

University of Kentucky

Institutional Biosafety Committee (IBC) Meeting

Date: 01APR026
Time: 12:02PM – 2:03PM
Location: Virtual Meeting via Zoom - <https://uky.zoom.us/j/81360084231>

Minutes

Call to Order

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

Attendance

IBC Members Present

Thomas Chambers (Local, Non-Affiliated Member)

Doug Harrison (Chairperson)

Cheryl Haughton (Animal Containment Expert)

Brandy Nelson (Institutional Member)

Carrie Shaffer (Institutional Member)

Delena Mazzetti (Biological Safety Officer)

Mike Mendenhall (Local, Non-Affiliated Member)

Amelia Pinto (Institutional Member)

Carol Pickett (Local, Non-Affiliated Member)

Arthur Hunt (Plant Containment Expert)

Delphine Malherbe (Laboratory Staff Representative)

Jan Smalle (Plant Containment Expert)

Yadi Wu (Institutional Member)

Regrets

Maria Landron (Local, Non-Affiliated Member)

Guests

Elizabeth Brooks (Administrative Support Associate I)

Robert Hayman (Assistant Biological Safety Officer)

Jeff Howell (IBC Administrative Professional II)

Audra Strahl (IBC Administrative Professional II)

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.

Approval of Previous Month's Meeting Minutes

[2026.03.04 IBC Meeting Minutes DRAFT.pdf](#)

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The previous month's minutes were approved. Thomas Chambers initiated the motion. Amelia Pinto seconded the motion. All members present (13) voted in favor.

Old Business

None.

New Business

Protocol Review

Resubmissions

PI: William de Souza

IBC Protocol Number: IBC-25-05

Protocol Title: Transmission dynamics and virus-host interactions of arboviruses

Protocol Type: Amendment

Amendment To: Personnel, Manipulations Planned, Organisms used in Research

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

Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: C. Haughton, A. Pinto, T. Chambers

Brief Project Overview:

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

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Chikungunya Virus (CHIKV) 181/25 vaccine strain (RG2-virus)/Vaccine-lab generated/; Mayaro Virus (MAYV) (RG2-virus)/Humans, animals, and insects (mosquitoes)/; Venezuelan Equine Encephalitis Virus (VEEV) TC83 vaccine strain (RG2-virus)/Vaccine-

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

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

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

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/Vero clone E6/; Animal/Vero cells/; Human/HEK293/; Insect/Aedes albopictus clone C6/36/; Human/HuH-7 /; Animal/Immune Cells/; Human/Immune Cells/; Animal/Immune Cells/; Human/human primary astrocytes /; Human/Human Umbilical Vein Endothelial Cells

Animal Use: No

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

Risk Assessment/Discussion:

This protocol amendment was originally reviewed at the March 4, 2026, IBC meeting and returned to the PI with significant revisions. In this revised version, Dr. de Souza has clarified PPE requirements for ABSL2 work with Mayaro virus in mice (yellow gown, bouffant cap, face shield, N95 respirator, double-gloves, and shoe covers). They have also identified three spaces in DLAR for this proposed work. Specifically, Combs CB26 will be used for ABSL2 housing of Mayaro virus infected mice and will be shared with other researchers housing animals at ABSL2. Combs CB27 will be used for ABSL2 procedures, including inoculations and necropsy, and has a certified Class II/B1 BSC available. Importantly, Combs CB24 has been identified as a dedicated space for behavioral testing of Mayaro virus infected mice using ABSL2 procedures. The proximity of these three locations minimizes transport risks as all work with Mayaro virus infected mice will take place in one corridor. Briefly, mice will be infected with Mayaro virus (MAYV), a RG2 virus. Anesthetized mice will be infected subcutaneously in the footpad with MAYV within a BSC in CB27. Biological samples will be obtained for downstream analysis (ex. PCR, flow cytometry), and all samples will be chemically inactivated (via TRIzol or paraformaldehyde) prior to removal from the BSC. Animals will be housed at ABSL2 housing in CB26. Beginning 45 days after infection, mice will undergo behavioral assessments (e.g. Barnes Maze, Morris Water Maze) in CB24. All behavioral testing equipment will be cleaned and disinfected with Lysol No Rinse Sanitizer in accordance

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with Animal Behavior Core SOPs after each use. At the conclusion of all behavioral experiments, CB24 will undergo VHP fogging by DLAR personnel. Infected mice will be housed in filtered microisolator cages and cages will only be opened within a certified BSC. Exterior of animal cages will be wiped with MB-10 prior to any movement. There is an IBC hold on corresponding IACUC 2025-4704.

IBC Discussion & Vote:

The amendment to IBC-25-05 (version 27.0) was provisionally approved pending modification with IBC Primary Reviewers final review :

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ANIMAL RESEARCH – Animals with Biohazards Table: Please update the PPE listed here to include a liquid impermeable layer and remove reference to N95 respiratory protection. Be sure PPE listed is congruent throughout.

DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Biohazardous Materials Transport Description: Remove reference to “triple-contained”.

LOCATIONS – Research Locations Table: Please update the PPE listed here to include a liquid impermeable layer for animal work and remove reference to N95 respiratory protection. Ensure PPE listed is congruent throughout the protocol.

SCIENTIFIC SUMMARY -

1. It is unclear whether the adoptive cell transfer is occurring in infected or uninfected mice. If this procedure is performed using uninfected mice, please clearly state this. If using infected mice, additional details are needed regarding how the cells are isolated and administered.
2. The descriptions of PPE are inconsistent throughout the protocol. Please clearly define the PPE that will be used for each procedure in the Scientific Summary and ensure congruency with descriptions in other sections of the protocol. Use of a liquid impermeable layer for animal work should be added.
3. Please provide a clear and thorough description of tissue isolation and collection procedures.
4. Under “B) Flow cytometry”, please include or clarify details associated with new mouse infection model. Are samples transported to Flow Cytometry fixed or live? If fixed, please specify the fixative and incubation time.
5. Under “D) Transport of biological samples:” please update the description to be congruent with what is stated in the “Biohazardous Materials Transport Description” in the Transport section of the protocol. Please also remove the “triple-contained” statement.
6. Please clarify what downstream assays are performed and the location where these assays take place. For example, are samples transported from DLAR to the main lab for PCR analysis, or is equipment for assays available in DLAR?

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Amelia Pinto initiated the motion. Thomas Chambers seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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Amendments

PI: Shuxia Wang

IBC Protocol Number: IBC-24-521

Protocol Title: Role of USF2, thrombospondin1, CD47 and PKG in the development of obesity and diabetes associated renal and vascular complications. Increasing cGMP signaling as an effective strategy for reno-protection during cisplatin chemotherapy; Role of SMPDL3B in obesity-associated non-alcoholic fatty liver disease; CD47 as a regulator of beige and brown fat function; Administrative Supplements for Research on Sexual and Gender Minority (SGM) Populations; Role of DHRSX in turner syndrome-related non-alcoholic fatty liver disease

Protocol Type: Amendment

Amendment To: Manipulations planned, Genetic constructs, Personnel

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

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

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

Brief Project Overview:

Role of USF2, thrombospondin1, CD47 and PKG in the development of obesity and diabetes associated renal and vascular complications. Increasing cGMP signaling as an effective strategy for reno-protection during cisplatin chemotherapy; Role of SMPDL3B in obesity-associated non-alcoholic fatty liver disease; CD47 as a regulator of beige and brown fat function; Administrative Supplements for Research on Sexual and Gender Minority (SGM) Populations; Role of DHRSX in turner syndrome-related non-alcoholic fatty liver disease.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Biohazardous Materials

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

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Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: upstream stimulatory factor 2/mouse/transcription factor/overexpression, silencing/rat mesangial cells /pAd/CMV/V5-DEST, pLenti6/V5-DEST/; CD47/mouse/membrane associated protein and a receptor for thrombospondin 1/silencing /mouse /N/A/; CD47/mouse/membrane associated protein and a receptor for thrombospondin 1/overexpression/mouse adipocytes/plenti-III/; GFP/mouse/Green fluorescence protein, working as a tracker/overexpression/rat mesangial cells/pLenti6/V5-DEST/; DHRSX/murine and human /dehydrogenase/reductase enzyme/overexpression, knockdown/murine and human hepatocytes/Ad5-CMV-DHRSX, AAV8-DHRSX-shRNA/; Cluh/mouse/RNA binding protein /overexpression /mouse/AAV8-mCluh/; UTX/murine/a histone H3K27 demethylase/overexpression, knockdown/mouse/Ad5-mUTX or AAV8-mUTX, AAV8-mUTX-shRNA/; GFP/mouse/Green fluorescence protein, working as a tracker /overexpression /mouse/AAV8-GFP/; TSP1 promoter/mouse/promoter reporter construct/To measure TSP1 promoter activity/Rat mesangial cells/pGL3-basic/; Cre/Mouse/Recombinase protein/overexpression/mouse/AD5CMV-Cre

Vector(s) [Vector Category/Vector Technical Name]: Adenovirus/pAd/CMV/V5-DEST/; Lentivirus/pLenti6/V5-DEST/; Lentivirus/pLenti-III/; Adenovirus/AD5-CMV-CRE/; Adenovirus/Ad5-CMV-DHRSX/; Adeno-Associated Virus (AAV)/AAV8-GFP/; Adeno-Associated Virus (AAV)/AAV8-DHRSX-shRNA/; Adeno-Associated Virus (AAV)/AAV8-mCluh/; Adeno-Associated Virus (AAV)/AAV8-mUTX-shRNA/; Adenovirus/Ad5-CMV-mUTX

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/293 HEK/; Human/293 A/; Animal/Mesangial cells/; Human/Mesangial cells/; Animal/proximal tubular cell/; Human/proximal tubular cell/; Animal/macrophage/; Animal/hepatocyte /; Human/hepatocyte /; Animal/macrophage/; Human/hepatic stellate cell/; Human/adipose progenitor cell /; Animal/WT-1 (brown preadipocytes)/; Animal/brown preadipocytes /; Animal/3T3-L1/; Human/iPSC/; Human/THP-1

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 - Adenovirus/tail vein/mouse restraint tube/ABSL2/lab coat, mask, glove/ABSL1/Yes/one time tail injection of Ad5-CMV-mUTX at $0.5-2 \times 10^9$ genomic particles (GP)/100 μ L /; Mouse/Viral Vector - Adeno-Associated Virus (AAV)/tail vein/mouse restraint tube/ABSL2/lab coat, mask, glove/ABSL1/Yes/one time tail vein injection of AAV-mUTX, AAV-mUTX-shRNA at $0.5-2 \times 10^9$ genomic particles (GP)/100 μ L /; Mouse/Naked Nucleic Acid-r/sDNA/IP injection/hand hold/ABSL1/Lab Coat, gloves, eye protection/ABSL1/No/CD47-ASO or control ASO (purchased from Ionis) will be intraperitoneally injected into obese mice (diet-induced or genetic obese mouse models) at a dosage of 10 μ M/20g body weight (in 750 μ l saline) thrice weekly for 8-10 weeks. Body weight, glucose tolerance or insulin sensitivity, adiposity distribution (by EchoMRI), food intake and energy expenditure (by TSE chamber), and core body temperature (Implantable Programmable Temperature Transponder) will be monitored. At the end of the study, mice will be euthanized, blood and adipose tissue will be collected for further analysis/; Mouse/Viral Vector - Adeno-Associated Virus (AAV)/tail vein/mouse restraint tube/ABSL2/lab coat, mask, glove/ABSL1/Yes/one time tail vein injection

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of AAV8-mCluh or AAV8-GFP (Vector Biolab) at $1-4 \times 10^{11}$ genomic particles/100 μ l; Mouse/Viral Vector - Adenovirus/nasal instillation /mouse restraint tube/ABSL2/lab coat, mask, glove/ABSL1/Yes/ 3×10^5 Ad-Cre will be administered into mice once by nasal instillation method.

Risk Assessment/Discussion:

Dr. Wang has submitted an amendment to add a new project involving AAV in mice. Specifically, Dr. Wang has added two AAV8 vectors (AAV8-mGluch and AAV8-GFP control) from Vector Biolabs that will be administered to mice via tail-vein injection. Mice will be restrained in a restraint tube during AAV injections, which will take place within a BSC in DLAR. Following AAV administration, mice will remain on an AMLN or LF diet for 8 weeks to allow progression from MASLD to MASH. Mice will undergo EchoMRI, glucose tolerance testing and insulin sensitivity testing. At the conclusion, mice will be sacrificed and tissues harvested for downstream assays including histology, RNA extraction, and qPCR. This work will be done using BSL1/ABSL1 containment and housing. PPE for AAV administration in mice includes a lab coat, mask, and gloves, in addition to use of BSC. Cluh is an RNA binding protein involved in regulation of mitochondria function. GFP is a fluorescent tag. The AAV vectors are designed specifically to target the mouse Cluh gene. The addition of this new project does not significantly alter the biohazardous risks associated with Dr. Wang's current work with biohazardous materials. There is an IBC hold on the corresponding IACUC protocol 2020-3563.

IBC Discussion & Vote:

The amendment to IBC-24-521 (version 24.0) was approved.

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Doug Harrison initiated the motion. Delphine Malherbe seconded the motion. All IBC members present (13) 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-25-118

Protocol Title: Plk1 in prostate cancer lineage plasticity

Protocol Type: Amendment

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

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

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

Primary Reviewers: C. Haughton, A. Pinto, T. Chambers

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Brief Project Overview:

Our research focuses on understanding how prostate cancer becomes more aggressive and stops responding to treatment. We study how tumor cells change their identity, a process called lineage plasticity, which can lead to a difficult-to-treat form known as neuroendocrine prostate cancer (NEPC). To study this, we use a special mouse model that allows prostate tissue to grow under the kidney capsule. In our experiments, we use mouse prostate epithelial cells (mPrECs) from a genetically engineered mouse strain called RPM (Rb1^{fl/fl}; Trp53^{fl/fl}; Myc^{LSL/LSL}). These prostate cells are genetically modified in the lab to either remove PLK1 or overexpress wild-type or mutant PLK1. The modified prostate cells are mixed with mouse urogenital sinus mesenchymal (UGSM) cells, which help support tissue growth. The mixture (called a graft) is then implanted under both kidney capsules of immunodeficient host mice such as NSG or SCID. In additional experiments, grafts will include prostate cells that knocking-down or overexpress specific genes we study — Plk1, NEUROD1, HOXB13, NANOG, BOP1, PHF2, BCL11A, WBP11, BRN2, and OCT4 — along with UGSM cells. To study the role of hormones in tumor growth, some mice will undergo surgical castration or receive a testosterone pellet implant.

Summary of Biohazard Materials & Manipulations:

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

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/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPRE; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; GFP/Jellyfish/Tag/Expression /human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPRE; mouse cells: mPrEC, UGSM, NIH3T3/FuCGW, pLV-EGFP-Cre/; 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/; Neurod1/Mouse and Human/Oncogene /Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPRE; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; HOXB13/Mouse and Human/Oncogene/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPRE;

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mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; Nanog/Mouse and Human/Oncogene/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; BOP1/mouse and human/Ribosome biogenesis protein/Expression/Knockdown/knockout/human PCa cells: LNCaP, mouse prostate epithelial cells (RPM)/pLV-puro, FU-CRW, pLKO.1, pGEX-4T-1/; PHF2/Mouse and Human/histone demethylase enzyme/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; BCL11A/Mouse and Human/transcription factor/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; WBP11/Mouse and Human/splicing factor and a regulatory component of the spliceosome/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; BRN2/Mouse and Human/Transcription factor that plays a key role in neuronal differentiation/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; 46299/Mouse and Human/transcription factor crucial for maintaining stem cell pluripotency in embryonic stem cells/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2, PGEX-4T-1/; AR/Mouse and Human/Androgen Receptor/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE; mouse cells: mPrEC, UGSM, NIH3T3/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2/; Cre/P1 bacteriophage/recombinase/Expression/mouse prostate epithelial cells/pLV-EGFP-Cre/; AARS/Mouse and Human/protein synthesis and cellular signaling/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE, NCI-H660, LASCPC-01, N2P1, N2P2; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2/; FOXA2/Mouse and Human/transcription factor/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE, NCI-H660, LASCPC-01, N2P1, N2P2; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2/; INSM1/Mouse and Human/transcription factor/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE, NCI-H660, LASCPC-01, N2P1, N2P2; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2/; LDHA/Mouse and Human/energy metabolism/Expression/Knockdown/knockout/human cells: LNCaP , C4-2, 22Rv1 ,PC3, NHPPrE, NCI-H660, LASCPC-01, N2P1, N2P2; mouse cells: mPrEC, UGSM, NIH3T3; E.coli: DH5alpha, BL21/pLKO.1 FuCRW, FuCRW, pLV-EGFP-Cre, LentiCRISPR v2/; Cas9/Streptococcus pyogenes/gene editing system/Expression/to knock-out GOI/human cells: LNCaP, C4-2, 22Rv1, PC3, NHPPrE/lentiCRISPR v2

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Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/FU-CGW/; Lentivirus/FU-CRW/; Plasmid/pCMV-VSV-G/; Plasmid/pGEX-4T-1/; Lentivirus/pLV-EGFP-Cre/; Lentivirus/lentiCRISPR v2/; Lentivirus/pLV-Luc/; Lentivirus/pLKO.1

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/NHPrE/; Animal/UGSM/; Human/NCI-H660

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/restrainer/gas anesthesia such as isoflurane/ABSL2/double-layered gloves, lab coats and eye protection/face mask/ABSL1/No/castration/; Mouse/Cells - Animal, genetically modified/SQ, subrenal capsule/restrainer/gas anesthesia/xylazine&ketamine/ABSL2/double-layered gloves, lab coats and eye protection/face mask/ABSL1/No/castration; sub-renal capsule implantation; Subcutaneous testosterone pellet 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/ABSL1/No/human tumor inoculation/; Mouse/Cells - Human, genetically modified/SQ/restrainer/gas anesthesia such as isoflurane/ABSL2/double-layered gloves, lab coats and eye protection/face mask/ABSL1/No/castration

Risk Assessment/Discussion:

Dr. Liu has submitted an amendment to add new lentivirus vector constructs and cells for work in animals. New lentivirus gene targets for overexpression and/or knockdown include FOXA2, INSM1, LDHA, and AARS. These new lentivirus constructs will be produced and used as previously described and approved to transduce mPreCs and UGSM cells using BSL2+ containment. These cells are washed a minimum of 3 times and administered to mice via renal subcapsular implantation (previously approved). They have also added two new cell lines, N2P2 and LASCPC-01, to be used in xenograft studies via subcutaneous implantation (previously approved). Administration of cells and tissues to mice will be done using ABSL2 containment and ABSL1 housing. All manipulations of animals and their tissues will remain the same as previously described. The addition of these new lentivirus vectors and cells does not significantly alter the existing biohazardous risks associated with this IBC protocol. There is an IBC hold on corresponding IACUC 2025-4631.

IBC Discussion & Vote:

The amendment to IBC-25-118 (version 20.0) was approved.

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Thomas Chambers initiated the motion. Amelia Pinto seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Stefan Stamm

IBC Protocol Number: IBC-25-37

Protocol Title: Regulation of alternative pre-mRNA processing in Prader Willi syndrome and tauopathies

Protocol Type: Amendment

Amendment To: Genetic constructs, Manipulations planned

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

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

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

Brief Project Overview:

Our laboratory studies alternative pre-mRNA splicing, a central process in human gene regulation. Almost all human genes undergo pre-mRNA splicing and with a few exceptions all genes are alternatively spliced, i.e. they form more than one isoform from a given piece of DNA, because the intermediate form, RNA, is spliced in different (alternative) ways. We focus on the deregulation of alternative splicing seen in human diseases, in particular in Prader-Willi syndrome and Alzheimer's disease. Prader-Willi syndrome is caused by the loss of gene expression that contains two clusters of snoRNAs (small nucleolar RNAs), SNORD115 and SNORD116. We found that these SNORDs regulate alternative splicing and gene expression of a number of genes.

The second disease that we are studying is Alzheimer's disease (AD), where alternative splicing of the microtubule associated protein tau is changed in the disease. We found that this gene creates a circular RNA through a backsplicing mechanism. This novel RNA could generate the tau protein deposits characteristic for Alzheimer's disease. Importantly we found that these circular RNAs are translated after undergoing RNA changes. We raised antisera against several circRNA-encoded proteins from the microtubule associated protein tau, amyloid precursor protein and presenelin genes. The work on AD is done in collaboration with Peter Nelson from the Center of Aging at UK.

We will also use Adeno-Associated Viruses to express these circular RNAs in cultured human neurons and mouse brain and test their ability to promote tau aggregation on to neurofibrillary tangles.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/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)]: serotonin receptor 2C/human, via addgene/membrane protein/cloned into pcDNA3.1 expressed in HEK293/Hela cells as a reporter/E. coli/Plasmid/; microtubule associated protein tau/human, via addgene/housekeeping gene/cloned into pcDNA3.1, expressed into HEK293 as a reporter/E. coli/Plasmid/; synaptophysin like protein 1/human, via addgene/membrane protein/cloned into pcDNA3.1, expressed into HEK293/E. coli/Plasmid/; tau circular RNAs (circTau)/human, via addgene/housekeeping gene/cloned into pcDNA3.1, expressed into HEK293/E. coli, mouse, human neuronal cells/Plasmid, AAV9/; HIPK3 circular RNAs/human, via Synbio or VectorBuilder/serine/threonine kinase/circRNA expression construct/E. coli, mouse, human neuronal cells/Plasmid, AAV9/

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pEGFP; Plasmid/pcDNA3.1; Plasmid/pCR2.1; Plasmid/pUC18; Adeno-Associated Virus (AAV)/AAV9

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Hela Cells; Human/HEK293 cells; Human/SH-SY5Y;

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Viral Vector - Adeno-Associated Virus (AAV)/Stereotactic Injection /Isoflurane Anesthesia/ABSL1/Gloves, Lab Coat, Eye Protection/ABSL1/No//

Risk Assessment/Discussion:

Dr. Stamm has submitted an amendment to add a new project with mice. Adeno-Associated Virus (AAV) vectors expressing HIPK3 circRNAs are produced by Vector Builder or Synbio. AAV vectors will be stereotactically injected into mouse hippocampus and entorhinal cortex under anesthesia. AAV vectors will also be used to transduce human neurons and HEK293 cells. 1-6 weeks after AAV administration, mice will be sacrificed and brains fixed for downstream analysis (Western blot, immunohistochemistry, etc.). The circRNAs are expected to induce neurofibrillary tangles (NFTs) in mice, leading to tauopathy. Because of the unique transgenes, these AAVs will be handled using BSL2 containment. Animals will be housed at ABSL1 containment. Work with animals in conjunction with biohazardous materials introduces new biohazardous risks to this IBC protocol. These risks are mitigated in several ways, including procurement of AAVs from 3rd party vendor, use of PPE including lab coat, eye protection, and gloves, anesthesia restraint of animals, and use of BSC for in vitro work.

IBC Discussion & Vote:

The amendment to IBC-25-37 (version 22.0) was approved pending minor modifications as listed below:

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RECOMBINANT AND/OR SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information Table: There are gene targets mentioned in the corresponding IACUC that are not listed in the Gene Information table here. Please ensure congruency between this IBC protocol and the corresponding IACUC. See the following text from the corresponding IACUC protocol:

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The constructs will express circTau12->7, circTau 12->10, circTau 9->5 and circTau 7->4, as well as a circGFP control. We will use CAG and Syn1 promoters that express ubiquitously and in neurons, respectively. If necessary for translation, we will co-inject with an AAV expressing ADAR1-p150 to induce circRNA editing that is necessary for translation in HEK293 cells.

SCIENTIFIC SUMMARY -

1. Statements regarding the AAVs not being known to be harmful to humans need to be modified to reflect potential risks to personnel associated with the specific gene inserts described.
2. It is noted that the AAVs are cloned by either Vector Builder or Synbio, but it is unclear if they are being produced in the lab or by a vendor. Please clarify. If the AAVs are produced in the lab, details on the production procedures and safety considerations need to be outlined.

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Mike Mendenhall initiated the motion. Yadi Wu seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Diego Lucero

IBC Protocol Number: IBC-25-97

Protocol Title: New Modulators of Lipoprotein Metabolism: From the liver to the vascular wall.

Protocol Type: Amendment

Amendment To: Genetic constructs

Applicable Guidelines & Regulations: NIH Guidelines Section III-E, NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-2, OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, NIH Guidelines Section IV-B-7, NIH Guidelines Section III-F-8, NIH Guidelines Section III-D-1, NIH Guidelines Section III-E-1, NIH Guidelines Section III-D-2

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

Primary Reviewers: D. Harrison, C. Pickett, A. Hunt

Brief Project Overview:

Heart disease is the leading cause of death in the United States and is driven by high levels of fats like cholesterol and triglycerides in the blood. These fats are transported through the bloodstream into particles called lipoproteins. When the systems for processing lipoproteins (metabolism) do not function properly, fats accumulate in blood, increasing the risk of heart disease.

Notably, evidence reveals that our knowledge of the regulation of lipoprotein metabolism is incomplete. For instance, the genetic cause cannot be identified in nearly half of the individuals with strong signs of inherited elevations of fats in the blood, limiting their access to early treatments to reduce their high risk for heart disease. Furthermore, a large proportion of patients with elevated blood lipoproteins either do

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not respond to current therapies or cannot tolerate them. Identifying new genes and proteins that control lipoprotein metabolism will improve the diagnosis of inherited disorders and uncover alternative therapeutic targets to reduce fats in the blood.

This project will identify and characterize new regulators of lipoprotein metabolism and understand how they contribute to the build-up of fat in the arteries. To achieve this goal, this project will combine cell-based assays (in vitro) and animal (in vivo) approaches, using tools like plasmids and lentiviruses. In addition, high-resolution imaging, lipid measurements, and the assessment of RNA and protein expression will be implemented.

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, Transfection, Use of Human Source Material(s), Use of infectious agents, Use of viral vectors, Transformation

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)]: sgRNA (LDLR)/human/Lipoprotein receptor (sgRNA)/knockout/human cells/Non-viral vector, plasmid (pNV-sgRNA-GFP)/; sgRNA (TAGLN) /human/actin-binding protein (sgRNA) /knockout /human cells /Non-viral vector, plasmid (pNV-sgRNA-GFP)/; sgRNA (MYLIP)/human/ubiquitinase (sgRNA) /knockout /human cells /Non-viral vector, plasmid (pNV-sgRNA-GFP)/; sgRNA (SYNRG) /human/adaptor protein (sgRNA) /knockout /human cells /Non-viral vector, plasmid (pNV-sgRNA-GFP)/; sgRNA (non-targeting) /human/Control (sgRNA)/knockout /human cells /Non-viral vector, plasmid (pNV-sgRNA-GFP)/; sgRNA (CLTB)/human/clathrin (sgRNA)/knockout /human cells /Non-viral vector, plasmid (pNV-sgRNA-GFP)/; sgRNA (CLTA)/human/clathrin (sgRNA) /knockout /human cells /Non-viral vector, plasmid (pNV-sgRNA-GFP)/; sgRNA (PACSIN3) /human/kinase substrate (sgRNA)/knockout /human cells /Non-viral vector, plasmid (pNV-sgRNA-GFP)/; sgRNA (HUNK) /human/kinase (sgRNA) /knockout /human cells /Non-viral vector, plasmid (pNV-sgRNA-GFP)/; 2 sgRNA (LDLR) /human/lipoprotein receptor (sgRNA)/knockout /human cells /Non-viral vector, plasmid (pRP[2gRNA]-EGFP-U6>hLDLR[gRNA1]-U6>hLDLR[gRNA2])/; 2 sgRNA (LCP1)/human/actin-binding protein (sgRNA)/knockout /human cells /Non-viral vector, plasmid (pRP[2gRNA]-EGFP-U6>hLCP1[gRNA1]-U6>hLCP1[gRNA2])/; 2 sgRNA (CFL1)/human/actin-binding protein (sgRNA)/knockout /human cells /Non-viral vector, plasmid (pRP[2gRNA]-EGFP-U6>hCFL1[gRNA1]-U6>hCFL1[gRNA2])/; 2 sgRNA (FCGRT)/human/immunoglobulin receptor (sgRNA) /knockout /human cells /Non-viral vector, plasmid (pRP[2gRNA]-EGFP-U6>hFCGRT[gRNA1]-U6>hFCGRT[gRNA2])/; sgRNA (Tagln) /mouse/actin-binding protein (sgRNA) /knockout /murine cells /Non-viral vector, plasmid (pRP[CRISPR]-EGFP-hCas9-U6>mTagln[gRNA])/; mCherry/human/reporter(mCherry)/overexpression of

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fluorescently tagged proteins/human cells/Non-viral, plasmid: pRP[Exp]-CMV+intron>EGFP/hLDLR[NM_000527.5]/; //((ns):P2A:mCherry/hCLTB[NM_007097.5]/; LIPG/human/lipase/overexpression of catalitically inactive form/human cells/non-viral vector, plasmid: pRP[Exp]-Neo-CMV>{hLIPG[NM_006033.4]*S169A}/; LIPG/human/lipase/overproduction of active enzyme/human cells/Non-viral vector, plasmid (pCMV6-Entry)/; empty/human/empty/negative control/human cells/Non-viral vector, plasmid (pCMV6-Entry)/; LDLR/human/lipoprotein receptor/express fluorescently tagger protein/human cells/non-viral vector, plasmid. /; Clta/mouse/clathrin/express fluorescently tagger protein/human cells/non-viral vector, plasmid. /; CLTB/human/clathrin/express fluorescently tagger protein/human cells/non-viral vector, plasmid. /; FCGRT/human/FCGRT: immunoglobulin receptor./overexpress FcRn/human cells /3rd generation lentivirus/; LIPG/human/lipase/overexpress endothelial lipase/human cells/3rd generation lentivirus/; shRNA (CD36)/human/cluster of differentiation 36/knockdown expression of CD36/human cells/3rd generation lentivirus/; shRNA (FCGRT)/human/immunoglobulin receptor/knockdown FCGRT expression/human cells/3rd generation lentivirus/; shRNA (SREBF2)/human/cholesterol sensor protein/knockdown SREBF2 expression/human cells/3rd generation lentivirus/; GFP/human/Reporter (GFP)/knockout/human cells/Non-viral vector, plasmid (pNV-sgRNA-GFP)/; Cas9 /Bacterial (Cas9)/nuclease (Cas9)/knockout /murine cells /Non-viral vector, plasmid (pRP[CRISPR]-EGFP-hCas9-U6>mTagln[gRNA]/; LDLR /human/lipoprotein receptor(LDLR)/overexpression of fluorescently tagged proteins/human cells/Non-viral, plasmid: pRP[Exp]-CMV+intron>EGFP/hLDLR[NM_000527.5](ns):P2A:mCherry/hCLTB[NM_007097.5]/; CLTA /human/clathrin(CLTA)/overexpression of fluorescently tagged proteins/human cells/Non-viral, plasmid: pRP[Exp]-CMV+intron>EGFP/hLDLR[NM_000527.5](ns):P2A:mCherry/hCLTB[NM_007097.5]/; B2M/human/B2M: histocompatibility complex/overexpress FcRn/human cells /3rd generation lentivirus/; shRNA (LRP1)/human/lipoprotein receptor/knockdown LRP1 expression/human cells/3rd generation lentivirus/; Cas9 /Bacterial (Cas9)/nuclease (Cas9)/knockout /murine and human cells /pLV[Exp]-CBh>hCas9/Hygro/; LAMP1/human/lysosomal marker/label lysosomal compartment/human cells /Baculovirus (BacMam 2.0)/; Rab7a/human/Late endosome marker/label late endosomal compartment/human cells /Baculovirus (BacMam 2.0)/; GFP/Aequorea victoria/Reporter (green fluorescent protein)/reporter/human cells /Baculovirus (BacMam 2.0)

Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/pLV[Exp]-Neo-CMV>GeneID/; Lentivirus/pLV[shRNA]-Hygro-U6>GeneID[shRNA#]/; Lentivirus/pLV[shRNA]-Bsd-U6>GeneID[shRNA#]/; Lentivirus/pLV[shRNA]-Neo-U6>GeneID[shRNA#]/; Lentivirus/pLV[Exp]-Neo-CMV+intron>GeneID1 (ns):P2A:GeneID2/; Plasmid/pRP[CRISPR]-EGFP-hCas9-U6>sgRNA/; Plasmid/pRP[Exp]-Neo-CMV-ORF/; Plasmid/pCMV6-Entry/; Plasmid/pNV-sgRNA-GFP/; Plasmid/pBa-LSS-GFP-LDLR/; Plasmid/PA-mCherry1-Clathrin-15/; Lentivirus/pLV[Exp]- CBh>hCas9/Hygro/; Baculovirus/CellLight® Late Endosomes-GFP *BacMam 2.0*/; Baculovirus/CellLight™ Lysosomes-GFP, BacMam 2.0

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HepG2 cells/; Human/Huh7 cells/; Human/T/G HA-VSMC cells/; Animal/J774A.1/; Animal/RAW264.7/; Animal/MOVAS/; Human/HeLa

Animal Use: Yes

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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/Human blood or other bodily fluids/intravenous/inhaled isoflurane (2-3%)/ABSL2/lab coat, gloves, protective eyewear/ABSL1/No/The intravenous injections of human or mouse LDL will be performed in the BSC located in the ABSL-2 room of the DLAR facility, following BSL-2 practices.

Risk Assessment/Discussion:

Dr. Lucero has submitted an amendment to add a new project utilizing pre-packaged BacMam (baculovirus vector) for use in cell culture. The baculovirus vector is derived from an insect virus that will replicate in insect cells but not mammalian cells. Although these vectors do not replicate in mammalian cells, they are still capable of transducing mammalian cells. The BacMam vector will be used to express LAMP1, Rab7a, and GFP in human cells. This work will be completed using BSL2 containment within a Class II BSC using lab coat, disposable gloves, and eye protection. After BacMam transduction, cells will be washed and subjected to imaging of live and fixed cells in the UK Light Microscopy Core facility. Any accidental exposures to the BacMam vectors would not be expected to result in sustained infection, transmission, or persistent transgene expression. LAMP1 and RAB7A are endogenous intracellular proteins involved in lysosomal and late endosomal trafficking, and GFP is a commonly used fluorescent reporter. Although use of this vector construct is new to the Lucero IBC protocol, it does not significantly increase associated biohazardous risks.

IBC Discussion & Vote:

The amendment to IBC-25-97 (v.40.0) was approved.

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Doug Harrison initiated the motion. Arthur Hunt seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Christopher Emfinger

IBC Protocol Number: IBC-25-103

Protocol Title: Unmasking conditional dependencies between key proteins influencing metabolic health.

Protocol Type: Amendment

Amendment To: Genetic constructs, Manipulations planned

Applicable Guidelines & Regulations: NIH Guidelines Section III-E-3, NIH Guidelines Section III-F-1, 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-1

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

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

Brief Project Overview:

Diabetes and related metabolic disorders burden millions of people worldwide. Many proteins regulating metabolism rarely act in isolation. This makes the consequences of altering these proteins (e.g. through drug treatments) difficult to predict, makes identifying new therapeutic targets difficult, and means not everyone will respond equally to the same therapies. Analysis of prior data nominated new potential regulators of metabolism but they and how they interact are poorly understood and consequently require validation experiments. The goal of this project is to identify the ways in which key genes regulating metabolism depend on one another to exert their effects on overall metabolic health. The project uses adenoviral and adeno-associated viral (AAV) vectors (both non-replicating) to deliver nucleic acid plasmids that alter abundance and/or function of these key genes. The adenoviral vectors will be used in cell culture and tissue culture experiments. The AAV vectors will be given to live mice. The studies will use loss of candidate genes to determine the influence of these genes on metabolic health both individually and acting together. Findings from these studies will further clarify processes that regulate metabolic health and may identify novel therapeutic targets for intervention in diabetes and its related disorders.

Summary of Biohazard Materials & Manipulations:

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

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)]: Transketolase (TKT)/Mouse (*Mus musculus*)/Enzyme/Knockdown/Mouse primary cells/Adenovirus/; Transketolase (TKT)/Human/Enzyme/Rescue experiment (over-expression)/primary cells from mice and humans/Adenovirus/; Transketolase (TKT), mutated nuclear localization sequence/Human/Enzyme/over-expression (rescue experiment)/Primary cells from mice and humans/Adenovirus/; Transketolase (TKT), mutant lacking enzymatic activity/Human/Enzyme/over-expression (rescue experiment)/primary cells from mice and humans/Adenovirus/; enhanced green fluorescent protein (eGFP)/*Aequorea Victoria*, mutated to enhance stability/fluorescence/tracking protein/expression/primary cells from mice and humans/Adenovirus/; mCherry red fluorescent protein/*Aequorea Victoria*, mutated to change fluorescent color/indicator protein/expression/primary cells from mice and humans/Adenovirus/; Dipeptidyl peptidase 8 (DPP8)/Mouse/Enzyme/knockdown/primary mouse cells/Adenovirus/; Dipeptidyl peptidase 8 (DPP8)/Human/Enzyme/knockdown/primary human cells/Adenovirus/; G-protein coupled receptor 180 (GPR180)/Mouse/Plasma membrane g-protein-coupled receptor/knockdown/primary mouse cells/Adenovirus/; G-protein-coupled receptor 180 (GPR180)/Human/plasma membrane g-protein-coupled receptor/knockdown/primary human cells/Adenovirus/; Transketolase

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(TKT)/Human/Enzyme/knockdown/primary human cells/Adenovirus/; H2B-Halo/Synthetic (Hybrid; human histone 2 B fused to haloalkane dehalogenase from Rhodococcus rhodochrous)/Enzyme/Transgenesis marker; converts dye precursors to active dyes for marking cells/Mouse/Adeno-associated Viral vector, serotype 8 (AAV8)/; DPP8/Mouse/Enzyme/gene activation/over-expression/primary mouse cells/Adenovirus/; G-protein coupled receptor 180 (GPR180)/Mouse/plasma membrane g-protein coupled receptor/gene activation/ over-expression/primary mouse cells/Adenovirus/; DPP8/Human/Enzyme/gene activation/over-expression/primary human cells/Adenovirus/; G-protein coupled receptor 180 (GPR180)/Human/plasma membrane g-protein coupled receptor/gene activation/ over-expression/primary human cells/Adenovirus/; DPP8/Mouse/Enzyme/gene activation/over-expression/live mice (in vivo expression)/Adeno-associated virus serotype 8 (AAV8)/; G-protein coupled receptor 180 (GPR180)/Mouse/plasma membrane g-protein coupled receptor/gene activation/ over-expression/live mice (in vivo)/Adeno-associated virus serotype 8 (AAV8)/; DPP8/Mouse/Enzyme/gene knockdown/live mice (in vivo expression)/Adeno-associated virus serotype 8 (AAV8)/; G-protein coupled receptor 180 (GPR180)/Mouse/plasma membrane g-protein coupled receptor/gene knockdown/live mice (in vivo)/Adeno-associated virus serotype 8 (AAV8)/

Vector(s) [Vector Category/Vector Technical Name]: Adenovirus/ad-m; Adenovirus/pAV[Exp]; Adeno-Associated Virus (AAV)/pAAV8

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/INS-1/832/; Human/HEK293T/; Animal/pancreatic islet beta cells/; Human/pancreatic islet beta cells/; Animal/mouse pancreatic islet/; Human/human pancreatic islet/; Animal/MIN6/; Human/EndoC- β H5/; Animal/AML12/

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)/Intraperitoneal, Retro-orbital/Hand restraint (scruff), inhalation anesthesia/ABSL1/Gloves, lab coat, eye protection/ABSL1/Yes/AAV injections performed in BSC in DLAR.

Risk Assessment/Discussion:

Dr. Emfinger has submitted an amendment to add a project administering AAV8 vectors to transgenic mice via intraperitoneal or retroorbital injection to deliver guide RNAs that will activate or deactivate gene targets (transketolase or TKT, DPP8, GPR180). AAVs are purchased from Vector Builder and diluted in a BSC for administration to mice. For intraperitoneal (IP) injection, mice will be restrained via scruffing restraint. For retroorbital injection, mice will be anesthetized via isoflurane. Personnel will wear lab coats, disposable gloves, and eye protection for these procedures. Mice will be housed at ABSL1 containment. Post-AAV administration, mice will undergo various procedures to measure plasma glucose and insulin levels, glucose and insulin tolerance tests, and mixed-meal tolerance tests. At the conclusion of experiment, mice will be sacrificed and tissues obtained for isolation of islets for insulin secretion measurement and calcium imaging (previously described and approved). The addition of this project does not increase the biosafety containment level, however work with animals in conjunction

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with AAV is new to this IBC protocol. Dr. Emfinger has also updated personnel. There is an IBC hold on corresponding IACUC 2025-465.

IBC Discussion & Vote:

The amendment to IBC-25-103 (version 27.0) was approved pending minor modifications as listed below:

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SCIENTIFIC SUMMARY – Please remove leftover comments reflecting no animal work as this amendment now introduces work with animals.

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Doug Harrison initiated the motion. Yadi Wu seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

New Protocols

PI: Austin Stone

IBC Protocol Number: IBC-25-186

Protocol Title: Randomized, Prospective, Double Blind, Multi-Center, Phase 3 Pivotal Clinical Trial to Compare Efficacy and Safety of CARTISTEM® and Surgical Comparator in Subjects with Knee Cartilage Lesions and Osteoarthritis

Protocol Type: New Protocol

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: D. Harrison, T. Chambers, B. Nelson

Brief Project Overview:

The goal of this clinical study is to see how well CARTISTEM® works and how safe it is for people who have severe pain in their knees caused by a condition called arthritis. CARTISTEM® is a treatment made from special cells called stem cells. Stem cells are cells that can take the form and function of other cells. We are comparing this treatment to a type of surgery called debridement, which is the standard treatment people who have severe pain in their knees caused by arthritis.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Biohazardous Materials

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Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.) /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. Stone has submitted a new IBC protocol for a Phase 3 clinical study of CARTISTEM, a drug product consisting of a combination of human umbilical cord blood-derived mesenchymal stem/stromal cells (hUCB-MSCs) and lyophilized sodium hyaluronate (HA) in study participants with osteoarthritis. Umbilical cord blood is tested for HIV, HBV, HCV, Syphilis, and WNV prior to manufacture. UK aims to enroll 10 study participants over 30 months. CARTISTEM is administered to study participants during knee surgery. After surgery, whole blood and urine will be collected. Urine will be processed at the UK Clinical Laboratory, and blood will be shipped back to the study sponsor. Samples are obtained and processed at the Turfland Orthopedic Surgery/Sports Medicine Clinic. Work with human specimens will be completed using BSL2 containment. The risks introduced in this clinical study do not exceed those that healthcare workers are likely to encounter during regular business.

IBC Discussion & Vote:

The protocol IBC-25-186 (version 8.0) was approved pending minor modifications as listed below:

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SCIENTIFIC SUMMARY –

1. It is unclear how reliable the control of stem cell differentiation is for the desired outputs. Is there a potential risk of tumorigenic or disease occurrence from the differentiation of the cells in CARTISTEM? Please clarify.
2. Please briefly describe product acceptance/rejection criteria. If these follow specific FDA guidance for acceptance/rejection, please cite these.

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Doug Harrison initiated the motion. Thomas Chambers seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Tyler Kirby

IBC Protocol Number: IBC-26-18

Protocol Title: Nuclear dynamics in skeletal muscle cells

Protocol Type: New Protocol

Amendment To: N/A

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

Maximum Containment Level: Biological Safety Level 2 (BSL2), Biological Safety Level 1 (BSL1), Animal Biological Safety Level 1 (ABSL1)

Primary Reviewers: C. Haughton, M. Mendenhall, D. Malherbe

Brief Project Overview:

This research project studies how muscle cells protect and repair their DNA over time. DNA is the instruction manual inside our cells, and keeping it healthy is important for normal muscle function and overall health. Problems with DNA maintenance can contribute to aging and disease.

The study will use muscle cells grown in the lab from both humans and mice. By comparing human and mouse muscle cells, researchers can better understand basic biological processes that are shared across species.

The main biohazard used in this work is adeno-associated virus (AAV). AAV is a commonly used research tool that helps scientists deliver specific genes into cells so they can study how those genes work. AAV is not known to cause disease in humans and is considered low risk when handled properly.

All experiments will be done in controlled laboratory spaces using approved safety practices. The goal of this research is to improve scientific understanding of how DNA is maintained in muscle cells, which may help guide future studies related to muscle health and disease.

Summary of Biohazard Materials & Manipulations:

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

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)]: Green fluorescent protein/Synthetic/Fluorescent Construct/Reporter for transduction/Mouse cells/pAAV/; mCherry2/Synthetic /Fluorescent construct/Reporter for transduction/Mouse cells/pAAV/; DN-Kash2/Synthetic/Dominant-negative protein/Disrupt the LINC complex in muscle cells/Mouse cells/pAAV/; DN-Kash2/Synthetic/Dominant-negative protein/Disrupt the LINC complex/Mouse cells/pPBv2-tetO/; LC3-mCherry/Human/Reporter/Track

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autophagosomes/Mouse cells/pAAV/; Lamin B1-GFP/Human/Reporter/Label the nuclear lamina for live cell imaging/mouse cells/pAAV/; H2B-GFP/Humans/Reporter/Track and label nuclei/Mouse cells/pAAV/ Vector(s) [Vector Category/Vector Technical Name]: Plasmid/Piggybac parental vector - constitutive /; Plasmid/Hyperactive Transposase/; Plasmid/kiCAP-AAV-MyoAAV1A/; Plasmid/pAdDeltaF6/; Plasmid/pAAV Gene vector/; Adeno-Associated Virus (AAV)/MyoAAV/; Plasmid/Piggybac parental vector - inducible

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/Mouse Embryonic Fibroblasts/; Animal/Primary myoblast/; Animal/C2C12/; Human/Induced Pluripotent Stem Cells/; Human/HEK293TN/

Animal Use: Yes

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

Risk Assessment/Discussion:

Dr. Kirby has submitted a new IBC protocol entitled Nuclear dynamics in skeletal muscle cells. Dr. Kirby's lab seeks to understand how muscle cells protect and repair DNA over time. Towards that aim, Dr. Kirby's laboratory utilizes human derived materials, including fresh skeletal muscle tissue and human induced pluripotent stem cells (iPSCs) and adeno-associated virus (AAV) transduction or PiggyBac transfection of iPSCs or muscle-differentiated progeny cells. Cells and tissues will be subjected to several downstream assays and manipulations, including flow cytometry and cell sorting, immunofluorescence, nuclear isolation for snRNA-seq, RNAscope, and proteomics. The work described will be completed using BSL2 containment. PPE will include laboratory coat, gloves, and eye protection. When working with fresh human muscle tissues, personnel will don a disposable gown and face shield or surgical mask with eye protection. Animals will be used to isolate primary myoblasts for transfection/transduction, but biohazardous materials will not be administered to animals. MyoAAV will be produced in Dr. Kirby's lab using triple transfection of HEK293T cells. AAV is harvested by freeze/thaw cycle and subsequent centrifugation to remove cellular debris. AAV production and work with human derived materials is completed within a BSC.

IBC Discussion & Vote:

The protocol IBC-26-18 (version 10.0) was returned for the modifications listed below:

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GENERAL INFORMATION – DOT/IATA Training: Shipping of hazardous materials is indicated, and Jeremy Pearson is listed as personnel responsible for shipping these materials, but a date of DOT/IATA training is not provided. Please ensure DOT/IATA training is completed and update the table accordingly.

CELL LINES – Cells in Use Table: Under the “Source” column for iPSC cells and Mouse Embryonic Fibroblasts, please specify the name of the collaborator where these cells were obtained.

DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Biohazardous Materials Transport Description: Personal Protective Equipment (PPE) should never be worn outside of the laboratory. Please

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update the description here to clarify that the exterior of secondary transport containers is wiped with an appropriate disinfectant prior to transport and remove reference to PPE worn during transport.

LOCATIONS – Research Locations Table: Please include entries for DLAR location(s) and flow cytometry.

LOCATIONS – Biological Safety Equipment: It is noted that paraformaldehyde will be used to fix cells in the BSC. A Chemical Fume Hood may be more appropriate for this procedure to protect personnel from chemical volatiles.

SCIENTIFIC SUMMARY -

1. Please briefly describe how primary myoblasts will be isolated from mice.
2. Additional details are needed for the MyoAAV purification steps. Please expand on the procedure used to remove bands from the centrifuge tube fractions including the risks involved and steps used to mitigate those risks. Describe how the MyoAAV is titrated. Please specify the use of safety cups/buckets for centrifugation of biohazardous materials.
3. In the “RNAscope on Muscle Fibers” section, please include a brief description of how the tissue is fixed, including fixative used and incubation time.
4. Remove references to AAVs being low risk and provide additional details about the transgenes used with MyoAAV and Piggybac. Include the biological function of these genes and associated risks of exposure to the specific gene inserts.

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Mike Mendenhall initiated the motion. Delphine Malherbe seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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Renewals

PI: Cheavar Blair

IBC Protocol Number: IBC-26-19

Protocol Title: Human Induced Stem Cell-Derived Cardiomyocytes as Models for Genetic Cardiomyopathies and Cardiotoxicity

Protocol Type: Renewal

Amendment To: N/A

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

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

Primary Reviewers: Y. Wu, C. Pickett, B. Nelson

Brief Project Overview:

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Our laboratory utilizes human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to investigate how alterations to sarcomeric structure and regulation contribute to cardiac dysfunction and heart failure. Our primary focus is elucidating the mechanisms by which cellular stressors, including pathogenic genetic mutations and cardiotoxic cancer therapies, drive disease pathogenesis. To this end, we employ a multifaceted experimental approach encompassing tissue culture, CRISPR-based genetic engineering, engineered hydrogel platforms for functional contractility assays, and molecular biology techniques to assess changes at the protein and transcriptional levels. The overarching goal of our research program is to define how dysregulation of sarcomeric function leads to heart failure, thereby informing the rational design of targeted therapeutic interventions.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Use of viral vectors, Use of Human Source Material(s), Proteomics, Transfection, PCR/qRT-PCR, Imaging/Microscopy, Bacterial culture, Cell culture, Creation of viral vectors, DNA/RNA isolation/purification

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)]: mKeima (tags mitochondria)/stony coral *Montipora*/tracking gene/expression/mammalian cell line/Lentivirus/; mCherry /synthetic /tracking gene/expression/mammalian cell line/AAV/; jGCaMP8m (calcium indicator)/*R. norvegicus* (rat), *G. gallus* (chicken); *A. victoria* (jellyfish)/tracking gene/expression/mammalian cell line/AAV/; ANKRD1/Synthetic guide RNA/Sarcomere regulation/knockout/mammalian cell line/AAV/; TCAP/Synthetic guide RNA/Sarcomere Regulation/Knockout/mammalian cell line/AAV/; dCas9-KRAB/Synthetic/Regulatory /knockdown/mammalian cell line/AAV/; Bmal1/Synthetic guide RNA/Circadian regulation/knockout & Over-expression/mammalian cell line/AAV/; Period (*Per1*)/Synthetic guide RNA/Circadian regulation/Knockout & over-expression/mammalian cell line/AAV/; REv-Erb/Synthetic guide RNA/Circadian regulation/Knockout & over-expression/mammalian cell line/AAV/; dSaCas9-KRAB/*S. aureus*/Regulatory/Knockdown/mammalian cell line/Lentivirus/

Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/pAAV-U6-gRNA-CBh-mCherry/; Lentivirus/pHAGE-mt-mKeima/; Adeno-Associated Virus (AAV)/pGP-AAV-CAG-FLEX-jGCaMP8m-WPRE/; Adeno-Associated Virus (AAV)/dCas9-KRAB-MeCP2/; Lentivirus/pLV hUbc-dSaCas9-KRAB-T2A-PuroR/

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/AICS-0075-085 - MONO-ALLELIC mEGFP-TAGGED ACTN2 WTC iPSC LINE (TAG AT C-TERM)/; Human/WTC11 GM25256/; Human/AICS-0030-022iPSC MONO-ALLELIC mEGFP-TAGGED MAP1LC3B WTC iPSC LINE (TAG AT N-TERM)/; Human/AICS-0090-391 MONO-ALLELIC TagBFP-TAGGED dCas9-KRAB WTC iPSC LINE/; Human/Duchenne Muscular Dystrophy (DMD) control cell line/; Human/Duchenne Muscular Dystrophy (DMD) genetic mutant cell lines/; Animal/Mouse heart cells with mutations in genes encoding cardiac function/; Animal/Mouse heart cells with mutations in genes that alter circadian rhythms/;

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Human/Hypertrophic Cardiomyopathy (HCM) genetic mutant cell lines;/ Human/HEK 293/

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. Blair has submitted a renewal of his IBC protocol entitled Human Induced Stem Cell-Derived Cardiomyocytes as Models for Genetic Cardiomyopathies and Cardiotoxicity. Dr. Blair's laboratory seems to investigate alterations in sarcomeric structure and regulation and how these contribute to cardiac dysfunction and heart failure. Towards that goal, Dr. Blair's laboratory utilizes human and rodent cells, adeno-associated virus (AAV) and lentivirus. Viral vectors are produced in Dr. Blair's laboratory. Lentivirus is prepared using a 3rd generation packaging system (pMDLg/pRRE, pRSV-Rev, pMD2.G), and AAV is prepared using Addgene's pAdDeltaF6 and pAAV2/9n plasmids. Viral particles are purified via ultracentrifugation, and risk is minimized by loading/unloading centrifuges tubes within the BSC. Centrifuge rotors will be cleaned and decontaminated after each use. Work with viral vectors does not involve the use of sharps, minimizing risk of accidental needlestick. Viral vectors are used to express fluorescent tracking proteins and to knockout or overexpress genes that regulate cardiomyocyte function and circadian regulation (overexpress and knockout *bmal1*, *period*, *Rev-Erb*, knockout *ANKRD1* and *TCAP*). Lentivirus production and work will utilize BSL2+ containment, whereas AAV work will be done using BSL2 containment. Dr. Blair's laboratory also using *E. coli* DH5alpha for cloning of plasmids using BSL1 containment. Dr. Blair's current protocol will expire on May 3, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-19 (version 10.0) was approved.

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Brandy Nelson initiated the motion. Carol Pickett seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Christopher Radka

IBC Protocol Number: IBC-26-23

Protocol Title: Bacterial lipid immune modulators

Protocol Type: Renewal

Amendment To: N/A

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

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

Primary Reviewers: C. Haughton, C. Shaffer, C. Pickett

Brief Project Overview:

Lipids are chemicals that cells can use to communicate with each other. These messages can tell cells what to do, where to go, and when to grow. Bacteria growing in the body use similar lipid messages to talk to the immune system. For decades, the bacterial lipid messages have been assumed to be counter productive and tell the immune system to attack the bacteria; however, under normal conditions, beneficial bacteria in our body (like intestinal bacteria that help us digest food) are not attacked by the immune system. My research studies bacterial lipid messages that cause the immune system to leave them alone. Studying how these new messages are made and how our cells process the message could lead to better probiotic formulations, new antibiotics, and new ways to treat intestinal inflammatory 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, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Propagation of infectious agents, Transformation, Use of Human Source Material(s), Use of infectious agents

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Staphylococcus aureus (RG2-bacteria)/Humans, animals/; Staphylococcus aureus (RG2-bacteria)/Humans, animals/; Enterococcus faecalis (RG2-bacteria)/Humans, animals/; Enterococcus faecium (RG2-bacteria)/Humans, animals/; Bifidobacterium longum (RG1-bacteria)/Humans, mammals/; Bifidobacterium bifidum (RG1-bacteria)/Humans, Mammals/; Bifidobacterium breve (RG1-bacteria)/Humans, Mammals/; Alistipes finegoldii (RG1-bacteria)/Humans, Mammals/; Non-Human Primate (NHP) Materials (RG2)/Non Human Primates/; 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)]: oleate hydratase/many bacteria/metabolic enzyme/expression/E. coli K12 derivative/pET vectors or pBluescript/; oleate hydratase/many bacteria/metabolic enzyme/expression/S. aureus/pPJ480/; acyl CoA synthetase/Homo sapiens/metabolic enzyme/expression/Pichia pastoris /pGAPZ/; peroxisome proliferator activated receptor/Homo sapiens/regulatory gene/expression/E. coli K12 derivative/pET vectors/; phospholipase/Homo sapiens/metabolic enzyme/expression/Pichia pastoris/pGAPZ/; ABC transporter/Homo sapiens/membrane receptor/expression/Pichia pastoris/pGAPZ/; CD36/Homo sapiens/scavenger receptor/expression/Pichia pastoris/pGAPZ/; green fluorescent protein/Aequorea Victoria/tracking/expression/Staphylococcus aureus/pJB38

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pET vectors/; Plasmid/pJB38/; Plasmid/pPJ480/; Plasmid/pBluescript/; Plasmid/pGAPZ

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Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/RAW 264.7 macrophages;
Animal/Bone marrow-derived macrophages

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/Staphylococcus aureus (RG2-bacteria)/Intra-muscular/Isoflurane/ABSL2/gloves, lab coat, eye protection/ABSL2/No/Inoculations and manipulations of infected animals will take place within a BSC.; Mouse/Enterococcus faecalis (RG2-bacteria)/oral gavage/manual hold/ABSL2/gloves, lab coat, eye protection/ABSL2/Yes/Inoculations and manipulations of infected animals will take place within a BSC.; Mouse/Enterococcus faecium (RG2-bacteria)/oral gavage/manual hold/ABSL2/gloves, lab coat, eye protection/ABSL2/Yes/Inoculations and manipulations of infected animals will take place within a BSC.; Mouse/Alistipes finegoldii (RG1-bacteria)/oral gavage/manual hold/ABSL2/gloves, lab coat, eye protection/ABSL2/Yes/Inoculations and manipulations of infected animals will take place within a BSC; Mouse/Bifidobacterium bifidum (RG1-bacteria)/oral gavage/manual hold/ABSL2/gloves, lab coat, eye protection/ABSL2/Yes/Inoculations and manipulations of infected animals will take place within a BSC; Mouse/Bifidobacterium breve (RG1-bacteria)/oral gavage/manual hold/ABSL2/gloves, lab coat, eye protection/ABSL2/Yes/Inoculations and manipulations of infected animals will take place within a BSC; Mouse/Bifidobacterium longum (RG1-bacteria)/oral gavage/manual hold/ABSL2/gloves, lab coat, eye protection/ABSL2/Yes/Inoculations and manipulations of infected animals will take place within a BSC.

Risk Assessment/Discussion:

Dr. Radka has submitted a renewal of his IBC protocol entitled Bacterial lipid immune modulators. Dr. Radka's laboratory studies bacterial lipids and how beneficial bacteria use lipids and how our cells process these messages with the hope that this understanding may lead to better probiotic formulations, new antibiotics, and new ways to treat intestinal inflammatory disease. Towards that goal, Dr. Radka's laboratory utilizes a number of RG1 and RG2 microorganisms, human and non-human primate materials, plasmid transfection, and an animal infection model. Staphylococcus aureus, Enterococcus faecalis, and Enterococcus faecium are all RG2 bacterial agents in use. RG1 bacteria include Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, and Allistipes finegoldii. These are generally considered "beneficial" bacterium from the intestinal microbiome. All manipulations of bacteria will be performed within a BSC using BSL2 containment. In addition to the use of a BSC, personnel will wear lab coat, disposable gloves, and eye protection. Lab strain E. coli (ex. DH5alpha) is used for expansion and purification of plasmid constructs using BSL1 containment. S. aureus will be transformed via electroporation to delete oleate hydratase (OhyA) gene or exchange OhyA with a defective version or homolog from a different organism. These manipulations are expected to reduce virulence or restore virulence to a OhyA knockout avirulent strain but not enhance virulence of S. aureus. There are two animal models described, a thigh infection model and intestinal colonization model. In the first, 50 ul of S. aureus is injected directly into the thigh muscle of anesthetized mice within a BSC in DLAR. Mice are housed at ABSL2 containment and euthanized 24 hours post-challenge for tissue collection and CFU enumeration. Handling of infected mice, including necropsy and tissues, will

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be done within a BSC. The second animal model will utilize oral gavage to introduce Bifidobacteria, Enterococci, or Allistipes with or without pretreatment of antibiotics to deplete the intestinal microbiome. Oral gavage will be done within a BSC in DLAR and mice are manually restrained for oral gavage. Animals are housed at ABSL2 containment and sacrificed 24 hours post infection for tissue extraction. Infected and uninfected animal tissues will be used for light and confocal microscopy and flow cytometry in the FCIM core facility. Handling of infected animal tissues will be done using BSL2 containment. Human and non-human primate tissues and blood will be utilized to quantify the lipid composition of these materials. Human and NHP materials will be treated with organics (chloroform, methanol, acetic acid, etc.) to extract lipids. Lastly, radiolabeled chemicals will be added to cells for lipid extractions and thin layer chromatography. Dr. Radka's current IBC protocol will expire on May 17, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-23 (version 8.0) was approved.

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Carrie Shaffer initiated the motion. Carol Pickett seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Wangxia Wang

IBC Protocol Number: IBC-26-27

Protocol Title: MicroRNA Regulation and Mitochondrial Function in Alzheimer's Disease and Traumatic Brain Injury

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-4, NIH Guidelines Section III-F-1, NIH Guidelines Section 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, M. Mendenhall, C. Shaffer

Brief Project Overview:

Alzheimer's disease (AD) is the most common form of dementia and currently affects millions of Americans. It is characterized by progressive memory loss and decline in thinking abilities, primarily in older adults. Despite decades of research, there is still no effective way to treat or prevent AD. Traumatic brain injury (TBI) is another major neurological disorder, affecting an estimated 1.7 million people each year in the United States. It is a leading cause of death and disability and carries a tremendous financial burden of more than \$75 billion annually in medical and indirect costs. Like AD, there are no effective

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treatments for TBI, partly because we still do not fully understand the damaging processes that occur in the brain soon after injury. Importantly, people who experience TBI are at significantly higher risk of later developing Alzheimer's disease and related dementias (AD/ADRD).

Our laboratory aims to better understand the biological mechanisms underlying both AD and TBI with a special focus on microRNAs (miRNAs), which are small molecules that help regulate gene activity, and mitochondria, which provide energy and support cell health. We study how miRNAs and mitochondria contribute to early and ongoing brain damage in AD/ADRD, as well as secondary injury processes that occur after the initial impact in TBI. We also investigate how TBI and other risk factors influence the development of AD/ADRD over time.

In addition to understanding these disease mechanisms, our research seeks new therapeutic strategies. One promising approach involves delivering small RNA molecules, such as miRNA mimics, inhibitors, or mRNAs, directly to the injured brain in rodent models. These molecules can be designed to switch off harmful genes or pathways that drive inflammation, cell damage, and long-term neurodegeneration in both TBI and AD/ADRD. Through this work, we hope to advance more effective methods for diagnosing, preventing, and treating these devastating neurological conditions.

The joint laboratories of Drs. Lane, Fan and Higashi are located in the Markey Cancer Center, 5th floor of the Lee T Todd Jr. Building, 789 S Limestone St. This facility comprises contiguous laboratory space including the tissue culture rooms (biosafety level II) containing biosafety cabinets, CO2 incubators, centrifuges and benches with washing facilities including eyewash. The laboratory area, including the corridors is only accessible to laboratory personnel, and is not shared with other researchers.

Analytical capabilities, especially Mass Spectrometry, Reverse Phase Protein Array (RPPA), Confocal Microscopy and Nanosight molecular size distribution analyzer are also located in this space. NMR is located in the basement of the building in a space designed for the purpose. No infectious or biohazardous agents leave the area, only extracted materials contained in appropriate vessels.

Summary of Biohazard Materials & Manipulations:

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

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)]: EGFP/jellyfish/Tracking/Expression/mammalian cells/bacteria/Plasmid/; GFP/jellyfish/Tracking/Expression/mammalian cells/bacteria/Plasmid/; mCherry/Discosoma/Tracking/Expression/mammalian cells/bacteria/Plasmid/;

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YFP/jellyfish/Tracking/Expression/mammalian cells/bacteria/Plasmid/;
RFP/Discosoma/Tracking/Expression/mammalian cells/bacteria/Plasmid/;
CFP/jellyfish/Tracking/Expression/mammalian cells/bacteria/Plasmid/;
Turquoise/jellyfish/Tracking/Expression/mammalian cells/bacteria/Plasmid/;
Kiema/Montipora/Tracking/Expression/mammalian cells/bacteria/Plasmid/;
LC3/Human/Autophagy/Expression/mammalian cells/bacteria/Plasmid/;
P62/Human/Autophagy/Expression/mammalian cells/bacteria/Plasmid/; UB
K63/Human/Ubiquitin/Expression/mammalian cells/bacteria/Plasmid/; TRIM32/Human/E3
ligase/Expression/mammalian cells/bacteria/Plasmid/;
TFEB/Human/Autophagy/Expression/mammalian cells/bacteria/Plasmid/;
LAMP1/Human/Autophagy/Expression/mammalian cells/bacteria/Plasmid/; P54/Human/RNA
helicase/Expression/mammalian cells/bacteria/Plasmid/; huPSS-1/Human/Lipid
synthase/signaling/Expression/mammalian cells/bacteria/Plasmid/; FIS/Human/Mitochondrial
fission/Expression/mammalian cells/bacteria/Plasmid/; UB/Human/Ubiquitin/Expression/mammalian
cells/bacteria/Plasmid/; miR-146a/Synthetic/Regulatory/Interference /mammalian
cells/rats/mice/N/A/; miR-223/Synthetic/Regulatory/Interference/mammalian cells/rats/mice/N/A/;
miR-155/Synthetic/Regulatory/Interference/mammalian cells/rats/mice/N/A/; miR-
124a/Synthetic/Regulatory/Interference/mammalian cells/rats/mice/N/A/; miR-142-
3p/Synthetic/Regulatory/Interference/mammalian cells/rats/mice/N/A/; miR-142-
5p/Synthetic/Regulatory/Interference/mammalian cells/rats/mice/N/A/; miR-
107/Synthetic/Regulatory/Interference/mammalian cells/rats/mice/N/A/; miR-
132/Synthetic/Regulatory/Interference/Mammalian cells/mouse/N/A/; miR-
150/Synthetic/Regulatory/Interference/mammalian cells/rats/mice/N/A/; Scrambled miRNA control
(also called scrambled control, or RNA scrambled control nucleic acids, or
scramble)/Synthetic/N/A/N/A/mammalian cells/rats/mice/N/A/; IRAK1/Human, mouse/Inflammatory
signaling/Expression/Mammalian cells/bacteria/Plasmid/; TRAF6/Human/mouse/Inflammatory
signaling/Expression/Mammalian cells/bacteria/Plasmid/; NLRP3/Human/mouse/Inflammasome
component/Expression/Mammalian cells/bacteria/Plasmid/; CEBPb/Human/mouse/Inflammatory
transcription factor /Expression/Mammalian cells/bacteria/Plasmid/; miR-
132/human/mouse/regulatory/Expression/mammalian cells/rats/mice/Plasmid/;
GRN/human/mouse/anti-inflammatory/lysosomal/Expression/mammalian cells/mouse/Plasmid/;
ARC/human/rat/mouse/RNA carrier/Expression/Mammalian cells/mouse/plasmids/;
SOD2/human/mouse/antioxidant/mitochondria/Expression/mammalian cells/mouse/Plasmid/;
IKKa/Human/mouse/Inflammatory signaling /Expression/Mammalian cells/bacteria/Plasmid
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pEGFP-LC3/; Plasmid/pDEST-mCherry-
LC3/; Plasmid/pDEST-mCherry-EGFP-LC3/; Plasmid/mCherry-mito-7/; Plasmid/pDsRed2-MitoTimer/;
Plasmid/paGFP-MT/; Plasmid/pEGFP-p62/; Plasmid/mCherry-p62/; Plasmid/YFP-p62/;
Plasmid/pDsRed2-mito-RFP/; Plasmid/pECFP-mito/; Plasmid/pcDNA-HA-UB K63/;
Plasmid/pmTurquoise2-Mito/; Plasmid/pCGN-HA-TRIM32/; Plasmid/pAc.GFP1-N1-TRIM32/;
Plasmid/pEGFP-N1-TFEB/; Plasmid/pmCherry-LAMP1/; Plasmid/pIND(SP1)-Mito-Kiema/; Plasmid/RFP-
P54-3UTR/; Plasmid/pEF-Tak-mCherry-huPSS-1/; Plasmid/pBaBe hygro-mCherry-GFP-FIS/;

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Plasmid/mCherry2-C1 (Control mCherry) /; Plasmid/pcDNA3.1(+)-hA5U.hGRN/; Plasmid/pcDNA3.1(+)-hA5U.hMIR132/; Plasmid/pcDNA3.1(+)-hA5U.hSOD2/; Plasmid/pET21a+RVG-hARC-StrepII/; Plasmid/pET21 a+Bl.hTFR1-hARC-StrepII-TAT-HIS/; Plasmid/pcDNA3.1(+)-hA5U.EGFP/; Plasmid/RFP-UB/; Plasmid/pRL-TK/; Plasmid/pET-21/; Plasmid/pcDNA3.1/; Plasmid/pGL3

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/BV-2/; Human/HeLa/; Human/PDZD8-KO-HeLa/; Human/SH-SY5Y/; Human/HEK293T/; Human/H4/; Human/SH-SY5Y-mito-CFP/; Human/SH-SY5Y-mito-RFP/; Human/hBMECs /; Human/hCMEC/D3/; Human/SH-SY5Y-HA-Ub/; Human/HMC3/; Human/MONO-ALLELIC mEGFP-TAGGED TUBA1B WTC iPSC LINE (TAG AT N-TERM)/; Human/MONO-ALLELIC mTagRFPT-TAGGED TUBA1B WTC iPSC LINE (TAG AT N-TERM, SECOND EXON)/; Human/Human Brain Vascular Pericytes/; Human/Human Astrocytes

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/Naked Nucleic Acid-r/sDNA/retro-orbital or intracerebroventricular injections /anesthesia/ABSL1/Lab coat, gloves, eye protection, surgical mask, head cover, surgical gown/ABSL1/No

Risk Assessment/Discussion:

Dr. Wang has submitted a renewal of her IBC protocol entitled MicroRNA Regulation and Mitochondrial Function in Alzheimer's Disease and Traumatic Brain Injury. Dr. Wang's laboratory focuses on microRNAs (miRNAs) and mitochondrial function contributes to the pathogenesis of neurodegenerative diseases, including Alzheimer's disease and related dementias, as well as traumatic brain injury (TBI). Human brain tissues and biofluids will be obtained from the UK Alzheimer's Disease Research Center to isolate RNA and protein or to be fixed for immunohistochemistry. They will also transfect mammalian cells with small RNA duplexes or protein and tracking genes via lipofectamine. Dr. Wang's laboratory is also studying BIONARC (Blood-brain barrier Infiltrating Optimized Neuron-targeting ARC Capsids) and its potential for delivery of gene-targeting miRNAs to down-regulate upstream activators of NF- κ B signaling to decrease the inflammatory response after TBI. An in-vitro BBB model will be constructed using human cells to evaluate the efficacy of these nanocarriers across the BBB. In a different project, Dr. Wang's lab will generate human iPSC-derived and patient-derived brain organoids. Organoids will be maintained for weeks to months to examine tau pathology development, microglial infiltration, synapse maturation, etc. They will also use a mouse model to test the efficacy of liposome/LNPs, peptide-based nanoparticle, and BIONARC delivery. Anesthetized mice will receive intracerebroventricular or retroorbital injection of miRNA/mRNA containing nanocarriers after induced brain injury. Animals will be euthanized at various timepoints and brains rapidly removed for isolation of RNA and protein. Animal work is completed using ABSL1 procedures/housing. Lastly, human and rodent brain tissues will be fixed for immunohistochemistry using formaldehyde or glutaraldehyde. Laboratory work with human source materials will take place using BSL2 containment. Lab personnel will wear lab coat, eye protection, and disposable gloves. Dr. Wang's current IBC protocol will expire on April 18, 2026.

IBC Discussion & Vote:

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The protocol renewal IBC-26-27 (version 6.0) was approved pending minor modifications as listed below:

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ANIMAL RESEARCH – Please select “Yes” in response to the question “Does your research involve the use of biohazardous materials in conjunction with live animals?”

SCIENTIFIC SUMMARY -

1. Please briefly describe the biological function and risks associated with the transgenes and make note of any particularly hazardous transgenes, if applicable.
2. Please expand on the procedure to remove bands from the centrifuge tube fractions including the risks involved and steps used to mitigate those risks.

*

Mike Mendenhall initiated the motion. Carrie Shaffer seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

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PI: Qing-Bai She

IBC Protocol Number: IBC-26-28

Protocol Title: Targeting Translational Control in Cancer Progression and Metastasis

Protocol Type: Renewal

Amendment To: N/A

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

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

Primary Reviewers: C. Haughton, Y. Wu, A. Pinto

Brief Project Overview:

Cancer develops when cells grow and divide out of control. Although treatments have improved, we still do not fully understand how cancer cells grow, spread to other organs, and resist therapy. Our research focuses on how cancer cells make proteins, which are the molecules that control how cells function. In many cancers, certain harmful proteins are produced in excess, helping tumors grow and survive. Our lab studies a key control system inside cells called the mTOR pathway. When this system becomes overactive, it drives the production of proteins that support tumor growth and spread. We have discovered that specific cancer-related factors, including a unique form of a protein called NRP1-ΔE4 and a viral protein found in Merkel cell carcinoma, can activate this pathway. We also study an enzyme

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called spermine synthase (SMS), which is often increased in colorectal cancer and helps cancer cells adapt their energy production to survive. This research uses biohazardous materials, including human cancer cells, viral proteins, and mouse tumor models. All experiments are conducted under strict safety guidelines and institutional oversight to protect researchers and the environment. By understanding how cancer cells depend on abnormal protein production and energy systems, our goal is to identify new treatment strategies. Ultimately, we aim to develop safer, more effective therapies that can be tailored to specific features of a patient's tumor.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Biohazardous Materials

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: NRP1/Human/Oncogene/Expression, knockdown, knockout/Human cells /pcDNA3, pCMV6, pLKO.1, pLenti6.3, PX459/; NRP1- Δ E4/Human/Oncogene/Expression, knockdown, knockout/Human cells /pcDNA3, pCMV6, pLKO.1, pLenti6.3, PX459/; MCV-sT/Merkel cell polyomavirus/Oncogene/Expression, knockdown/Human cells /pCMV6, pLKO.1, pLenti6.3/; eIF4E/Human/Translation initiation factor/Expression, knockdown/Human cells/pCMV6, pLKO.1, pLenti6.3/; 4E-BP1/Human/Translation repressor/Expression, knockdown/Human cells/pcDNA3, pCMV6, pLKO.1, pLenti6.3/; Survivin/Human/Cell survival/Expression, knockdown, knockout/Human cells/pCMV6, pLKO.1, PX459/; eIF5A/Human/Translation initiation factor/Expression, knockdown, knockout/Human cells/pcDNA3, pCMV6, pLKO.1, pLenti6.3, PX459/; Snail/Human/EMT driver/Expression, knockdown, knockout/Human cells /pcDNA3, pCMV6, pLKO.1, pLenti6.3, PX459/; Slug/Human/EMT driver/Expression, knockdown, knockout/Human cells /pcDNA3, pCMV6, pLKO.1, pLenti6.3, PX459/; Firefly luciferase/Firefly/Cell tracking/Expression/Human cells/pcDNA3, pLenti6.3/; GFP/Jellyfish Aequorea victoria/Cell labeling/Expression/Human cells/pcDNA3, pLenti6.3/; SMS/Human/Polyamine biosynthetic enzyme/Expression, knockdown, Knockout/Human cells/pcDNA3, pCMV6, pLKO.1, pLenti6.3, PX459/; MTIF2/Human/Mitochondrial translational regulator/Expression, Knockdown, Knockout/Human cells/pcDNA3, pCMV6, pLKO.1, pLenti6.3, PX459

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pcDNA3/; Plasmid/pCMV6/; Lentivirus/pLKO.1/; Plasmid/PX459/; Lentivirus/pLenti6.3

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HEK293T/; Human/HCT116/; Human/DLD-1/; Human/HT29/; Human/T84/; Human/HCT15/; Human/RKO/; Human/SW403/;

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Human/SW620/; Human/SW480/; Animal/MC38/; Animal/CT26/; Human/MKL-1/; Human/MKL-2/; Human/WaGa/; Human/MCC26/; Human/MCC13/; Human/UIISO

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Cells - Human, genetically modified/IV-tail vein or subq into organs/anesthesia/ABSL2/disposable glove, shoe cover, lab coat, head cover and surgical gown and mask/ABSL1/No//; Mouse/Cells - Human, non-modified/IV-tail vein or subq into organs/anesthesia/ABSL2/disposable glove, shoe cover, lab coat, head cover and surgical gown and mask/ABSL1/No

Risk Assessment/Discussion:

Dr. She has submitted a renewal of their IBC protocol entitled Targeting Translational Control in Cancer Progression and Metastasis. Dr. She's laboratory seeks to better understand the mTOR pathway in tumorigenesis and metastatic progression. Dr. She's laboratory performs cell culture work using BSL2 containment within a certified BSC. Mammalian expression vectors encoding NRP1, NRP1- Δ E4, MCV-sT, SMS, eIF4E, survivin, snail, slug, 4E-BP1, eIF5A, MTIF2, firefly luciferase and GFP are used to transiently or stably transfect mammalian cells for downstream studies including PCR and Sanger sequencing. Dr. She's laboratory will also produce lentiviral vectors expressing shRNAs targeting various genes of interest using a 3rd generation packaging system. Supernatant is collected and used directly for transduction. Lentivirus is not concentrated or purified. Work with lentivirus requires BSL2+ containment, including use of disposable gloves, dedicated laboratory coat, and eye protection. The 3rd generation lentiviral vectors in use will express, knockdown, or knockout oncogenes, translation factors, cell survival genes, and others. Use of oncogenic lentiviral vectors introduces additional risk to personnel in case of accidental exposure. Stably transduced cells will be used in xenograft and metastatic tumor models in mice. Lentivirus-transduced cells are washed at least 3 times prior to subsequent experiments, including animal work. Anesthetized mice will be administered both modified and non-modified cells via tail vein or subcutaneous injection using ABSL2 experimental procedures within a BSC. Animals administered lentivirus-transduced cells will be housed at ABSL2 containment for 72 hours, after which they are moved to ABSL1 housing. Mice will undergo bioluminescent imaging. At the conclusion of experiment, mice are sacrificed and tissues harvested, fixed in 10% NBF, and subjected to subsequent histological analysis. Dr. She's current IBC protocol will expire on April 18, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-28 (version 8.0) was approved pending minor modifications as listed below:

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ANIMAL RESEARCH – Animals with Biohazards

1. In the table: Please correct “subQ” to “intraperitoneal” for injection into organs.

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2. Please update the Special Procedures for the genetically modified injection entry to specify that animals are housed at ABSL2 for 72 hours post injection before moving to ABSL1 housing.

SCIENTIFIC SUMMARY -

1. Please remove reference to the use of the UV germicidal lamp from the summary.
2. In Specific Aims 3, please correct the first sentence: Athymic nude, Balb/c, C57BL/6L, and NSG mice will be used to generate xenograft models by “subcutaneous injection,” to “intraperitoneal injection.”
3. Please specify the PPE used during work with cell lines and E.coli strains versus with lentivirus.
4. Please specify what procedure is utilized for cell lysis and describe when biohazardous materials are inactivated.
5. Include the procedures in place that minimize the risk of needlestick while handling animals.
6. In the “Use of lentiviral vectors in cultured cells and animal studies” section, please describe the procedure for positive clone selection and expansion. Please also describe what specific assays will be conducted with these positive clones.
7. Regarding use of lentiviral vectors, please describe what clones are being preserved and what in vivo and in vitro assays will be done with them.
8. In Specific Aim 3, please clarify the length of time between injection of genetically modified cells and live animal imaging.

*

Amelia Pinto initiated the motion. Yadi Wu seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

*

PI: Lance Johnson

IBC Protocol Number: IBC-26-30

Protocol Title: Effects of genetic and environmental factors on metabolism and cognition in mice

Protocol Type: Renewal

Amendment To: N/A

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

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

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

Brief Project Overview:

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Our laboratory is interested in how certain genetic and environmental factors predispose the brain to cognitive impairments later in life, with a special emphasis on the gene Apolipoprotein E (APOE). The E4 polymorphism of APOE is the strongest genetic risk factor for late onset Alzheimer's Disease (AD) and is carried by approximately 25% of the human population. We employ unique mouse models to analyze and develop treatments for age-related cognitive impairment and AD. Our distinct mouse models increase our understanding of brain function during healthy aging and disease, and allow for the development of therapeutic interventions to mitigate or even reverse neurological impairments. The translational nature of our research program is evident in our development of metabolic and cognitive tests in humans based on the cognitive tests we use in mice.

SCIENTIFIC SUMMARY Overview & Justification

The proposed experiments examine the effect of APOE genotype on the transport and metabolism of key energy substrates within the periphery and brain. APOE genotype is a very important predictor of body mass, cognitive impairment and dementia. Since mice do not express the human apoE isoforms, the mouse apoE locus is targeted replaced with the various human apoE isoforms, thereby remaining under control of the endogenous mouse apoE promoter. This results in a physiologically relevant pattern and level of apoE expression [Sullivan et al., 1997; Knouff et al., 1999; Sullivan et al., 1998; Johnson et al., 2014]. These mice are commonly used in investigating the effect of the various apoE isoforms on cellular and physiological function [Kim et al., 2009]. There are definitive sex discrepancies regarding body fatness and adipose distribution and cognition [Lin et al., 2015; Holland et al., 2013]. Therefore, it is important to compare males to females. In order to stimulate weight gain and to mirror the diet consumed by the typical person [Johnson et al., 2014], mice in several experiments will be fed a high fat diet (HFD; 60% calories from fat), beginning at various points of the lifespan. Additionally, to study diet-gene interactions, several experiments will employ different diets with varying caloric makeup (i.e. 5-95% calories from carbohydrates vs 5-95% calories from fat). Human apoE mice are needed for these experiments to measure the uptake and utilization of energy substrates in the brain. Mice provide several advantages: a) murine metabolism is similar enough to that of humans to allow extrapolation of the results; b) Their small size allows the use of less space; c) their abbreviated lifespan allows for the study of changes in metabolism and cognition across the lifespan; and d) behavior studies can be performed to test changes in cognition. Cell models will provide insight into metabolic processes at the cellular level, but do not adequately reflect tissue/organ level and whole body physiology, and are not realistic in regards to studies of cognition. No less sentient species is available and humans are not ideal as the proposed experiments would be unethical in humans. In one set of experiments, we will measure substrate uptake in the brain and peripheral tissues of mice expressing human apoE, as well as in primary and immortalized mouse cell lines, using radiolabeled molecules. We hypothesize that E4 contributes to cognitive impairment through a metabolic abnormality in which a preference toward fatty acid oxidation results in an inherent inefficiency to utilize glucose. In this set of experiments, we will measure substrate utilization in mice expressing human apoE. The proposed experiments will provide a new understanding of substrate utilization in E4+ individuals, and offer insight into potential apoE effects on cerebral energy responses.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: enhanced GFP (EGFP)/jellyfish/tracking/expression/mouse/AAV8, AAV BR1/; Thyroxine-binding globulin (TBG) /mouse/regulatory gene; promoter (liver specific)/expression/mouse/AAV8/; codon-improved Cre recombinase (iCre)/bacteriophage P1/enzymatic protein/expression/mouse/AAV8, AAV BR1/; Perilipin 2 (PLIN2)/Homo sapiens (human)/Lipid droplet-associated protein (perilipin family); lipid metabolism / lipid storage regulator/Interference (RNAi knockdown via siRNA)/Human/None (synthetic siRNA duplex; lipid-based transfection reagent such as RNAiMAX is the delivery method, not a vector)/; Perilipin 2 (PLIN2)/Mus musculus (mouse)/Lipid droplet-associated protein (perilipin family); lipid metabolism / lipid storage regulator/Interference (RNAi knockdown via siRNA)/Mouse/None (synthetic siRNA duplex; lipid-based transfection reagent such as RNAiMAX is the delivery method, not a vector)/; CAG promoter/mouse/regulatory gene (ubiquitously expressed)/expression/mouse/AAV8, AAV BR1/ Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/AAV8-TBG-EGFP-T2A-iCre (VB1725) /; Adeno-Associated Virus (AAV)/AAV8-TBG-eGFP (VB1743)/; Adeno-Associated Virus (AAV)/AAV(BR1)-CAG-iCre-T2A-EGFP (SL116089)/; Adeno-Associated Virus (AAV)/AAV(BR1)-CAG-EGFP (SL116035)/

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/primary astrocytes/; Animal/primary neurons/; Animal/immortalized astrocytes/; Human/immortalized astrocytes/; Animal/primary microglia/; Human/ioMicroglia APOE 4/4 C112R/C112R/; Human/ioMicroglia Male/; Human/E3 and E4 astrocytes; isogenic iPSC lines from human fibroblasts/; Human/JIPSC1000 Parent (APOE3/3) iPSCs/; Human/human immortalized cell lines/; Human/JIPSC1154 A (APOE2/2) iPSCs/; Human/JIPSC1162 A (APOE3/3) iPSCs/; Human/JIPSC1150 A (APOE4/4) iPSCs/; Human/JIPSC1264 A (APOE3/3 Ch) iPSCs/; Human/JIPSC1146 B (APOE2/2) iPSCs/; Human/JIPSC1268 B (APOE3/3) iPSCs/; Human/JIPSC1142 B (APOE4/4) iPSCs/

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)/Retro-orbital injection/2-3% isoflurane anesthesia/ABSL1/gloves, lab coat, safety glasses/ABSL1/No//

Risk Assessment/Discussion:

Dr. Johnson has submitted a renewal of his IBC protocol entitled Effects of genetic and environmental factors on metabolism and cognition in mice. In this renewal, Dr. Johnson's laboratory is interested in better understanding how genetic and environmental factors may predispose the brain to cognitive

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impairments. They are specifically interested in the Apolipoprotein E (APOE) gene. The E4 polymorphism of this gene is the strongest genetic risk factor for late onset Alzheimer's disease. Towards this aim, Dr. Johnson's laboratory utilizes a number of human derived materials, including brain tissues, blood, serum, plasma, and CSF from the UK Pathology Core Laboratory, PBMC samples from the Norton Neuroscience Institute, and immortalized cells from 3rd party vendors. Brain tissues will be sectioned, fixed in 4% paraformaldehyde, and cryoprotected in sucrose. Brain sections will be used for histological staining and immunohistochemistry. Blood, serum, CSF, plasma and PBMCs will be used to isolate proteins for Western blot or ELISA. Cells will also be subjected to downstream RNA sequencing and metabolomic analyses. All work with human derived materials will be completed at BSL2 containment and includes use of a certified BSC and laboratory coat, gloves, and eye protection. Dr. Johnson's laboratory also utilizes adeno-associated virus (AAV) vectors from a 3rd party vendor for delivery of Cre and/or EGFP in mice. AAV preparations are diluted within a BSC and transported to DLAR for retroorbital delivery in anesthetized mice. Mice will be injected at 2- or 6-month timepoints to investigate APOE isoform switching. Prior to euthanasia, mice will undergo behavioral testing at 8 months of age. Upon study end, brain, liver, blood, and other tissues will be obtained for histological staining, immunohistochemistry, and molecular assays including Western blot, ELISA, RNA isolation, and PCR or sequencing. Animal work will be conducted using ABSL1 experimental procedures, and animals will be housed at ABSL1 containment. The specific transgenes (Cre and EGFP) do not present significant risk to personnel should they be accidentally exposed. Dr. Johnson's current IBC protocol will expire on April 24, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-30 (version 10.0) was approved pending minor modifications as listed below:

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LOCATIONS – Research Locations Table: The currently approved IACUC indicates that animals will be transported to the Animal Behavior Core and /or the Light Microscopy Core after AAV administration. Please update the locations accordingly.

SCIENTIFIC SUMMARY –

1. Please provide a description of how cells are removed from centrifuge tubes after centrifugation to remove the band fractions from the density gradient.
2. Please specify where animals will undergo behavioral testing.

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Mike Mendenhall initiated the motion. Carrie Shaffer seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

*

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PI: John D'Orazio

IBC Protocol Number: IBC-26-32

Protocol Title: Culture and transfection of human cell lines

Protocol Type: Renewal

Amendment To: N/A

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

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

Primary Reviewers: T. Chambers, A. Pinto, D. Malherbe

Brief Project Overview:

Our laboratory is interested in understanding why certain people have a higher risk of cancer than other individuals. Our main area of focus involves collecting mutation data from patients that have been diagnosed with pediatric cancer at UK (and have volunteered to have their genome sequenced for potential mutations in a panel of cancer-causing genes) and exploring whether the observed mutations contributed to their cancer formation. We have focused primarily on the p53 gene, as it is both the most commonly mutated gene in cancers and the most commonly mutated gene in the population we are studying. Our goal with this project is to discover how mutations lead to higher mutation rates and cancer.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, Cell culture, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Transfection, Use of viral vectors, Use of Human Source Material(s), Transformation, Use of infectious agents

Transport: Yes

Materials Transported: Biohazardous Materials

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Luciferase/Firefly, Renilla/Fluorescent protein/Use in reporter assays/Cell Lines/pcDNA3.1/pGL4.38[Luc2P/p53 RE/Hygro]/pGL4.75[hRluc/CMV]/; p53/Human/Transcriptional regulator/Expression in Cell Culture/Cell Lines/pLenti-C-mGFP-P2A-Puro, Lenti vector with C-terminal mGFP tag and P2A-Puro/; p53 T253I/Human/Transcriptional regulator (mutant)/Expression in Cell Culture/Cell Lines/pLenti-C-mGFP-P2A-Puro, Lenti vector with C-terminal mGFP tag and P2A-Puro (with SNP mutated from WT)/; p53 C176Y/Human/Transcriptional regulator (mutant)/Expression in Cell Culture/Cell Lines/pLenti-C-mGFP-P2A-Puro, Lenti vector with C-terminal mGFP tag and P2A-Puro (with SNP mutated from WT)/; p53 R213X/Human/Transcriptional regulator (mutant)/Expression in Cell Culture/Cell Lines/pLenti-C-mGFP-P2A-Puro, Lenti vector with C-terminal mGFP tag and P2A-Puro (with truncated gene product mutated from WT)/; p53 C141Y/Human/Transcriptional regulator

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(mutant)/Expression in Cell Culture/Cell Lines/pLenti-C-mGFP-P2A-Puro, Lenti vector with C-terminal mGFP tag and P2A-Puro (with SNP mutated from WT)/; p53 R337H/Human/Transcriptional regulator (mutant)/Expression in Cell Culture/Cell Lines/pLenti-C-mGFP-P2A-Puro, Lenti vector with C-terminal mGFP tag and P2A-Puro (with SNP mutated from WT)/; GFP/Aequorea victoria/Fluorescent protein/Use to detect transgenes/Cell lines/Various plasmids and lenti-plasmids

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pcDNA3.1/; Plasmid/pIRES-EGFP-p53 WT GFP/; Plasmid/pIRES-EGFP-p53 T253I GFP/; Plasmid/pGL4.38[Luc2P/p53 RE/Hygro]/; Plasmid/pGL4.75[hRluc/CMV]/; Lentivirus/pLenti-C-mGFP-P2A-Puro

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/B16/; Animal/Pam212/; Animal/3T3/; Human/Phoenix /; Human/HCT116/; Human/DLD1/; Human/HT-29/; Human/A375/; Human/HIEC-6/; Human/SK-MEL2/; Human/hTERT transformed primary melanocytes/; Human/HEK 293/; Human/NCM-356/; Human/PT-2377/; Human/PT-93/; Human/PT-130/; Human/SAOS2/; Human/HEK-293 p53 -/- (CRISPR deleted)/; Human/A549

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. D'Orazio has submitted a renewal of his IBC protocol entitled Culture and transfection of human cell lines. Dr. D'Orazio's laboratory focuses on collecting mutation data from patients that have been diagnosed with pediatric cancer at UK and exploring whether the observed mutations contributed to their development of cancer. They focus primarily on the p53 gene. The work in Dr. D'Orazio's lab requires the use of human cell lines and lentivirus vector to express wild type and mutant p53. Lentivirus particles are purchased from Origene and will not be produced in Dr. D'Orazio's laboratory, significantly lowering risk. These lentiviral constructs will be used to infect p53-defective CRISPR-deleted HEK293 cells (obtained from Ubigene). Expression will be verified by immunoblot and screened for p53 expression using commercially available p53 antibodies. The p53 signaling pathway will be studied by exposing cells to DNA damaging agents such as cisplatin or doxorubicin. Lentivirus transduced cells will be subjected to Western blotting. RIPA buffer will be used to lyse cells for protein extraction. Additional experiments involving lentivirus-transduced cells include flow cytometry and confocal microscopy. Cells will be fixed in 75% ethanol for flow cytometry or 10% PFA for confocal microscopy. Work with lentivirus is conducted at BSL2+ containment and includes work exclusively within a BSC and dedicated lab coat, gloves, and eye protection. P53 is a tumor suppressor gene present in nearly all tissues, but exposure to mutant p53 may pose additional risk. Dr. D'Orazio's current IBC protocol will expire on May 10, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-32 (version 8.0) was approved pending minor modifications as listed below:

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RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Vector Information Table: Please review the entries' last columns, "Will DNA Integrate into the host genome." Do all the plasmid vectors actually integrate into the host genome?

LOCATIONS – Biological Safety Equipment: Please update the expired certification date for the biosafety cabinets in use.

SCIENTIFIC SUMMARY - Please update references from "BSL2+ tissue culture hood" to "biological safety cabinet."

*

Thomas Chambers initiated the motion. Amelia Pinto seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

*

PI: Christopher Richards

IBC Protocol Number: IBC-26-33

Protocol Title: Identification of novel cancer therapies

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-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 - Enhanced (BSL2+)

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

Brief Project Overview:

Ovarian cancer (OC) and lung cancer (LC) are among the most commonly occurring cancers worldwide. In the United States, OC is the fifth leading cause of cancer death among women and LC remains one of the most deadly forms of cancer. In Kentucky, rates of incidence and death from lung cancer are the highest in the nation. Although recent studies have led to a greater understanding of OC and LC development and progression, treatments have not proven to be effective in clinical trials. In children, acute myeloid leukemia (AML) makes up about 15 percent of childhood leukemia, and osteosarcoma (OS) is the most common bone cancer in children. While standard chemotherapy treatments have greatly improved the overall survival of children with AML and OS, there have been limited improvements in therapies in recent decades.

Therefore, it is critically important to identify new therapies for these cancers. A powerful method for better identifying novel therapies is through precision medicine, where the unique characteristics of a patient's tumor are used to tailor tumor-specific treatment. The aims of this proposed research are to 1) identify a panel of drugs that target key genes involved in AML, LC, or OC and test their ability to inhibit

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cancer cell growth, 2) establish and characterize the genetic profiles of xenografts generated from freshly isolated AML and OC tissue and commercially available cancer cell lines, 3) identify novel therapies specific for each unique tumor profile that inhibit cell growth in OC, LC, pediatric AML and pediatric OS xenograft models, and 4) test a novel drug delivery therapeutic in OC and OS cell lines and xenograft models. The research plans described in this proposal should identify novel therapeutic strategies which can be taken to clinical trials, improving current treatment regimens.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Biohazardous Materials, Animals

Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human; Escherichia coli K12 MG1655 (RG1-bacteria)/bacteria

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Nrf2/Homo sapiens/Oncogene/Silencing/N/A/N/A/; Keap1/Homo sapiens/Tumor Suppressor/Silencing/N/A/N/A/; ERBB2/Homo sapiens/Oncogene/Silencing/N/A/N/A/; ERBB3/Homo sapiens/Oncogene/Silencing/N/A/N/A/; ERBB4/Homo sapiens/Oncogene/Silencing/N/A/N/A/; EGFR/Homo sapiens/Oncogene/Silencing/N/A/N/A/; ABCB1/Homo sapiens/Membrane protein/Silencing/N/A/N/A/; KRT81/Homo sapiens/Structural/Silencing/N/A/N/A/; rFLuc/Luciola cruciate/Tracking gene/Expression/N/A/pLL-CMV-rFLuc-T2A-GFP-mPGK-Puro/; GFP/Aequorea victoria/Tracking gene/Expression/N/A/pLL-CMV-rFLuc-T2A-GFP-mPGK-Puro/; SLCO2B1/Homo sapiens/Membrane protein/Silencing/N/A/N/A/; SLC22A4/Homo sapiens/Membrane protein/Silencing/N/A/N/A/; BCL2/Homo sapiens/Mitochondrial protein/Silencing/N/A/N/A/; BCL-xL/Homo sapiens/Mitochondrial protein/Silencing/N/A/N/A/; CHOP/DDIT3/Homo sapiens/cytoplasmic and nuclear protein/Silencing/N/A/N/A/; GBP1/Homo sapiens/Endoplasmic Reticulum protein/Silencing/N/A/pLentiCRISPRv2-puro /; GBP5/Homo sapiens/Endoplasmic Reticulum protein/Silencing/N/A/pLentiCRISPRv2-puro /; CD40/Homo sapiens/Membrane protein/Silencing/N/A/pLentiCRISPRv2-puro /; NFkB/Homo sapiens/Nuclear protein/Silencing/N/A/pLentiCRISPRv2-puro /; RASD2/Homo sapiens/Cytoplasmic protein/Silencing/N/A/pLentiCRISPRv2-puro /; clyA/E. coli/Transmembrane/expression/cell lines/sfGFP-pBAD/; ICOS/Mus musculus/surface receptor/expression/cell lines/pCMV-3-Flag. pLenti-C-mGFP-P2A-Puro/; DCT (encodes Trp2)/Mus musculus/enzyme/expression/cell lines/sfGFP-pBAD/; PDCD1 (encodes PD1)/Homo sapiens; Mus musculus/surface receptro/expression/cell line/pCMV-3-N-Flag/; //sfGFP-pBAD/; //pLenti-C-mGFP-P2A-Puro/; SIRP alpha/Homo sapiens/surface receptor/expression/cell lines/pCMV6-entry/; Kcna3/Mus musculus/Voltage gated membrane channel/expression/cell lines/pLenti-C-mGFP-P2A-Puro/; Cas9/Streptococcus

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pyogenes/endonuclease/expression/cell lines//; ICAM1/Homo sapiens; Mus musculus/surface receptor/expression/cell lines/pLenti-C-mGFP-P2A-Puro

Vector(s) [Vector Category/Vector Technical Name]: Naked nucleic acid/siRNA/; Lentivirus/pLL-CMV-rFLuc-T2A-GFP-mPGK-Puro/; Lentivirus/pLenti-C-mGFP-P2A-Puro/; Lentivirus/pLentiCRISPRv2-puro/; Plasmid/pcdna3.1/; Plasmid/sfGFP-pBAD/; Plasmid/pET45/; Plasmid/pCMV-3-Flag/; Plasmid/pCMV3-SP-N-Flag/; Plasmid/pCMV6-Entry

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HCT116/; Human/HT29/; Human/pt 93/; Human/pt 130/; Human/pt 2387/; Human/pt 2377 LM/; Human/pt 2377 PT/; Human/Jackson Labs OC PDX derived cells/; Human/Charles River Lab OC PDX derived cells/; Human/Caov-3/; Human/OVCAR-3/; Human/UWB1.289/; Human/SW 626/; Human/A549/; Human/Univ. of Miami OC cell lines/; Human/UK OC cell lines/; Human/UK lung cancer cell lines/; Human/H226/; Human/H1299/; Human/H1563/; Human/UK057/; Human/UK022/; Human/UK062/; Human/UK032/; Human/UK068/; Human/Monocytes/; Human/Macrophages/; Human/TOV21G/; Human/HOS (KRIB)/; Human/143B/; Animal/RAW 264.7/; Human/MV4-11/; Human/M07e/; Human/THP-1/; Human/OV-90/; Human/SKOV-3/; Human/OVCAR-8/; Human/Normal human ovarian epithelial cells/; Human/FaDu/; Human/UM-SCC-47/; Human/CAL 27/; Animal/STOSE/; Human/iPSC 802-3G/; Animal/K7M2

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/intravenous, intraperitoneal, subcutaneous injection, intratibial injection/isoflurane anesthesia, mechanical restrainer for tail vein injections/ABSL2/disposable gown, gloves, head cover, shoe covers, eye protection, surgical mask/ABSL1/No/Administration of biohazardous materials to mice will take place within a BSC. Following implantation/injection of cells there are no special handling procedures and animals can be handled at ABLS1/; Mouse/Cells - Human, genetically modified/intravenous, intraperitoneal, subcutaneous injection, intratibial injection/isoflurane anesthesia, mechanical restrainer for tail vein injections/ABSL2/disposable gown, gloves, head cover, shoe covers, eye protection, surgical mask/ABSL1/No/Administration of biohazardous materials to mice will take place within a BSC. Following implantation/injection of cells there are no special handling procedures and animals can be handled at ABLS1/; Mouse/Tissue - Human (ex. PDX tumor tissue)/intravenous, intraperitoneal, subcutaneous injection, intratibial injection/isoflurane anesthesia, mechanical restrainer for tail vein injections/ABSL2/disposable gown, gloves, head cover, shoe covers, eye protection, surgical mask/ABSL1/No/Administration of biohazardous materials to mice will take place within a BSC. Following implantation/injection of cells there are no special handling procedures and animals can be handled at ABLS1

Risk Assessment/Discussion:

Dr. Richards has submitted a renewal of his IBC protocol entitled Identification of novel cancer therapies. Dr. Richards' laboratory is identifying new therapies for ovarian cancer, lung cancer, acute myeloid leukemia, osteosarcoma, and oral cancer. Both established and primary human cell lines will be

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used initially to assess sensitivity to novel or known therapeutic compounds. Work with human cells is completed using BSL2 containment within a certified BSC. The genetic background and proteomic analysis of cells will be used to select potentially beneficial drug combinations. Hypothetical tumor drivers or key nodes of synergistic pathways will be knocked down using siRNA and lipid-based transfection or lentiviral based CRISPR/Cas9 knockdown. Once single or drug combinations that effectively inhibit growth are identified, cells will be exposed to treatment plans to mimic those used to treat patients to induce therapeutic resistance. After which, NGS sequencing by the UK Oncogenomics Core will be performed to identify likely mutation drivers of resistance. siRNA will be used to knockdown a variety of genes thought to be related to resistance of cancer cells to standard therapies. Monocyte lineage cell lines (macrophages, AML cells, iPSCs, and T cells) will be transduced using a lentivirus vector to overexpress or knockdown genes of interest, including GBP1, GBP5, CD40, NF- κ B, and RASD2. Lentiviral vectors are purchased from Vector Builder (CRISPR/Cas9) or System Biosciences. Knockout M1 and M2 macrophages will be used to generate vesicles via N2 cavitation. All work with lentivirus vectors will be conducted in a certified BSC under BSL2+ conditions. Transduced cells will be sent to the FCIM core for enrichment. FCIM personnel are informed that cells are live and have been transduced with lentivirus. Expanded cells will be used for tumor xenograft experiments in mice. Anesthetized mice will be injected with tumor cells via IP injection in a BSC in DLAR under ABSL2 containment. Tumor growth will be monitored weekly via IVIS imaging. Animals will be housed at ABSL1 housing. Ovarian tissue obtained from UK Hospital will be used for implantation in anesthetized mice or generating 3D cultures to assess sensitivity to various drug regimens. In a different project, tumor cells will be harvested from xenografts to generate exosome-like vesicles to be loaded with anti-cancer therapeutics. Similarly, programmed macrophage derived engineered vesicles (P-MEVs) and bacterial engineered vesicles will be developed and used similarly. Once tumors are established, exosomes, P-MEVs, and bacterial engineered vesicles will be administered to mice. Bacteria engineered vesicles are developed using *E. coli* MG1655, a non-pathogenic K12 derivative. Lastly, human blood samples will be used to extract circulating cell free RNA or DNA from plasma and analysis at the UK Oncogenomics Core. Dr. Richard's current IBC protocol will expire April 12, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-33 (version 8.0) was approved pending minor modifications as listed below:

*

PERSONNEL: Thompson, Landon J is listed in the IACUC protocol as handling Human Source Cell Line Or Transplantable Tumor/NHP specimen but is not listed in the IBC protocol personnel list. Please update personnel list accordingly.

INFECTIOUS AGENTS – Infectious Agent(s): Please remove *E. coli* from the table.

RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information Table: Please update the table to include entries for the “Host(s),” rather than, “N/A.” For example, should “cell lines” be listed as the host for siRNA constructs used for gene silencing?

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LOCATIONS – Research Locations Table: Please add an entry to reference the Light Microscopy Core facility used for animal imaging after tumor cell/PDX implant.

*

Doug Harrison initiated the motion. Brandy Nelson seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

*

PI: Cindy Burklow

IBC Protocol Number: IBC-26-37

Protocol Title: A Plant Genomics Approach to Drug Discovery

Protocol Type: Renewal

Amendment To: N/A

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

Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: A. Hunt, J. Smalle, C. Shaffer

Brief Project Overview:

The purpose of our research is to improve production of plant-derived compounds with potential value as drugs or nutrients. These improvements are achieved by randomly switching on (or off) the plants' own genes using plant cell culturing techniques. Gene activation is performed using a section of DNA from a common plant virus known as a "promoter" sequence. When randomly inserted into the plant's DNA, this promoter activates genes near its insertion point. We create a large population of plant cell cultures containing these mutations and select the "best" cultures based upon the biological activity they contain. Selected cultures may over-produce the original plant compounds to give increased yields of a valuable plant chemical, or may be producing a completely new plant compound not seen in the unmanipulated plant. We currently apply this approach to plants with known medicinal value, as well as plants native to our geographic region which we have discovered to possess previously unknown potential value as drug candidates. All of our work is done using plant cell cultures under carefully controlled laboratory conditions where any manipulated plant tissue can be confined and destroyed when experiments are completed. We have used cell cultures derived from *Lobelia cardinalis*, *Artemisia annua*, and *Salvia miltiorrhiza* to identify novel compounds contained in plants. To identify plants containing novel bioactivity, we test extracts from these plants in cell cultures derived from mice and human cancer cells performed under appropriate biosafety conditions. Alternately, we express a human protein that is a drug target or involved in a disease process whose expression allows a survival selection in that culture. Using a survival selection, we can focus on cultures that may contain compounds that interact with the expressed drug target or protect cultures from the expressed pathological protein. Our

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approach to finding new active compounds in plants is more rapid than traditional plant drug discovery and offers advantages related to intellectual property and patenting novel compounds or genetically optimized plants as sources of useful compounds. The biosafety level of our plant cell culturing is bsl-1. Our human neuroblastoma cell culture work is bsl-2.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, Genetics, DNA/RNA isolation/purification, Transformation, Plant work, Use of Human Source Material(s)

Transport: No

Materials Transported: N/A

Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human; Rhizobium rhizogenes (RG1-bacteria)/Plants

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: human dopamine transporter (hDAT)/human/receptor/expression/Lobelia cardinalis/pCambia1301; KanR kanamycin resistance /bacterial/selection/selection/lobelia cardinalis/pSKI074

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pcambia1301; Plasmid/pSKI074

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Plant/Lobelia cardinalis plant hairy root culture; Human/SH-SY5Y neuroblastoma cells; Plant/salvia mirtiorhizza plant hairy root culture; Plant/Polygonum multiflorum plant hairy root culture; Plant/Artemisia annua plant hairy root culture

Animal Use: Yes

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

Risk Assessment/Discussion:

Dr. Burklow has submitted a renewal of her IBC protocol entitled A Plant Genomics Approach to Drug Discovery. Dr. Burklow's laboratory aims to improve production of plant-derived compounds with potential value as drugs or nutrients. Three primary approaches are utilized. In the first, Dr. Burklow's laboratory aims to identify native plants that contain metabolites with previous unknown bioactivity with potential value by high-throughput pharmacological screening of a plant extract library. In the second approach, bioactivity is optimized using Natural Products Genomics (NPG) technology. This involves activation tagging mutagenesis mediated by Rhizobium rhizogenes. This is completed primarily in hair root culture lines but also in explants. In the third approach, target proteins are expressed in plant cell cultures. Extracts are used for in vitro or cell-based screens using human SH-SY5Y neuroblastoma cells. Work is completed under BSL2 containment and includes use of a certified BSC and lab coat, eye protection, and disposable gloves. Dr. Burklow's current IBC protocol will expire on April 4, 2026.

IBC Discussion & Vote:

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The protocol renewal IBC-26-37 (version 8.0) was approved pending minor modifications as listed below:

*

SCIENTIFIC SUMMARY – Please make a statement to clarify that any new gene targets that are identified will be added as an amendment.

*

Arthur Hunt initiated the motion. Jan Smalle seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

*

PI: Mariana Nikolova-Karakashian

IBC Protocol Number: IBC-26-40

Protocol Title: B23-4155: Liver acute phase response and aging

Protocol Type: Renewal

Amendment To: N/A

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

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

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

Brief Project Overview:

The process of aging is associated with increased responsiveness of the cells in the liver to cytokines. The consequences of these alterations involve more severe reaction to small inflammatory challenges to the organism which in some occasions leads to increased mortality among elderly. The goal of our work is to decipher the cellular mechanism for this hyperresponsiveness. To achieve this, we use liver cells and alter the expression of specific genes that we hypothesize might contribute to the hyperresponsiveness.

According to our National Institute of Health (NIH)-funded hypothesis, genes involved in sphingolipid metabolism, specifically sphingomyelinases are involved in the aging hyperresponsiveness. Thus we either overexpress the specific proteins, neutral sphingomyelinase1 (NSMase1), Neutral sphingomyelinase-2 (NSMase2) and Acid sphingomyelinase (ASMase), (all rat sequences) or depress their expression using interference deoxyribonucleic acid (DNA). We are also going to overexpress genes that catalyze the opposite enzyme reaction, namely the conversion of ceramide to sphingomyelin. These are sphingomyelin synthases and there are three forms, sms1, sms2 and sms3. At the end, the response to infection stimuli will be tested in vitro and in vivo.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Smpd-1 (Acid Sphingomyelinase)/Mus musculus/Enzymatic Protein Metabolic Protein/Expression/Cell Culture Rats Mice/Adenoviral/; Smpd-2 (Neutral Sphingomyelinase)/Mus musculus/Enzymatic Protein /Expression/Cell Culture Rats Mice/Adenoviral/; Smpd-3 (Neutral Sphingomyelinase 2)/Mus musculus/Enzymatic Protein Metabolic Protein Membrane Protein/Expression/Cell Culture Rats Mice/Adenoviral/; Foxo-1/Homo Sapiens/Regulatory Gene/Expression/Cell Culture/Plasmid/; GFP/Aequorea victoria/Tracking Gene/Tracking efficiency of transfection/infection/Cell Culture Rats Mice/Adenoviral, Plasmid/; shRNA (small hairpin RNA against neutral sphingomyelinase-2)/Mus Musculus/Rattus Norvegicus/Silencer/Silencing gene expression of smpd-3/Cell Culture Rats Mice/Adenoviral, Plasmid/; Sms-1 (Shingomyelin synthase 1)/Homo Sapiens/Enzymatic Protein Metabolic Protein/Expression/Cell Culture /Plasmid/; Sms-2 (Shingomyelin synthase 2)/Homo Sapiens/Enzymatic Protein Metabolic Protein/Expression/Cell Culture /Plasmid/; SmsR (Shingomyelin synthase related)/Homo Sapiens/Enzymatic Protein Metabolic Protein/Expression/Cell Culture /Plasmid

Vector(s) [Vector Category/Vector Technical Name]: Adenovirus/pAdTrack/; Adenovirus/pAdEasy-1/; Plasmid/pBluescript/; Plasmid/pENTR/U6/; Plasmid/pCMVSPORT6/; Plasmid/pT7CFE1-Chis/; Plasmid/pclmammalian expression vector/; Plasmid/pT7CFE1-CHis/; Plasmid/pT7-FLAG-2/; Plasmid/pET-32a (+)/; Plasmid/pET-26b (-)/; Plasmid/pcDNA3.1/V5-His-TOPO/; Plasmid/pcDNA3.1/V5-His-TOPO-lacZ/; Plasmid/pAcGFP-CI/; Plasmid/pmStrawberry

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/293 HEK/; Human/HEK293IL-IR/; Human/HepG2/; Human/HAEC/; Human/Niemman Pick fibroblast/; Animal/H1h4E/; Animal/Hep6c/; Animal/Rat hepatocytes/; Animal/AML-12 cells/; Animal/Mouse hepatocytes

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]: Mice/Adenovirus/Tail vein injection/ABSL2/ Lab Coat, Disposable Gloves, Eye Protection, Disposable Gown, Shoe Covers/Booties, Face Shield, and Surgical Mask/ABSL2/No; Rat/Adenovirus/Tail vein injection/ABSL2/ Lab Coat, Disposable Gloves, Eye Protection, Disposable Gown, Shoe Covers/Booties, Face Shield, and Surgical Mask/ABSL2/No

Risk Assessment/Discussion:

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Dr. Nikolova-Karakashian has submitted a renewal of her IBC protocol entitled Liver acute phase response and aging. This laboratory seeks to understand the cellular mechanism for hyperresponsiveness of liver cells to cytokines related to aging. Liver cells are manipulated to alter the expression of target genes thought to contribute to this effect. Dr. Nikolova-Karakashian's laboratory uses an adenovirus vector to overexpress NSMase1, NSMase2 and ASMase and to silence NSMase2 by shRNA in isolated hepatocytes as well as in mice and rats. Adenovirus vectors are obtained from ViraQuest and not produced in Dr. Nikolova-Karakashian's laboratory. Hepatocytes isolated from rats or mice are transduced with adenovirus vectors and subjected to Western blot, PCR, and other downstream assays. Adenovirus vectors delivering shRNAs will be used to silence expression of rat NSMase-2 in hepatocytes. All work with adenovirus will be done under BSL2+ containment within a designated BSC and includes dedicated lab coat, disposable gloves, and eye protection. In addition to in vitro work, adenovirus vectors will be administered to rats and mice to overexpress or silence NSMase-2. Animals are restrained in tail vein restraint device within a certified BSC and administered adenovirus using ABSL2 procedures. Upon euthanasia, liver and serum will be obtained for same in vitro assays. Replication Competent Virus (RCV) testing of adenoviral stocks is completed by ViraQuest. The lab also utilizes mammalian expression vectors for overexpression of Foxo-1, sms1, sms2, and sms3 in HepG2 and HEK293 cells. This work is completed using BSL2 containment within a certified BSC using lab coat, eye protection, and disposable gloves. Dr. Nikolova-Karakashian's current IBC protocol will expire on April 17, 2026.

IBC Discussion & Vote:

The protocol renewal IBC-26-40 (version 8.0) was approved pending modification with IBC Primary Reviewers final review:

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ANIMAL RESEARCH – IACUC 2019-3411 expired and has not yet been replaced with an IACUC that involves biohazardous materials. Please remove 2019-3411 and add “pending”.

ANIMAL RESEARCH – Animals with Biohazards: Please complete the Animals with Biohazards table to reflect work described in this IBC protocol.

INFECTIOUS AGENTS - Use of needles in conjunction with human sourced materials, human pathogens, or intentionally infected animals: Please review the PPE selected here to ensure it is congruent with the PPE descriptions in the Scientific Summary and Research Locations sections of the protocol. Is all of the PPE listed here actually in use?

SCIENTIFIC SUMMARY:

1. Please edit the summary for consistent description of PPE.
2. Please include a brief description of emergency response procedures in case of radiation spill or exposure.
3. Please include a description of isolation of hepatocytes. What is “standard procedure”?
4. Remove the phrase "BSL2+ compatible fashion" and clarify requirements for BSL2+ containment.

*

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Delphine Malherbe initiated the motion. Brandy Nelson seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

*

PI: Peter Nagy

IBC Protocol Number: IBC-26-42

Protocol Title: Key role of gel condensate in the formation of viral replication organelle

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-5, NIH Guidelines Section III-E, NIH Guidelines Section III-E-2, NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-3

Maximum Containment Level: Biological Safety Level 1 (BSL1)

Primary Reviewers: J. Smalle, A. Hunt, C. Pickett

Brief Project Overview:

Numerous plant RNA viruses cause major losses in food production. Plant RNA viruses cause changes in host protein expression levels, host membrane structures and many cellular processes. The central part of pathogenesis of RNA viruses is genome replication that leads to the production of abundant viral progenies that can start new infections. The PI has developed highly tractable Tomato bushy stunt tobravirus (TBSV) and yeast as a model host to study the roles of host factors in viral replication. The TBSV RNA used in yeast is a noninfectious RNA replicon, which cannot survive in nature, thus minimizing the environmental risks. TBSV cherry strain infects cherry plant- no symptoms, and noninfectious in tomato. CIRV infects orchids and causes ringspot on leaves. TBSV and CIRV do not have known vectors in nature and transmitted via mechanical damage of plants. DI-72 RNA does not code for any proteins and it is not possible to reconstitute into infectious genome via recombination or rearrangement.

Summary of Biohazard Materials & Manipulations:

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

Transport: Yes

Materials Transported: Plants

Infectious Agent(s)/Natural Host(s): Carnation Italian Ringspot Virus (CIRV) (RG1-virus)/Broad, first detected in commercial carnations, will infect many species of fruit trees and vines; Tomato Bushy Stunt Virus (TBSV) (RG1-virus)/crop vegetables and ornamental plants

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Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Rpn11/Nicotiana benthamiana and Arabidopsis/enzymatic protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; ATG8/Nicotiana benthamiana and Arabidopsis/housekeeping protein/expression/Nicotiana benthamiana/pGD/; Cdc48/Nicotiana benthamiana and Arabidopsis/housekeeping protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; Xpo1/Nicotiana benthamiana and Arabidopsis/housekeeping protein/expression/Nicotiana benthamiana/pGD/; PK1/Nicotiana benthamiana and Arabidopsis/metabolic protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; Pfk1/Nicotiana benthamiana and Arabidopsis/metabolic protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; Adh1/Nicotiana benthamiana and Arabidopsis/metabolic protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; Pdc1/Nicotiana benthamiana and Arabidopsis/metabolic protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; p33/Tomato bushy stunt virus/membrane protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; p92/Tomato bushy stunt virus/membrane protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; GFP/Aequorea Victoria/tracking protein/expression/Nicotiana benthamiana and S. cerevisiae/pGD/; Deg1/Nicotiana benthamiana and Arabidopsis and S. cerevisiae/house keeping protein/expression/Nicotiana benthamiana and S. cerevisiae /pGD/; Tomato bushy stunt virus genome expression via agrobacterium /Tomato bushy stunt virus/plant virus/expression/Nicotiana benthamiana/pGD/; Carnation Italian ringspot virus genome expression via agrobacterium/Carnation Italian ringspot virus/plant virus/expression/Nicotiana benthamiana/pGD

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pGD/; Plasmid/pMAL/; Plasmid/pGAD/; Plasmid/pESC/; Plasmid/pHisGBK/; Plasmid/pGD-RFP

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. Nagy has submitted a renewal of his IBC protocol entitled Key role of gel condensate in the formation of viral replication organelle. Dr. Nagy's laboratory seeks to understand the roles of different host factors in RNA virus replication. Dr. Nagy has developed a Tomato bushy stunt tombusvirus (TBSV) and yeast model to study these host factors. Most of the work described in Dr. Nagy's IBC protocol uses the noninfectious viral RNA replicon model in yeast. This work is completed using BSL1 containment. Agrobacterium infiltration will be used to induce transient expression in N. benthamiana. No biohazardous materials infectious to humans are utilized in this IBC protocol. Infected and agrobacterium infiltrated plants are grown exclusively in the laboratory or plant growth room. PPE for the work described includes lab coat, disposable gloves, and eye protection. Dr. Nagy's current IBC protocol will expire on April 18, 2026.

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IBC Discussion & Vote:

The protocol renewal IBC-26-42 (version 8.0) was approved.

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Jan Smalle initiated the motion. Arthur Hunt seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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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).

Guo, Houfu Structure and function studies of lysyl hydroxylases Amendment BSO 04/01/26 IBC-24-70 (v.41.0)

Kuhs, Krystle Biomarkers of cancer risk and recurrence Amendment BSO 04/01/26 IBC-24-105 (v.25.0)

Despa, Florin Cardiovascular consequences of diabetes; electrical remodeling in heart disease Amendment BSO 03/31/26 IBC-25-72 (v.30.0)

Guo, Zhenheng Roles of Bmal1, iPLA2, MR, and GLP-1R in cardiovascular diseases Amendment BSO 03/31/26 IBC-24-358 (v.29.0)

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 of Diabetes and Methotrexate Anti-inflammatory Therapy (IRB 17-0439-F6A/E-IRB 44558) Renewal BSO 03/30/26 IBC-26-13 (v.10.0)

Sachdeva, Rahul Translational spinal cord stimulation studies New BSO 03/27/26 IBC-26-26 (10.0)

Liu, Chunming Wnt signaling in colon cancer Amendment BSO 03/26/26 IBC-25-10 (v.22.0)

Smalle, Jan Plant growth regulation and secondary metabolites Amendment BSO 03/26/26 IBC-25-167 (v.17.0)

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Campbell, Kenneth Cellular level contractile function in human heart failure Amendment BSO 03/25/26 IBC-24-482 (v.60.0)

Kagan, Isabelle Distribution of Slafractonia leguminicola (formerly Rhizoctonia leguminicola) in red clover and search for resistant red clover varieties, and mycotoxin analysis Amendment BSO 03/24/26 IBC-25-17 (v.20.0)

Katz, Douglas KB801-01: A Phase 1/2, Multicenter, Double-Masked, Placebo-Controlled Study of KB801 in Subjects with Stage 2 or 3 Neurotrophic Keratitis Amendment BSO 03/24/26 IBC-25-150 (v.18.0)

Czuba, Lindsay Mechanisms of Regulation of Bile Acid Homeostasis Amendment BSO 03/24/26 IBC-25-102 (v.14.0)

Staton, Michele STOP Study Amendment BSO 03/23/26 IBC-25-155 (v.12.0)

Frolenkov, Gregory Mechanoelectrical and Electromechanical Transduction in Auditory Hair Cells Amendment BSO 03/20/26 IBC-24-496 (v.24.0)

Brainson, Christine Defining epigenetic vulnerabilities of lung cancer and lung disease Amendment BSO 03/20/26 IBC-25-85 (v.32.0)

Kent-Dennis, Coral Exploring health-protective effect of food-derived bioactive compounds Renewal BSO 03/20/26 IBC-26-21 (v.6.0)

Jaramillo, Anel Neurobiology of anxiety and alcohol-use disorder Amendment BSO 03/18/26 IBC-25-185 (v.18.0)

Elsayed, Hossam Multiomics Analysis of Human Myometrial Cells in Response to LPS Stimulation New BSO 03/16/26 IBC-25-117 (v.10.0)

Bocklage, Therese Markey Cancer Center General Banking Protocol Renewal BSO 03/16/26 IBC-25-174 (v.12.0)

Chakravarti, Ritu Immune Mechanisms of Autoimmune Diseases Amendment BSO 03/12/26 IBC-24-522 (v.45)

Fry, Christopher Cellular regulators of skeletal muscle plasticity Amendment BSO 03/12/26 IBC-25-74 (v.28.0)

Stumpf, Elizabeth Stumpf Lab Protocol Amendment BSO 03/12/26 IBC-25-92 (v.22.0)

Zarate, Yuri 307-201: A Phase 1/2 Open-Label, Dose-Escalation Study to Determine the Safety and Efficacy of BMN 307, an Adeno-Associated Virus Vector-Mediated Gene Transfer of Human Phenylalanine Hydroxylase in Subjects with Phenylketonuria and Plasma Phe Levels >600 umol/L Closure BSO 03/12/26

McClintock, Tim B24-4373: Molecular Biology of Olfaction Amendment BSO 03/10/26 IBC-24-324 (v.13.0)

Weisleder, Noah Modifying gene expression in vitro and in vivo Amendment BSO 03/09/26 IBC-24-499 (v.70.0)

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Gensel, John Lipid Biomarkers after Spinal Cord Injury Amendment BSO 03/05/26 IBC-25-46 (v.30.0)

Emfinger, Christopher Unmasking conditional dependencies between key proteins influencing metabolic health. Amendment BSO 03/05/26 IBC-25-103 (v.19.0)

Shaddox, Luciana Protocol Title: 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. Understanding the association between buprenorphine and oral health outcomes 6. Advancing Salivary Biomarker Development and Utility in Periodontitis 7. Targeting Health Disparities: Engineering Antimicrobial and Remineralizing Dental Resins for Therapeutic and Preventive Care in Underserved Communities Amendment BSO 03/05/26 IBC-24-349 (v.73.0)

Protocols Meeting Registration Requirements

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

Emfinger, Christopher Unmasking conditional dependencies between key proteins influencing metabolic health. Amendment IBC 04/01/26 IBC-25-103 (v.29.0)

Gipson-Reichardt, Cassandra Glutamate, Neuroinflammation, Acetylcholine, HIV and Addiction Renewal IBC 03/26/26 IBC-25-181 (v.21.0)

Feola, David Mechanisms of Alternative Macrophage Activation Renewal IBC 03/24/26 IBC-26-29 (v.12.0)

Black, Esther Predicting response to therapeutic agents in lung cancer Renewal IBC 03/18/26 IBC-26-15 (v.16.0)

Kunz, Ashlan B23-4292: Mechanistic Insights to Combating Multidrug-resistant Gram-negative Bacterial Infections Amendment IBC 03/18/26 IBC-24-55 (v.21.0)

Helmy, Yosra Evaluation of the efficacy of different antibiotic alternatives against infectious pathogens Amendment IBC 03/16/26 IBC-25-12 (v.34.0)

Temel, Ryan Knockdown and overexpression of genes in mice and nonhuman primates using antisense oligonucleotides (ASO) Renewal IBC 03/16/26 IBC-26-07 (v.10.0)

Zarate, Yuri 307-201: A Phase 1/2 Open-Label, Dose-Escalation Study to Determine the Safety and Efficacy of BMN 307, an Adeno-Associated Virus Vector-Mediated Gene Transfer of Human Phenylalanine Hydroxylase in Subjects with Phenylketonuria and Plasma Phe Levels >600 umol/L Renewal IBC 03/12/26

Tsodikov, Oleg Biochemical and structural studies of enzymes and DNA binding proteins Renewal IBC 03/11/26 IBC-26-02 (v.8.0)

Lane, Andrew Environmental Systems Biochemistry Renewal IBC 03/11/26 IBC-26-22 (v.12.0)

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IBC Training

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

Adjournment

Douglas Harrison moved to adjourn the meeting at 2:03PM. Thomas Chambers seconded the motion. All members present (13) voted in favor.

APPROVED