

# University of Kentucky

## Institutional Biosafety Committee (IBC) Meeting

Date: 04MAR026  
Time: 12:02PM – 1:26PM  
Location: Virtual Meeting via Zoom - <https://uky.zoom.us/j/82304184575?jst=1>

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## Minutes

### Call to Order

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

### Attendance

#### IBC Members Present

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

#### Regrets

Brandy Nelson (Institutional Member)

#### Guests

Elizabeth Brooks (Administrative Support Associate I)	Audra Strahl (IBC Administrative Professional II)
Robert Hayman (Assistant Biological Safety Officer)	Melissa Hollifield (Animal Compliance Manager)
Jeff Howell (IBC Administrative Professional II)	Kathryn Childress (Temporary STEPS Office and Clerical)
	Marisa McGrath (Guest)

#### 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

[2026.02.04 IBC Meeting Minutes DRAFT.pdf](#)

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

### Old Business

None

### Protocol Review

#### *Amendments*

#### **PI: Ashlan Kunz**

IBC Protocol Number: IBC-24-55

Protocol Title: B23-4292: Mechanistic Insights to Combating Multidrug-resistant Gram-negative Bacterial Infections

Protocol Type: Amendment

Amendment To: Laboratory Location(s), Organisms used in research, Personnel, Manipulations planned

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

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

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

#### *Brief Project Overview:*

Our research focuses on finding better ways to treat infections caused by difficult-to-treat bacterial strains with varying genotypes and phenotypes. These infections can be very serious and are becoming harder to treat because the bacteria are becoming resistant to many antibiotics. We want to improve the use of standard-of-care and novel antibiotics, which can still work against these bacteria in some cases. Additionally, this work will identify why certain bacterial strains acquire treatment-emergent resistance and how that can be prevented. To do this, we will test different strains of bacteria in the lab and test various antibiotic treatments. We will also look at the bacteria's genes to see how they react to the treatments. Our ultimate goal is to find the most effective antibiotic treatment that kills the bacteria effectively without letting them become resistant. This research is essential because it can help clinicians choose the right antibiotics to treat these infections and improve patient quality of life. It can also provide insights for dealing with other antibiotic-resistant bacteria. In addition to laboratory testing, some antibiotic regimens will be evaluated in a neutropenic mouse lung infection model using *Stenotrophomonas maltophilia* under ABSL-2 containment to better understand how treatments perform in a living system and how resistance can emerge during therapy.

#### *Summary of Biohazard Materials & Manipulations:*

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

Transport: Yes

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Materials Transported: Biohazardous Materials, Animals

Infectious Agent(s)/Natural Host(s): *Acinetobacter baumannii* (RG2-bacteria)/The environment; Humans/; *Acinetobacter* spp. (RG2-bacteria)/The environment; Humans/; *Burkholderia cepacia* (RG1-bacteria)/The Environment; Humans/; *Citrobacter freundii* (RG1-bacteria)/The Environment; Humans/; *Enterobacter cloacae* (RG2-bacteria)/The Environment; Humans/; *Escherichia coli* (RG2-bacteria)/The Environment; Humans/; *Klebsiella pneumoniae* (RG2-bacteria)/The Environment; Humans/; *Klebsiella* spp. (RG2-bacteria)/The Environment; Humans/; *Klebsiella oxytoca* (RG2-bacteria)/The Environment; Humans/; *Proteus mirabilis* (RG2-bacteria)/The Environment; Humans/; *Providencia rettgeri* (RG2-bacteria)/The Environment; Humans/; *Pseudomonas aeruginosa* (RG2-bacteria)/The Environment; Humans/; *Serratia marcescens* (RG2-bacteria)/The Environment; Humans/; *Stenotrophomonas maltophilia* (RG2-bacteria)/The environment, 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: 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/*Stenotrophomonas maltophilia* (RG2-bacteria)/Intranasal/Anesthesia/ABSL2/Personnel will wear appropriate ABSL-2 personal protective equipment, including disposable lab coat or gown, disposable gloves, and eye protection. Additional PPE such as surgical mask, double gloves, and protective sleeves may be used during inoculation, cage changes, or other high-contact procedures involving infected animals or contaminated materials./ABSL2/Yes/All work with infected animals will be conducted under ABSL-2 containment in accordance with institutional biosafety policies. Animal cages will be handled as biohazardous, and cage changes will be performed within a certified biological safety cabinet. Soiled bedding, cages, and disposable materials will be treated as biohazardous waste and autoclaved prior to disposal. Work surfaces and equipment will be disinfected with institutionally approved disinfectants after use. Transport of infected animals will occur using secure, covered containers within designated ABSL-2 areas. Personnel will follow standard ABSL-2 practices to minimize exposure risk.

### *Risk Assessment/Discussion:*

Dr. Kunz has submitted an amendment to her current IBC protocol, IBC-24-55, entitled *Mechanistic Insights to Combating Multidrug-resistant Gram-negative Bacterial Infections*. In this amendment, Dr. Kunz is adding a project to infect mice intranasally with *Stenotrophomonas maltophilia*, a RG2 bacteria that is considered a "newly emerging pathogen of concern". *S. maltophilia* is a gram-negative multi-drug resistant organism (MDRO) that is an opportunistic pathogen of immunocompromised individuals. Neutropenia will be induced, and anesthetized mice will be administered *S. maltophilia* intranasally within a BSC located in DLAR. Infected animals will be housed in microisolator cages within ABSL-2 containment. Antimicrobials will be administered to mice intraperitoneally for up to 72 hours. Mice will be euthanized and tissue samples collected for microbiologic, immunologic, and pharmacokinetic

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analyses. All manipulations of infected animals will take place within a BSC using disposable lab coat or gown, disposable gloves, and eye protection. All downstream assays and manipulations will take place using BSL-2 containment and practices, including use of a BSC and appropriate PPE as described previously. There is an IBC hold on the corresponding IACUC protocol, 2026-4742.

### *IBC Discussion & Vote:*

The amendment to IBC-24-55 (version 17.0) was approved pending minor modifications as listed below:

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**ANIMAL RESEARCH:** The IACUC originally listed intratracheal as the route of administration instead of intranasal. Is there a way to ensure the training of staff that will be doing these infections? It is very easy to misjudge isoflurane anesthesia with intranasal infections and have a scenario where the mice are only lightly anesthetized such that they flick their snout or wipe their snout with their paws and disperse the inoculum.

From the IACUC: On Day 0, neutropenic mice will be infected intranasally under isoflurane anesthesia with *S. maltophilia*. Mice will be anesthetized as described above and placed in a supine position. Bacterial inocula will be prepared immediately prior to use and administered dropwise to the external nares using a calibrated pipette, allowing the mouse to inhale the inoculum naturally. The inoculum will be administered slowly (in small drops with brief pauses) to minimize aspiration and ensure inhalation. The total inoculation volume will not exceed 50  $\mu\text{L}$  per mouse (typically administered as approximately 25  $\mu\text{L}$  per nostril), targeting approximately  $1 \times 10^7$  CFU per mouse (range  $10^5$ – $10^7$  CFU as applicable to pilot optimization).

Anesthesia described in the IACUC (Nose cone for intranasal?): Animals will be placed individually into an induction chamber and anesthetized with up to 4% isoflurane in oxygen until loss of righting reflex is achieved. Following induction, anesthesia will be maintained at approximately 1–3% isoflurane via a nose cone as needed to maintain an appropriate depth of anesthesia.

**SCIENTIFIC SUMMARY:** Please indicate that the anesthesia (according to the IACUC) is isoflurane anesthesia.

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Thomas Chambers initiated the motion. Carrie Shaffer 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: William de Souza**

IBC Protocol Number: IBC-25-05

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

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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-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

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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:*

Dr. de Souza has submitted an amendment to his current IBC protocol, IBC-25-05, entitled *Transmission dynamics and virus-host interactions of arboviruses*. In this amendment, Dr. De Souza updates personnel and adds an animal infection project. 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. 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. 30 and 60 days after infection, mice will undergo long-term cognitive and motor assessments (e.g. Barnes Maze, Morris Water Maze) in the UK Animal Behavior Core. Previous data confirms viral 100% viral clearance in mice at 7 days post infection, and we will confirm the non-infectious state of all mice via plaque assay prior to behavioral studies in the Animal Behavior Core facility. The addition of animal work is new for Dr. De Souza's IBC protocol and introduces additional risk of exposure, however these risks are mitigated by ensuring mice are anesthetized during inoculation to avoid accidental needlesticks, working with the BSC, and wearing appropriate PPE including lab coat, disposable gloves, eye protection, and shoe covers. There is an IBC hold on the corresponding IACUC protocol, 2025-4704.

### *IBC Discussion & Vote:*

The amendment to IBC-25-05 (version 23.0) was returned for the modifications listed below:

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### ANIMAL RESEARCH – Animals with Biohazards Table:

1. The Scientific Summary also describes intravenous adoptive cell transfers or depleting antibodies, but details are unclear. If these are biohazardous materials, such as human cells or genetically modified animal or human cells, an additional entry is needed to reflect these materials.
2. Please review the PPE requirements for these animal procedures in DLAR facilities for accuracy as disposable gown, arm covers, and double gloving may also be required in the PPE list.
3. In the Special Practices/Procedures column of the table, it is indicated that 10% bleach will be used to decontaminate work surfaces. Is this accurate? Will you bring 10% bleach to DLAR or utilize the DLAR provided disinfectant (typically MB-10)?

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INFECTIOUS AGENTS – Laboratory Procedures: The PPE selections include both Lab Coat and Disposable Gown. Is this accurate? Will any additional PPE be required for the proposed animal work in DLAR, such as Shoe Covers that were referenced in the Animals with Biohazards table?

LOCATIONS – Research Locations Table: Please include DLAR facility location(s).

### SCIENTIFIC SUMMARY -

1. Please review the disinfectants utilized in DLAR and update, as needed. Will you be transporting freshly prepared 10% bleach to DLAR to decontaminate work surfaces? Will you utilize DLAR provided disinfectant (often MB-10)?
2. Please specify that the exterior of microisolator cages will be thoroughly wiped with an appropriate disinfectant prior to being removed from the BSC.
3. Please clarify the location of necropsy. Will necropsies be completed within a BSC in DLAR? Within a BSC in your laboratory? Presumably, you will be transporting infectious materials to/from DLAR and your laboratory. Please include a description of how these materials are transported safely between locations.
4. There is significant concern regarding the proposal to remove Mayaro virus–infected mice from ABSL-2 containment for behavioral testing in a shared core facility. As written, the protocol does not sufficiently address the biosafety risks associated with transporting infected animals outside of containment. Mayaro virus replication dynamics, viremia, and the potential for viral shedding can vary considerably based on mouse strain, inoculation route, and dose. The use of plaque assays on serum samples alone is not sufficiently sensitive to confirm that animals are non-infectious at the time of transfer. A more sensitive detection method—such as quantitative PCR validated for the specific mouse strain and inoculation parameters—may be required to ensure that infected animals do not pose a risk of environmental contamination or exposure. Please provide a detailed plan describing how you will confirm the absence of viral shedding and ensure that Mayaro virus–infected mice pose minimal risk to personnel and other animals when transferred to the Animal Behavior Core. This plan should address:
  - a. The specific assays to be used, including sensitivity, limits of detection, and justification for suitability
  - b. The time points and sample types to be collected and tested
  - c. The criteria for determining when animals can be safely removed from ABSL-2 containment
  - d. Any transport procedures, engineering controls, or decontamination steps to prevent exposures during movement between facilities
  - e. A clear assessment of risk and how it will be mitigated
5. If adequate risk mitigation cannot be established to remove Mayaro-infected animals from ABSL2 containment, please consider conducting behavioral testing within a Biological Safety Cabinet (BSC) or other appropriate primary containment device.

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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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### *New Protocols*

#### **PI: Anthony Sinai**

IBC Protocol Number: IBC-26-17

Protocol Title: Molecular and Cell Biology of *Toxoplasma gondii*

Protocol Type: New Protocol

Amendment To: N/A

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

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

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

#### *Brief Project Overview:*

Toxoplasmosis is a disease that infects both humans and animals. The infection is associated with cats and the consumption of undercooked meat most notably pork and lamb in the US. Despite over a third of the population being infected, disease is only observed in patients with weakened immune systems such as those with HIV-AIDS and patients undergoing cancer chemotherapy. *Toxoplasma* as an organism is an excellent model for the study of Apicomplexan parasites (which include the malaria parasite) and pathogen-host interaction. Among the early interests of the laboratory has been the dissection of the parasite and host factors involved in the establishment of the infection and progression of disease. A major current focus of the laboratory is on the poorly understood life-long chronic stage of the infection which is the primary cause of symptomatic disease in the context of a weakened immune system. Our group has been responsible for revealing a previously unrecognized level of metabolic activity that has direct implications in the development of new treatments that target this challenging infection in patients.

#### *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, Proteomics, Transfection, Use of Human Source Material(s), Use of infectious agents

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Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): *Toxoplasma gondii* (RG2-protozoa)/Humans, all warm blooded animals including marine mammals; *Toxoplasma gondii* (RG2-protozoa)/Humans, all warm blooded animals including marine mammals; *Toxoplasma gondii* (RG2-protozoa)/Humans, all warm blooded animals including marine mammals; *Toxoplasma gondii* (RG2-protozoa)/Humans, all warm blooded animals including marine mammals; *Toxoplasma gondii* (RG2-protozoa)/Humans, all warm blooded animals including marine mammals; *Toxoplasma gondii* (RG2-protozoa)/Humans, all warm blooded animals including marine mammals; *Toxoplasma gondii* (RG2-protozoa)/Humans, all warm blooded animals including marine mammals - all warm blooded animals. Note: most of the KO parasite lines have reduced virulence or are avirulent in vivo (mouse model); *Toxoplasma gondii* (RG2- protozoa)/Humans, all warm blooded animals including marine mammals - all warm blooded animals. Note: most of the KO parasite lines have reduced virulence or are avirulent in vivo (mouse model); Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: *Toxoplasma* (Tg) Laforin/*Toxoplasma* cDNA/Glucan phosphatase/Protein expression /*E. coli*/pET family; TgLaforin –HA/*Toxoplasma* genomic DNA/Glucan phosphatase/gene tagging in *Toxoplasma*/*E. coli* to derive and *Toxoplasma*/pLIC-HA-DHFR; TgOTUD3A/*Toxoplasma* cDNA/deubiquitinase/protein expression/*E. coli*/PET family; TgOTUD3A-HA Epitope tagging/*Toxoplasma* cDNA /deubiquitinase/gene tagging in *Toxoplasma*/*E. coli* to derive and *Toxoplasma*/PLIC-HA DHFR; TgOTUD1B/*Toxoplasma* cDNA/deubiquitinase/protein expression/*E. coli*/pET family; TgOTUD1C/*Toxoplasma* cDNA/deubiquitinase /protein expression /*E. coli*/pET family; TgOTUD5A/*Toxoplasma* cDNA/deubiquitinase/protein expression/*E. coli*/pET family; pCAS9GFP- Laforin/synthetic DNA/glucan phosphatase/gene KO/*E. coli* and *Toxoplasma*/PCAS9-GFP; pCas9GFP- OTUD3A/synthetic DNA/deubiquitinase/gene KO/*E. coli* and *Toxoplasma*/PCAS9-GFP; pCas9GFP- OTUB1/synthetic DNA /deubiquitinase/gene KO/*E. coli* and *Toxoplasma*/PCAS9-GFP; pCAS9GFP- OTUD5/synthetic DNA/deubiquitinase/gene KO/*E. coli* and *Toxoplasma*/PCAS9-GFP; pCas9GFP- Myr1/synthetic DNA/translocation channel structural protein/gene KO/*E. coli* and *Toxoplasma*/PCAS9- GFP; pCas9GFP-UDP-gPPP/synthetic DNA/UDP glucose/pyrophosphorylase, Starch biosynthesis/gene KO/*E. coli* and *Toxoplasma*/PCAS9-GFP; Luciferase/Firefly (commercial constructs)/reporter gene/gene expression reporter/*E. coli* *Toxoplasma* and mammalian cells/promega pLUC; TgATG1/TgATG6/TgATG8 (genes in the autophagic cascade). Additional genes in this pathway will be cloned in the future./*Toxoplasma gondii* Type I generated by cloning of cDNA's/gATG1-kinase. TgATG6 and TGATG8 are structural proteins/Expression in *E. coli* and *Toxoplasma*/*E. coli* and *Toxoplasma*/pET and pminiGFP; *Toxoplasma* genes involved in the manipulation of NFkB upon infection. Including- TgIKK, HSP100. and 2 hypothetical genes of unknown function mutated in mutant lines A4-21 and A4-28/All from *Toxoplasma gondii* strain RH and for TgIKK T. *gondii* strain ME49 in addition. /TgIKK-kinase activity with IkappaB kinase activity. HSP100 heat shock protein homologous to *E. coli* clpB. Activities of A421 and A428 are not known as they are annotated as hypothetical ORF's /Expression of wild type and mutant proteins in

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Toxoplasma. expression of protein fragments and intact proteins in E. coli for generation of antigens and activity evaluations. /E coli and Toxoplasma/PminiGFP and PTUBX-DHFR; Genes identified in a proteomic screen for proteins at the Toxoplasma parasitophorus vacuole membrane. including (annotation numbers in the genome data base given): 53.m03103, 583.m00021, 59.m0006, 80.m02161, 50.m02408, 44.m00024, 4.m00024, 44.m02803, 583.m0910, 641.m01538, 50.m03408, 55.m04701, Additional genes from this data set (70 novel genes identified) will be cloned and characterized. Specific focus is on the genes annotated as hypothetical for which no function can be assigned 44.m02803/Toxoplasma cDNA /Hypothetical ORF and enzymatic activities including E2 UB ligase structural proteins and SOD/Expression of epitope tagged proteins in Toxoplasma/E coli and ToxoplasmaPLIC -HADHFR; TgALG7Toxoplasma gondii ALG7. ALG7 is the first enzyme in the N-linked glycosylation pathway/Glycosyltransferase/N- linked glycosylation in Toxoplasma/E coli and Toxoplasma/pMiniGFP; TgUDP-GPP Crispr KO construct/synthetic CRISPR targeting TgUDP-GPP/Carbohydrate activating enzyme/Knock out starch synthesis in Toxoplasma Toxoplasma/pCAS9-GFP; TgSS Crispr KO construct/synthetic CRISPR targeting TgSS / Starch Synthase/Starch synthesis/Knock out starch synthesis in Toxoplasma /Toxoplasma/pCASp-GFP

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pBluescript; Plasmid/pET family; Plasmid/pQE30; Plasmid/pGEM family; Plasmid/pTUB-XFlag; Plasmid/pminiGFP; Plasmid/pHALIC-DHFR (and gene specific derivatives); Plasmid/pLIC-GFP-DHFR; Plasmid/pCas9-GFP (and gene specific derivatives); Plasmid/pCDNA3/3.1 (and its derivatives)

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Human foreskin fibroblasts (HFF); Human/HeLa Human carcinoma; Human/Vero; Human/CHO; Animal/wild type mouse embryonic fibroblasts MEF/ 3T3 cells from various sources; Animal/p65/RelA KO MEF; Animal/IKKalpha KO MEF; Animal/IKK beta KO MEF; Animal/IKKa/b DKO MEF; Animal/CHO-beta gal omega; Animal/MEF-NFkB GFP (multiple lines); Human/HeLa-cytocGFP

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/Toxoplasma gondii (RG2- protozoa)/IP/anesthesia/ABSL2/lab coat+gown, gloves, eye protection, face mask, shoe covers/ABSL2/No/Handing of infected animals including infection and recovery of tissues conducted in BSL2 cabinet

### *Risk Assessment/Discussion:*

Dr. Sinai has submitted a new IBC protocol entitled *Molecular and Cell Biology of Toxoplasma gondii*. Dr. Sinai previously had IBC approval for work with *T. gondii* but closed his previous IBC protocol in February 2025. In this IBC protocol, Dr. Sinai proposes in vitro and in vivo projects utilizing *T. gondii*, a RG2 protozoa. Gloves, lab coat, and eye protection will be worn for work with *T. gondii*, with some procedures requiring additional PPE (ex. Sleeve covers during tissue cyst purification). BSL2 practices will be utilized, including use of a Class II BSC. Centrifugation is required for pelleting of parasites and will utilize sealable "Aerosolve" buckets. Buckets will be loaded/unloaded within the BSC and wiped with disinfectant prior to being removed from the BSC. Manipulations with needles are required and care is

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taken to minimize risk of sharps injury, including use of Luer lock syringes, immediate disposal of syringes, and no re-sheathing of needles. Additional manipulations include imaging of live, infected cells in the HKRB Microscopy Core and flow cytometry in the HKRB FCIM facility, both of which have IBC approval for BSL2 practices and procedures. A French press is also utilized to isolate and purify parasite organelles in a dedicated space. The French pressure cell is loaded within a BSC and transported to the French Press. "Do Not Enter" signage is posted for the duration of the French press procedure. For this work, a lab coat, disposable gloves taped to the lab coat cuff, full-face shield, and N95 respiratory protection is required. Mice will also be infected with *T. gondii*. Anesthetized mice will be injected IP within a BSC. Infected animals are manipulated and housed at ABSL2 containment. At the conclusion of animal work, tissues will be harvested for additional downstream analyses. It is important to note that a mutant DHFR gene is used as a drug selection marker for making stable transgenic *T. gondii*, however this confers resistance to pyrimethamine, which is commonly used to treat *T. gondii* infections in humans. Pyrimethamine-resistant *T. gondii* remains sensitive to sulfonamides, clindamycin, azithromycin, and atovaquone. Tetracycline and arprinocid are alternative therapeutic options, although they have not been tested in vivo. Laboratory personnel are trained to follow incident response procedures and contact UK Worker's Care in the event of potential exposure.

### *IBC Discussion & Vote:*

The protocol IBC-26-17 (version 8.0) was approved pending minor modifications as listed below:

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### SCIENTIFIC SUMMARY:

1. Please briefly discuss the goals and objectives of the proposed experiments described here.
2. Please note that any new proposed gene manipulations will be included in a future IBC amendment.

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Carol Pickett initiated the motion. Carrie Shaffer 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: Oleg Tsodikov**

IBC Protocol Number: IBC-26-02

Protocol Title: Biochemical and structural studies of enzymes and DNA binding proteins

Protocol Type: Renewal

Amendment To: N/A

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

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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)

*Primary Reviewers: M. Mendenhall, A. Hunt, C. Shaffer*

### *Brief Project Overview:*

This research is aimed at discovery of new ways to overcome bacterial infections and cancer, by investigating enzymatic and other protein targets in bacteria and cancer cells that are essential for bacterial survival inside and outside of the human host as well as cancer development, respectively. The research is conducted on several proteins that are all nonhazardous and that are expressed in *Escherichia coli* BL21(DE3) cells and purified from these cells. Upon purification, the *E. coli* cells are destroyed (DNA is sheared and cells are broken) both mechanically (sonication) and by applying 10% bleach. Plasmids that encode these genes are *E. coli* expression vectors (pEt19b, pEt28a, pEt22b from Novagen). The plasmid constructs are engineered by PCR from non-hazardous pure genomic DNA from different bacteria. This genomic DNA is free from bacterial contamination. This genomic DNA has no ability to transform other organisms. The proteins are: CtcP and CtcQ that are halogenase and reductase enzymes, Eis, human transcription factors Fli1 and a highly similar factor Erg. These proteins are all non-hazardous, and will be obtained as recombinant purified proteins and handled in the test tube. The coding DNA for these proteins is also minimal risk. Human immortalized cell cultures will be used to verify toxicity of mithramycin derivatives against breast cancer cell lines.

### *Summary of Biohazard Materials & Manipulations:*

Manipulations Planned: Bacterial culture, Cell culture, DNA/RNA isolation/purification, Genetics, PCR/qRT-PCR, Transformation, 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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: *Homo sapiens* fli1/cDNA clone, human/Friend leukemia integration 1 transcription factor/expression/*E. coli* top10, BL21(DE3) /pEt-19b (bacterial expression); *Mycobacterium tuberculosis* dnaG/genomic DNA from *M. tuberculosis* H37Rv/DNA primase/expression/*E. coli* top10, BL21(DE3)/pEt-19b (bacterial expression); *Kitasatospora aurofaciens* ctcP/genomic DNA from *Kitasatospora aurofaciens*/enzyme/expression/*E. coli* top10, BL21(DE3)/pEt-19b (bacterial expression); *Kitasatospora aurofaciens* ctcQ/genomic DNA from *Kitasatospora aurofaciens*/enzyme/expression *E. coli* top10, BL21(DE3)/pEt-19b (bacterial expression)

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pET-28a; Plasmid/pET-19b; Plasmid/pET-22b

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Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HepG2; Human/HS MCF7; Human/T-47D; Human/MDA MB468

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. Tsodikov has submitted a renewal of his IBC protocol entitled *Biochemical and structural studies of enzymes and DNA binding proteins*. Dr. Tsodikov's lab seeks to identify new ways to overcome bacterial infections and cancer by investigating enzymatic and protein targets in bacteria and cancer cells. Target proteins will be expressed in E. coli BL21 and purified for enzymatic and DNA binding assays, as well as crystallization experiments. They will also utilize human cell cultures to verify toxicity of mithramycin derivatives in breast cancer cell lines. Work will be done using BSL2 containment and includes the use of gloves, lab coats, and eye protection. Human cells are manipulated inside a BSC. Plates are sealed with paraffin or 3M sealing tape prior to centrifugation. Dr. Tsodikov's current IBC protocol expires on April 17, 2026.

### *IBC Discussion & Vote:*

The protocol renewal IBC-26-02 (version 6.0) was approved.

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Arthur Hunt initiated the motion. Mike Mendenhall 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: Esther Black**

IBC Protocol Number: IBC-26-15

Protocol Title: Predicting response to therapeutic agents in lung cancer

Protocol Type: Renewal

Amendment To: N/A

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

Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: Y. Wu, M. Landron, D. Harrison

### *Brief Project Overview:*

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Lung cancer is the leading killer of all cancers and Kentucky has the highest rate of incidence and mortality from this dreaded disease. Although lung cancer is the most preventable cancer by smoking cessation or never smoking, those who do not make this choice will likely become the population that most needs the best available treatment for lung cancer. It is important to acknowledge that great strides have been made in treatment options that extend the lifespan of those afflicted. However, five-year survival rates are very low. We propose that survival rates can be increased if therapeutic options are assessed when a patient is diagnosed with lung cancer. Thus, we believe that by using molecular diagnostics and patient outcomes data facilitates a reasoned choice that gives the patient the best opportunity of increased survival time and lessens the potential toxic effects of treatment.

### *Summary of Biohazard Materials & Manipulations:*

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

Transport: No

Materials Transported: N/A

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)]: SHP2/human/phosphatase/ectopic expression/bacteria and human cells/pET28 and pIRES2

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pET28; Plasmid/pIRES2

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Beas2b; Human/A431; Human/A549; Human/H1650; Human/PC9; Human/H1975; Human/H3255; Human/Hcc827; Human/H322; Human/H2122; Human/H358; Human/H460; Human/UKY29; Human/H661

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. Black has submitted a renewal of her IBC protocol entitled *Predicting response to therapeutic agents in lung cancer*. Dr. Black's laboratory seeks to understand how molecular diagnostics can predict patient benefit from specific therapies. Towards this aim, Dr. Black's laboratory utilizes human cells to express SHP2 (and mutants of SHP2) via plasmid transfection. Transfected cells will be collected and analyzed for RNA and protein expression. Transfected cells may also be sent to the UK FCIM core facility for cell sorting and/or counting. His-tagged SHP-2 will be expressed in BL21 E. coli for pulldown experiments. Work with human cells will be done using BSL2 containment utilizing a BSC. Biohazardous waste

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generated is described as handled and disposed of according to UK Research Safety guidance. Dr. Black's current IBC protocol will expire on April 17, 2026.

### *IBC Discussion & Vote:*

The protocol renewal IBC-26-15 (version 8.0) was approved pending minor modifications as listed below:

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### SCIENTIFIC SUMMARY:

1. Reports of pathogen testing from commercial cell sources should not be relied upon to determine the safety of cell lines. Please note that these cells will be treated as if they are potentially infectious and remove reference to commercial pathogen testing.
2. Clarify whether centrifugation is being done inside a biosafety cabinet. If on the bench, add details about the risk mitigation steps in place. More information regarding centrifuge safety is online (<https://researchsafety.uky.edu/biological-safety/laboratory-equipment/centrifuges>).
3. Please either eliminate the discussion of UV disinfection or specify that 10% bleach/70% ethanol is the primary method of disinfection.
4. Specify whether cells used for flow cytometry will be fixed or unfixed. If unfixed, add information about the safe transport of cells and disinfection of equipment after use.
5. Please remove vague language "...unlikely this will be necessary..." regarding flow cytometry.

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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

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### **PI: Andrew Lane**

IBC Protocol Number: IBC-26-22

Protocol Title: Environmental Systems Biochemistry

Protocol Type: Renewal

Amendment To: N/A

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

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

Primary Reviewers: M. Landron, Y. Wu, A. Pinto

### *Brief Project Overview:*

The Markey Cancer Center has established a Center for Environmental Systems Biochemistry, which has a focus on translational cancer research, but also other common diseases. The research is supported by

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grants from the National Institutes of Health, including the Cancer Center P30 Support grant and NIGMS P20 COBRE as a support facility as well as others. The common thread of the research is metabolism, the activity that all cells do all of the time. The metabolic activity of cells is often perturbed in diseases, especially cancers, but also in diabetes and during infections among other common diseases. Therefore the metabolic output is a direct measure of the health of the cells, and can be used to understand fundamental biochemistry, for discovering biomarkers for developing diagnostics tests, determine the response to treatments including surgery, radiotherapy or drugs, as well as providing specific information about new drug targets for developing novel therapies. The laboratory works with cells in culture and tissues from human patients who have given permission for the research. A particular approach the laboratory uses is to provide cells or human subjects with a non-radioactive tracer, and follow the fate of this tracer as it is metabolized. The metabolism is monitored using state of the art analytical instrumentation, especially nuclear magnetic resonance and mass spectrometry. Because we identify novel drug targets using this approach, we also need to overexpress and purify the proteins or nucleic acids to carry out detailed studies of the properties of the molecules and how they interact with new drugs in the test tube. The standard means for achieving this is either chemical synthesis (nucleic acids) or producing large amounts of protein in a suitable host, usually *Escherichia coli* that has been modified specifically for this purpose. For testing ideas about how the target functions in the cell, we also use a new technology for deleting the protein, so-called shRNA targeting which is achieved by inserting a gene via a viral carrier into the cells, which leads to depletion of the desired protein, which can then be tested for its effects in a very direct manner. Genes are also ablated using CRISPR technology.

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: 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): 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)]: pyruvate carboxylase/human/metabolic enzyme/shRNA mRNA

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knockdown/A549, H1299 cells/Lentiviral plasmid/; O-GlcNAc transferase/human/metabolic enzyme/shRNA mRNA knockdown/A549, H1299 cells/Lentiviral plasmid/; glycogen phosphorylase/human/metabolic enzyme/shRNA mRNA knockdown/A549, H1299 cells/Lentiviral plasmid/; pyruvate carboxylase/human, mouse/metabolic enzyme/expression in cells /E. coli BL21(DE3)/pET vector 22b/; Mlu binding protein I (Mbp1)/yeast/DNA binding domain/expression in E. coli/E. coli/pET vector 22b/; Cre recombinase/P1

Bacteriophage/enzyme/recombination/mice/adenovirus/; FlpO/yeast/enzyme/inversion, "flip"/mice/adenovirus/; mcherry/sea anemone/tracking/expression/mice/adenovirus/; O-GlcNAc transferase/human, mouse/metabolic enzyme/expression in cells/E. coli BL21(DE3)/pET vector 22 Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pET vector 22b /; Plasmid/pMDLg/pRRE, pRSV-Rev, and pMD2.G/; Lentivirus/PLKO.1/; Lentivirus/plentiCRISPRv2/; Lentivirus/pLVhUbC-Cas9-T2A-GFP

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/A549/; Human/H1299/; Human/293T/; Human/MDAMB231/; Human/MCF-7/; Human/HMEC/; Human/NHBE/; Human/ZR-75-1/; Human/PC9/; Human/H2030/; Human/Hcc827/; Human/UOK268/; Human/UOK265/; Human/p493-6/; Human/PLC-1/; Human/BEAS-2B/; Human/BAST/; Human/LCC9/; Human/Jurkat/; Human/IMR90/; Human/UOK269/; Human/human monocytes

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. Lane has submitted a renewal of his IBC protocol entitled *Environmental Systems Biochemistry*. The Center for Environmental Systems Biochemistry was established by the Markey Cancer Center to focus on translational cancer research and other common diseases. The laboratory focuses on the metabolic activity of cells, often disrupted in diseases including cancer, diabetes, infections, etc., and seeks to discover new biomarkers, drug targets, and more. The laboratory receives patient both cancerous and non-cancerous patient tissues as well as patient blood and urine samples for extraction and later on NMR and mass spectrometry. Patient blood samples will be used to separate plasma for mass spectrometry or isolation of monocytes for differentiation into macrophages for additional downstream analysis. All human source materials are handled using BSL2 containment, including use of masks, gloves, lab coats, and eye protection. Human tissues will also be fixed in 4% formalin and embedded in paraffin blocks for staining and imaging. Dr. Lane's laboratory will also express target proteins in lab strain E. coli for purification and characterization. Proteins of interest include Mbp1, pyruvate carboxylase, glycogen phosphorylase and glycogen synthase. They will also utilize lentivirus vectors for shRNA targeting and CRISPR/Cas. Lentivirus will be produced in Dr. Lane's laboratory utilizing a 3<sup>rd</sup> generation packaging system. The lentiviral vectors in use will silence metabolic genes of importance in cancer cell proliferation and survival or express metabolic enzymes that are normally expressed. Work with lentivirus vectors and lentivirus transduced cells will take place using BSL2+ containment and includes the use of a BSC for all manipulations. Dr. Lane's current IBC protocol will expire March 21, 2026.

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### *IBC Discussion & Vote:*

The protocol renewal IBC-26-22 (version 10.0) was approved pending minor modifications as listed below:

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**INFECTIOUS AGENTS – Infectious Agents Table:** Please edit the “Symptoms of Exposure” field to read “Symptoms synonymous with bloodborne pathogens such as HIV, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, or other unknown infectious materials.”

**SCIENTIFIC SUMMARY:** Please note that after disinfecting biological safety cabinet (BSC) surfaces with 10% bleach, the surfaces will be wiped with 70% ethanol to remove residual chlorine and corrosive damage.

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Amelia Pinto 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: David Feola***

IBC Protocol Number: IBC-26-29

Protocol Title: Mechanisms of Alternative Macrophage Activation

Protocol Type: Renewal

Amendment To: N/A

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

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

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

### *Brief Project Overview:*

The work in this laboratory investigates various implications of modulating the immune response to infectious diseases in order to define mechanisms of host defense against pathogens, identify novel therapeutic targets for intervention, and improve outcomes in patients infected with a variety of microorganisms. Our primary focus is on the characteristics of macrophages, and specifically their role in the regulation of inflammation and coordination of tissue remodeling and repair. Alternatively activated macrophages (AAM) have been described as a functionally distinct macrophage subset that differs from classically activated macrophages (CAM). AAM are anti-inflammatory and involved in down regulation of inflammation, debris scavenging, and tissue remodeling. The role of this cell type in the

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response to gram-negative bacterial infection or viral infection has not been studied. It has recently been demonstrated that the azalide antimicrobial agent azithromycin (AZM) will polarize macrophages to an AAM-like phenotype.

In addition, AZM has been shown to improve morbidity in patients with cystic fibrosis (CF), presumably due to its anti-inflammatory properties. CF causes progressive, life-threatening lung disease due in part to repeated bacterial infections that contribute to chronic inflammation, leading to lung damage and functional decline. In the end stages of the disease, 90% of patients become colonized by the bacteria *Pseudomonas aeruginosa* that cannot be cleared. The inflammatory response is excessive and dysregulated in both mouse CF models of *P. aeruginosa* infection and in human disease. Our work will utilize AZM to investigate the role of AAM in both the immune response to *P. aeruginosa* infection and the fibrosis that subsequently develops in the lungs. In addition, we are investigating the mechanisms by which the lungs respond to the pulmonary fungal pathogen *Pneumocystis murina*, in which macrophage phenotype has been shown to be important in the response. Human sputum and blood cells will be utilized in translational studies in order to extend our investigations towards human endpoints.

An additional new direction for this work is to evaluate the delivery of AZM using nanoparticles called liposomes. By incorporating AZM into liposomes (L-AZM), we can target delivery of the drug directly to the immune cells. We are investigating macrophage uptake of AZM in liposomes, how they traffic to the site of inflammation (in this case, the lungs), and how the pharmacokinetics of AZM change when delivered via liposome in the context of infection.

### *Summary of Biohazard Materials & Manipulations:*

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, Flow cytometry/Cell sorting, PCR/qRT-PCR, Propagation of infectious agents, Transfection, Use of Human Source Material(s), Use of infectious agents

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): *Pseudomonas aeruginosa* (RG2-bacteria)/Humans, animals, plants; *Pneumocystis murina* (RG1-fungus)/Mice; 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)]: firefly luciferase/*Photinus pyralis*/luminescent tracking protein/expression in animals/mice/nanoparticle

Vector(s) [Vector Category/Vector Technical Name]: Nanoparticle/lipid nanoparticle

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/J774; Human/U-937; Human/NIH/3T3; Animal/Organs; Human/Primary Human Cells

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/*Pseudomonas aeruginosa* (RG2-bacteria)/intratracheal/Isoflurane anesthesia/ABSL2/labcoat, gloves, oversleeves, safety glasses/ABSL2/Yes/work in a biosafety cabinet; housed in microisolator cages; Mouse/*Pneumocystis murina* (RG1-fungus)/Intratracheally/Isoflurane anesthesia/ABSL2/Lab coat, gloves, oversleeves, safety glasses/ABSL2/Yes/work in a biosafety cabinet; housed in microisolator cages; Mouse/Nanoparticle-r/sNA/IV/anesthesia/ABSL1/gloves, lab coat, eye protection, surgical mask/ABSL1/No/N/A

### *Risk Assessment/Discussion:*

Dr. Feola has submitted a renewal of his IBC protocol entitled *Mechanisms of Alternative Macrophage Activation*. Dr. Feola's laboratory focuses on the characteristics of macrophages and their role in the regulation of inflammation and coordination of tissue remodeling and repair. Towards that goal, Dr. Feola's laboratory utilizes in vitro experiments using human cells, an in vivo mouse model of *P. aeruginosa* infection, and human clinical samples. Mice will be infected with *P. aeruginosa* (a RG2 bacteria) or *Pneumocystis murina* (a RG1 fungus) via intratracheal inoculation within a BSC. Mice are anesthetized for inoculations. Infected mice will be administered L-AZM or L-mRNA (firefly luciferase) via retro-orbital injection (under anesthesia) and later imaged via IVIS imaging in the UK Light Microscopy Core. After imaging, animals will be sacrificed and tissues excised for further imaging and downstream analysis. *P. aeruginosa* is an opportunistic pathogen that can cause local infections, respiratory tract infections, endocarditis, urinary tract infections, and other symptoms. *P. murina* is not known to cause disease in humans but does cause pneumonia in mice. Mouse infections will be conducted using ABSL2 containment and housing. Dr. Feola's laboratory also utilizes human blood and sputum samples. Work with these materials carries risk of exposure to bloodborne pathogens and other unknown human pathogens. Work with these materials will take place within a BSC using BSL2 practices. Dr. Feola's current IBC protocol will expire on April 17, 2026.

### *IBC Discussion & Vote:*

The protocol renewal IBC-26-29 (version 6.0) was approved pending minor modifications as listed below:

\*

### SCIENTIFIC SUMMARY:

1. Please briefly describe disposal of bedding and special handling of cages from *P. aeruginosa* infected mice.
2. Please clarify where organs are harvested prior to imaging. Is this done in a BSC?
3. Please specify the incubation time for formalin fixation prior to flow cytometry.
4. Remove redundant phenotyping verbiage.

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Delphine Malherbe 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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### *Resubmissions*

**PI: Cassandra Gipson-Reichardt**

IBC Protocol Number: IBC-25-181

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

Protocol Type: Renewal

Amendment To: N/A

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

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

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

### *Brief Project Overview:*

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

### *Summary of Biohazard Materials & Manipulations:*

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

Transport: Yes

Materials Transported: Animals

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

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

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

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

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

Animal Use: Yes

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

### *Risk Assessment/Discussion:*

This protocol renewal was reviewed at the February 4, 2026, IBC meeting and was returned to the PI for significant revisions. Dr. Gipson-Reichardt has resubmitted this IBC renewal for IBC review. Dr. Gipson-Reichardt's laboratory utilizes AAV vector constructs and Eco-HIV in rats. AAVs expressing DREADDs, reporters, CRE, or shRNA are obtained from Addgene and administered to anesthetized rats via intracranial injection. Some rats will also be administered antisense or control sequence morpholinos. Rats will then undergo a number of downstream behavioral assessments and ultimately peptide/cellular analysis following sacrifice. AAVs will be handled using BSL1/ABSL1 containment. A second project utilizes EcoHIV, a rodent-specific HIV in which the gp120 coding region has been replaced with gp80

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from ecotropic murine leukemia virus. EcoHIV cannot infect human cells in culture and poses minimal risk to personnel. Chimeric EcoHIV will be produced and utilized using BSL2 containment. This includes work within a BSC. Rats will be administered EcoHIV through intravenous administration through a catheter port to establish infection. Because administration of EcoHIV via catheter port does not require the use of needles, rats are manually restrained. EcoHIV infected rats will undergo a number of downstream assays and/or manipulations, including cART therapy and behavioral testing prior to euthanasia and analysis of spleen and brain tissues. This project will be completed using ABSL2 procedures and containment.

### *IBC Discussion & Vote:*

The protocol renewal IBC-25-181 (version 13.0) was approved pending minor modifications as listed below:

\*

GENERAL INFORMATION – Manipulations Planned: Please review the manipulations listed here and update, as needed. Ensure that all manipulations selected here are described in the Scientific Summary below. Please remove “Viral culture”.

### SCIENTIFIC SUMMARY:

1. Please update the Scientific Summary here to ensure that all manipulations selected in the General Information – Manipulations Planned section are described here.
2. Please expand the description of downstream assays and manipulations. Please clearly identify the animal tissues or sample types that will be used in each downstream assay and describe how they are processed/utilized. For each downstream assay/manipulation, clarify what work will take place within a BSC vs. on the open lab bench. Please clearly identify the potential biohazard risks associated with each assay/manipulation, and detail how these risks are mitigated.

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

\*

Conflicts of Interest: None

\*

### *Amendments*

#### **PI: Jill Turner**

IBC Protocol Number: IBC-25-62

Protocol Title: Pharmacogenetics of Nicotine Dependence

Protocol Type: Amendment

Amendment To: Genetic constructs

Applicable Guidelines & Regulations: NIH Guidelines Section IV-B-7, UK Administrative Regulation 6.3,

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UK Administrative Regulation 6.9, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-1, NIH Guidelines Section III-F-1, OSHA 29 CFR 1910.1030  
Maximum Containment Level: Biological Safety Level 2 (BSL2), Animal Biological Safety Level 1 (ABSL1)

*Primary Reviewers: C. Haughton, D. Harrison, B. Nelson, M. Mendenhall*

### *Brief Project Overview:*

Nicotine is the most highly addictive and highly abused drug in the world. Although there are a number of pharmacotherapies for smoking cessation, quitting success rate remains less than 20%. The proposed research will be directed towards a pharmacogenetic understanding of nicotine dependence; using state of the art genomic and functional techniques. Specifically, genetically modified mice will be used alongside adeno-associated viruses (AAVs) in these experiments to discern the necessity of specific proteins in the acquisition of drug dependence. This project will not only help us better understand why current treatment success rates are so low, but it will identify new pharmacological targets for smoking cessation and perhaps indicate how tailoring these drugs to an individual's genetics can improve quite rates.

### *Summary of Biohazard Materials & Manipulations:*

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

Transport: Yes

Materials Transported: Biohazardous Materials, Animals

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Cre Recombinase/PI bacteriophage/enzymatic protein/knockdown/mouse/Adeno-associated virus/; Red Fluorescent Protein/synthetic construct/reporter/tracking gene/expression/mouse/Adeno-associated virus/; GCamp6f/synthetic construct/calcium reporter/expression/mouse/AAV/; ErbB4 - 1 and 2/synthetic construct/guide for cas9 to the ErbB4 locus/knockdown/mouse/AAV/; scr sgRNA/synthetic construct/control guide for cas9/control/mouse/AAV/; EGFP/synthetic construct/reporter/tracking gene/expression/mouse/AAV

Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV), AAV9.CMV.PI.Cre.rBGAdeno-Associated Virus (AAV), AAV9.CMV.TurboRFP.WPRE.rBGAdeno-Associated Virus (AAV), AAV9.Syn.GCaMP6f.WPRE.SV40Adeno-Associated Virus (AAV), AAV9.CMV.sgRNA.rBGAdeno-Associated Virus (AAV), AAV9.CMV.scr.rBGAdeno-Associated Virus (AAV), AAV9.CMV.EGFPAdeno-Associated Virus (AAV), AAV9.CMV.CreAdeno-Associated Virus (AAV), AAV-CreAdeno-Associated Virus (AAV), AAV-RFPAdeno-Associated Virus (AAV), AAV-sgRNA (scr)Adeno-Associated Virus (AAV), AAV-sgRNA (ErbB4-1)Adeno-Associated Virus (AAV), AAV-sgRNA (ErbB4-2)Adeno-Associated Virus (AAV), AAV5.hSyn.DIO.hM3Dq,

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

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/Viral Vector – Adeno-Associated Virus (AAV)/Intracranial Injection/Anesthesia/Stereotaxic Apparatus/ABSL1/Lab Coat, Booties, Gloves, Face Mask/ABSL1/No//  
*Risk Assessment/Discussion:*

Dr. Turner has submitted an amendment to her current IBC protocol, IBC-25-62, entitled *Pharmacogenetics of Nicotine Dependence*. In this amendment, Dr. Turner has added a new AAV construct, AAV5.hSyn.DIO.hM3Dq, for intracranial injections of mice. Dr. Turner is already approved for the same procedures in mice utilizing numerous AAV constructs. This new AAV construct will be handled using BSL1/ABSL1 containment as previously described and approved. This new AAV construct does not encode any particularly hazardous transgenes and does not significantly alter the biohazardous risks associated with Dr. Turner's current IBC protocol. There is an IBC hold on the corresponding IACUC protocol, 2021-3779.

### *IBC Discussion & Vote:*

The amendment to IBC-25-62 (version 18.0) was approved pending minor modifications as listed below:

\*

SCIENTIFIC SUMMARY: Please briefly describe the purpose of the new viral vector construct added in this amendment. Include whether there are any additional hazards to consider given the transgene and details of downstream assays, if applicable.

\*

Mike Mendenhall initiated the motion. Doug Harrison 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: Ryan Temel**

IBC Protocol Number: IBC-26-07

Protocol Title: Knockdown and overexpression of genes in mice and nonhuman primates using antisense oligonucleotides (ASO)

Protocol Type: Renewal

Amendment To: N/A

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

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

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

### Brief Project Overview:

The Temel lab studies metabolic diseases that impact the liver and cardiovascular system. To determine the disease mechanisms and to develop new therapies to prevent or treat the diseases, our lab currently injects mice and previously injected monkeys with antisense oligonucleotide (ASO) drugs that decrease the amount or activity of a specific protein or RNA. ASOs are chemically created in the lab from synthetic nucleic acids and therefore are considered a biohazardous material. The monkey species that we previously used for our research is the cynomolgus monkey, which can carry and transmit human pathogenic microbes such as shigella bacteria, hepatitis A virus, salmonella bacteria, and herpes B virus. While we have no current or future plans to conduct studies in live monkeys, our lab stores and analyzes monkey tissues, fluids, and feces. The Temel lab uses procedures approved by the University of Kentucky Internal Biosafety Committee to reduce the risk of exposure to ASOs and transmission of pathogens carried by monkey tissues.

### Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), DNA/RNA isolation/purification, PCR/qRT-PCR, Immunohistochemistry, Imaging/Microscopy, Histology

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

Infectious Agent(s)/Natural Host(s): Non-Human Primate (NHP) Materials (RG2)/cynomolgus monkey

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: ATP binding cassette transporter G8/mouse/membrane protein, sterol transporter/knockdown/mouse/naked nucleic acid; Non-coding control/mouse/N/A/Negative control/mouse/naked nucleic acid; microRNA-128-1/primate/Regulatory RNA/knockdown/cynomolgus monkey tissue/naked nucleic acid; microRNA-22/primate/Regulator RNA/knockdown/cynomolgus monkey tissue/naked nucleic acid; LIM Domain And Actin Binding 1/mouse/cytoskeleton-associated protein/knockdown/mouse/naked nucleic acid; Angiopoietin-like 3 (Angptl3) mouse Lipase inhibitor/knockdown mouse naked nucleic acid; Sterol O-acyltransferase 2 (Soat2) mouse Lipid synthesis/ knockdown mouse naked nucleic acid

Vector(s) [Vector Category/Vector Technical Name]: Naked nucleic acid/Antisense Oligonucleotides

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

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Naked Nucleic Acid-r/sDNA/IP, SC/Manual restraint/ABSL1/Lab coat, eye protection, disposable gloves/ABSL1/No

### Risk Assessment/Discussion:

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Dr. Temel has submitted a renewal of his IBC protocol entitled *Knockdown and overexpression of genes in mice and nonhuman primates using antisense oligonucleotides (ASO)*. While Dr. Temel's laboratory previously utilized non-human primates (NHPs) in conjunction with ASOs targeting miR-22 and miR-128-1, their work now focuses on ASOs in mice targeting mRNAs of ATP binding cassette transporter 8, LIM domain and actin binding 1, angiotensin-like 3, and sterol O-acyltransferase 2. ASOs will be administered to mice via IP or SQ injection. Samples obtained from mice treated with ASOs will undergo a variety of downstream assays using BSL1 containment. Samples obtained from previous work with NHPs will also be analyzed. Lipid content will be examined and lipids extracted, RNA extracted via Trizol, and various tissues will be formalin fixed. Work with NHP samples will be completed using BSL2 containment and procedures. Work with NHP samples introduced the risk of exposure to possible zoonotic pathogens, including *Shigella*, Hepatitis A virus, *Salmonella*, and Herpes B virus. Herpes B virus is of particular concern because human infection is often fatal, even with antiviral therapy and supportive care. Staff are trained to recognize and report potential exposures and seek treatment immediately. Dr. Temel's current IBC protocol will expire on April 21, 2026.

### *IBC Discussion & Vote:*

The protocol renewal IBC-26-07 (version 8.0) was approved.

\*

Doug Harrison initiated the motion. Cheryl Haughton 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: Vincent Cassone**

IBC Protocol Number: IBC-26-01

Protocol Title: Circadian rhythms in metagenomes and microbiota

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-2, 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 (BSL2)

*Primary Reviewers: J. Smalle, T. Chambers, A. Pinto*

### *Brief Project Overview:*

Our lab has published data on the influence of the gastrointestinal tract on circadian rhythms of behavior (Cassone et al. 2017). Recent bioinformatics data we have collected using the human microbiome assembly have led us to believe that certain gastrointestinal flora may be sensitive to melatonin

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signaling. These include *Klebsiella aerogenes* (formerly called *Enterobacter aerogenes*), which we have established to 1) express increased motility in the presence of pM-nM concentrations of the hormone, 2) express circadian patterns of motility, and 3) entrain circadian patterns to subtle 1°C cycles of temperature (Paulose et al. 2016 a,b; Paulose et al. 2019). We have also shown that melatonin increases genes associated with fimbrial attachment and decreases in genes related to biofilm formation using an RNASeq protocol (Graniczkowska and Cassone, 2021; Graniczowska et al. 2022). Further, we have shown that *K. aerogenes* expresses rhythms in multiple different processes (Graniczkowska et al. 2023). We have also conducted a study to determine melatonin binding sites in *K. aerogenes* (Stasel et al. unpublished).

In the immediate future, we are awaiting data from another RNASeq study of gene expression over circadian time. We will validate the top rhythmic sequences using qPCR. We will then collate the gene and protein sequences that 1) are expressed rhythmically, 2) that entrain to temperature cycles, 3) the respond to melatonin, 4) that bind melatonin. Those sequences that are found on all of these lists will be considered top candidate genes for knocking out and/or knock-down by a lambda-red protocol to determine whether these genes are necessary for circadian rhythms and/or melatonin sensitivity. Once knocked down, we will test each knockout/down strain in several assays, including : 1) bioluminescence rhythms in our Lumicycle system, 2) cell division rhythms in our chemostat system, 3) sensitivity to melatonin in motility, and 4) sensitivity to melatonin in biofilm formation. If these successfully block one or several of these functions, we will complement them to restore function.

We are also interested in seeing if other gastrointestinal bacteria express circadian rhythms and/or respond to melatonin. We will use the same techniques as above on the following organisms: *Salmonella enterica*, *Acetobacter baumani*, *Pseudomonas aeruginosa* and *E. coli*.

### *Summary of Biohazard Materials & Manipulations:*

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

Transport: Yes

Materials Transported: Biohazardous Materials

Infectious Agent(s)/Natural Host(s): *Klebsiella* spp. (RG2-bacteria)/Humans, animals, plants; *Bacillus subtilis* (RG1-bacteria)/Humans, animals; *Escherichia coli* (RG2-bacteria)/Humans, animals; *Pseudomonas aeruginosa* (RG2-bacteria) Humans, animals, plants; *Acinetobacter baumannii* (RG2-bacteria) Humans, animals; *Clostridiales* spp. (RG2-bacteria) Humans, animals *Lactobacillus acidophilus* (RG1-bacteria)/humans, animals; *Salmonella enterica* (RG2-bacteria)/human, animals

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: motor stator protein A/*E. aerogenes*/motility/expression/*E. aerogenes*/Plasmid (non-integrating)/; Deophospho coa kinase/*E. aerogenes*/metabolism/expression/*E. aerogenes*/Plasmid (non-integrating)/; Filamenting temperature-sensitive mutant Z/*E. aerogenes*/cell division/expression/*E. aerogenes*/Plasmid (non-integrating)/; Manganese Transporter type H/*E.*

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aerogenes/membrane protein/expression/E. aerogenes/Plasmid (non-integrating)/;  
Luciferase/firefly/tracking gene/expression/E. aerogenes, E. coli DH5alpha/Plasmid (non-integrating)

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/PRG19/; Plasmid/pZE27mc3/;  
Plasmid/pRG19/; Plasmid/pKM200/; Plasmid/pKD46

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/Organoids from C57Bl mice (already deceased and obtained from culled mice)

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. Cassone has submitted a renewal of his IBC protocol entitled Circadian rhythms in metagenomes and microbiota. Dr. Cassone's laboratory seeks to examine melatonin sensitivity and circadian rhythmicity of numerous RG2 bacterial pathogens, including Klebsiella spp., B. subtilis, E. coli, P. aeruginosa, A. baumannii, Clostridiales spp., and L. acidophilus (a RG1 bacteria). Work with these organisms will be done using BSL2 containment, including utilizing a BSC for aerosol-generating procedures. While previously approved for work with animals in conjunction with these organisms, Dr. Cassone's laboratory is shifting to an ex vivo model using gastrointestinal and bladder tissues from previously euthanized mice. Organoids will be infected with a bioluminescent K. aerogenes and later imaged. They will also generate new reporter strains via plasmid electroporation and tetracycline selection. Lab staff and students will wear nitrile gloves, eye protection, and lab coats. Biohazardous waste is described as being handled and disposed of in accordance with UK Research Safety guidance. Dr. Cassone's current IBC protocol will expire on March 27, 2026.

### *IBC Discussion & Vote:*

The protocol renewal IBC-26-01 (version 6.0) was approved pending minor modifications as listed below:

\*

**GENERAL INFORMATION – Manipulations planned:** The marked manipulations include Human Source Material(s) but no materials are described anywhere in the protocol. Please remove “Use of Human Source Material(s)” if not applicable.

**INFECTIOUS AGENTS – Infectious Agents table:** Please update the table to identify the source(s) for Klebsiella spp.

**LOCATIONS – Research Locations:** In the “Procedures” field for Gluck 446 entry, reference is made to Dr. Carrie Shaffer’s lab in 435 Gluck. Room 435 Gluck is office space, not laboratory space. Please update to list the lab space.

**SCIENTIFIC SUMMARY:** Please include a description of basic bacterial culture and propagation. Clearly identify what work will take place within a BSC vs. what work will take place on the open lab bench.

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Thomas Chambers initiated the motion. Jan Smalle seconded the motion. All IBC members present (12) voted in favor of the motion. Carrie Shaffer abstained from voting.

\*

Conflicts of Interest: Carrie Shaffer is a collaborator referenced on this IBC protocol. Carrie Shaffer abstained from voting on this protocol.

\*

### 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).*

Stumpf, Elizabeth, Stumpf Lab Protocol, Amendment, BSO, 03/04/26, IBC-25-92 (v.16.0)

Grondin, Richard, Long-term effects of wildtype huntingtin lowering in the primate corticostriatal tract and thalamus, Renewal, BSO, 03/04/26, IBC-26-20 (v.12.0)

Chwatko, Malgorzata, Polyhydroxyalkanoates generation using varying microbes, Amendment, BSO, 03/03/26, IBC-25-48 (v.19.0)

Xu, Mei, Center for Clinical and Translational Science: Biomarker Analysis Laboratory, Renewal, BSO, 03/02/26, IBC-26-03 (v.12.0)

Yamasaki, Tritia, Neuroscience Biobank, Amendment, BSO, 02/27/26, IBC-24-476 (v.32.0)

Lovell, Mark, B23-4170: Studies of Alzheimer's Disease Pathology, Renewal, BSO, 02/27/26, IBC-26-11 (v.10.0)

Yamasaki, Tritia, Synuclein seeding activity as a biomarker in Parkinson Disease, Amendment, BSO, 02/27/26, IBC-24-477 (v.37.0)

Helsley, Robert, Macronutrient metabolism in Cardiometabolic Disease, Amendment, BSO, 02/27/26, IBC-25-78 (v.28.0)

Papazoglou, Ioannis, Neuronal response to hypoglycemia, Amendment, BSO 02/26/26, IBC-24-35 (v.51.0)

Testa, Stephen, B23-4202: Development of Novel Catalytic RNA Reactions, Renewal, BSO, 02/26/26, IBC-26-25 (v.8.0)

Liu, Xiaoqi, Enhancing the efficacy of prostate cancer therapy, Amendment, BSO, 02/25/26, IBC-24-355 (v.51.0)

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Pearson, Kevin, Effects of trans-resveratrol on diet induced obesity in nonhuman primates and analysis of insulin signaling in human tissue/primary cells, Amendment, BSO, 02/25/26, IBC-24-425 (v.16.0)

Cai, Weikang, Understanding astrocytes and microglia functions in neurological diseases., Amendment, BSO, 02/24/26, IBC-24-408 (v.82.0)

Iragavarapu, Chait, B24-4332: 23-NHL-34-JJ-PMC: A Phase 1b Multicenter, Open-label, Study of JNJ-90009530, an Autologous Anti-CD20 CAR-T Cell Therapy in Adult Participants with Relapsed or Refractory B-cell Non- Hodgkin Lymphoma, Closure, BSO, 02/23/26

Chattopadhyay, Saurabh, Innate immune responses to virus infection, Amendment, BSO, 02/19/26, IBC-24-108 (v.57.0)

Kampourakis, Thomas, Molecular characterization and modulation of cardiac myofilament regulatory proteins, Amendment, BSO, 02/19/26, IBC-24-406 (v.31.0)

Venditto, Vincent, Venditto Lab Biosafety, Amendment, BSO, 02/18/26, IBC-25-33 (v.34.0)

Velez Ortega, A. Catalina, Molecules and Mechanisms of Endogenous Hearing Protection and Plasticity, Renewal, BSO, 02/18/26, IBC-26-08 (v.8.0)

Bauer, John, KCH Office of Pediatric Research, Renewal, BSO, 02/18/26, IBC-26-09 (v.10.0)

Delisle, Brian, Delayed Rectifier K Channel Biogenesis is Unveiled in Models of Long QT Syndrome/Transcriptional Regulation of KCNH2 Circadian Clock Regulation of Myocardial Ion Channel Expression and Function, Amendment, BSO, 02/18/26, IBC-24-381 (v.24.0)

Chwatko, Malgorzata, Polyhydroxyalkanoates production using microbes, New, BSO, 02/17/26

Kolodziej, Andrew, BICARD: Biopsy Collection and Repository Database, Closure, BSO, 02/16/26

Graf, Gregory Lipid metabolism in obesity and its related diseases, Amendment, BSO, 02/16/26, IBC-24-93 (v.43.0)

Shaddox, Luciana, 1. Analysis of Host-Biofilm Interactions: A Novel Polymicrobial model 2: Evaluation of different risk factors on periodontal disease and caries 3: COVID-19 testing using Saliva and Nasal swabs 4. Analyze and characterize the oral microbiome in the presence COVID-19 in the Saliva and Dental plaque samples 5. 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, 02/16/26, IBC-24-349 (v.67.0)

Suzuki, Yasuhiro, Molecular and cellular mechanisms of host resistance against Toxoplasma gondii, Amendment, BSO, 02/16/26, IBC-24-421 (ver. 23.0)

Johnson, Lance, Effects of genetic and environmental factors on metabolism and cognition in mice, Amendment, BSO, 02/16/26, IBC-24-183 (v.28.0)

Gandhapudi, Siva, Pre-clinical studies of viral and cancer vaccines in mice, Amendment, BSO, 02/11/26, IBC-24-443 (v.43.0)

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Brainson, Christine, Defining epigenetic vulnerabilities of lung cancer and lung disease, Amendment, BSO, 02/09/26, IBC-25-85 (v.26.0)

Crocker, Ellen, Testing the pathogenicity and virulence of the bacterial pathogen *Xylella fastidiosa* on various shade trees, Amendment, BSO, 02/06/26, IBC-24-504 (v.19.0)

Richards, Christopher, B22-4089: HKRB Microscopy Core Facility, Renewal, BSO, 02/06/26, IBC-25-161 (v.10.0)

Lucero, Diego, New Modulators of Lipoprotein Metabolism: From the liver to the vascular wall., Amendment, BSO, 02/06/26, IBC-25-97 (v.32.0)

### Protocols Meeting Registration Requirements

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

Garcia, Erin, Mechanisms of competition and cooperation in *Burkholderia* species, Renewal, IBC, 02/27/26, IBC-26-05 (v.12.0)

Jaramillo, Anel, Neurobiology of anxiety and alcohol-use disorder, Renewal, IBC, 02/25/26, IBC\_25-185 (v. 12.0)

Zhan, Chang-Guo, Development of Cocaine-Metabolizing Enzymes, Renewal, IBC, 02/25/26, IBC-26-06 (v.10.0)

Bradley, Carl, B22-4128: Research on endemic plant pathogens of agronomic field crops in Kentucky, Renewal, IBC, 02/13/26, IBC-25-159 (v.13.0)

Plattner, Rina, Role of Abl Family Kinases in Solid Tumors, Amendment, IBC, 02/13/26, IBC-24-474 (v.39.0)

Pinto, Amelia, Risk group two virus protocol, Amendment, IBC, 02/13/26, IBC-24-83 (v.64.0)

Stewart, Andrew, Gene Therapy Approaches to Induce and Control Neuronal Growth in Rodents With Spinal Cord Injuries, Renewal, IBC, 02/13/26, IBC-26-04 (v.10.0)

Tackenberg, Michael C., Genetic, molecular, and environmental determinants of circadian period length and output phase., Amendment, IBC, 02/13/26, IBC-25-130 (v.26.0)

Flythe, Michael, B22-4062: The ecology and physiology of anaerobic bacteria in grazing animals, Renewal, IBC, 02/13/26, IBC-26-10 (v.12.0)

Goehring, Lutz, Equid (alpha)herpesvirus epidemiology, transmission, pathophysiology, vaccinology, latency & reactivation, Amendment, IBC, 02/11/26, IBC-25-02 (ver. 33.0)

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, New, IBC, 02/10/26, IBC-25-150

Kikani, Chintan, Epigenetic control of muscle stem cell function by PASK-Wdr5 signaling, Renewal, IBC, 02/06/26, IBC-25-154 (v.13.0)

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Andres, Douglas, Ras Family Protein Signaling, Amendment, IBC, 02/06/26, IBC-24-28 (v.22.0)

Alilain, Warren, Understanding the role of FGF receptors in recovery after spinal cord injury, Renewal, IBC, 02/05/26, IBC-25-162 (v.12.0)

Howe, Daniel, B23-4138-M: Biology of Coccidian Parasites, Renewal, IBC, 02/05/26, IBC-25-136 (v.11.0)

### IBC Training

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

### New Business

None.

### Adjournment

Douglas Harrison moved to adjourn the meeting at 1:26PM. Thomas Chambers seconded the motion. All members present (13) voted in favor.

APPROVED