ Yes/ BSL2 Barn practices & procedures

Risk Assessment/Discussion:

Dr. Helmy has submitted an amendment to her current IBC protocol entitled Evaluation of the efficacy of different antibiotic alternatives against infectious pathogens. In this amendment, Dr. Helmy has updated lab personnel, added four new RG2 bacterial agents, and added two new projects utilizing these new biohazardous agents. In the first new project, Dr. Helmy's laboratory will be conducting surveillance studies for food contamination in ground beef, ground poultry/turkey, pork, and seafood. Specifically, they will be isolating *Shigella flexneri*, *Aeromonas* spp., and *Vibrio* spp. from these materials. Enriched media will be used to select for these bacteria which will be confirmed via PCR. All work with these materials will be completed within the BSC until inactivation prior to PCR using BSL2 practices. Lab personnel will wear lab coat or disposable gown, gloves, and eye protection. The second project will

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utilize *Helicobacter pylori* and *Shigella* spp. to evaluate probiotic candidate strains and probiotic-derived cell-free supernatants identified from a previous experiment have the same antimicrobial activity against these two bacterial pathogens. Like the previous project, all work with these materials will be completed within a BSC using BSL2 practices, including use of lab coat or disposable gown, eye protection, and disposable gloves. Dr. Helmy already has IBC approval for work with several RG1 and RG2 bacteria pathogens. The addition of these new RG2 bacteria does not significantly alter the biohazardous risks associated with the work described in this IBC protocol.

IBC Discussion & Vote:

The amendment to IBC-25-12 (version 43.0) was approved pending minor modifications as listed below:

*

SCIENTIFIC SUMMARY: Please acknowledge that all samples are considered to contain infectious material and will be handled using BSL2 procedures, and that it may be possible to propagate bacterial agents that you are not specifically looking for when isolating bacterial agents from food samples.

*

Amelia Pinto initiated the motion. Carol Pickett seconded the motion. All IBC members present (11) voted in favor of the motion.

*

Conflicts of Interest: None

*

New Protocols

PI: Insija Selene

IBC Protocol Number: IBC-26-71

Protocol Title: NCI-CIRB-S2419: Phase III Double-Blinded Trial of Immune-Based Therapy with a Live Biotherapeutic MO-03 or Placebo for Frontline Therapy of Advanced Clear Cell Renal Cell Carcinoma [BioFront Trial]

Protocol Type: New Protocol

Amendment To: N/A

Applicable Guidelines & Regulations: 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 (BSL2)

Primary Reviewer: D. Harrison, C. Pickett, B. Nelson

Brief Project Overview:

This study is a double-blind placebo controlled randomized trial comparing MO-03 in combination with any immuno-oncology-based combination against the standard-of-care immuno-oncology-based treatment regimen, for patients with advanced renal cell carcinoma (RCC). MO-03 is a strain of the bacterium *Clostridium butyricum* (live bacterial product, LBP), an anaerobic butyric acid-producing

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bacterium. It's used clinically in Japan to treat and prevent infectious and non-infectious diarrhea and other GI disorders caused by dysbiosis of the gut microbiome. As a butyric acid producer, it restores gut microbiota and mucosal function by inhibiting enteric pathogens; it promotes Bifidobacteria growth and regulates mucosal immunity. It is taken daily, orally to maintain high levels in the GI tract and will go to undetectable levels within a week of therapy cessation.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Human Clinical Trial, Use of Human Source Material(s)

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Clostridium butyricum MIYAIRI 588 strain (CBM588) (RG1-bacteria)/Human; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: N/A

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

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

Animal Use: No

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

Risk Assessment/Discussion:

Dr. Selene has submitted a new IBC protocol application for a clinical study entitled Phase III Double-Blinded Trial of Immune-Based Therapy with a Live Biotherapeutic MO-03 or Placebo for Frontline Therapy of Advanced Clear Cell Renal Cell Carcinoma. This clinical trial seeks to understand whether adding a probiotic therapy, MO-03, to the usual treatment for advanced kidney cancer can help make existing therapies more effective, can help people with advanced kidney cancer live longer, and to identify any side effects of combining these therapies can cause. MO-03 is a live probiotic strain of *Clostridium butyricum* that is an orally administered, butyrate-producing bacterium that promotes gut microbial balance, enhances *Bifidobacterium* growth, and regulates mucosal immunity. It is commonly used in Japan and has an established safety profile for use in gastrointestinal disorders, but it does not have any regulatory approvals from US agencies (ex. FDA). It is also being studied in Japan as a potential adjunct to immunotherapies. MO-03 is manufactured in Japan and imported by Osel, Inc. Approximately 18 patients will be enrolled at the University of Kentucky over the next 3 years. MO-03 will be received and stored by the UK IDS Pharmacy. UK IDS will dispense MO-03 and transport it to the administering physician. Study participants will be instructed by the administering physician and/or nurse on how to take the capsules, as well as discuss sample collection and scheduling. Whole blood, stool samples, and archival tumor tissues will be obtained for downstream analysis by the study sponsor. This work will be conducted using BSL2 practices and procedures and in accordance with the Study Sponsor's instructions.

IBC Discussion & Vote:

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The protocol IBC-26-71 (version 6.0) was approved pending minor modifications as listed below:

*

SCIENTIFIC SUMMARY:

1. Please describe the precautions, instructions, or guidance provided to patients to minimize the risk of transmission or exposure to close contacts during at-home treatment. Additionally, please discuss the potential risks that close contacts may encounter if exposure occurs, including whether patients are advised to monitor for specific signs or symptoms of adverse events in those individuals and to seek medical attention if such symptoms develop.
2. Will any personal protective equipment be utilized by patients when collecting their stool samples at home?

*

Doug Harrison initiated the motion. Brandy Nelson seconded the motion. All IBC members present (11) voted in favor of the motion.

*

Conflicts of Interest: None

*

Renewals

PI: Melissa Morgan

IBC Protocol Number: IBC-26-47

Protocol Title: General Operations of the Food Microbiology Laboratory (Renewal 2026)

Protocol Type: Renewal

Amendment To: N/A

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: M. Landron, A. Pinto, D. Harrison

Brief Project Overview:

The general operation of the laboratory utilizes three distinct types of experimentation: non-microbial analysis, microbial discovery, and microbial challenges.

Non-microbial analysis includes studies which investigate physical and chemical traits in non-inoculated foods and beverages. These experiments employ analytical measurements such as pH, water activity (aw), water phase salt, percent salt, and percent moisture to characterize foods and beverages.

Microbial discovery includes studies which investigate the natural non-inoculated microflora found in the environment, in food/beverage production areas, and the food/beverage products or original ingredients. These experiments employ various microbial enumeration methods of the samples including the use of general agar plates, selective agar plates, and Petrifilms.

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Microbial challenges include studies which investigate the survival and potential growth of various microbial cultures that are purposefully inoculated into foods and beverages. These experiments are used to validate antimicrobials, heat/cooking treatments, cooling/refrigeration treatments, and storage conditions. Microbial maintenance in the 221 laboratory includes creating working culture stocks for experimental use and frozen culture stocks for long term storage.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, Imaging/Microscopy, Use of infectious agents

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): *Aspergillus flavus* (RG2-fungus)/ Humans, animals, plants/ ; *Aspergillus niger* (RG2-fungus)/ Humans, animals, plants/ ; *Candida albicans* (RG2-fungus)/ Humans/ ; *Fusarium* spp. (RG2-fungus)/ Humans, animals, plants/ ; *Geotrichum candidum* (RG1-fungus)/ Humans, animals, plants/ ; *Saccharomyces cerevisiae* (RG1-fungus)/ Ripe fruits/ ; *Achromobacter viscosus* (RG2-bacteria)/ Humans, animals/ ; *Acinetobacter* spp. (RG2-bacteria)/ Humans/ ; *Acinetobacter* spp. (RG2-bacteria)/ Humans/ ; *Acinetobacter* spp. (RG2-bacteria)/ Humans/ ; *Aerococcus viridans* (RG2-bacteria)/ lobsters, humans/ ; *Alcaligenes faecalis* (RG2-bacteria)/ Humans, animals/ ; *Bacillus cereus* (RG2-bacteria)/ Humans, animals/ ; *Bacillus cereus* (RG2-bacteria)/ Humans, animals/ ; *Bacillus cereus* (RG2-bacteria)/ Humans, animals/ ; *Bacillus cereus* (RG2-bacteria)/ Humans, animals/ ; *Bacillus cereus* (RG2-bacteria)/ Humans, animals/ ; *Bacillus cereus* (RG2-bacteria)/ Humans, animals/ ; *Bacillus coagulans* (RG1-bacteria)/ Humans, animals, plants/ ; *Bacillus licheniformis* (RG1-bacteria)/ Humans, animals, plants/ ; *Bacillus megaterium* (RG1-bacteria)/ N/A/ ; *Bacillus megaterium* (RG1-bacteria)/ N/A/ ; *Bacillus mycoides* (RG1-bacteria)/ Humans, animals, plants/ ; *Bacillus mycoides* (RG1-bacteria)/ Environment/ ; *Bacillus subtilis* (RG1-bacteria)/ Humans, animals/ ; *Bacillus subtilis* (RG1-bacteria)/ Humans, animals/ ; *Bacillus subtilis* (RG1-bacteria)/ Humans, animals/ ; *Bacillus subtilis* (RG1-bacteria)/ Humans, animals/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Clostridiales* spp. (RG2-bacteria)/ Humans/ ; *Cronobacter sakazakii* (RG2-bacteria)/ Humans/ ; *Cronobacter sakazakii* (RG2-bacteria)/ Humans/ ; *Cronobacter sakazakii* (RG2-bacteria)/ Humans/ ; *Enterobacter cloacae* (RG2-bacteria)/ Humans, animals, plants/ ; *Enterobacter cloacae* (RG2-bacteria)/ Humans, animals, plants/ ; *Enterococcus faecium* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus faecium* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus faecium* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus faecalis* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus faecalis* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus faecalis* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus faecalis* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus faecalis* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus faecalis* (RG2-bacteria)/ Humans, animals/ ; *Enterococcus hirae* (RG2-bacteria)/ Humans, animals/ ; *Escherichia coli* (RG2-bacteria)/ Humans, animals/ ; *Escherichia coli* (RG2-bacteria)/ Humans,

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bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Salmonella enterica (RG2-bacteria)/ Humans, animals/ ; Serratia marcescens (RG2-bacteria)/ Humans, animals, plants/ ; Shewanella putrefaciens (RG2-bacteria)/ Human/ ; Shigella boydii (RG2-bacteria)/ Humans, animals/ ; Shigella flexneri (RG2-bacteria)/ Humans, higher primates/ ; Shigella sonnei (RG2-bacteria)/ Humans, animals/ ; Staphylococcus arlettae (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus aureus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus auricularis (RG1-bacteria)/ Humans, animals/ ; Staphylococcus auricularis (RG1-bacteria)/ Humans, animals/ ; Staphylococcus capitis (RG2-bacteria)/ Humans, animals/ ; Staphylococcus capitis (RG2-bacteria)/ Humans, animals/ ; Staphylococcus caprae (RG2-bacteria)/ Humans, animals/ ; Staphylococcus carnosus (RG1-bacteria)/ Humans, animals/ ; Staphylococcus carnosus (RG1-bacteria)/ Humans, animals/ ; Macrocoocus caseolyticus (RG1-bacteria)/ Humans, animals/ ; Staphylococcus chromogenes (RG1-bacteria)/ Humans, animals/ ; Staphylococcus chromogenes (RG1-bacteria)/ Humans, animals/ ; Staphylococcus cohnii (RG1-bacteria)/ Humans, animals/ ; Staphylococcus cohnii (RG1-bacteria)/ Humans, animals/ ; Staphylococcus cohnii (RG1-bacteria)/ Humans, animals/ ; Staphylococcus cohnii (RG1-bacteria)/ Humans, animals/ ; Staphylococcus epidermidis (RG1-bacteria)/ Humans/ ; Staphylococcus epidermidis (RG1-bacteria)/ Humans/ ; Staphylococcus equorum (RG1-bacteria)/ Humans, animals/ ; Staphylococcus gallinarum (RG1-bacteria)/ Humans, animals/ ; Staphylococcus gallinarum (RG1-bacteria)/ Humans, animals/ ; Staphylococcus haemolyticus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus hominis (RG1-bacteria)/ Humans, animals/ ; Staphylococcus hyicus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus hyicus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus intermedius (RG2-bacteria)/ Humans, animals/ ; Staphylococcus intermedius (RG2-bacteria)/ Humans, animals/ ; Staphylococcus kloosii (RG1-bacteria)/ Humans, animals/ ; Mammaliicoccus lentus (RG2-

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bacteria)/ Humans, animals/ ; Staphylococcus lugdunensis (RG2-bacteria)/ Humans, animals/ ; Staphylococcus lugdunensis (RG2-bacteria)/ Humans, animals/ ; Staphylococcus lugdunensis (RG2-bacteria)/ Humans, animals/ ; Staphylococcus lugdunensis (RG2-bacteria)/ Humans, animals/ ; Staphylococcus lugdunensis (RG2-bacteria)/ Humans, animals/ ; Staphylococcus lugdunensis (RG2-bacteria)/ Humans, animals/ ; Staphylococcus saprophyticus (RG2-bacteria)/ Humans, animals/ ; Staphylococcus schleiferi (RG2-bacteria)/ Humans, animals/ ; Staphylococcus schleiferi (RG2-bacteria)/ Humans, animals/ ; Staphylococcus schleiferi (RG2-bacteria)/ Humans, animals/ ; Staphylococcus schleiferi (RG2-bacteria)/ Humans, animals/ ; Staphylococcus sciuri (RG2-bacteria)/ Humans, animals/ ; Staphylococcus simulans (RG2-bacteria)/ Humans, animals/ ; Staphylococcus warneri (RG2-bacteria)/ Humans, animals/ ; Staphylococcus xylosus (RG1-bacteria)/ Humans, animals/ ; Streptococcus agalactiae (RG2-bacteria)/ Humans, animals (particularly cattle)/ ; Streptococcus agalactiae (RG2-bacteria)/ Humans, animals (particularly cattle)/ ; Streptococcus dysgalactiae (RG1-bacteria)/ Humans, animals/ ; Streptococcus gordonii (RG2-bacteria)/ Humans, animals (particularly cattle)/ ; Streptococcus pyogenes (RG2-bacteria)/ Humans/ ; Streptococcus uberis (RG1-bacteria)/ Humans, mammals/ ; Streptococcus uberis (RG1-bacteria)/ Humans, mammals/ ; Weisella viridescens (RG1-bacteria)/ Humans

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

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

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

Animal Use: No

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

Risk Assessment/Discussion:

Dr. Morgan has submitted a renewal of her IBC protocol entitled General Operations of the Food Microbiology Laboratory (Renewal 2026). Her IBC protocol covers the research activities of the Food Microbiology Laboratory and the class activities for the Food Microbiology teaching laboratory course FSC530. Research activities are broken down into 3 distinct experiment types. The first experiment type does not involve biohazardous materials and seeks to explore the different characteristics of food and beverages examining pH, water activity, and percent moisture. The second experiment type studies food and beverage samples and seeks to discover different microorganisms naturally occurring within the original product. Experiment type 3 involves the microbial challenge of food and beverage items to validate efficacy of antimicrobials, cooking times and temperatures, cooling times and temperatures, and storage conditions. A long list of RG1 and RG2 bacteria and fungi are utilized for this work, including: *A. flavus*, *A. niger*, *C. albicans*, *Fusarium spp.*, *G. candidum*, *S. cerevisiae*, *A. viscosus*, *Acinetobacter spp.*, *A. viridans*, *A. faecalis*, *B. cereus*, *B. coagulans*, *B. licheniformis*, *B. megaterium*, *B. mycoides*, *B.*

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subtilis, *Clostridiales* spp., *C. sakazakii*, *E. cloacae*, *E. faecium*, *E. faecalis*, *E. hirae*, *E. coli*, *E. coli* (entero-hemorrhagic), *K. pneumoniae*, *L. acidophilus*, *L. brevis*, *L. delbruckii*, *L. fermentum*, *L. pentosus*, *L. plantarum*, *L. rhamnosus*, *L. lactis*, *Leuconostoc* spp., *L. innocua*, *L. monocytogenes*, *M. lacticum*, *M. luteus*, *M. morganii*, *P. polymyxa*, *Pediococcus* spp., *P. vulgaris*, *P. rettgeri*, *P. aeruginosa*, *P. fluorescens*, *S. enterica*, *S. marcescens*, *S. putrefaciens*, *S. boydii*, *S. flexneri*, *S. sonnei*, *S. arlettae*, *S. aureus*, *S. auricularis*, *S. capitis*, *S. caprae*, *S. carnosus*, *M. caseolyticus*, *S. chromogenes*, *S. cohnii*, *S. epidermidis*, *S. equorum*, *S. gallinarum*, *S. haemolyticus*, *S. hominis*, *S. hycius*, *S. intermedius*, *S. kloosii*, *M. lentus*, *S. lugdunensis*, *S. schleiferi*, *S. sciur*, *S. simulans*, *S. warneri*, *S. xylosus*, *S. agalactiae*, *S. dysgalactiae*, *S. gordonii*, *S. pyogenes*, *S. uberis*, and *W. viridescens*. Food and beverage samples are inoculated with a variety of RG1 and RG2 microorganisms for validation testing and other downstream manipulations. For all of the research experiments described, BSL2 practices and procedures will be followed. PPE will include laboratory coats, disposable gloves, and eye protection. Aerosol-producing procedures, such as vortexing, will be done within a BSC. The teaching laboratory explores agar plating techniques, biochemical tests, and BSL1 and BSL2 procedures. The following microorganisms will be used in the teaching laboratory: *Bacillus cereus*, *Bacillus subtilis*, *Bacillus polymyxa*, *Candida albicans*, *Clostridial* sp., *Enterobacter cloacae*, *Enterococcus faecalis*, *E. coli*, *E. coli* O157, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides* sub. *mesenteroides*, *Leuconostoc mesenteroides* sub. *cremoris*, *Listeria innocua*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Proteus vulgaris*, *Saccharomyces cerevisiae*, *Salmonella enterica*, *Salmonella enterica* serovar *typhimurium*, *Salmonella enterica* serovar *enteritidis*, *Serratia marcescens*, *Shigella flexneri*, *Streptococcus* sp. (demonstration only), *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Weissella viridescens*. Prior to starting lab work, all students must complete Biological Safety training provided by the Department of Research Safety. Students will wear disposable gloves, eye protection, and disposable lab coats for all laboratory work. Microorganisms are grouped into different teaching units based on similar characteristics or the specific biochemical testing to be performed, and students are required to read laboratory procedures for each prior to class. Dr. Morgan's current IBC protocol will expire on July 25, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-47 (version 8.0) was approved pending minor modifications with final review by IBC primary reviewers:

*

DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Transport:

1. Please update the description of the secondary container to include that the container is shatterproof.
2. Specify how agents/samples are transported between locations, for example, by hand, on a cart, in a vehicle, etc.

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1. Please edit the summary to remove redundant information and ensure congruency across the protocol. For example, disinfection and transport descriptions are repeated multiple times.
2. The overall procedures involving handling, propagating, and testing bacterial agents are not well described. Include information about how agents are routinely handled and propagated. What equipment is in use? How are those prepared and disinfected?
3. For experimental assays, please include where they are being performed (e.g. biological safety cabinet, open lab bench). Please note that all potentially aerosol generating procedures with biohazardous materials (e.g. pipetting, opening tubes, vortexing, etc.) will be performed within a BSC.
4. Please describe PPE in use for the assays being performed.
5. Add information about the procedures in place if glassware is broken during the propagation or experimentation with infectious agents/biohazards.

*

Doug Harrison initiated the motion. Amelia Pinto seconded the motion. All IBC members present (11) voted in favor of the motion.

*

Conflicts of Interest: None

*

PI: Natalia Martinez

IBC Protocol Number: IBC-26-50

Protocol Title: Burley and Dark Tobacco Disease Management

Protocol Type: Renewal

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

Maximum Containment Level: Biological Safety Level 2 - Plants (BSL2-P)

Primary Reviewers: J. Smalle, A. Hunt, D. Harrison

Brief Project Overview:

Our tobacco breeding program at U. of Kentucky is looking into finding new tobacco lines that have resistance to several diseases and better chemistry and in the long term make these lines available to tobacco farmers.

Diseases cause major losses to Kentucky tobacco crops each year. One of the most important objectives of our tobacco breeding program is the development of disease resistant cultivars. This involves evaluating breeding lines of burley and dark tobacco under pathogen pressure in greenhouse and field experiments. Virulent isolates of bacteria, fungi and viruses are continually being collected from the major production areas of Kentucky and are maintained in the laboratory for use in greenhouse and field screening nurseries as needed.

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Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, Field trial(s), Plant work, Propagation of infectious agents, Use of infectious agents

Transport: Yes

Materials Transported: Biohazardous Materials, Plants

Infectious Agent(s)/Natural Host(s): *Ralstonia solanacearum* (race 1, biovar 1) (RG1-bacteria)/ Tobacco and other solanaceous crops (tomato, pepper)/ ; *Thielaviopsis basicola* (RG1-fungus)/ Wide host range, especially species of Fabaceae, Solanaceae, Cucurbitaceae. / ; *Phytophthora nicotianae* (RG1-fungus)/ Wide host range including tobacco, tomato, onion, ornamentals, cotton, pepper and citrus/ ; Tobacco Mosaic Virus (TMV) (RG1-virus)/ Wide host range, especially tobacco and other Solanaceae/ ; *Fusarium oxysporum* f.sp. *nicotianae* (RG1-fungus)/ Tobacco/ ; *Pseudomonas syringae* pv. *tabaci* (RG1-bacteria)/ tobacco/ ; *Cercospora nicotianae* (RG1-fungus)/ Tobacco

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

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

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

Animal Use: No

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

Risk Assessment/Discussion:

Dr. Martinez has submitted a renewal of the IBC protocol entitled Burley and Dark Tobacco Disease Management. Dr. Martinez's lab works to find new tobacco lines that have resistance to several tobacco pathogens with better chemistry. Their long-term goal is to make these lines available to tobacco farmers. The IBC protocol describes screening tobacco for resistance to a variety of pathogens, including *Cercospora nicotianae*, causal agent of frog eye leaf spot, *Pseudomonas syringae* p.v. *tabaci* causal agent of angular leaf spot, *Phytophthora nicotianae* Race 1 and 0 causal agent of black shank, *Thielaviopsis basicola* causal agent of black root rot (BRR), Tobacco Mosaic Virus (TMV) often referred to as mosaic, soil-borne fungus *Fusarium oxysporum* f.sp. *nicotianae*, causal agent of Fusarium wilt (FT), and *Ralstonia solanacearum* race 1 biovar 1, causal agent of Granville (bacterial) wilt. Of note, *Ralstonia solanacearum* is on the USDA PPQ Select Agents and Toxins list. However, the strain in use (*R. solanacearum* race 1, biovar 1) is excluded from Select Agent regulations. This specific isolate is imported from North Carolina, and Dr. Martinez has a current PPQ permit for this agent. The procedures for each plant pathogen are very similar - Inoculum is produced in the laboratory and transported to the greenhouse for inoculation of tobacco plants. Disease progression is followed for a few weeks, and plants and materials are destroyed via autoclave. While they have previously been approved for field work, they have currently suspended field work with pathogens and will amend their IBC protocol prior to starting field work in the future. All work will be completed using BSL2-P containment and according to PPQ permit conditions, where applicable. None of the plant pathogens in use are known to cause disease in humans. The current IBC protocol will expire on July 28, 2026.

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IBC Discussion & Vote:

The protocol renewal IBC-26-50 (version 9.0) was approved.

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Arthur Hunt initiated the motion. Jan Smalle seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Mark Farman

IBC Protocol Number: IBC-26-51

Protocol Title: Clonal variation in expression as a mechanism of pathogenic variability in the rice blast fungus

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: NIH Guidelines Section III-E-2, NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-2, NIH Guidelines Section III-F-7, NIH Guidelines Section IV-B-7, UK

Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA Act of 1970 Clause 5(a)(1)

Maximum Containment Level: Biological Safety Level 2 - Plants (BSL2-P)

Primary Reviewers: A. Hunt, J. Smalle, B. Nelson

Brief Project Overview:

The ascomycete fungus *Magnaporthe oryzae* (formerly *Magnaporthe grisea*) is most well known as the causal agent of a devastating disease of rice known as “blast”. However, there are other host specialized populations of the fungus which infect other crops including wheat, oats, barley, and millet, as well as forage and turf grasses. Fungal isolates from rice and other grass hosts are taxonomically described as *Magnaporthe oryzae* while isolates from crabgrass are classified as *Magnaporthe grisea*.

Rice blast has the greatest economic impact because it occurs in all rice growing regions of the world (at least 85 countries) and it is estimated that the quantity of rice lost annually to blast could feed more than 60 million people <http://www.agribusinessweek.com/rice-disease-seriespart-2-rice-blast/>.

Use of resistant rice cultivars continues to be the most effective (and environmentally friendly) means of controlling rice blast. However, resistance tends to be overcome with alarming rapidity. It is common for newly developed, resistant rice cultivars to succumb to rice blast within just two years of their initial deployment. This is because, for any given resistance gene, there is a strong possibility that a pathogen population capable of defeating it already exists. In addition, spontaneous changes in specificity can occur, allowing incompatible strains to gain virulence on rice cultivars that were formerly resistant. This ability of the pathogen to overcome new resistance genes represents the most significant challenge to long term control of rice blast and, indeed, the many other crop and turf diseases caused by *M. oryzae*.

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Host plant resistance to *M. oryzae* usually follows a gene for-gene relationship in which each gene for resistance requires the presence of a corresponding gene in the pathogen for the resistance phenotype to be expressed. These pathogen genes are known as avirulence (or AVR) genes because their presence causes the fungus to be avirulent to a plant containing the cognate resistance genes. These gene-for-gene interactions explain why host resistance eventually breaks down because, if resistance requires the presence of specific genes in the pathogen, the pathogen simply has to lose or mutate those genes to cause disease on a formerly resistant plant.

Now, it might seem counterintuitive for pathogens to produce proteins that elicit host defense, and one would expect such proteins to have been eliminated/modified through selection. However, without the appropriate host receptor, these proteins often facilitate disease by suppressing the host's basal resistance mechanisms. Due to this ability to effect changes in the host cell's physiology, these proteins are termed "effectors."

A possible new mechanism for pathogenic adaptation: The Farman lab studies the genetic mechanisms underlying *M. oryzae*'s pathogenic variability. Recently, my lab members and those of my collaborator, Barbara Valent, conducted a large-scale, functional genomics project to identify candidate Magnaporthe effector proteins. We used high throughput methods to generate autofluorescent protein fusions to monitor the localization patterns of over 500 secreted proteins during host penetration and growth in planta. This project produced a number of new and exciting findings. Our most significant discovery was that many (but not all) secreted proteins were heterogeneously expressed among genetically identical individuals – i.e. strong fluorescence was observed in one or more clonal spores/colonies but was absent in others.

Initially, we suspected that the phenomenon was an experimental artifact associated with the use of transgenic lines. However, this does not appear to be the case because of its reproducibility among independent transformants. In addition, we found that many proteins, including histone 2B, BAS4 and a glucosyltransferase, to name a few, which were universally and evenly expressed in all fungal colonies and in all fungal transformants. Therefore, we suspect that *M. oryzae* genes are subdivided into at least two classes: ones with exceptionally heterogeneous (noisy) expression (putative "contingency" genes) and others whose expression tends to be more consistent (housekeepers).

Clonal variation in gene expression is a well-documented phenomenon but only recently has it been proposed to have important biological roles. A comprehensive study of protein levels in single yeast cells revealed that the proteins with the noisiest expression tended to be ones whose products responded to environment change. This led to the idea that gene expression heterogeneity could serve roles in development, adaptation, and evolution; and this phenomenon has been termed "bet hedging" because heterogeneous gene expression serves to spread the risk of encountering a harsh environment among individuals within the population.

Our discovery of heterogeneous protein expression in Magnaporthe leads us to suspect that bet hedging is also at play in this fungus. More specifically, we hypothesize that clonal variation in gene expression serves to spread the risk of host recognition among different fungal individuals, thereby increasing the likelihood of infection success on variable and unpredictable plant genotypes.

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Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, DNA/RNA isolation/purification, Genetics, Imaging/Microscopy, PCR/qRT-PCR, Plant work, Propagation of infectious agents, Transformation, Use of infectious agents

Transport: Yes

Materials Transported: Plants, Biohazardous Materials

Infectious Agent(s)/Natural Host(s): *Magnaporthe oryzae* (RG1-fungus)/wide variety of monocot plants including rice, millet, and arabidopsis

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Hph/*Streptomyces* sp./selectable marker/expression in *M.oryzae*/*Magnaporthe oryzae*/pBht2/3/4/; Phleo/*Streptomyces* sp./selectable marker/expression in *M.oryzae*/*Magnaporthe oryzae*/pBht2/3/4/; GFP/*Aequoria victoria*/Tracking protein/expression in *M.oryzae*/*Magnaporthe oryzae*/PBht2/3/4/; RFP/*Discosoma* sp./Tracking protein/expression in *M.oryzae*/*Magnaporthe oryzae*/PBht2/3/4/; KANMX4 (G418r)/*Streptomyces* sp./selectable marker/expression in *M.oryzae*/*Magnaporthe oryzae*/pBht3/; AvrPiz-t/*Magnaporthe oryzae* avirulence/effector/protein/expression in *M.oryzae* /*Magnaporthe oryzae*/pBht2/3/4/; AvrPita /*Magnaporthe oryzae* avirulence/effector/protein/expression in *M.oryzae*/*Magnaporthe oryzae*/PBht2/3/4/; mCHERRY::NLS/*Discosoma* sp. /nucleus-targeted tracking protein/expression in *M.oryzae* /*Magnaporthe oryzae*/PBht2/3/4/; Piz-t /*Oryza sativa* /disease resistance protein/expression in rice /*Magnaporthe oryzae*/pBht2/3/4/; AvrPi9 /*Magnaporthe oryzae* avirulence/effector/protein /expression in *M.oryzae*/KO in *M. oryzae* /*Magnaporthe oryzae*/PBht2/3/4, pMY200/; MoHP1/*Magnaporthe oryzae*/chromatin modification/KO in *M. oryzae* /*Magnaporthe oryzae*/pMY200/; MoSET1 /*Magnaporthe oryzae*/chromatin modification/KO in *M. oryzae* /*Magnaporthe oryzae*/pMY200/; MoSET3 /*Magnaporthe oryzae*/chromatin modification/KO in *M. oryzae* /*Magnaporthe oryzae*/pMY200/; MoDMT1/*Magnaporthe oryzae* /DNA methylation /KO in *M. oryzae*/*Magnaporthe oryzae*/pMY200/; TERT/*Magnaporthe oryzae*/Telomerase/KO in *M. oryzae*/*Magnaporthe oryzae*/pMY200/; SIR2 /*Magnaporthe oryzae* /chromatin modification/KO in *M. oryzae*/*Magnaporthe oryzae*/pMY200/ Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pBht2/; Plasmid/pCB1004/; Plasmid/pBht3 /; Plasmid/pBht4 /; Plasmid/pFPNL1 /; Plasmid/pCAMBIA1300/; Plasmid/PMY200/

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. Farman has submitted a renewal of his IBC protocol entitled Clonal variation in expression as a mechanism of pathogenic variability in the rice blast fungus. Dr. Farman's lab seeks to understand the genetic mechanisms underlying the pathogenic variability of the fungus *Magnaporthe oryzae*, causal agent of the disease rice blast. The IBC protocol describes 4 specific experimental aims that utilize similar procedures and assays. They will challenge rice cultivars with *P. oryzae* strains that carry the

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AvrPI-ta and Avr-Piz-t avirulence effectors, excise lesions, and extract RNA for RNAseq library construction and HiSeq sequencing at Duke University. Once avirulence alleles are identified, they will generate fluorescent fusion proteins for introduction into *P. oryzae* via Agrobacterium-mediated transformation. They will also knockout epigenetic regulators in *P. oryzae* via Agrobacterium-mediated transformation. Lastly, they will grow fungal cultures and extract genomic DNA to construct sequencing libraries. All fungal manipulations will take place in a dedicated BSC using BSL2-P practices and procedures. A dedicated containment suite will be used for whole-plant spray inoculations. The suite is maintained under negative pressure and is used exclusively for this work. No other plants are grown in this location while this experiment is ongoing. Transgenic rice plants will be grown in greenhouse and growth chamber locations. No field work is planned. The fungal pathogen in use is not known to cause disease in humans. Dr. Farman's current IBC protocol will expire on July 5, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-51 (version 9.0) was approved pending minor modifications as listed below:

*

LOCATIONS – Research Locations table: Is work actively taking place in the Seed House and Greenhouse locations despite upcoming demolition plans? If these spaces are not being used, please remove them.

SCIENTIFIC SUMMARY: Please briefly comment on whether there is any expected risk associated with knocking out genes in the fungus that may affect global expression.

*

Arthur Hunt initiated the motion. Jan Smalle seconded the motion. All IBC members present (11) voted in favor of the motion.

*

Conflicts of Interest: None

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PI: Jessica Santollo

BC Protocol Number: IBC-26-56

Protocol Title: Estrogen Receptor Regulation of Fluid Intake

Protocol Type: Renewal

Amendment To: N/A

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

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

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

Brief Project Overview:

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Estrogens acting through brain estrogen receptors influence a variety of behaviors, including how much we drink and eat. While there are a number of different estrogen receptors in the brain our previous work demonstrates that activation of specific estrogen receptor subtypes selectively influences fluid and food intake. Specifically, we have shown that pharmacological activation of estrogen receptor alpha (ER α) decreases food intake and water intake stimulated by the hormone Angiotensin II (AngII).

Pharmacological activation of estrogen receptor beta (ER β) and G-protein coupled estrogen receptor 1 (GPER-1) has no effect on food intake or water intake but decreases saline intake stimulated by the hormone AngII. Furthermore, analysis of drinking patterns revealed that ER β and GPER-1 inhibit saline intake in discrete ways. Specifically ER β decreases saline intake through changes in orosensory (taste) feedback while GPER-1 decreases saline intake through changes in postingestive feedback (enhances satiety signals). We are now interested in understanding which nuclei in the brain are necessary for these effects of estrogens. Because highly selective estrogen receptor antagonists are not commercially available or reliable our goal is to knock down different estrogen receptors using AAV siRNA in areas of the brain that control fluid intake. We will then ask the question of whether disruption of estrogen receptor expression influences the inhibitory effect estrogens have on AngII-stimulated fluid intake. We use the Long Evans rat as our experimental model.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Use of viral vectors, DNA/RNA isolation/purification
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)]: shRNA targeting Estrogen Receptor alpha [ER α]/rat/Transcription Factor/Inserted into Brain Nuclei of Rat for gene knockdown/rat/AAV/; shRNA targeting Estrogen receptor beta [ER β]/rat/Transcription Factor/Inserted into Brain Nuclei of Rat for gene knockdown/rat/AAV/; shRNA targeting G-protein coupled estrogen receptor 1 [GPER1]/rat/Transmembrane Receptor Protein/Inserted into Brain Nuclei of Rat for gene knockdown/rat/AAV/; GFP/Aequorea victoria/Tracking gene/Inserted into Brain Nuclei of Rat for gene tracking/rat/AAV/; hM3D/human/Transmembrane Receptor Protein/Inserted into Brain Nuclei of Rat for gene expression/rat/AAV/; mCherry/Aequorea victoria/Tracking gene/Inserted into Brain Nuclei of Rat for gene tracking/rat/AAV/; GCaMP6/Aequorea victoria/Tracking gene/Inserted into Brain Nuclei of Rat for gene tracking/rat/AAV/

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pZac2.1/; Plasmid/pZac2.1 derivatives/; Adeno-Associated Virus (AAV)/AAVs derived from pZac2.1 derivatives/; Adeno-Associated Virus (AAV)/pAAV.Syn.GCaMP6f.WPRE.SV40/; Adeno-Associated Virus (AAV)/AAV8-CAMPKII-hM3d-mCherry/
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

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Practices & Procedures]: Rat/Viral Vector - Adeno-Associated Virus (AAV)/Brain injection/Isoflurane in stereotax /ABSL1/Standard PPE = Gloves, Lab Coat, Eye Protection, Head Wear (cap)/ABSL1/No/

Risk Assessment/Discussion:

Dr. Santollo has submitted a renewal of her IBC protocol entitled Estrogen Receptor Regulation of Fluid Intake. Dr. Santollo's laboratory seeks to understand whether disruption of estrogen receptor expression influences the inhibitory effect estrogens have on AngII-stimulated fluid intake. Towards that goal, Dr. Santollo's lab uses adeno-associated virus (AAV) vectors in rats. The AAVs in use were originally constructed by the UK Genetic Technologies Core facility, and the Santollo lab has enough stock remaining for future planned use. The AAV vectors have been engineered to knockdown expression of Estrogen Receptor alpha, Estrogen Receptor beta, and G-protein couple estrogen receptor 1 in rat brains. The AAVs also encode tracking genes (GFP, mCherry, and GCaMP6) and hM3D for expression. Rats are anesthetized and AAVs are injected via stereotaxic injection. Animals are allowed to recover for a few hours and are returned to ABSL1 housing. Upon experiment completion, animals are euthanized and brains removed for fixation and immunohistochemistry. Work with AAV will be done using BSL1/ABSL1 practices and procedures. Dr. Santollo's current IBC protocol will expire on July 19, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-56 (version 10.0) was approved pending minor modifications as listed below:

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RECOMBINANT AND/OR SYNTHETIC NUCLEIC ACID MATERIALS – Vector Information table: Please review and clarify whether DNA will integrate into the host genome for the vectors listed. For example, the response for pZac2.1 states “Yes”, but the response for pZac2.1 derivatives contradicts this by stating “No.”

RECOMBINANT AND/OR SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information table: Under “Use of Construct” for hM3D, expression is stated. Will hM3D also be knocked down, or is it only being overexpressed? Please update accordingly.

SCIENTIFIC SUMMARY:

1. Please define AngII and E2 and describe potential risks of exposure to personnel.
2. Please briefly expand the description of gene function in the event of accidental exposure to AAV constructs in use.

*

Mike Mendenhall initiated the motion. Amelia Pinto seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Olga Tsyusko-Unrine

IBC Protocol Number: IBC-26-60

Protocol Title: Toxicity of environmental contaminants

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-4, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), UK Administrative Regulation 6.3, UK Administrative Regulation 6.9
Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: C. Pickett, M. Landron, M. Mendenhall

Brief Project Overview:

This project utilizes the model organism a nematode *Caenorhabditis elegans* to assess toxicity and the mechanisms of toxicity of various environmental contaminants including metals and engineered nanomaterials, per- and polyfluoroalkyl substances (PFAS), and water disinfection-by-products. The nematodes are raised in petri dishes containing K-agar gel and they feed on a lawn of non-pathogenic bacteria grown on the agar gel. The nematodes are exposed to each contaminant (nanomaterials, per- and polyfluoroalkyl substances, or disinfection-by-products) in a liquid medium at different concentrations. Among nanomaterials are one-dimensional (e.g., zinc oxide, silver, gold, titanium oxide) and 2D nanomaterials (e.g., hydroxyapatites, phosphorene and hexagonal boron nitride) as well as other types of nanoparticles. The contaminants may exhibit varying degrees of toxicity. Adverse effects on nematode survival, growth, behavior, and reproduction are studied. Some strains of *C. elegans* are used which contain the gene for green fluorescent protein (GFP). This gene, which comes from a species of jelly fish (*Aequoria victoria*) is used to report the expression of normal *C. elegans* genes. These strains are used widely throughout the world without any problems. The risks of using GFP reporter and mutant *C. elegans* strains are very minimal because the mutant can only survive under very specific conditions, which are provided in the laboratory, and these are not parasitic nematodes. Additionally, no genetic modifications will be performed in our lab. We are also interested in responses of *C. elegans* after exposure to pathogenic bacteria *Klebsiella pneumoniae* under different environmental stressors (PFAS) and nanomaterials. All work involving this pathogenic bacteria which will be fed to *C. elegans* will be conducted in BSC II. Finally, the toxicity of water disinfection-by-products will be studied in *C. elegans* and also in the mammalian cell culture, using Chinese Hamster Ovary cells. The cells will be ordered from ATCC supplier and won't be genetically modified. The cell toxicity assays will be conducted in cell growth medium in 96-well plates using CO2 incubator.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

Infectious Agent(s)/Natural Host(s): *Klebsiella pneumoniae* (RG2-bacteria)/Human

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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/Chinese Hamster Ovary (CHO)

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Invertebrate - Non-Insect/Non-Arthropod/*Klebsiella pneumoniae* (RG2-bacteria)/Close contact (on same plate)/N/A/ABSL2/Disposable lab coat, surgical mask, eye protection, disposable gloves/ABSL2/Yes/K. *pneumoniae* will be incorporated into agar on plate for *C. elegans* feeding. All related procedures with *K. pneumoniae* on plates will be conducted in the BSC./;

Invertebrate - Non-Insect/Non-Arthropod/Naked Nucleic Acid-r/sDNA/Close contact (liquid culture)/N/A/ABSL1/Lab coat, eye protection, disposable gloves/ABSL1/No/ssRNA/dsRNA will be added to liquid culture of *C. elegans* in microcentrifuge tubes. Procedure is done on the benchtop under a PCR Workstation.

Risk Assessment/Discussion:

Dr. Tsyusko-Unrine has submitted a renewal of her IBC protocol entitled Toxicity of environmental contaminants. Dr. Tsyusko-Unrine's lab uses a nematode *C. elegans* model to study the toxicity of various environmental contaminants. Transgenic *C. elegans*, obtained from the Caenorhabditis Genetics Center, are reared and exposed to different contaminants. Adverse effects on nematode survival, growth, behavior, and reproduction are studied. A second project will study the effects of the RG2 bacteria *K. pneumoniae* on *C. elegans*. Work with *C. elegans* and *K. pneumoniae* will be done in a Class II BSC. Agar plates with *C. elegans* will be inoculated with *K. pneumoniae*. 8-24 hours after exposure, nematodes will be transferred to tubes containing environmental contaminants and monitored for survival. RNA will be extracted from both non-infected and infected nematodes to examine the molecular mechanisms of nematode recovery from infection as a result of environmental contaminant treatments. Lastly, *C. elegans* will be exposed to ssRNAs extracted from seed mucilage. Dr. Rodriguez Lopez will extract ssRNAs from basil, chia, and flax plants, and provide these to Dr. Tsyusko-Unrine's lab to evaluate the effects on *C. elegans* gene expression. Dr. Tsyusko-Unrine's current IBC protocol will expire on July 25, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-60 (version 9.0) was approved pending minor modifications as listed below:

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SCIENTIFIC SUMMARY:

1. The proposed work involving exposure of *C. elegans* to ssRNAs extracted from seed mucilage is not sufficiently described. Please expand on the details of this project and potential risks of exposure to ssRNAs, or remove the description of this project if it is not actively planned.

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2. It is stated that mutant strains of *C. elegans* can only survive under specific conditions in the laboratory, but it is unclear if you are providing the conditions required for survival. Please clarify.
3. Please provide a description of how *Klebsiella* contamination is avoided when nematodes are washed off the plates and transferred to tubes. Will the surface of the tubes be disinfected prior to removal from the BSC after this transfer process is complete?

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Mike Mendenhall initiated the motion. Carol Pickett seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Hongyan Zhu

IBC Protocol Number: IBC-26-62

Protocol Title: Genetic and molecular analysis of root-nodule symbiosis in legumes

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: NIH Guidelines Section III-E-2, 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 - Plants (BSL1-P)

Primary Reviewers: A. Hunt, J. Smalle, B. Nelson

Brief Project Overview:

The availability of nitrogen in the soil is a major limiting factor for agricultural production. Although the use of industrial fertilizers has contributed substantially to feeding the world, the production of nitrogen fertilizers requires fossil fuels, and excessive fertilizers cause serious environmental pollution. In contrast, plants of the legume family can make their own nitrogen fertilizer by forming symbiosis with nitrogen-fixing soil bacteria, called rhizobia. This symbiosis culminates in the formation of specialized root organs, called nodules, within which the bacteria convert atmospheric nitrogen into ammonia for use by the plant. The research of the Zhu lab is to identify plant genes that regulate the nodulation and nitrogen fixation processes, with the ultimate goal to improve nitrogen fixation in sustainable agriculture.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: DNA/RNA isolation/purification, Bacterial culture, Genetics, PCR/qRT-PCR, Plant work, Transformation, Imaging/Microscopy

Transport: No

Materials Transported: N/A

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of

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Construct/Host(s)/Vector(s)]: Not known yet/Medicago/not known yet/Plasmid/Medicago/pCambia1305.1/; Cas9/S. pyogenes/DNA endonuclease/Developing gene knockout constructs to validate gene function/Agrobacterium/Hairy root transformation methods using pKSE401 Vector/; Medtr7g0231181/Medicago/Nodule-Specific C2H2 Transcription Factor/Hairy Root Transformation/Medicago, Red Clover/pCambia1305.1/

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pCambia1305.1; Plasmid/pKSE401

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Plant/Medicago truncatula; Plant/Red Clover

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. Zhu has submitted a renewal of his IBC protocol entitled Genetic and molecular analysis of root-nodule symbiosis in legumes. Dr. Zhu's lab seeks to identify plant genes that regulate the nodulation and nitrogen fixation processes in legumes, with the goal of improving nitrogen fixation in sustainable agriculture. Towards that goal, candidate genes will be tested by cloning the genes into pCambia vectors and using hairy root transformation methods to transform them into roots. Hairy root transformation involves the transformation of expression vectors into Agrobacterium rhizogenes or Agrobacterium tumefaciens (depending on the plant host), which is then inoculated onto roots. Transgenic plants are grown in a growth chamber or in the laboratory. Transformed roots will be imaged. No greenhouse or field work is planned. Work with these materials will be done using BSL1-P containment and requires the use of lab coat, eye protection, and gloves. None of the materials in use are pathogenic to humans. Dr. Zhu's current IBC protocol will expire on August 9, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-62 (version 8.0) was approved.

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Jan Smalle initiated the motion. Arthur Hunt seconded the motion. All IBC members present (11) voted in favor of the motion.

*

Conflicts of Interest: None

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PI: Houfu Guo

IBC Protocol Number: IBC-26-66

Protocol Title: Structure and function studies of lysyl hydroxylases

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-D-4

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Institutional Biosafety Committee (IBC) Meeting

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

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

Brief Project Overview:

Collagen-modifying enzymes modify collagen to regulate the structure, signaling, and functions of tissues. When collagen modifications go awry, it can lead to various inherited and acquired diseases. One particular collagen-modifying enzyme of interest is lysyl hydroxylase 2 (LH2), which has been identified as a potential therapeutic target for conditions like fibrosis and cancer, which are major contributors to natural deaths in the US.

Unfortunately, we currently lack a comprehensive understanding of LH2's structure and function, which hinders the development of LH2-based therapies. To address this gap, we aim to investigate LH2 in the context of matrix biology and diseases. Our preliminary studies have revealed that LH2 has two important activities. First, it acts as a telopeptidyl lysyl hydroxylase (t-LH), creating stable collagen crosslinks known as hydroxylysine aldehyde-derived collagen crosslinks (HLCCs), which are vital for maintaining the strength of load-bearing structures in our bodies. Second, we have discovered that LH2 also possesses a previously unknown collagen glucosyltransferase (GGT) activity, which modifies collagen to regulate cell adhesion and mobility.

Through our investigations, we have gained insights into the unique structural features of LH2 and its enzymatic activities. We have identified electrostatic interactions between LH2 and collagen that determine its t-LH activity and the formation of HLCCs. Additionally, we have found that LH2b, an alternative form of LH2, acts as a collagen GGT. LH2b forms a dimer, binds to a molecule called UDP-glucose cooperatively, and modifies specific regions of collagen.

Based on these findings, we propose that LH2 b's distinct structural features and enzymatic activities allow it to modify collagen and influence disease progression. To test this hypothesis, we will introduce specific LH2b mutations into mouse models to gain a deeper understanding of how LH2b alters collagen and drives the progression of diseases.

Recently, we are interested in testing our hypotheses in simpler systems including the budding yeast, cyanobacteria, and cyanobacteria phage.

The insights gained from this research will contribute to the development of LH2-based therapies for conditions like fibrosis and cancer. By unraveling the intricacies of LH2's structure-function relationship, we hope to advance our knowledge of matrix biology and ultimately improve treatment options for these diseases.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Animals

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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)]: LH2WT/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2L735D/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2L665E/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2a WT/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2b WT/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2D115E/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2aD115E/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2bD115E/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2C279S/C282S/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2E54A/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2K222A/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2K438A/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2Y231E/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2R495E/Human/enzymatic protein/expression/Chinese hamster ovary cells/pSGHP1/; LH2C279S/C282S/Human/enzymatic protein/Knockin/Human lung cancer cell line /pLKO.1 puro/; LH2Y231E/Human/enzymatic protein/Knockin/Human lung cancer cell line /pLKO.1 puro/; LH2R495E/Human/enzymatic protein/Knockin/Human lung cancer cell line /pLKO.1 puro/; LH2C279S/C282S/Mouse/enzymatic protein/Knockin/Mouse lung cancer cell line /pLKO.1 puro/; LH2Y231E/Mouse/enzymatic protein/Knockin/Mouse lung cancer cell line /pLKO.1 puro/; LH2R495E/Mouse/enzymatic protein/Knockin/Mouse lung cancer cell line /pLKO.1 puro/; LH2Δexon13a/Human/enzymatic protein/Knockin/Human lung cancer cell line /pLKO.1 puro/; LH2Δexon13a/Mouse/enzymatic protein/Knockin/Mouse lung cancer cell line /pLKO.1 puro/; LH2W75A/Human/enzymatic protein/Knockin/Human lung cancer cell line /pLKO.1 puro/; LH2W75A/Mouse/enzymatic protein/Knockin/Mouse lung cancer cell line /pLKO.1 puro/; LH2K735E/Human/enzymatic protein/Knockin/Human lung cancer cell line /pLKO.1 puro/; LH2K735E/Mouse/enzymatic protein/Knockin/Mouse lung cancer cell line /pLKO.1 puro/; LH2D689A/Human/enzymatic protein/Knockin/Human lung cancer cell line /pLKO.1 puro/; LH2D689A/Mouse/enzymatic protein/Knockin/Mouse lung cancer cell line /pLKO.1 puro/; GLT25D3/Mouse/enzymatic protein/Knockout/Mouse osteoblast cell line /pLKO.1 puro/; GLT25D2/Mouse/enzymatic protein/Knockout/Mouse osteoblast cell line /pLKO.1 puro/; GLT25D1/Mouse/enzymatic protein/Knockout/Mouse osteoblast cell line /pLKO.1 puro/; PGGHG/Mouse/enzymatic protein/expression/E coli/pET28/; LH2C279S/C282S/Human/enzymatic protein/expression/E coli/pET28/; LH2Y231E/Human/enzymatic protein/expression/E coli/pET28/; LH2R495E/Human/enzymatic protein/expression/E coli/pET28/; R655//; L593/Acanthamoeba polyphaga mimivirus/enzymatic protein/expression/E coli/pCOLADuet-1/; LH3 WT/Human/enzymatic protein/expression/E coli/pCOLADuet-1/; MimiColII/Human/matrix protein/expression/E coli/pETDuet-1/; GLT25D1/Amphimedon queenslandica/enzymatic protein/expression/E coli/pETDuet-1/; R699/Acanthamoeba polyphaga mimivirus/enzymatic protein/expression/E coli/pET28/;

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L71/Acanthamoeba polyphaga mimivirus/structural protein/expression/E coli/pET28/;
L230Y831W/Acanthamoeba polyphaga mimivirus/enzymatic protein/expression/E coli/pET28/;
Cre/Bacteriophage/enzymatic protein/expression/Mouse/pLenti-Ubc-PGK/; GFP/Aequorea victoria (jellyfish)/tracking gene/expression/Mouse/pLenti-Ubc-PGK/; LH2WT/Mouse/enzymatic protein/expression/Mouse/pLenti-Ubc-PGK
Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/Lentivirus Ubc-Cre_Pgk-GFP/;
Lentivirus/Lentivirus Ubc-Cre_Pgk-LH2WT/; Lentivirus/Lentivirus Ubc-Cre_Pgk-LH2EP/;
Lentivirus/Lentivirus Ubc-Cre_Pgk-LH1WT/; Lentivirus/Lentivirus Ubc-Cre_Pgk-LH1RR/;
Lentivirus/Lentivirus Ubc-Cre_Pgk-Tp53R172H/; Lentivirus/Lentivirus MMP9 shRNAs/;
Lentivirus/Lentivirus control shRNA/; Lentivirus/Lentivirus Ubc-Cre_Pgk-FLAG-LH2a-WT/;
Lentivirus/Lentivirus Ubc-Cre_Pgk-FLAG-LH2b-WT/; Lentivirus/Lentivirus Ubc-Cre_Pgk-FLAG-LH2b-Y231E/; Lentivirus/Lentivirus Ubc-Cre_Pgk-FLAG-LH2b-R495E/; Baculovirus/Flashbac Baculovirus vector/; Baculovirus/pBacPAK9-LH2/; Plasmid/pET28/; Plasmid/pMDLg/pRRE/; Plasmid/pCOLADuet-1/; Plasmid/pETDuet-1/; Plasmid/pRSV/REV/; Plasmid/pMD2.G/; Plasmid/pLKO.1 puro/; Lentivirus/pLenti-Ubc-PGK/; Plasmid/pDS190/; Plasmid/p406TDH3/; Plasmid/pBacPAK9/; Plasmid/pSGHPI
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/ExpiCHO-S Cells/; Human/HEK293-T/; Human/H460 (lung cancer cells)/; Human/A549 (lung cancer cells)/; Animal/KC2 (lung cancer cells)/; Animal/KC4 (lung cancer cells)/; Insect/Tni (High Five derivative) cells/; Animal/344SQ (lung cancer cells)/; Animal/MC3T3-E1 (osteoblast)/; Animal/393P (lung cancer cells)/; Human/H358/; Human/Expi293 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/Cells - Animal, genetically modified/Subcutaneous and Intratracheal injection/manual scruffing and Anesthesia/ABSL2/Lab coat or Disposable gown, Disposable gloves, Eye protection, and Surgical mask/ABSL2/No//; Mouse/Cells - Human, genetically modified/Subcutaneous Intratracheal injection/manual scruffing and Anesthesia/ABSL2/Lab coat or Disposable gown, Disposable gloves, Eye protection, Surgical mask/ABSL2/No//; Mouse/Viral Vector - Lentivirus/intratracheal instillation/Both Chemical (Anesthesia) and Physical Restraint/ABSL2/Lab coat or Disposable gown, Disposable gloves, Eye protection, Face shield, and Surgical mask/ABSL2/Yes/Mice will be kept at ABSL2 caging for 72 hours, during which agent might be shed.

Risk Assessment/Discussion:

Dr. Guo has submitted a renewal of their IBC protocol entitled Structure and function studies of lysyl hydroxylases. Dr. Guo's laboratory explores collagen modification and how incorrect collagen modification can lead to various inherited and acquired diseases. They are particularly interested in lysyl hydroxylase 2 (LH2) and its potential as a therapeutic target for fibrosis and cancer. Towards that aim, Dr. Guo's laboratory will utilize a lentivirus vector expressing Cre recombinase to generate two different spontaneous lung cancer models in mice. Lentivirus will be delivered to mice intratracheally to anesthetized mice via catheter within a BSC. Personnel will wear lab coat or disposable gown, disposable gloves, eye protection and surgical mask or face shield for this work. 3rd generation lentivirus is purchased from a 3rd party commercial vendor and will not be produced in Dr. Guo's lab. Mice

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administered lentivirus will be housed at ABSL2 housing for a minimum of 72-hours post administration, after which they will be moved to ABSL1 housing. Tumor burden will be monitored by MRI imaging. Upon euthanasia, mice will be necropsies and lung tissues weighed, visible lung tumors counted, and collagen glycosylation and crosslinks will be analyzed. Lentivirus will also be used to generate knockdown cell lines for subcutaneous and orthotopic lung cancer models in mice. The lentivirus used for generation of stable knockdown cell lines will be produced in Dr. Guo's laboratory. Lentivirus production and transduction will take place using BSL2+ practices and procedures. All work will take place in a class II BSC. Stably transduced cells will be used for in vitro cell proliferation assays, cell migration/invasion assays, and lung cancer models in mice. Cells will be injected intrathoracically or subcutaneously to anesthetized mice within a BSC in DLAR. Transduced cells will have been passaged for at least 10 generations prior to administration to mice. Procedures will be completed at ABSL2, and mice will be housed at ABSL1 housing. Dr. Guo's laboratory will also produce recombinant proteins in *E. coli* and mammalian cells. Recombinant proteins will be produced in *E. coli* using BSL1 procedures and practices. Human cells will be handled using BSL2 procedures and practices. They will also use *S. cerevisiae* to generate recombinant proteins at BSL1. *Synechococcus* and *synechococcus* phage species, found in marine environments, will be collected from ocean water. *Synechococcus* will be cultured and *synechococcus* phage introduced. Ultimately, phage proteins will be analyzed using SDS-PAGE. *Synechococcus* and *synechococcus* phage are not considered pathogenic and are handled at BSL1. They will also produce recombinant protein via baculovirus vector. The baculovirus vector in use cannot transfect mammalian cells and will be handled at BSL1. Dr. Guo's current IBC protocol will expire on August 14, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-66 (version 8.0) was approved pending minor modifications as listed below:

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GENERAL INFORMATION – DOT/IATA Training: It is implied that shipping of biohazardous materials may be carried out. If this is accurate, at least one person on the protocol requires DOT/IATA Training. Please verify whether you are shipping biohazardous materials, and if so, provide the DOT/IATA training date.

GENERAL INFORMATION: Manipulations Planned: Chromatography and mass spectrometry procedures are mentioned in the Scientific Summary. If these manipulations are done in the lab, please add “Proteomics” as an additional selection under Manipulations Planned.

LOCATIONS – Research Locations table: The corresponding IACUC indicates that animals will also be housed and handled in the BBSRB DLAR facility in addition to the BioPharm facility. Please update to include all animal research locations.

SCIENTIFIC SUMMARY:

1. Please expand on additional proteins that will be produced and downstream assays that will be performed with the produced proteins.
2. Please include details on the type of chemical disinfectants being used and contact time.

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3. Details on where downstream applications for in vitro proliferation and cell migration assays take place should be included.

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Doug Harrison initiated the motion. Delphine Malherbe seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Daniel Lee

IBC Protocol Number: IBC-26-67

Protocol Title: Modulation of Arginine Metabolism and Polyamines to Mitigate Alzheimer's Disease Pathology

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-1, 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 (BSL2), Animal Biological Safety Level 1 (ABSL1)

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

Brief Project Overview:

We study metabolic disruptions in an amino acid called arginine and how that contributes to accumulation of proteins known to cause Alzheimer's and other dementias. We have a particular focus on trying to repair these disruptions with an ultimate goal of treating dementias like Alzheimer's disease.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Cell culture, DNA/RNA isolation/purification, Immunohistochemistry, 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)]: Arginase 1 (Arg1)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Arginase mutant (ArgM)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Spermidine synthase (SRM)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Spermine synthase (SMS)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Spermidine spermine N acetyl transferase1 (SSAT1)/ Human/ Enzyme/ Expression/ Mice/

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pTrAAV9/ ; Ornithine decarboxylase (OCD1)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Ornithine decarboxylase antizyme (OAZ1)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Ornithine decarboxylase antizyme inhibitor (AZIN2) aka Arginine Decarboxylase (ADC)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Argmatinase (AGMAT)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Green fluorescent protein (GFP)/ Jellyfish/ Tracking/ Expression/ Mice/ pTrAAV9/ ; GPRC6a/ Human, mouse/ Structural/ expression, silencing via shRNA, silencing via siRNA plasmid/ Mice, mammalian cells/ pTrAAV9, siRNA/ ; CASTOR1/ Mouse, human/ Structural/ expression, silencing via shRNA, silencing via siRNA plasmid/ Mice, mammalian cells/ pTrAAV9, siRNA/ ; CASTOR mutant (I126)/ Mouse/ Structural/ Expression/ Mice/ pTrAAV9/ ; Arginine deiminase (ADI)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Peptidyl arginine deiminase 2 (PAD2)/ Human/ Enzyme/ Expression, silencing via shRNA/ Mice/ pTrAAV9/ ; Peptidyl arginine deiminase 4 (PAD4)/ Human/ Enzyme/ Expression, silencing via shRNA/ Mice/ pTrAAV9/ ; Peptidyl arginine deiminase 4 mutant D350A (PAD4m)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Tau Full Length/ Human/ Structural/ Expression/ Mice/ pTrAAV9/ ; Tau C-terminal truncated/ Human/ Structural/ Expression/ Mice/ pTrAAV9/ ; Alpha synuclein/ Human/ Structural/ Expression/ Mice/ pTrAAV9/ ; GPRC6a mutant (KY Indel)/ Human, mouse/ Structural/ Expression/ Mice/ pTrAAV9/ ; Polyamine-modulated Factor 1 (PMF1)/ Human/ Enzyme/ Expression/ Mice/ pTrAAV9/ ; Tau N-terminal truncated/ Human/ Structural/ Expression/ Mice/ pTrAAV9/ ; SLC38A9/ Mouse, human/ Structural/ Silencing via shRNA, siRNA/ Mice, mammalian cell culture/ pTrAAV9, siRNA/ ; GFP/GPRC6a/ Jellyfish, human/ Tracking, structural/ Expression, silencing via shRNA/ Mice/ pTrAAV9/ ; GFP/Scramble/ Jellyfish, human/ Tracking, structural/ Expression, silencing via shRNA/ Mice/ pTrAAV9/ ; synGFP/ Jellyfish/ Tracking/ Expression/ Mice/ AAV-PHP.eB/ ; Peptidyl arginine deiminase 4 (synPADi4)/ Human/ Enzyme/ Expression/ Mice/ AAV-PHP.eB/ ; Peptidyl arginine deiminase 4 (synPADi4m)/ Human/ Enzyme/ Expression/ Mice/ AAV-PHP.eB/ ; Cre recombinase/ Bacteria/ Enzyme/ Expression/ Mice/ pTrAAV9 Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/pTrAAV9; Adeno-Associated Virus (AAV)/AAV-PHP.eB

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/ SH-SY5Y/ ; Human/ M17/ ; Human/ C3/ ; Animal/ HT22/ ; Animal/ H11/ ; Animal/ HAPI/ ; Animal/ N2a/ ; Human/ iN2a-tau/ ; Animal/ N2a-ssGT/ ; Human/ HEK293T/ ; Human/ HEK293T-TBS/ ; Human/ iHEK-WT/ ; Human/ iHEK-DK/ ; Human/ iHEK-DK/ ; Human/ iHEK-WD/ ; Human/ iHEK-PD

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)/Injection into brain/anesthesia/ABSL1/Lab coat/gown, gloves, eye protection, surgical mask/ABSL1/No

Risk Assessment/Discussion:

Dr. Lee has submitted a renewal of his IBC protocol entitled Modulation of Arginine Metabolism and Polyamines to Mitigate Alzheimer's Disease Pathology. In Dr. Lee's laboratory, they work to understand metabolic disruptions in arginine metabolism and how that contributes to accumulation of proteins known to cause Alzheimer's and other dementias. Their focus is to work to repair these disruptions in

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order to treat Alzheimer's and other dementias. The biohazardous materials used towards that goal include human tissues, human cells, and adeno-associated virus (AAV) vectors. Human tissues with known diagnoses of Alzheimer's and other related dementias will be used for biochemical analysis including Western blot and immunohistochemistry and/or immunofluorescent labeling. Human cells will be cultured and used for proof of principle experiments for drug treatments. BSL2 practices and procedures are used for work with human source materials and includes use of a lab coat, disposable gloves, and eye protection. Work will be completed within a BSC until biohazardous materials are inactivated via lysis buffer. AAV vectors will be purchased/obtained from collaborators or commercial vendors and will not be produced in the Lee lab. AAVs will be used for in-vivo stereotaxic injections. AAVs are administered to anesthetized mice via stereotaxic injection. After euthanasia, brain tissue will be harvested for immunohistochemistry and biochemical analysis including Western blot, RNA sequencing, and HTRF. AAV work in mice will be done using ABSL1 procedures and housing. Dr. Lee's current IBC protocol will expire on August 24, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-67 (version 6.0) was approved pending minor modifications as listed below:

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PERSONNEL: Christopher Saunders and Lucas J Knepp are listed in IACUC 2021-3776 as handling recombinant and/or synthetic nucleic acid materials, but they are not listed in the IBC protocol personnel list. Please ensure personnel lists are congruent.

LOCATIONS – Research Locations table:

1. IACUC protocol 2020-3609, 2021-3776, and 2020-3620 indicate that behavior testing occurs in several locations including Todd building DLAR and HKRB building RBC. Please add these locations to the table.
2. IACUC protocol 2020-3620 indicates that EchoMRI and Sable Promethion testing will be done in BBSRB DLAR. Please add these locations to the table.

SCIENTIFIC SUMMARY:

1. Please add some detail about the kinds of assays being performed after AAV administration and where they will be performed, for example, benchtop, biosafety cabinet, chemical fume hood, etc. What kinds of tissues are being assayed? Fixed, frozen, unfixed, etc.
2. Add information about the length of time in animal experiments between the administration of viral vectors and euthanasia.

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Delphine Malherbe initiated the motion. Brandy Nelson seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Carrie Shaffer

IBC Protocol Number: IBC-26-69

Protocol Title: B23-4242-M: Molecular Mechanisms of Bacterial Secretion Systems

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-2, NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-3, NIH Guidelines Section III-F-4, OSHA 29 CFR 1910.1030

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

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

Brief Project Overview:

Research projects in the Shaffer lab focus on defining molecular mechanisms underlying host-microbe interactions that influence human, animal, and plant disease. We are particularly interested in exploring the function and architecture of bacterial type IV secretion system (T4SS) nanomachines that are used by many diverse human and animal pathogens to establish host colonization. Our research employs innovative reporter cell lines, high-resolution microscopy, chemical biology, and biochemical approaches to investigate the mechanism by which T4SS activity delivers diverse molecular cargo to host cells. We also study various aspects of microbial cell biology including the biogenesis and architecture of bacterial cell surface structures assembled at the host-microbe interface. We aim to (i) delineate how the T4SS apparatus assembles in response to target cell contact, (ii) resolve the topology of T4SS machinery in various architectural states, (iii) identify and characterize small molecule inhibitors of T4SS function in diverse bacterial systems, (iv) understand the molecular mechanisms that orchestrate and trigger T4SS cargo transport across the bacterial cell envelope, and (v) investigate how translocated substrates regulate host cell biology. Specific projects in the Shaffer lab apply interdisciplinary approaches to understand how bacteria build and use protein secretion machines to colonize and survive within diverse host environments. In future work, we will leverage this knowledge to design new therapeutic strategies to combat infectious disease.

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, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Propagation of infectious agents, Transfection, Transformation, Use of Human Source Material(s), Use of infectious agents, Viral culture, Proteomics

Transport: Yes

Materials Transported: Biohazardous Materials, Animals

Infectious Agent(s)/Natural Host(s): *Acinetobacter baumannii* (RG2-bacteria)/Humans;/ *Agrobacterium tumefaciens* (RG1-bacteria)/Amphotropic. *Arabidopsis*, walnuts, grape vines, stone fruits, nut trees,

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sugar beets, horse radish, and rhubarb. /; *Escherichia coli* (RG2-bacteria)/Humans, animals/; *Helicobacter pylori* (RG2-bacteria)/Humans, animals/; *Legionella pneumophila* (RG2-bacteria)/Humans, experimental infection in animals/; *Pseudomonas aeruginosa* (RG2-bacteria)/Humans, animals, plants/; *Rhodococcus equi* (RG2-bacteria)/Humans, animals/; *Streptococcus equi* subsp. *equi* (RG2-bacteria)/Humans, animals/; *Enterobacter aerogenes* (RG2-bacteria)/Humans, animals, plants/; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human (human cell lines)/; *Salmonella enterica* (RG2-bacteria)/Humans, animals/; *Wolbachia pipientis* (RG1-bacteria)/Insects and nematodes/; *Corynebacterium uterequi* (RG1-bacteria)/Horses/; *Streptococcus anginosus* (RG2-bacteria)/Humans/; Epstein-Barr virus (EBV) (RG2-virus)/humans/

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: cytotoxin-associated gene A (cagA)/*Helicobacter pylori*/oncoprotein that is translocated from *H. pylori* to host gastric epithelial cells/epitope-tagged version of CagA used for western blot analysis in vitro/*H. pylori*, *E. coli* DH5alpha/pGEM T-easy/; cytotoxin-associated gene H (cagH)/*Helicobacter pylori* /type IV secretion system protein (structural)/epitope-tagged version of CagH (CagH-HA) used for western blot analysis in vitro/*H. pylori*, *E. coli* DH5alpha/pADN/; other cag genes/*Helicobacter pylori* /type IV secretion system components/various uses (protein purification, western blot, microscopy)/*H. pylori*, *E. coli* DH5alpha/pADN/; pRL-SV40P/Renilla Luciferase/tracking gene/Renilla SV40 promoter (no enhancer) reporter gene for normalization of luciferase experiments/human cell lines, *E. coli* DH5alpha/vector backbone pRL-TK (Addgene)/; pcDNA3.1-Hygro(+)-PSTCD-cGAS-delta DBD /human/cytoplasmic protein/Mammalian expression of mutant cGAS with deletion of DNA binding domain fused to PSTCD epitope tag/human cell lines, *E. coli* DH5alpha/pcDNA3.1 (Addgene)/; IFN-Beta_pGL3/luciferase/tracking gene/IFN-beta promoter driving luciferase. Use in IFN reporter assay./human cell lines, *E. coli* DH5alpha/pGL3 (Addgene)/; pUNO1-hSTING/human/endoplasmic reticulum/innate immune protein/expression of human stimulator of interferon genes (STING)/human cell lines, *E. coli* DH5alpha/pUNO1 (Invivogen)/; pUNO1-hcGAS-HA3x/human/innate immune receptor cGAS (cytoplasmic protein)/expression of human cGAS with 3 tandem HA epitope tags/human cell lines, *E. coli* DH5alpha/pUNO1 (Invivogen)/; pUNO1-hcGAS-AA/human/innate immune receptor cGAS (cytoplasmic protein)/expression of catalytically inactive human cGAS/human cell lines, *E. coli* DH5alpha/pUNO1 (Invivogen)/; mScarlet reporter/engineered/synthetic monomeric fluorescent protein/tracking/reporter gene/Used to monitor DNA injection into host cells by *H. pylori* cag T4SS activity/*H. pylori*, *E. coli* DH5alpha, human cell lines/pLifeAct_mScarlet-i_N1/; (Plasmid #85056, Addgene)/; mNeonGreen reporter/engineered/synthetic monomeric fluorescent protein/tracking/reporter gene/Used to monitor DNA injection into host cells by *H. pylori* cag T4SS activity/*H. pylori*, *E. coli* DH5alpha, human cell lines/LifeAct-mNeonGreen/; / (Plasmid #98877, Addgene)/; NanoLuciferase reporter/engineered/synthetic nanoluciferase protein/tracking/reporter gene/Used to monitor DNA injection into host cells by *H. pylori* cag T4SS activity/*H. pylori*, *E. coli* DH5alpha, human cell lines/pNL1.1.CMV[Nluc/CMV] (Promega)/; pNL3.2.NF-kB-RE/engineered/synthetic nanoluciferase protein/reporter/Used to monitor NF-kB activation in transfected cells that are infected with *H. pylori*. Vector contains five copies of an NF-kB response element (NF-kB-RE) that drives transcription of a destabilized form of NanoLuc® luciferase, an engineered small (23.3kDa) luciferase fusion protein.

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/human/pNL3.2.NF- κ B-RE (Promega)/; HA-MYD88/human/Innate immune system component/Used to overexpress HA epitope-tagged MYD88 protein in transfected cells to analyze downstream TLR signaling in *H. pylori* infected cell lines/human cell lines, *E. coli* DH5alpha/pCMV-HA-MyD88 (Plasmid #12287 commercially available from Addgene)/; pGL4.54[luc2/TK]/engineered/synthetic firefly luciferase protein/reporter/Vector contains the luc2 gene encoding firefly luciferase (Fluc) from *Photinus pyralis* expressed from the HSV-TK promoter, a viral promoter that provides low levels of expression in mammalian cells. This vector is used as a constitutive expression control vector. /human cell lines, *E. coli* DH5alpha/pGL4.54[luc2/TK] (Promega)/; pMAL-c6T (malE gene)/*E. coli*/recombinant protein expression vector/Expression of recombinant maltose-binding protein (MBP) fusions in the bacterial cytoplasm for small scale purifications using amylose resin./*E. coli* BL21/pMAL-c6T (New England Biolabs)/

Vector(s) [Vector Category/Vector Technical Name]: Plasmid, pADNPlasmid, pcDNA3Plasmid, pRL-SV40PPlasmid, IFN-Beta_pGL3 Plasmid, pGEM T-easyPlasmid, pUNO1Plasmid, LifeAct-mNeonGreenPlasmid, pLifeAct_mScarlet-i_N1Plasmid, pNL1.1.CMV[Nluc/CMV]Plasmid, pNL3.2.NF- κ B-REPlasmid, pCMV-HA-MyD88Plasmid, pGL4.54[luc2/TK]

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/AGS (ATCC® CRL-1739™)/; Human/HEK293 (HEK-Blue™ hTLR9)/; Human/HEK293 null (HEK-Blue™ Null1 cells)/; Human/U937 (ATCC® CRL-1593.2™)/; Human/5637 (ATCC® HTB-9™)/; Human/AGS NF- κ B luciferase reporter/; Human/HEK293T (ATCC® CRL-3216™)/; Human/Human Primary Stomach Epithelial Cells (H-6039)/; Human/AGS-EBV/; Human/Caco-2 (ATCC® HTB-37)/; Human/A549 (ATCC® CRM-CCL-185)/; Human/3dGRO™ Stomach Gastric Organoids, Age 60 (SCC344)/; Animal/J774A.1 (ATCC® TIB-67)/; Animal/Equine Organoid Models/; Human/293-Dual™ Null Cells (293d-null)/; Human/293-Dual™ hSTING-R232 Cells (293d-r232)/; Human/HEK-Dual™ cGas Cells (hkd-cgas)/; Insect/Drosophila Schneider 2 (S2) cells/; Animal/Murine organoid models

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/*Helicobacter pylori* (RG2-bacteria)/orogastric lavage/Isoflurane anesthesia and hand restraint/ABSL2/Lab coat, disposable gloves, eye protection, shoe covers/booties/ABSL2/No/Infections will be performed in a Biological Safety Cabinet (BSC); mice will be housed in microisolator cages marked with a cage card to indicate infection status

Risk Assessment/Discussion:

Dr. Shaffer has submitted a renewal of her IBC protocol entitled Molecular Mechanisms of Bacterial Secretion Systems. Dr. Shaffer's lab is interested in the function and architecture of bacterial type IV secretion system (T4SS) nanomachines used to establish host colonization. Several pathogenic and non-pathogenic microorganisms are used to study bacterial secretion system biology, including *Helicobacter pylori* (RG2), *Wolbachia pipientis* (RG1), *Legionella pneumophila* (RG2), *Agrobacterium tumefaciens* (RG1), *Escherichia coli* (RG2), *Pseudomonas aeruginosa* (RG2), *Klebsiella aerogenes* (RG2), *Rhodococcus equi* (RG2), *Acinetobacter baumannii* (RG2), *Streptococcus agalactiae* (RG2), *Salmonella enterica* (RG2), *Cornebacterium uterequi* (RG1), and *Streptococcus equi* (RG2). Dr. Shaffer also utilizes a gastric epithelial cell line that's been transformed with a GFP-expressing recombinant Epstein-Barr virus (EBV)

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to study co-infection with *H. pylori* impacts the development of gastric cancers. All work with bacteria that may generate potential aerosols and work studying bacteria-host cell interactions will be completed within a BSC using BSL2 practices and procedures. An animal model of *H. pylori* infection is also utilized. Anesthetized mice will be administered *H. pylori* inoculum via feeding needle within a BSC in DLAR. Mice will be housed at ABSL2 housing throughout the duration of the experiment. Mice will be imaged in the Light Microscopy Core (LMC) facility in HKRB. Upon euthanasia, stomach contents will be removed and tissues dissected into thirds for downstream analysis. Recombinant DNA technologies will be used to generate knock-out, mutant, and genetically complemented strains of microorganisms in use, specifically *E. coli*, *K. aerogenes*, and *H. pylori*. A variety of human, animal, and insect cell lines are used to generate organoid models that will be cultured under BSL2 containment. Dr. Shaffer's current IBC protocol will expire on July 19, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-69 (version 10.0) was approved pending minor modifications as listed below:

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INFECTIOUS AGENTS – Infectious Agent(s) table:

1. Under the “Amount Produced” column, some of the entries list <5ml, which seems notably smaller than that of other agents. Please review and update for accuracy, if necessary.
2. The Scientific Summary describes *Klebsiella aerogenes*, but the Infectious Agents table lists *Enterobacter aerogenes*. Please update with appropriate name for congruency.

SCIENTIFIC SUMMARY:

1. The Scientific Summary describes *Klebsiella aerogenes*, but the Infectious Agents table lists *Enterobacter aerogenes*. Please acknowledge that these are the same bacterial agent that was reclassified.
2. Please specify the chemical agent and exposure time for tissue fixation.
3. In descriptions of waste disposal for cultures, solid biohazardous waste disposal is described but liquid biohazardous waste disposal is not. If you are generating liquid biohazardous waste from bacterial cultures, please describe how this is done.
4. Please briefly provide additional transport details for the transport of biohazardous material from Gluck to BBSRB. Will this be done in a vehicle or on foot?

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Delphine Malherbe initiated the motion. Carol Pickett seconded the motion. All IBC members present (11) voted in favor of the motion.

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Conflicts of Interest: None

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Other Business

Delena Mazzetti reminded IBC members the next scheduled IBC meeting will occur on August 12, 2026.

Incident Review

None.

Protocol Issued Registration Numbers

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

Cantu, Edward MULTICENTER VALIDATION OF THE PROSPECTIVE REGISTRY OF OUTCOMES IN PATIENTS ELECTING LUNG TRANSPLANTATION (PROPEL) COHORT STUDY New BSO 06/30/26 IBC-26-58 (v.12.0)

DeVries, Zach B24-4361: Bed Bug Rearing Amendment BSO 06/29/26 IBC-24-86 (v.13.0)

Venditto, Vincent Venditto Lab Biosafety Amendment BSO 06/23/26 IBC-25-33 (v.42.0)

Chattopadhyay, Saurabh Innate immune responses to virus infection Amendment BSO 06/22/26 IBC-24-108 (v.69.0)

Sompol, Pradoldej Oxidative Stress and Related Neurovascular Dysfunction in Alzheimer's Disease and Related Dementias Amendment BSO 06/17/26 IBC-25-42 (v.31.0)

Zhu, Qingzhang Adipocyte function in metabolism, inflammation and fibrosis during obesity Amendment BSO 06/17/26 IBC-24-493 (v.52.0)

Helsley, Robert Macronutrient metabolism in Cardiometabolic Disease Amendment BSO 06/16/26 IBC-25-78 (v.34.0)

Suzuki, Yasuhiro Molecular and cellular mechanisms of host resistance against *Toxoplasma gondii* Amendment BSO 06/15/26 IBC-24-421 (v.31.0)

McLaurin, Kristen Neurocognitive Impairments Resulting from Adolescent Prescription Opioid Use Disorder Amendment BSO 06/12/26 IBC-24-37 (v.48.0)

Temel, Ryan Knockdown and overexpression of genes in mice and nonhuman primates using antisense oligonucleotides (ASO) Amendment BSO 06/12/26 IBC-26-07 (v.16.0)

Morford, Lorri University of Kentucky (UK) Center for the Biologic Basis of Oral/Systemic Diseases (CBBO/SD): Hereditary Genetics/Genomics Core 1. Prospective Investigation of External Apical Root Resorption (EARR) and Genetic Markers (IRB 09-0181-F6G/E-IRB 45268) 2. Combined Genetic and Cephalometric Analysis to Study Class III Malocclusion in Families From Brazil and Colombia (IRB 09-0398-F1V/E-IRB 45272) 3. Analysis of Genetic Variations Associated With Both Hypodontia (Naturally Missing Teeth) and Other Medical Conditions (IRB 10-0499-F6A/E-IRB 44209) 4. Genetics of Class III Malocclusion in US Caucasians (IRB 14-0446-F6A/E-IRB 45270) 5. Biosignatures of periodontitis: Effects

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of Diabetes and Methotrexate Anti-inflammatory Therapy (IRB 17-0439-F6A/E-IRB 44558) Amendment BSO 06/10/26 IBC-26-13 (v.16.0)

Geisler, Caroline Neuroendocrine Control of Systemic Metabolism and Energy Balance Amendment BSO 06/09/26 IBC-24-445 (v.44.0)

Emfinger, Christopher Unmasking conditional dependencies between key proteins influencing metabolic health. Amendment BSO 06/09/26 IBC-25-103 (v.35.0)

Jaramillo, Anel Neurobiology of anxiety and alcohol-use disorder Amendment BSO 06/09/26 IBC-25-185 (v.30.0)

Czuba, Lindsay Mechanisms of Small of Molecule Transport and Metabolism Amendment BSO 06/09/26 IBC-25-102 (v.26.0)

Hunt, Arthur B24-4363: Characterization of messenger RNA polyadenylation in plants Amendment BSO 06/04/26 IBC-24-341 (v.13.0)

Cai, Weikang Understanding astrocytes and microglia functions in neurological diseases. Amendment BSO 06/04/26 IBC-24-408 (v.88.0)

Stewart, Andrew Gene Therapy Approaches to Induce and Control Neuronal Growth in Rodents With Spinal Cord Injuries Amendment BSO 06/04/26 IBC-26-04 (v.18.0)

de Souza, William Transmission dynamics and virus-host interactions of arboviruses Amendment BSO 06/04/26 IBC-25-05 (v.41.0)

Cormier, Lindsay Chemotherapeutic development towards cancer Amendment BSO 06/04/26 IBC-25-45 (v.22.0)

Shaddox, Luciana 1. Analysis of Host-Biofilm Interactions: A Novel Polymicrobial model 2: Evaluation of different risk factors on periodontal disease and caries 3: COVID-19 testing using Saliva and Nasal swabs 4. Analyze and characterize the oral microbiome in the presence COVID-19 in the Saliva and Dental plaque samples 5. A new SiC-coated anodized titanium surface to evaluate osteointegration and peri-implantitis progression: an animal model 6. Understanding the association between buprenorphine and oral health outcomes 7. Advancing Salivary Biomarker Development and Utility in Periodontitis Amendment BSO 06/04/26 IBC-24-349 (v.79.0)

Campbell, Kenneth Cellular level contractile function in human heart failure Amendment BSO 06/03/26 IBC-24-482 (v.68.0)

Protocols Meeting Registration Requirements

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

Richards, Christopher Identification of novel cancer therapies Amendment IBC 06/30/26 IBC-26-33 (v.24.0)

Yang, Eddy Novel combination therapies to treat cancer Renewal IBC 06/29/26 IBC-26-54 (v.10.0)

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Stamm, Stefan Regulation of alternative pre-mRNA processing in Prader Willi syndrome and tauopathies
Amendment IBC 06/29/26 IBC-25-37 (v.35.0)

Li, Xiangang The role of SR-BI in sepsis and in therapy-induced immune activation Amendment IBC
06/24/26 IBC-24-96 (v.31.0)

Tong, Sheng Development of magnetic-responsive nanomaterials for disease treatment Amendment IBC
06/11/26 IBC-25-47 (v.46.0)

Harrison, Douglas B23-4205-M: Roles of JAK Signaling in Drosophila Development Renewal IBC 06/10/26
IBC-26-49 (v.10.0)

Rodgers, David B23-4189: Cavities in Choline Acetyltransferase and Neuromuscular Disorders Renewal
IBC 06/10/26 IBC-26-52 (v.7.0)

IBC Training

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

Adjournment

Doug Harrison moved to adjourn the meeting at 1:44PM. Delena Mazzetti seconded the motion. All members present (11) voted in favor.

APPROVED