

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

Date: 01OCT2025  
Time: 12:03 PM – 1:28 PM  
Location: Virtual Meeting via Zoom - <https://uky.zoom.us/j/878884765220>

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## Minutes

### Call to Order

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

### Attendance

#### IBC Members Present

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

#### Regrets

Carol Pickett (Local, Non-Affiliated Member)

#### Guests

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

#### 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

[2025.09.03 IBC Meeting Minutes DRAFT.pdf](#)

Thomas Chambers initiated a motion to approve the meeting minutes from the September 3<sup>rd</sup>, 2025, IBC meeting. Amelia Pinto seconded the motion. All IBC members present (12) voted in favor of the motion. Jan Smalle arrived late and was not present to vote.

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## Old Business

None.

## Protocol Review

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

## Resubmissions

None.

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## Amendments

PI: Ka Wing Fong

IBC Protocol Number: IBC-24-385

Protocol Title: Understanding the mechanisms underlying advanced prostate cancer and finding novel therapies

Protocol Type: Amendment

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

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

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

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

## Brief Project Overview:

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

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

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

Transport: Yes

Materials Transported: Biohazardous Materials, Animals

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: TRIM28 (WT, S473A, S473D)/human/oncogenes, cell cycle/cell division/expression, knockdown, silencing/human cells/pLVX, pLenticrisprV2, HA-Flag, pLKO; AR/human/oncogenes, cell cycle/cell division/overexpression, knockdown/human cells/pLVX, pLKO; SETDB1/human/oncogenes, cell cycle/cell division/expression, knockdown, silencing/human cells/pLVX, pLKO.1, pLenticrisprV2; E2F1-wildtype and mutants(E132 or  $\Delta$ TA)/human/oncogenes, cell cycle/cell division/expression, knockdown, silencing/human cells/pLVX, pLKO.1, pLenticrisprV2; CCDC83/human/oncogenes, cell cycle/cell division/expression, knockdown, silencing/human cells/pLVX, pLKO.1, pLenticrisprV2; RB/human/tumor suppressor knockdown, silencing/human cells/pLKO.1 and pLenticrisprV2; LDHA/human/oncogenes, cell cycle/cell division/knockdown, silencing/human cells/pLenticrisprV2; RSK/human/oncogenes, cell cycle/cell division/silencing/human cells/pLenticrisprV2; luciferase/firefly/reporter/expression/human cells/pLVX; SETDB1/Human/Oncogene/silencing gene expression/NA/It is purchased from IDT DNA technology as anti-sense DNA oligonucleotide ready to use; TRIM28/Human/Oncogene/silencing gene expression/NA/It is purchased from IDT DNA technology as anti-sense DNA oligonucleotide ready to use; RHOB/human/tumor suppressor/silencing/human cells/pLKO.1; USP21/human/oncogenes, cell cycle/cell division/expression, knockdown silencing/human cells/pLVX, pLKO.1, pLenticrisprV2, HA-Flag; FLVCR1 (Feline leukemia virus subgroup C cellular receptor 1) /Feline/oncogene, phospholipid metabolism/knockdown/human cells/pLKO.1

Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/pLVX; Lentivirus/pLKO.1; Lentivirus/pLenticrisprV2; Lentivirus/HA-Flag

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HEK293T; Human/22Rv1; Human/C4-2B; Human/DU145; Human/LAPC4; Human/LNCaP; Human/PC-3; Human/VCaP; Human/LuCaP35CR; Human/LuCaP96CR; Human/NCI-H660; Human/N2P1; Human/LuCaP 145.1; Human/LuCaP77CR; Human/C4-2B EnzR; Human/22Rv1 EnzR; Human/C4-2R; Human/MR49F

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Cells - Human, genetically modified/Subcutaneously injection, intraprostatic injection/Chemical Isoflurane (1-4% in O<sub>2</sub>, inhaled-nosecone)/ABSL2/disposable gloves, surgical mask, head cover, surgical gown, eye protection/ABSL2/Yes; Mouse/Patient-Derived Xenograft Materials (Human)/Subcutaneously injection/Chemical Isoflurane (1-4% O<sub>2</sub>, inhaled nosecone)/ABSL2/disposable gloves, surgical mask, head cover, surgical gown, eye protection/ABSL2/Yes; Mouse/Cells - Human, non-modified/subcutaneously injection and intraprostatic injection/chemical isoflurane (1-4% in O<sub>2</sub>, inhaled nosecone)/ABSL2/disposable gloves, surgical mask, head cover, surgical gown, eye protection/ABSL2/Yes; Mouse/Naked Nucleic Acid-r/sDNA/Intraperitoneal injection/chemical isoflurane (1-4% in O<sub>2</sub>, inhaled-nosecone)/ABSL2/disposable gloves, surgical mask, head cover, surgical gown, eye protection/ABSL2/Yes

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### *Risk Assessment/Discussion:*

Dr. Fong has submitted an amendment to IBC protocol IBC-24-385, entitled Understanding the mechanisms underlying advanced prostate cancer and finding novel therapies. In this amendment, Dr. Fong has added a new project to investigate the function of FLVCR1 (Feline leukemia virus subgroup C cellular receptor 1) in prostate cancer. FLVCR1 will be knocked down via 3rd generation lentivirus in human prostate cancer cells and administered to mice via subcutaneous injection or tail vein injection to induce tumor formation. Tumor volumes will be measured and excised at endpoint for RNA and protein extraction. Dr. Fong acknowledges that FLVCR1 has oncogenic potential and will take extra steps to communicate increased hazard to personnel by training all personnel prior to beginning work and posting additional signage to indicate ongoing work with oncogene-bearing lentivirus. The lentivirus is packaged using 3rd generation packaging plasmids, decreasing the potential for inadvertently generating replication-competent lentivirus. Dr. Fong's lab will not be concentrating lentivirus which also minimizes risk. The primary risks associated with this amendment include parenteral inoculation or aerosol exposure to the lentivirus in use. Risks are mitigated via minimizing the use of sharps or needles to the greatest extent possible, performing all work with lentivirus in a Biological Safety Cabinet (BSC), and wearing appropriate PPE including lab coat, disposable gloves, and eye protection. Biohazardous waste will be decontaminated and disposed of according to UK Research Safety guidelines. All work with this new lentivirus construct will be performed at BSL2+ containment, as previously described and approved. Dr. Fong's laboratory is currently approved to work with several oncogenic lentiviral vectors. The addition of this new lentivirus vector does not significantly alter the risk associated with work already approved under Dr. Fong's IBC protocol. There is an IBC hold on corresponding IACUC 2025-4675 related to this amendment.

### *IBC Discussion & Vote:*

The amendment to IBC-24-385 (version 53.0) was approved pending minor modifications as listed below:

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#### ANIMAL RESEARCH – Animals with Biohazards table:

1. For the Naked Nucleic Acid entry, ABSL Experimental Procedures and ABSL Housing are listed as ABSL2. Please correct both to ABSL1.
2. Please update the entry for “Cells – Human, genetically modified” to add IV administration (tail vein) as a route of administration.
3. Please ensure the PPE is congruent between the Scientific Summary and the Animals with Biohazards table. The Scientific Summary currently states, "PPE (lab coat, gloves, goggles) will be employed to minimize the biohazard risks." but the Animals with Biohazards table lists the PPE as "disposable gloves, surgical mask, head cover, surgical gown, eye protection."

#### SCIENTIFIC SUMMARY -

1. Please clarify whether the new FLVCR1 gene acts as an oncogene on its own. What is the relationship of this gene to oncogenesis?
2. The new text initially states, “we will knockdown FLVCR1 using lentiviral vector” and later says “the lentivirus vector in use does express known oncogenes”. These statements are contradictory. Please update the text to clarify. Are you expressing a known oncogene or knocking down FLVCR1?
3. Please clearly specify the method of restraint for each route of administration described in the new text – subcutaneous and tail vein injection.

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Doug Harrison initiated the motion. Yadi Wu seconded the motion. All IBC members present (12) voted in favor of the motion. Jan Smalle arrived late and was not present to vote.

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Conflicts of Interest: None

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PI: Pavel Ortinski

IBC Protocol Number: IBC-25-29

Protocol Title: Neurobiology of Cocaine Seeking

Protocol Type: Amendment

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

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

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

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

*Brief Project Overview:*

The experiments in this proposal will investigate how exposure to fentanyl or cocaine changes neuronal activity in brain areas that control addictive behaviors. In these experiments we will study rat behavior and record activity of neurons in brain slices or in vivo. Additionally, we will measure physiological parameters, such as blood pressure. This will allow us to gain insight into brain mechanisms that underlie development of drug dependence. The expression levels of specific genes in the brain of living rats will be increased or decreased using non-replicating lentiviruses and adeno-associated viruses (AAV). These viruses are engineered to safely deliver their cargo to target cells and are obtained through commercial sources. In addition to manipulating expression levels of specific genes viral vectors will incorporate fluorescent tags. The fluorescent signal will allow identification of cells manipulated by the virus as targets for recordings of neuronal activity. Development of new therapeutic treatments for cocaine abuse relies on understanding the effects of cocaine in the brain. These experiments will allow us to evaluate how specific cell types are involved in the development and expression of drug use disorders.

*Summary of Biohazard Materials & Manipulations:*

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

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

Infectious Agent(s)/Natural Host(s): EcoHIV (RG2-virus)

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: GCamp6f/Synthetic construct/ca reporter/expression/rat/adeno-associated virus; GIPC1/ rattus, rattus norvegicus, Fisher/scaffolding protein/knockdown/rat/lentivirus; GFP/Synthetic

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construct/reporter/tracking gene/expression/rat/adeno-associated virus, lentivirus; hM3D (aka GFAP hM3D)/Synthetic construct/modified G protein coupled receptor (Gq coupled)/expression/rat/adeno-associated virus; hM4D (aka GFAP hM4D) /Synthetic construct /modified G protein coupled receptor (Gi coupled) /expression/rat/adeno-associated virus; KCNQ2 /rattus, rattus norvegicus, Fisher/voltage-gated potassium channel/knock-down/rat/adeno-associated virus; KCNQ3/rattus, rattus norvegicus, Fisher /voltage-gated potassium channel/knock-down/rat/adeno-associated virus; KCNQ4/rattus, rattus norvegicus, Fisher/voltage-gated potassium channel/knock-down/rat/adeno-associated virus; KCNQ5/rattus, rattus norvegicus, Fisher/voltage-gated potassium channel/knock-down/rat/adeno-associated virus; mCherry/Discosoma (Addgene)/reporter/tracker gene/expression/rat/adeno-associated virus

Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/pZac2.1 gfaABC1D-lck-GCaMP6f (AAV5); Adeno-Associated Virus (AAV)/helf (AAV5); Adeno-Associated Virus (AAV)/pAAV.GfaABC1D.PI.Lck-GFP.SV40 (AAV5); Adeno-Associated Virus (AAV)/ AAV9.Syn.GCaMP6f.WPRE.SV40; Lentivirus/ LV-sh-GIPC1; Lentivirus/LV-shNC; Lentivirus/LV-shKCNQ2; Lentivirus/LV-shKCNQ3; Lentivirus/ LV-shKCNQ4; Lentivirus/LV-shKCNQ5; Lentivirus/LV-shScrambled

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]: Rat/Viral Vector - Adeno-Associated Virus (AAV)/intracranial/stereotaxic frame, isoflurane anesthesia/ABSL1/eye protection, gloves, lab coat, surgical gown, face protection/ABSL1/No; Rat/Viral Vector – Lentivirus/intracranial/stereotaxic frame, isoflurane anesthesia/ABSL2/eye protection, gloves, lab coat, surgical gown, face protection/ABSL2/No; Rat/EcoHIV (RG2-virus)/tail vein/stereotaxic frame, isoflurane anesthesia/ABSL2/eye protection, gloves, lab coat, surgical gown, face protection/ABSL2/No

### *Risk Assessment/Discussion:*

Dr. Ortinski has submitted an IBC amendment to his IBC protocol entitled Neurobiology of Cocaine Seeking. In this amendment, Dr. Ortinski has added a collaborator and updated AAV gene constructs. Specifically, Dr. Ortinski has added an AAV construct expressing mCherry. Dr. Ortinski's protocol currently describes the use of similar AAV constructs for intracranial administration to rats. mCherry is a reporter gene and does not present significant risk to personnel. AAV constructs will be handled at BSL1/ABSL1 containment as previously described. The procedures utilizing this new AAV-mCherry are the same as previously described and approved for other AAV constructs. AAV constructs are obtained from Addgene. This new AAV-mCherry does not significantly alter the biohazardous risks associated with this IBC protocol.

### *IBC Discussion & Vote:*

The amendment to IBC-25-29 (version 25.0) was approved pending minor modifications as listed below:

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DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Animal Transport Description: Please clearly specify the “surgery room” location.

DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE - Biohazardous Materials Transport Description: Please clearly specify the “surgery room” location and clarify that virus will be transported in a closed, secure-lidded, shatter-proof, leak-proof secondary container.

SCIENTIFIC SUMMARY:

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1. Please specify the surgery location for clarity.
2. The term “retrovirus,” is used as an all-inclusive term in several places in the Scientific Summary. Please use specific terms for which viral vectors are being used and remove references to “retrovirus” from the protocol unless it is specifically being used.
3. If EcoHIV is being used, it can remain in the protocol but if there is currently no intention of using it, it should be removed from the protocol to avoid confusion.

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Thomas Chambers initiated the motion. Amelia Pinto seconded the motion. All IBC members present (12) voted in favor of the motion. Jan Smalle arrived late and was not present to vote.

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Conflicts of Interest: None

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PI: Brandon Logeman

IBC Protocol Number: IBC-25-99

Protocol Title: Sex and physiological State dependent molecular characterization of circuits gov

Protocol Type: Amendment

Amendment To: Proteins produced

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

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

Primary Reviewers: B. Nelson, Y. Wu, J. Smalle

*Brief Project Overview:*

This project seeks to understand how differences in brain gene expression and protein composition influence innate behaviors.

*Summary of Biohazard Materials & Manipulations:*

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

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)]: GFP/Aequorea victoria/fluorescent protein/transfection/human and mouse cells/pCMV-GFP /; GCaMP8s/Aequorea victoria/fluorescent Ca<sup>2+</sup> sensor/neural activity monitor/mouse cells/pAAV-hSyn-jGCaMP8s

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pCMV-GFP; AAV-hSyn-jGCaMP8s

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HEK293; Animal/Neuro-2a

Animal Use: Yes

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

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Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Viral Vector - Adeno-Associated Virus (AAV)/intracranial/stereotaxic/ABSL1/disposable gown, gloves, eye protection, surgical mask/ABSL1/No/All procedures involving recombinant AAV will be conducted using BSL-2 practices and PPE. Personnel will wear a disposable lab coat or surgical gown, double gloves, and eye/face protection (safety glasses or face shield). Masks will be worn during injection and craniotomy procedures to reduce splash or aerosol exposure. Outer gloves will be changed immediately if contaminated and prior to leaving the procedure area.

### *Risk Assessment/Discussion:*

Dr. Logeman has submitted an amendment to add a project to produce recombinant proteins in E. coli BL21. Dr. Logeman is seeking to establish this technique in his lab and only intends to produce GFP (green fluorescent protein) with a 6x Histag fused to the N-terminus. This work will be done using BSL1 containment. Personnel will wear lab coat, disposable gloves, and eye protection. All biohazardous waste generated will be decontaminated and disposed of according to UK Research Safety guidelines. The risks associated with this new project are minimal and can safely be mitigated using BSL1 containment.

### *IBC Discussion & Vote:*

The amendment to IBC-25-99 (version 34.0) was approved.

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Yadi Wu initiated the motion. Brandy Nelson seconded the motion. All IBC members present (12) voted in favor of the motion. Jan Smalle arrived late and was not present to vote.

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Conflicts of Interest: None

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### *New Protocols*

PI: Bonnie Firestein

IBC Protocol Number: IBC-25-104

Protocol Title: Regulation of Dendritic Morphology and Function by Cypin, the Main Guanine Deaminase

Protocol Type: New Protocol

Amendment To: N/A

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

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

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

### *Brief Project Overview:*

Dendritic spines are sites of communication in excitatory neurons. Loss of these spines and swelling along dendrites and axons at the area of injury occurs due to traumatic brain injury (TBI), and this spine loss is correlated

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with the behavioral deficits that accompany TBI. Spontaneous or rehabilitative improvements in function following injury are accompanied by a reemergence of dendritic spines. Very little is known about the molecular mechanisms and identities of molecular participants in dendritic spine loss and reemergence, with regard to TBI. Recently, we showed that two proteins, postsynaptic density-95 (PSD-95) and cytosolic PSD-95 interactor (cypin), play opposing roles in mediating the morphological changes that occur in response to sublethal NMDA receptor activation, a secondary effect of TBI. Cypin promotes small and numerous varicosity formation and is necessary for NMDA-mediated spine retraction, and most importantly, confers protection from NMDA-induced neurotoxicity. PSD-95 mediates the opposite effects. Given this role that cypin plays in mediating synaptic contacts among neurons and the resulting 'wiring' of the circuit, this appears an ideal candidate to therapeutically target to rewire damaged circuits after traumatic brain injury. Since the majority of our data have come from studies on cultured neurons, we now propose to move our studies into the intact animal. The value of such a study would be considerable, as it could provide information that could ultimately be used to influence dendritic spine density after insult in a way that could enhance the outcome of rehabilitative training. We therefore hypothesize that resident postsynaptic proteins participate in the synaptic deconstruction/reconstruction that occurs as dendritic spines retract and reemerge after TBI. As such, we will determine the effects of altering cypin or PSD-95 levels on dendrite and spine morphology and neural circuit dynamics *in vitro* and *in vivo* in response to TBI. We will also assess neurobehavioral recovery from TBI when cypin or PSD-95 levels are altered or when novel cypin activators are administered to the animals. The knowledge gained from these experiments could then be used to develop therapies to treat TBI-induced brain injury.

Picrotoxin will be used to antagonize GABAA receptor mediated neuronal signaling *in-vitro* and *in effect* block inhibitory signaling for electrophysiological recordings. The use of this agent will allow us to identify the mechanism by which PSD-95 and cypin act to influence recovery after TBI and general development. alpha-Bungarotoxin will be used to specifically label nAChR in developing Neuromuscular Junctions. This agent will allow us to specifically identify areas of reformation of the Neuromuscular Junction and identify methods or materials which will allow us to reform this vital structure.

The hypothesis is that altering synaptic and neuronal proteins early in development alters the patterning of the cortex. We use cDNAs to increase or decrease these proteins.

Adeno-associated viruses (AAV) will be used to introduce Cre in our cypin floxP/floxP mice, and effectively induce knock out of cypin.

U1 adaptors will be used to knock down cypin in wild type mice. This approach is an alternative to AAV induced knock down.

One promising avenue for intervention development for patients with schizophrenia involves N-methyl-D-aspartate glutamate receptor (NMDAR) hypofunction, which has been consistently implicated in the etiology of schizophrenia [12-14]. However, most treatment development efforts have continued to focus on the dopamine and serotonin systems. Interestingly, decreased serum levels of D-serine, an NMDAR full agonist, are observed in subjects with schizophrenia [15], supporting the idea that D-serine is a viable drug for the treatment of schizophrenia. However, results of D-serine treatment have been mixed, leading to the question of why some patients respond to treatment while others do not. Nitric Oxide Synthase 1 Adaptor Protein (NOS1AP) plays an important role in the regulation of NMDAR signaling and has been implicated in susceptibility for schizophrenia [16, 17]. High levels of NOS1AP mRNA [16] and protein [18] are observed in postmortem dorsolateral prefrontal cortex of subjects with schizophrenia, and this overexpression is thought to result in NMDAR hypofunction [19]. Our preliminary data suggest that 1) treatment with D-serine decreases NOS1AP levels in human iPSC-derived neurons, 2) treatment with D-serine decreases NOS1AP levels in the cortex of male, but not female, rats, and 3) NOS1AP protein can be detected in buccal cells from humans. Thus, we hypothesize that D-serine is a viable

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treatment for patients harboring schizophrenia-associated alleles in the NOS1AP gene, specifically males (or a subgroup of male patients). We will make use of our novel human buccal cell diagnostic assay to test this hypothesis.

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

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

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Guanine Deaminase (GDA or Cypin)/Homo sapiens / Mus musculus/Cytoskeletal remodeling / Guanine metabolism/Overexpression, knockdown, fluorescent tagging/Human cells, rat, mouse, neurons/Lentivirus (pHUUG-Cypin-shRNA, FG12.Cypin-EGFP), AAV (pAAV[Exp]-CMV>mGda:IRES:EGFP:WPRE, pAAV[Exp]-Syn1>mGda:IRES:EGFP:WPRE, pAAV[Exp]-CBh>mGda:IRES:EGFP:WPRE, AAV-CamKII-Cypin, AAV-PHP.eB-Cypin); AHCYL1/Homo sapiens / Mus musculus/Metabolic enzyme complex / protein interactor/Expression, region mapping, interaction assays/HEK293T cells for packaging, Mouse/rat for delivery /AAV (AAV-PHP.eB), Lentivirus (pLV-CMV, pLV-U6); PSD-95 (DLG4)/Homo sapiens / Mus musculus/Synaptic scaffolding protein/Overexpression and tagging/E. coli, HEK 293, neurons/Plasmids (pSuper-GFP); Klotho/Homo sapiens / Mus musculus/Aging-associated factor/Overexpression/Mouse, rat, neurons, cell lines/Lentivirus (FGF12.Cypin-EGFP); CamKII (a-subunit)/Mus musculus/Kinase (signaling pathway)/Neuronal promoter (for targeting excitatory neurons)/Mouse (in vivo), neurons/AAV1.CamKII.eGFP, AAV-CamKII-Cre; Protein kinase sensors (e.g., AKAR4a, AKAR4?)/Synthetic / engineered/Kinase activity biosensors/FRET-based live imaging of kinase activity/HEK293T, neurons/Lentivirus (pRK5 series), plasmid transfection; GNSTM-3-RVG-10-Lamp2b-HA/Synthetic/Fusion protein for exosome targeting/Exosome modification (capsid targeting)/HEK293T for packaging; Mouse/Rat for delivery/AAV (PHP.eB, rh10 capsids); Vinculin/Homo sapiens/Cytoskeletal protein/Fluorescent tagging (VinTi, VinTS, VinVenus, etc.)/Neurons, HEK293T, SH-SY5Y/Lentivirus (pRRL series: VinTi, VinTS, VinVi, VinVenus, etc.); nNOS (NOS1, Nitric Oxide Synthase)/Homo Sapiens / Mus musculus/Neuronal Constitutive - Nitric oxide synthase regulator/Fluorescent tagging/E. coli, HEK 293, COS-7 cells, rats (in-utero electroporation)/Plasmid (pEGFP-C1, pEGFP-N1, pIRES-GFP, pCAG-GFP, pGE2hrGFPII, pSuper-GFP); CPE (Carboxipeptidase)/Homo sapiens / Mus musculus/Protein-coding gene/Fluorescent tagging/E. coli, HEK 293, COS-7 cells, Neuro2A cells, mice (in utero electroporation)/Plasmid (pEGFP-C1, pEGFP-C1-CPE, pEGFP-C1-CPE-H114A,E117A)

Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/pAAV[Exp]-CBh>EGFP:WPRE;/ Adeno-Associated Virus (AAV)/pAAV[Exp]-CBh>mGda[NM\_010266.2]:IRES:EGFP:WPRE;/ Adeno-Associated Virus (AAV)/pAAV[Exp]-CMV>mGda[NM\_010266.2]:IRES:EGFP:WPRE;/ Adeno-Associated Virus (AAV)/pAAV[Exp]CMV>EGFP:WPRE;/ Adeno-Associated Virus (AAV)/pAAV[Exp]-Syn1>mGda[NM\_010266.2]:IRES:EGFP:WPRE;/Adeno-Associated Virus (AAV)/pAAV[Exp]-Syn1>EGFP:WPRE;/ Plasmid/pEGFP-C1, pEGFP-N1, pIRES-GFP, pCAG-GFP, pGE2hrGFPII, pSuper-GFP;/ Lentivirus/pLV-CMV, pLV-U6;/ Adeno-Associated Virus (AAV)/AAV hSyn1-WRPE-HGHpA;/ Plasmid/pGEXT-1;/ Lentivirus/pHUUG-GST-shRNA, pHUUG-Cypin-shRNA;/ Lentivirus/pRRL-VinTi, pRRL-VinVi, pRRL-VinTS, pRRL-TSMod, pRRL-VinVenus, pRRL-empty;/ Plasmid/pRK5-tAKAR4alpha, pRK5-tAKAR4alpha T391A, pRK5-tAKARalpha, pRK5-tAKARalpha T391A, pRK5tAKAR4-gamma;/ Adeno-Associated Virus (AAV)/GNSTM-3-RVG-10-Lamp2b-HA;/ Plasmid/All pEGFP, pIRES, pSuper, pGEXT vectors;/ Plasmid/pMAL;/ Plasmid/pET28a;/ Adeno-Associated Virus

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(AAV)/AAV1.CamKII.eGFP/; Adeno-Associated Virus (AAV)/CamKII.HI.eGFP-Cre/; Lentivirus/FG12.EGFP, FG12.Cypin-EGFP/

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/Embryonic Kidney 293 cells/; Human/SH-SY5Y human neuroblastoma/; Animal/Rat Hippocampal and Cortical Neurons /; Animal/Rat spinal cord neurons/; Animal/Mouse hippocampal and cortical neurons/; Animal/Mouse spinal cord neurons/; Animal/Cos7 cells/; Animal/PC12 cells/; Animal/Neuro-2A/

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Viral Vector – Adeno-Associated Virus (AAV)/Intra-Cranial Injections/Isoflurane-anesthesia/ABSL1/Lab coat, gloves and eye protection/ABSL1/No/None/; Mouse/Viral Vector - Adeno-Associated Virus (AAV)/Intracerebroventricular (ICV)/Stereotaxic frame (surgery under anesthesia)/ABSL1/Lab coat, disposable surgical gown, disposable gloves, surgical mask/ABSL1/No//; Mouse/Viral Vector - Adeno-Associated Virus (AAV)/Intravenous (IV)/Tail vein injection restrainer device/ABSL1/Lab coat, disposable surgical gown, disposable gloves, surgical mask/ABSL1/No//; Mouse/Viral Vector - Adeno-Associated Virus (AAV)/Intrahippocampal stereotaxic injection (surgery under anesthesia)/Stereotaxic frame/ABSL1/Lab coat, disposable surgical gown, disposable gloves, surgical mask/ABSL1/No//; Mouse/Viral Vector - Lentivirus/Intracerebroventricular (ICV)/Stereotaxic frame (surgery under anesthesia)/ABSL2/Lab coat, disposable surgical gown, disposable gloves, surgical mask/ABSL2/No//; Mouse/Plasmid/In-utero electroporation/Isoflurane Anesthesia/ABSL1/Lab coat, disposable surgical gown, disposable gloves, surgical mask/ABSL1/No//; Rat/Plasmid/In-utero electroporation/Isoflurane Anesthesia/ABSL1/Lab coat, disposable surgical gown, disposable gloves, surgical mask/ABSL1/No/

### *Risk Assessment/Discussion:*

Dr. Firestein is a new PI to UK and has submitted an IBC protocol ahead of her arrival for her project entitled Regulation of Dendritic Morphology and Function by Cypin, the Main Guanine Deaminase, and Other Proteins. Dr. Firestein's laboratory seeks to understand how cypin regulates synaptic protein composition, cellular metabolism, and neuronal recovery after traumatic brain injury (TBI) while testing various therapeutic strategies including viral vectors, exosome-mediated RNA delivery, and small molecule modulators. They will utilize E. coli DH5alpha for plasmids preparation and cloning using BSL1 containment. They also intend to utilize adeno-associated virus (AAV) and lentivirus vectors. Some AAVs will be purchased directly from vendors, however they intend to produce one specific AAV vector with GNSTM-3-RVG-10-Lamp2b-HA using triple transfection of HEK293T cells. AAV production will be done at BSL2 containment as plasmids are transfected into HEK293T cells for packaging. Once constructed, AAVs will be handled at BSL1 containment. Purchased AAVs will be administered to mice via intracranial injection or intravenous injection. Animals administered AAV will be housed at ABSL1 containment. The AAVrh10 packaged in house will be used for generation of exosomes for siRNA delivery. These exosomes with siRNA targeting Preso will be administered to animals via intravenous tail vein injection. 3rd generation lentivirus vectors will be packaged in house. Lentivirus packaging and downstream transductions will be done using BSL2+ containment exclusively in a Biological Safety Cabinet (BSC). Lentivirus is concentrated via centrifugation, and centrifuge tubes are loaded/unloaded into safety rotors within a BSC. Lentivirus vectors will be administered to animals via stereotaxic injection, and animals will be housed at ABSL2 containment for a minimum of 72 hours post administration. After 72 hours have passed, animals will be moved to ABSL1 containment. After viral delivery, animals will undergo several downstream assays and procedures, including TBI and neurobehavioral testing, according to their corresponding IACUC protocol (pending). The transgenes that will be expressed or knocked down via viral vectors described in this IBC protocol are not oncogenic, toxic, or otherwise known to be particularly hazardous. The AAVs and lentivirus vectors will also be used to transduce human and animal cells in culture.

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Transduced cells will undergo downstream assays including excitotoxicity, protein extraction, snRNA-seq nuclei preparation, and metabolomics. All samples will be manipulated inside a BSC until inactivation via treatment with TRIzol, RIPA buffer, or 4% paraformaldehyde fixation, depending on the assay. Once samples are inactivated, they can be safely handled using BSL1 containment. Lastly, Dr. Firestein's laboratory will also receive buccal swabs from patients and controls for RNA and protein isolation. All work with human source materials will be done at BSL2 containment. Personal protective equipment (PPE) used throughout all laboratory work will include laboratory coat, disposable gloves, and eye protection. BSL2+ work with lentivirus will utilize a dedicated lab coat, and double gloves will be worn when working with TRIzol. All biohazardous waste generated throughout this work will be decontaminated and disposed of according to UK Research Safety guidance. The primary risks associated with the work described here are parenteral inoculation or aerosol inhalation, and potential exposure to bloodborne pathogens when working with buccal swabs obtained from patients. These risks are mitigated using isoflurane anesthesia in animals for all viral administration, appropriate PPE including lab coat, gloves, and eye protection, and restricting work with biohazardous materials to a BSC until inactivation.

*\*\*Jan Smalle arrived at 12:23pm during discussion of Dr. Firestein's IBC protocol\*\**

### *IBC Discussion & Vote:*

The protocol IBC-25-104 (version 6.0) was approved pending minor modifications as listed below:

\*

**GENERAL INFORMATION:** Lay Description - Please simplify the lay description of your research. For example, the paragraph at the start of the Scientific Summary section would be more appropriate in this location.

**PERSONNEL:** Some of the personnel have been given multiple role assignments. Please only assign one role (Co-Investigator or Staff) for each person on the protocol.

**LOCATIONS:** Research Locations Table – Please make note of the location where animal manipulations are performed. If specific room numbers are unknown at this time, indicate “Pending DLAR Assignment”.

**SCIENTIFIC SUMMARY:** Consider inserting some of the existing text from the Lay Summary into the Scientific Summary here to fill in more details and provide necessary context.

\*

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

\*

Conflicts of Interest: None

\*

**PI: Zhonglin Hao**

IBC Protocol Number: IBC-25-109

Protocol Title: 25-LUN-146-GPX (ONC-003): A Phase 1/2 Open-Label, Dose-Escalation and Clinical Response Study of Quaratusugene Ozeplasmid in Combination with Osimertinib in Patients with Advanced, EGFR-Mutant, Metastatic Non-Small Cell Lung Cancer who have Progressed after Treatment with Osimertinib

Protocol Type: New Protocol

Amendment To: N/A

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

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Regulation 6.9, OSHA 29 CFR 1910.1030

Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: D. Harrison, B. Nelson, M. Landron

### *Brief Project Overview:*

Genprex is developing quaratusugene ozeplasmid (also known as REQORSA®) a DNA-lipid complex containing a plasmid vector expressing the suppressor gene TUSC2 in advanced lung cancer. This phase 1/2 study aims to identify the maximum tolerated dose and recommended dose of the study agent, estimate the overall response rate in patients, and to follow and compare the progression-free survival of patients treated with the study agent as compared to those treated with platinum-based chemotherapies.

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

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

Transport: Yes

Materials Transported: Biohazardous Materials

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: TUSC2 (formerly known as FUS1) Wild-type/Human/Tumor Suppressor/Over-expression/Human Research Participants/ Quaratusugene ozeplasmid; Neomycin/Kanamycin Resistance Cassette/Synthetic/(Antibiotic) Aminoglycoside Resistance Selection Cassette/Selection/Human Research Participants/ Quaratusugene ozeplasmid

Vector(s) [Vector Category/Vector Technical Name]: Nanoparticle/Quaratusugene Ozeplasmid (REQORSA®) (pLJ143/KGB2/TUSC2)/Genprex/Transfection/Lipofection/No

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

Animal Use: No

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

### *Risk Assessment/Discussion:*

Dr. Hao has submitted an IBC protocol for his clinical research study entitled A Phase 1/2 Open-Label, Dose-Escalation and Clinical Response Study of Quaratusugene Ozeplasmid in Combination with Osimertinib in Patients with Advanced, EGFR-Mutant, Metastatic Non-Small Cell Lung Cancer who have Progressed after Treatment with Osimertinib. This clinical study will utilize Quaratusugene ozeplasmid (AKA REQORSA) to deliver DNA plasmid expressing the TUSC2 tumor suppressor gene to patients with advanced, EGFR-mutant, metastatic non-small cell lung cancer and does not replicate or integrate into the genome. Quaratusugene ozeplasmid is a liquid suspension containing DNA plasmid expressing the TUSC2 gene encapsulated in positively charged lipoplex prepared using 1,2-dioleoyloxy-3-(trimethylammonium) propane- $\gamma$ -chlorine:Cholesterol (DOTAP-Cl:Cholesterol) liposome solution. Plasmid is generated by manufacturer in genetically modified E. coli, purified, and formulated into lipid nanoparticles. Study product is tested for identify, purity, safety, and sterility prior to release. Each lot will be accompanied by a Certificate of Analysis to be provided to the investigator. 4 study participants will be enrolled at UK. Study drug will be shipped by the study sponsor to the UK Investigational Drug Service (IDS) Pharmacy where it will be stored until time for preparation. Study drug will be thawed and prepared by IDS Pharmacy staff according to study sponsor instructions and transported to the UK Precision Medicine Center (PMC) for IV administration to study participants. All study related waste (syringes, absorbent materials, etc.) will be disposed of as regulated medical waste per UK Healthcare guidelines. Study participants will be observed in the PMC unit

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for 6 hours following administration of the first three doses prior to discharge. Follow-up patient samples will be obtained according to the study Schedule of Activities in the UK PMC unit and transported in leak-proof, shatter-proof, secure-lidded, secondary container to the UK Biospecimen Procurement and Translational Pathology (BPTP) laboratory for packaging and shipment to the study sponsor. The risks to healthcare workers (HCW) handling this study drug and patient samples is no greater than that which HCWs would encounter in a hospital setting working with patients. The primary risk is exposure to potential human pathogens. The primary risk of Quaratusugene ozeplasmid exposure for HCW is accidental needle-stick during preparation or administration, and the risks would be minimal considering the likely small amount of study product that an individual may be exposed to via needle-stick. Work with patient samples will be done using universal precautions and BSL2 laboratory containment. HCWs will wear disposable gown, disposable gloves, face shield or protective eyewear, and surgical mask when administering study drug to patients.

### *IBC Discussion & Vote:*

The protocol IBC-25-109 (version 6.0) was approved pending minor modifications as listed below:

\*

RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information Table: Please indicate the gene source for the Neomycin/Kanamycin entry.

LOCATIONS-Research Location Table: Booties/Shoe Covers are listed in this section but not listed under the PPE in the Infectious Agents section. Please update these sections to be congruent.

\*

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

\*

Conflicts of Interest: None

\*

### *Renewals*

PI: Sylvie Garneau-Tsodikova

IBC Protocol Number: IBC-25-111

Protocol Title: Chemoenzymatic studies of aminoglycoside-resistance enzymes towards new drugs and Investigation and Redesign of Nonribosomal Peptide Synthetases

Protocol Type: Renewal

Amendment To: N/A

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

Maximum Containment Level: Biological Safety Level 2 (BSL2)

Primary Reviewers: D. Malherbe, D. Harrison, M. Landron

*Brief Project Overview:*

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The work that is performed in my laboratory has two goals:

1. to develop new aminoglycoside antibiotics and to understand the mechanisms of resistance to these antibiotics.
2. to decipher how biosynthetic enzymes from the natural products thiocoraline and kutzneride pathways function and to develop novel more effective enzymes.

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

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

Transport: No

Materials Transported: N/A

Infectious Agent(s)/Natural Host(s): *Acinetobacter baumannii* (RG2-bacteria); *Aspergillus flavus* (RG2-fungus); *Aspergillus nidulans* (RG2-fungus); *Aspergillus terreus* (RG2-fungus); *Bacillus anthracis*, Sterne 34F2 (RG2-bacteria); *Bacillus cereus* (RG2-bacteria); *Bacillus subtilis* (RG1-bacteria); *Campylobacter jejuni* (RG2-bacteria); *Candida albicans* (RG2-fungus); *Candida glabrata* (RG1-fungus); *Candida parapsilosis* (RG1-fungus); *Citrobacter amalonaticus* (RG1-bacteria); *Citrobacter freundii* (RG1-bacteria); *Cryptococcus neoformans* (RG2-fungus); *Enterobacter aerogenes* (RG2-bacteria); *Enterobacter cloacae* (RG2-bacteria); *Enterobacter* spp. (RG2-bacteria); *Enterococcus faecalis* (RG2-bacteria); *Enterococcus faecium* (RG2-bacteria); *Escherichia coli* (RG2-bacteria); *Gordonia bronchialis* (RG2-bacteria); *Haemophilus influenzae* (RG2-bacteria); *Issatcheukia orientalis* (aka *Candida krusei*) (RG1-fungus); *Klebsiella oxytoca* (RG2-bacteria); *Klebsiella pneumoniae* (RG2-bacteria); *Klebsiella* spp. (RG2-bacteria); *Listeria monocytogenes* (RG2-bacteria); *Micrococcus luteus* (RG1-bacteria); *Mycobacterium abscessus* (RG2-bacteria); *Mycobacterium avium* subsp. *avium* (RG2-bacteria); *Mycobacterium intracellulare* (RG2-bacteria); *Mycobacterium kansasii* (RG2-bacteria); *Mycobacterium marinum* (RG2-bacteria); *Mycobacterium parascrofulaceum* (RG2-bacteria); *Mycobacterium smegmatis* (RG2-bacteria); *Mycobacterium tuberculosis* H37RA (RG2-bacteria); *Pseudomonas aeruginosa* (RG2-bacteria); *Salmonella enterica* (RG2-bacteria); *Serratia marcescens* (RG2-bacteria); *Shigella flexneri* (RG2-bacteria); *Staphylococcus aureus* (RG2-bacteria); *Staphylococcus epidermidis* (RG1-bacteria); *Streptococcus pyogenes* (RG2-bacteria); *Mycobacterium tuberculosis* mc2 (RG2-bacteria); *Candida auris* (RG2-fungus); *Yersinia pestis* (avirulent) (RG2-bacteria); *Candida duobushaemulonii* (RG2-fungus); *Candida haemulonii* (RG2-fungus); *Candida krusei* (RG2-fungus); *Candida lusitanae* (RG2-fungus); *Kodameae ohmeri* (RG2-fungus); *Kodameae ohmeri* (RG2-fungus) *Streptomyces* spp. (RG1-bacteria); *Actinomadura verrucosospora* (RG1-bacteria); Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: *Eis* and its homologues/ *Mycobacterium tuberculosis* H37Rv, MC2, *smegmatis*, *parascrofulaceum*, *kansasii*, *haciacum*, *intracellulare*, *avium* subs., *Tsukamurella paurometabola*, *Kocuria rhizophila*, *Gordonia bronchialis*, and *Anabaena variabilis* genomic DNA / enzymatic protein/ Used to purify protein for enzymatic assay/protein; target promoter for overexpression / *E. coli* TOP10 and BL21(DE3); *Mycobacterium tuberculosis* MC2 Strains/ pET14b, pET28a, Int-pET19b-pps; Aminoglycoside acetyltransferases, phosphotransferases, nucleotidyltransferases (AAC(2')-Ic, AAC(3)-IV, AAC(3)-Ib, AAC(3)-Ib/AAC(6')-Ib', AAC(6')/APH(2''), AAC(6')-Ib', ANT(4'), ANT(3'')/AAC(6'), AAC(3)-Ia) / AAC(2')-Ic (*Mycobacterium tuberculosis* H37Rv genomic DNA) AAC(3)-IV (*E. coli* genomic DNA) AAC(3)-Ib (*Pseudomonas aeruginosa* genomic DNA) AAC(3)-Ib/AAC(6')-Ib' (*Pseudomonas aeruginosa* genomic DNA) AAC(6')/APH(2'') (*Enterococcus* genomic DNA) AAC(6')-Ib' (*Pseudomonas aeruginosa* genomic DNA) ANT(4') (*S. aureus* genomic DNA) ANT(3'')/AAC(6') (*Serratia marcescens*

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genomic DNA) AAC(3)-Ia (*Serratia marcescens* genomic DNA)/ enzymatic protein/ Used to purify protein for enzymatic assay/ *E. coli* TOP10 and BL21(DE3)/ pET (22b, 23a, 28a, 19b), pGEM-T; Enzymes from the thiocoraline biosynthetic gene cluster (TioF, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, X) / *Micromonospora* sp. ML1 genomic DNA / enzymatic protein/ Used to purify protein for enzymatic assay / *E. coli* TOP10 and BL21(DE3) ybdz::aac(3)iv/ pET22b, pET28a, pACYC-Duet1, pGS-21a, Int-pET19b-pps; Enzymes from the kutzneride biosynthetic gene cluster (KtzJ and KtzH) / *Kutzeria* sp. 744 genomic DNA / enzymatic protein / Used to purify protein for enzymatic assay/ *E. coli* TOP10 and BL21(DE3) ybdz::aac(3)iv/ pET28a, pACYC-Duet1; MtbH-like proteins (MbtH, Ecm8, CouY, PA2412) / MbtH (*M. tuberculosis* genomic DNA) Ecm8 (*Streptomyces lasaliensis* genomic DNA) CouY (*Streptomyces rishiriensis* genomic DNA) PA2412 (*Pseudomonas aeruginosa* genomic DNA)/ enzymatic protein/ Used to purify protein for enzymatic assay / *E. coli* TOP10 / pET15b, pET16b, pACYC-Duet1; Mycobacterium proteins: HupB, Sir2, Rv3034c/ *M. tuberculosis* H37Rv and *M. smegmatis* MC255 genomic DNA/ enzymatic protein / Used to purify protein for enzymatic assay/ *E. coli* TOP10 and BL21(DE3)/ pET28a, pET17b, pET22b, pET38b, Int-pET19b-pps; LinB / *S. aureus* genomic DNA / enzymatic protein/ Used to purify protein for enzymatic assay/ *E. coli* TOP10 and BL21(DE3) / pET28a; Chloramphenicol resistance enzymes (phosphotransferase, nitroreductase, acetyltransferase)/ CNR = nitroreductase (*H. influenzae* genomic DNA) CPT = phosphotransferase (*S. venezuelae* genomic DNA) CATI = acetyltransferase (plasmid containing Cam resistance marker)/ enzymatic protein/ Used to purify protein for enzymatic assay / *E. coli* TOP10 and BL21(DE3)/ pET28a, pET19b

Vector(s) [Vector Category/Vector Technical Name]: Plasmid/ pET vectors (14b, 15b, 16b, 17b, 19b, 22b, 23a, 28a, 38b) ; Plasmid/ pACYC-Duet1 ; Plasmid/ pGEM-T ; Plasmid/pGS-21a ; Plasmid/ Int-pET19b-pps

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/ HEK-293 ; Human/ BEAS-2B ; Human/ A549 ; Animal/ J774a.1 ; Human/ Hep G2; Human/ HaCat

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]: Insect - IACUC Exempt/ *Acinetobacter baumannii* (RG2-bacteria)/ injection/ secured tips/ ABSL2/ lab coat, disposable gloves, eye protection/ ABSL2/ Yes/Use of BSC, Petri plate lids housing *G. mellonella* (wax worms) taped shut

### *Risk Assessment/Discussion:*

Dr. Garneau-Tsodikova has submitted a renewal of her IBC protocol entitled Chemoenzymatic studies of aminoglycoside-resistance enzymes towards new drugs and Investigation and Redesign of Nonribosomal Peptide Synthetases. Dr. Garneau-Tsodikova's laboratory utilizes several Risk Group 2 (RG2) bacterial and fungal pathogens with the goal of developing new antibiotics and understanding mechanisms of antibiotic resistance. The primary risks associated with this IBC protocol include exposure to the pathogenic bacterial and fungal agents utilized, including: *Acinetobacter baumannii* (RG2-bacteria), *Aspergillus flavus* (RG2-fungus), *Aspergillus nidulans* (RG2-fungus), *Aspergillus terreus* (RG2-fungus), *Bacillus anthracis*, Sterne 34F2 (RG2-bacteria), *Bacillus cereus* (RG2-bacteria), *Bacillus subtilis* (RG1-bacteria), *Campylobacter jejuni* (RG2-bacteria), *Candida albicans* (RG2-fungus), *Candida glabrata* (RG1-fungus), *Candida parapsilosis* (RG1-fungus), *Citrobacter amalonaticus* (RG1-bacteria), *Citrobacter freundii* (RG1-bacteria), *Cryptococcus neoformans* (RG2-fungus), *Enterobacter aerogenes* (RG2-bacteria), *Enterobacter cloacae* (RG2-bacteria), *Enterobacter* spp. (RG2-bacteria), *Enterococcus faecalis* (RG2-bacteria), *Enterococcus faecium* (RG2-bacteria), *Escherichia coli* (RG2-bacteria), *Gordonia bronchialis* (RG2-bacteria), *Haemophilus influenzae* (RG2-bacteria), *Issatchekia orientalis* (aka *Candida krusei*) (RG1-fungus), *Klebsiella oxytoca* (RG2-bacteria), *Klebsiella pneumoniae* (RG2-bacteria), *Klebsiella* spp. (RG2-bacteria), *Listeria monocytogenes* (RG2-bacteria), *Micrococcus luteus* (RG1-bacteria), *Mycobacterium abscessus* (RG2-

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bacteria), *Mycobacterium avium* subsp. *avium* (RG2-bacteria), *Mycobacterium intracellulare* (RG2-bacteria), *Mycobacterium kansasii* (RG2-bacteria), *Mycobacterium marinum* (RG2-bacteria), *Mycobacterium parascrofulaceum* (RG2-bacteria), *Mycobacterium smegmatis* (RG2-bacteria), *Mycobacterium tuberculosis* H37RA (RG2-bacteria), *Pseudomonas aeruginosa* (RG2-bacteria), *Salmonella enterica* (RG2-bacteria), *Serratia marcescens* (RG2-bacteria), *Shigella flexneri* (RG2-bacteria), *Staphylococcus aureus* (RG2-bacteria), *Staphylococcus epidermidis* (RG1-bacteria), *Streptococcus pyogenes* (RG2-bacteria), *Mycobacterium tuberculosis* mc2 (RG2-bacteria), *Candida auris* (RG2-fungus), *Yersinia pestis* (avirulent) (RG2-bacteria), *Candida duobushaemulonii* (RG2-fungus), *Candida haemulonii* (RG2-fungus), *Candida krusei* (RG2-fungus), *Candida lusitanae* (RG2-fungus), *Kodameae ohmeri* (RG2-fungus), *Kodameae ohmeri* (RG2-fungus), *Streptomyces* spp. (RG1-bacteria), and *Actinomyces verrucosospora* (RG1-bacteria). This list includes 4 attenuated strains – *Bacillus anthracis* Sterne 34F2 (live, attenuated strain lacking pXO2 plasmid used for production of veterinary anthrax vaccine), *Mycobacterium tuberculosis* H37RA and mc2, and *Yersinia pestis* CO92. While the WT parent of each of these requires BSL3 containment, and two of the WT parent strains are Select Agents, the attenuated strains are excluded from the Select Agent regulations (<https://www.selectagents.gov/sat/exclusions/hhs.htm> and <https://www.selectagents.gov/sat/exclusions/overlap.htm>). All work with these agents that requires manipulations that might expose pathogens to the environment (ex. Plating, transferring, harvesting, or otherwise opening plates, tubes, etc.) will be done in a BSC using BSL2 containment. Gloves, lab coat, and eye protection will be worn for this work. Biohazardous waste generated in this work is decontaminated and disposed of according to UK Research Safety guidelines. Dr. Garneau-Tsodikova's lab also utilizes a *Galleria mellonella* (aka wax moths/wax worms) model to test antimicrobial compounds. Worms are restrained and injected with 10-25 microliters of *A. baumannii*, followed by a second injection 30 minutes later with PBS control or compound to be tested. All work is done in a BSC using PPE as previously described. Worms are housed in large petri plates with lids taped shut in a dedicated incubator and will be monitored for life/death. Manipulations of worms after infection are minimal and only involve observation. All materials are disposed of as solid biohazardous waste. The only BSL1 work in Dr. Garneau-Tsodikova's laboratory is cloning of biosynthetic and antibiotic resistance enzymes into pET vectors for expression in non-pathogenic, lab-strain *E. coli*. Antibiotic resistance genes are never inserted into pathogenic bacterial species. All other work described on this IBC protocol is performed using BSL2 containment as described above. Dr. Garneau-Tsodikova's current IBC protocol will expire on December 6, 2025.

### IBC Discussion & Vote:

The protocol IBC-25-111 (version 9.0) was approved pending minor modifications as listed below:

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RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Vector Information Table: Are plasmids expected to integrate into the genome of the host? Please revise.

RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information Table: Please review the gene hosts currently listed (ex. Top10, BL21 *E. coli*, *Mycobacterium tuberculosis* MC2 strains) and update as needed. For example, *Bacillus subtilis* is listed as a strain utilized as part of a host-vector system elsewhere but is not listed here as a host.

### SCIENTIFIC SUMMARY:

1. Please specify that only one bacterial agent will be manipulated at any one time to minimize potential for cross-contamination.
2. Please very clearly specify work that will be performed now vs. future work planned.

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3. Please clearly specify whether human and mammalian cells are exposed to compounds isolated from bacterial agents or the bacteria themselves.
4. Will any work with live biohazardous materials be performed outside of the biological safety cabinet? The summary currently states there are “other steps,” performed in 466A.
5. The use of double-gloves is suggested for work in the biological safety cabinet. Is this a suggestion for laboratory personnel or is this standard practice when working in the biological safety cabinet?
6. Include verbiage that soil and water samples being collected may possibly carry pathogenic bacteria and other agents and that there is minimal risk of exposure due to splash and/or aerosolization during collection.

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

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Conflicts of Interest: None

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PI: Ling Yuan

IBC Protocol Number: IBC-25-116

Protocol Title: Plant Metabolic Engineering

Protocol Type: Renewal

Amendment To: N/A

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

Maximum Containment Level: Biological Safety Level 1 - Plants (BSL1-P)

Primary Reviewers: A. Hunt, J. Smalle, M. Mendenhall

*Brief Project Overview:*

My research interests are centered in two main areas: (i) mechanistic study of transcription factors (TFs) for plant metabolic pathway regulation, and (ii) metabolic engineering of tobacco plants for harm reduction. The research in my lab addresses some of the key issues in molecular biology and protein biochemistry and contributes to the development of new-crop opportunities, an important area in the agricultural economies of Kentucky and the nation.

We clone genes from plants and ligate them into bacterial (pET, pBS, pNFYP, pCYFP, pCAMBIA, pKYLX), fungal (yeast) (pAD, pBD), or plant (pCAMBIA, pKYLX) vectors for DNA sequencing and expression. We produce recombinant proteins in *E. coli* cells, and purify these proteins. We also transform DNA vectors into plants to generate transgenic plants. In a typical experiment, we clone a transcription factor (TF) gene, from tobacco, periwinkle or *Arabidopsis*, and ligate the gene into a yeast one-hybrid or two-hybrid vector (pAD, pBD) that allows the detection of DNA-protein or protein-protein interactions. Such modified vectors are transformed into yeast cells that are subsequently plated on selection agar plates. Colonies are picked and assayed for ability to survive in the pre-determined selection media. The TF gene might also be ligated into an *Agrobacterium* T-DNA binary vector (pCAMBIA, pKYLX) for introduction into *Agrobacterium*. The transformed *Agrobacterium* is used to infect plant tissue, the *Agrobacterium* transfers the vector into plants, leading to the generation of transgenic plants.

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We apply sterile techniques in our work environment and routinely use bleach to clean glassware used for cell culture. All used cultures, cells, transgenic plants and media are put in biohazard bags and autoclaved.

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

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

Transport: Yes

Materials Transported: Plants

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Glabrous 3 (GL3); Enhancer of GL3 (EGL3); Glabrous 1 (GL1); Arabidopsis thaliana/Transcription factor (TF)/Regulatory protein/Protoplast; expression; Plant/; expression;Protein-protein interaction; E. coli; Agrobacterium; tumefaciens; Saccharomyces cerevisiae/PBS; pCAMBIA1300; pBD; pA7-nYFP; Transparent Testa Glabra 1 (TTG1), Transparent Testa 8 (TT8)/Arabidopsis thaliana/Transcription factor (TF)/Regulatory protein/Protein-protein interaction/Agrobacterium; tumefaciens; /pCAMBIA1300; Catharanthus roseus WRKY1 (CrWRKY1); Octadecanoid-; derivative responsive Catharanthus AP2-domain (ORCA2), ORCA3, ORCA4, ORCA5; ORCA6; MYC2; Repressor of MYC2 (RMT1), bHLH iridoid synthesis 1 (BIS1), BIS2, BIS3; Catharanthus roseus/TF/Regulatory proteins/Protoplast/; expression/; Expression in hairy roots/; Protein-protein interaction; E. coli ; Agrobacterium; rhizogenes/; Saccharomyces cerevisiae/; /pBS, pAD/ Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pBlueScript II (pBS)/; Plasmid/pUC/; Plasmid/pCAMBIA 1300 pCAMBIA 2300/; Plasmid/pAD, pBD/; Plasmid/pTA7001/; Plasmid/pKYLX80/; Plasmid/pKYLX71/; Plasmid/pRGEB31/; Plasmid/pET29b/; Plasmid/pGEX-4T/; Plasmid/pA7-cYFP pA7-nYFP/; Plasmid/pSATN-nEYFP-N1/; Plasmid/pMAL-p5X pMAL-c5X/; Plasmid/pGADT7/; Plasmid/pGBKT7/; Plasmid/pCAMBIA1300-nLUC, pCAMBIA1300-cLUC/

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Plant/Nicotiana tabacum; Plant/Catharanthus roseus  
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. Yuan has submitted a renewal of their IBC protocol entitled Plant Metabolic Engineering. In Dr. Yuan's laboratory, they clone plant genes into bacterial and plant vectors for DNA sequencing and expression. Recombinant proteins are produced using non-pathogenic, lab-strain E. coli and purified for downstream analysis. Transgenic plants (Arabidopsis and tobacco) and transgenic hairy roots are also produced via Agrobacterium mediated transformation. The biohazardous materials described in Dr. Yuan's IBC protocol do not pose a significant risk to humans. The primary risks associated with this work is unintended release of transgenic plant materials. All transgenic plants are grown in sterile culture, growth chambers, or greenhouses. No field work is planned. Transgenic plants in the greenhouse are not grown to flowering stages. When allowed to flower in growth chambers, flower heads are covered to prevent seed dispersal. Seeds are stored in a dedicated cabinet for transgenic seeds. Transgenic plant materials are autoclaved prior to disposal. Transgenic C. roseus hairy roots are also created to evaluated gene expression. All work with transgenic hairy roots is done in the laboratory. No infectious or pathogenic agents are utilized in this project. Work is completed using BSL1-P containment and laboratory members wear lab coat, disposable gloves, and eye protection or face shield. All biohazardous waste generated is decontaminated or devitalized according to UK Research Safety guidelines. Dr. Yuan's current IBC protocol will expire on October 3, 2025.

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### *IBC Discussion & Vote:*

The protocol IBC-25-116 (version 9.0) was approved pending minor modifications as listed below:

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**PLANTS:** Transgenic Plants Table – It is indicated that transgenic *Arabidopsis* and *Catharanthus roseus* will be generated, and there are many different genes being expressed. Please update the transgenic plants table to include all strains of transgenic plants.

**PERMITS:** An APHIS permit for plant pathogens is attached but these infectious agents are not described in the protocol. Please either update the protocol to include the details of these pathogens or remove the attached APHIS permit.

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

\*

Conflicts of Interest: None

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**PI: Amanda Adams**

IBC Protocol Number: IBC-25-121

Protocol Title: Study of Immunology of Equids

Protocol Type: Renewal

Amendment To: N/A

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

Maximum Containment Level: Biological Safety Level 2 (BSL2)

*Primary Reviewers: C. Shaffer, T. Chambers, A. Hunt*

### *Brief Project Overview:*

Aged horses and even more so aged horses with Pituitary Pars Intermedia Dysfunction (PPID) and those with Equine Metabolic Syndrome (EMS) are thought to experience immunosuppression as a side effect of the disease. However, to date, this increased susceptibility to infection has not been explored thoroughly and there is no definitive answer as to whether or not horses with PPID are indeed immunosuppressed. Horses that are immunocompromised typically exhibit frequent bacterial and fungal infections, particularly respiratory and integumentary infections, in addition to other inflammatory diseases of the respiratory system (Kane & Steward, 2009; Miller, M.A., 2016). In order to investigate the potential immunosuppression experienced by horses with PPID, peripheral blood samples will be obtained from aged horses with PPID and EMS and healthy control non-PPID and non-EMS horses of similar age and younger horses as comparisons.

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

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

Transport: No

Materials Transported: N/A

Infectious Agent(s)/Natural Host(s): Escherichia coli (RG2-bacteria)/Mammal/; Rhodococcus equi (RG2-bacteria)/Equine/; Streptococcus equi subsp. zooepidemicus (RG2-bacteria)/Equine/; Equine Herpesvirus (RG1-virus)/Equine/; Equine Influenza Virus (EIV) (RG1-virus)/Equine/; Streptococcus equi subsp. equi (RG2-bacteria)/Equine

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

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

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/peripheral blood mononuclear cells; Animal/bronchoalveolar lavage (BAL) samples

Animal Use: Yes

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

### *Risk Assessment/Discussion:*

Dr. Amanda Adams has submitted a renewal of her IBC protocol entitled Study of Immunology of Equids. In this protocol, blood will be obtained from healthy horses and transported back to the laboratory where it will be stimulated with bacteria or equine viral pathogens within a Biological Safety Cabinet (BSC). Personnel will wear lab coat, gloves, and eye protection. After stimulation, tubes will be incubated, centrifuged using sealed rotors, and plasma aliquoted for downstream ELISA for measurement of myeloperoxidase produced by neutrophils. This protocol utilizes several pathogenic or zoonotic bacteria including Escherichia coli (RG2-bacteria), Rhodococcus equi (RG2-bacteria), Streptococcus equi subsp. zooepidemicus (RG2-bacteria), Streptococcus equi subsp. equi (RG2-bacteria), and equine viral pathogens including Equine Influenza Virus (EIV) (RG1-virus) and Equine Herpesvirus (RG1-virus). EIV and EHV were grown by previous collaborators and will not be propagated in Dr. Adam's laboratory. All work described here will be done using BSL2 containment, regardless of the infectious agent in use, with personnel wearing lab coat, gloves, and eye protection. Work will be conducted inside a BSC until materials are inactivated for downstream analysis. Dr. Adam's current IBC protocol will expire on October 4, 2025.

### *IBC Discussion & Vote:*

The protocol IBC-25-121 (version 9.0) was approved pending minor modifications as listed below:

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### SCIENTIFIC SUMMARY:

1. It is noted that peripheral blood mononuclear cells (PBMCs) will be isolated, plated, and stimulated by bacteria before the cells are fixed. However, there is no description of what is done with the supernatant after the PBMCs are isolated. Please update to include details of how supernatant is decontaminated and disposed of.
2. It is unclear whether bacterial agents will be propagated in the lab. Please clarify and provide details of propagation, if applicable.

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Carrie Shaffer initiated the motion. Arthur Hunt seconded the motion. All IBC members present (12) voted in favor of the motion. Tom Chambers abstained from voting.

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Conflicts of Interest: Dr. Thomas Chambers previously provided frozen Equine influenza virus (EIV) stocks to Dr. Adams and opted to abstain from voting.

PI: Emilia Galperin

IBC Protocol Number: IBC-25-123

Protocol Title: Regulation of MAPK activity by EGF receptor endocytosis

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9

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

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

### *Brief Project Overview:*

The extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling pathway plays an essential role in several critical steps of embryonic development and tumor progression. It also controls critical cellular functions such as apoptosis, motility, and differentiation. Kinases and phosphatases of this pathway have been extensively studied and targeted therapeutically. However, the mechanisms that determine the signal specificity and orchestrate the diverse biological outcomes of ERK1/2 signaling are still poorly understood. Scaffold proteins are key players in the ERK1/2 signaling pathway that are thought to integrate incoming signals and deliver signaling specificity. Yet, their role in signal propagation and the mechanisms of their action are still unknown. The long-term goal of our research is to reveal how scaffold proteins are involved in the biological processes regulated by the ERK1/2 cascade. In this way, new therapeutic strategies can be developed to more specifically target this cascade without affecting other essential biological functions. In our studies, we utilize lentiviruses that express fluorescently tagged proteins and shRNA. Our research protocol involves recombinant DNA techniques, the use of human and animal cell lines, the use of BSL-2 infectious pathogens, and zebrafish. To minimize risk and maintain a safe working environment, we will follow the procedures outlined below.

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

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

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

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

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Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: EGFR/Human/signal transducing protein/expression in cell culture/cell culture (human)/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; M-Ras/Human/signal transducing protein/expression in cell culture/cell culture (human)/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; C-Raf/Human/kinase/expression in cell culture/cell culture (human)/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; Shoc2/Human/signaling modulator/expression, knockdown, knockout/human cell/zebrafish cell/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; pLVTHM; PSMC5/Human/protein ubiquitilation/expression, knockdown, knockout/human cell/zebrafish cell/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; pLVTHM; USP7/Human/protein ubiquitilation/expression, knockdown, knockout /human cell/zebrafish cell/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; pLVTHM; VCP/Human/protein degradation/expression in cell culture/cell culture (human)/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; HUWE1/Human/protein ubiquitilation/expression, knockdown, knockout/human cell/zebrafish cell/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; pLVTHM; RFP/jellyfish/protein visualization/expression in cell culture/cell culture (human)/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; GFP/jellyfish/protein visualization/expression in cell culture/cell culture (human)/pcDNA3.1; ptaqRFP; pRSETa; pCMV-FLAG10; Rab5/human/intracellular trafficking protein/expression in cell culture/cell culture (human) GFP, taqRFP; Rab7/human/intracellular trafficking protein/expression in cell culture/cell culture (human) GFP, taqRFP; Rab11/human/intracellular trafficking protein/expression in cell culture/cell culture (human)/GFP, taqRFP; Rab4/human/intracellular trafficking protein/expression in cell culture/cell culture (human)/GFP, taqRFP

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

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

Animal Use: Yes

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

### *Risk Assessment/Discussion:*

Dr. Galperin has submitted a renewal of her IBC protocol entitled Regulation of MAPK activity by EGF receptor endocytosis. Dr. Galperin's laboratory utilizes transient plasmid expression systems in mammalian cells and protein expression in non-pathogenic, lab-strain E. coli. Mammalian cells are transfected with plasmid vectors and utilized for several downstream manipulations including immunofluorescence, microscopy, and Western blot. All work with cells will be done in a Biological Safety Cabinet (BSC) using BSL2 containment. Materials will be inactivated via fixation or lysis prior to working with materials at BSL1 containment. Dr. Galperin's laboratory also utilizes replication-deficient lentivirus vectors for transduction of mammalian cells. Transduced cells will be utilized for the same downstream analysis as previously described, including immunofluorescence, microscopy, and Western blotting. Live cell imaging of lentivirus transduced cells will be done at least 14 days post-transduction or six-cell passages. Lentivirus will be packaged in Dr. Galperin's laboratory using 2nd generation packaging plasmids and will express and/or knock down Shoc2, HUWE1, PSMC5, and USP7. Lentivirus is concentrated via centrifugation using a sealed swinging-bucket rotor. Tubes are only opened in the BSC. Lentivirus work is done using BSL2+ containment. Laboratory members are trained specifically in virus production and will wear dedicated lab coat, gloves, and eye protection. Dr. Galperin's laboratory also utilizes transgenic zebrafish. Mutant zebrafish lines are created by Dr. Galperin's laboratory using CRISPR/Cas9. Embryos are injected at the one-cell stage with Cas9 endonuclease and the guide RNAs. Genes targeted included Shoc2, HUWE1, USP7, and PSMC5. They will also overexpress target genes by administering mRNA to developing zebrafish embryos and knockdown target genes by administering morpholinos. Zebrafish are housed using the Aquarius Fish System,

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which is a self-contained system without any direct output to the water supply. All drains located in fish housing rooms will be fitted with drains as well to prevent escape. All biohazardous waste generated is decontaminated and disposed of according to UK Research Safety guidelines. Dr. Galperin's current IBC protocol will expire on January 5, 2026.

### *IBC Discussion & Vote:*

The protocol IBC-25-123 (version 6.0) was approved pending minor modifications as listed below:

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**GENERAL INFORMATION – Lay Summary:** Please remove reference to “BSL-2 infectious pathogens”. Consider replacing this term with “viral vectors”.

**RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information table:** Please update the “Vector(s)” column to specify which gene constructs will be modified via lentivirus vector.

**DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Animal Transport Description:** Please confirm that DLAR will receive frozen zebrafish carcasses for disposal.

**DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Biohazardous Materials Transport Description:** Please update description to include transport of other materials mentioned (ex. plates for microscopy, cells, etc.)

### **SCIENTIFIC SUMMARY:**

1. The summary states that the default biosafety level is BSL2+ but this is not necessary for all procedures, like plasmid cloning. Please clearly state the biological safety level (BSL) for each distinct procedure. For instance, expressing tagged proteins of interest in lab strain E. coli via plasmid can be safely done using BSL1 containment.
2. What are the “mentioned vectors” in the “Experiments with transient expression in tissue culture cells”. Please be specific.
3. Please clearly identify oncogenes and acknowledge the risk of exposure to oncogenic lentivirus. What additional steps are taken to mitigate this risk?
4. Signage for oncogene usage and viral vector usage should be described in the summary and used in the laboratory.

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

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Conflicts of Interest: None

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**PI: Christopher Norris**

IBC Protocol Number: IBC-25-124

Protocol Title: B22-4043-M: Neurovascular pathology in Alzheimer's disease and vascular cognitive impairment

Protocol Type: Renewal

Amendment To: N/A

Applicable Guidelines & Regulations: NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-3, NIH

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

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

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

### *Brief Project Overview:*

Vascular contributions to cognitive impairment and dementia (VCID) are a major cause of dementia, both alone and co-morbid with Alzheimer's disease (AD) and AD Related Dementias (ADRD). Astrocytic end-feet are physically anchored to the vascular basement membranes via dystroglycan complexes. At the end-feet are potassium and water channels Kir4.1 and aquaporin 4 (AQP4), as well as monocarboxylic acid transporters (MCTs). We have found that cerebral small vessel disease (cSVD) induced by hyperhomocysteinemia (HHcy) resulted in degeneration and dissociation of the astrocytic end-feet and an associated loss of AQP4 and Kir4.1 from the end-feet. This suggests that the end-feet are particularly susceptible to vascular injury and as such may mediate neuronal dysfunction and neurodegeneration associated with dementia. We have observed increased activity of matrix metalloproteinases (MMPs), in particular MMP9, coincident with astrocytic end-foot degeneration. We therefore hypothesize that MMP9 is necessary and sufficient for astrocytic end-foot degeneration and associated neurovascular coupling, and metabolic and ionic dyshomeostasis. We will use live brain imaging (microscopically and whole brain MRI) to track the neurovascular astrocyte changes over time. In order to track these changes we need to use AAV vectors to introduce fluorescent labels into the astrocytes so they can be visualized under the microscope. We also use transgenic animals to increase MMP9 expression in astrocytes to test whether that is sufficient to uncouple the end-feet from the blood vessels, and use MMP9 knockout animals to determine how removal of this gene affects this process.

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

Manipulations Planned: Animal work (breeding, surgeries, etc.), Imaging/Microscopy

Transport: Yes

Materials Transported: Animals, Biohazardous Materials

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: MMP9/mouse/enzymatic/expression in mice/mice/AAV 2/5/;

Cre/unknown/enzymatic/to delete floxed genes/mouse/AAV 2/5/; reverse transactivator/unknown/transcriptional activator/to initiate expression in presence of doxycycline/mouse/AAV 2/5/; EGFP or VIVIT-

EGFP/unknown/fluorescent tracker/to marker astrocytes/mouse/AAV 2/5/; GCaMP 6/8/unknown/fluorescent tracker responsive to Ca<sup>2+</sup>/to track Ca<sup>2+</sup> increases/mouse/AAV 2/5/; mCherry or VIVIT-

mCherry/unknown/fluorescent tracker/to mark astrocytes/mouse/AAV 2/5/

Vector(s) [Vector Category/Vector Technical Name]: Adeno-Associated Virus (AAV)/AAV 2/5 GCaMP6/8; Adeno-Associated Virus (AAV)/AAV 2/5 EGFP or VIVIT-EGFP; Adeno-Associated Virus (AAV)/AAV 2/5 mCherry or VIVIT-mCherry

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

Animal Use: Yes

Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Viral Vector - Adeno-Associated Virus (AAV)/intracranial injection/anesthesia/ABSL1/gloves, labcoat, eye protection/ABSL1/No

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### *Risk Assessment/Discussion:*

Dr. Norris has submitted a renewal of his IBC protocol entitled Neurovascular pathology in Alzheimer's disease and vascular cognitive impairment. This IBC protocol involves human source materials (human blood and CSF) as well as adeno-associated virus (AAV) vectors for intracranial injection to anesthetized mice. The transgenes inserted into AAV are not oncogenic, toxic, or otherwise known to be hazardous and include MMP9, Cre, reverse transactivator, EGFP, VIVIT-EGFP, GCaMP 6/8, mCherry, and VIVIT-mCherry. Mice will undergo two-photon imaging and MRI. AAVs are obtained from a vendor, Biohippo, and not produced in Dr. Norris' laboratory. Work with AAV in animals will be done using ABSL1 containment. Animals will be housed at ABSL1. Personnel will wear lab coat, disposable gloves, and eye protection. The primary risk is accidental needlestick during AAV administration to mice, and this risk is significantly mitigated by ensuring mice are anesthetized during injections. Dr. Norris current IBC protocol will expire on October 18, 2025.

### *IBC Discussion & Vote:*

The protocol IBC-25-124 (version 5.0) was approved pending minor modifications as listed below:

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ANIMAL RESEARCH - IACUC Protocol Number(s): Please review and verify that the IACUC protocol number listed (2022-4065) is the correct IACUC protocol associated with this IBC registration.

ANIMAL RESEARCH - Animals with biohazardous materials: Under the prompt asking if this registration involves the use of biohazardous materials in conjunction with animals, two selections are made. The first indicates this work involves the use of recombinant or synthetic nucleic acids in animals and the other selection states "Not Applicable" which contradicts the first selection. Please unselect the "Not Applicable" box.

### SCIENTIFIC SUMMARY:

1. Please describe how human plasma and CFS will be processed between receipt and ELISA/histological examination. Include whether this is being done in a BSC or on the open bench.
2. Please specify the source of the AAV constructs in use.

\*

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

\*

Conflicts of Interest: None

\*

PI: Ken Fields

IBC Protocol Number: IBC-25-128

Protocol Title: Molecular Pathogenesis of Chlamydia spp.

Protocol Type: Renewal

Amendment To: N/A

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

Guidelines Section III-F-1, NIH Guidelines Section III-F-4, NIH Guidelines Section III-F-5, NIH Guidelines Section

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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, C. Shaffer, D. Harrison

### *Brief Project Overview:*

Species in the Chlamydia genus are important bacterial pathogens that cause disease in humans. Pathogenic species include Chlamydia trachomatis, the agent of sexually transmitted disease and blinding trachoma, and Chlamydia pneumoniae, a common agent of respiratory infections. There are also Chlamydia species that specifically infect small animals and these are commonly used to model human disease. Species include Chlamydia muridarum which infects mice and Chlamydia caviae which is specific for guinea pigs. All chlamydial species are obligate intracellular and can only grow within mammalian cells. Survival is dependent on secretion of anti-host proteins termed “effectors” that manipulate the host cell to allow chlamydial growth and evasion of productive immune responses. The goal of this project is to understand how chlamydial effector proteins facilitate chlamydial survival. Since these effectors are required for full virulence, our overall goal is to establish how they function during infection. We therefore propose to explore the cellular microbiology of Chlamydia. This will be accomplished by performing experiments using both tissue-culture infection and murine infection models.

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

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

Transport: Yes

Materials Transported: Biohazardous Materials

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

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: tmeA (CT694)+E33A44B4:E32B4:E35B4:E33B4:EB4:E33/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; tmeB (CT695)/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; trpA/tryptophan biosynthesis/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; cdu1/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; cdu2/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; lda1/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; lda2/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; lda3/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; tsp/putative effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; cpaf/putative effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; tepP/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; tarP/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1,

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pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ChlaDub1/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ChlaDub2/secreted effector/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; scc1/chaperone/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; copN/regulatory protein/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 mycBioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; cdsF/secretion apparatus/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; copB/secretion apparatus/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; cdsD/secretion apparatus/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; cdsU/secretion apparatus/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct053/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct847/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct711/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct875/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct620/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct621/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct622/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct161/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct338/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct429/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct105/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct142/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct696/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct421/unknown function/expression/Chlamydia/HeLa cells//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC/; ct166/putative glycosyltransferase/expression/Chlamydia/HeLa cells/E.coli/pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC, pET-15b/; tc0437/putative glycosyltransferase/expression/Chlamydia/HeLa cells/E.coli//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC, pET-15b/; tc0438/putative glycosyltransferase/expression/Chlamydia/HeLa cells/E.coli//pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC, pET-15b/; tc0439/putative glycosyltransferase/expression/Chlamydia/HeLa cells/E.coli/pCompAll, pEGFP-C3, pmCherry-C1, pcDNA3.1 myc-BioID, p3X-Flag-CMV-10, pBOMB4-tet-mCherry, pFlag-CTC, pET-15b/; gfp/Clontech/expression/HeLa, Chlamydia/; mcherry/clontech/expression/HeLa, Chlamydia/; birA/E. coli/proximity labeling/HeLa/;

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ahnak/ATCC/structure/functionstudies/HeLa/; C9 transposase/Ian Clarke/random mutagenesis/Chlamydia Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pCompAll/; Plasmid/p3X-Flag-CMV-10/; Plasmid/pEGFP-C3/; Plasmid/pGFP-SW2/; Plasmid/pSUMC-bla-gfp-sbfl/; Plasmid/pcDNA3.1 myc-BiOD/; Plasmid/pBOMB4-tet-mCherry/; Plasmid/pFlag-CTC/; Plasmid/pmCherry-C1/; Plasmid/pSU-CRE/; Plasmid/pSW2GFPNiggCDS2/; Plasmid/pET-15b/; Plasmid/pKW-RiboJ-CRE/; Plasmid/pKW-Spec-RiboJ-ccdB/; Plasmid/pTnQ-GFP-Spec/; Plasmid/pBOMB-sRNA/; Plasmid/pSUMC/; Plasmid/pSUMC-CTX/; Plasmid/pSUMC-4.0/; Plasmid/pSU-GFPNigg/; Plasmid/pSU-SW2GFPNiggCDS2/; Plasmid/pKW-CMori/; Plasmid/pKW-L2ori/; Plasmid/pOri-Tn

Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HeLa 229; Human/A2EN; Human/HEK293; Human/A549; Animal/McCoy; Animal/RAW264.7

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/Chlamydia muridarum (RG1-bacteria)/Intravaginally/Anesthesia/ABSL2/Lab coat, disposable gloves, eye protection/ABSL2/Yes/; Mouse/Chlamydia trachomatis (RG2-bacteria)/Intravaginally/Anesthesia/ABSL2/Lab coat, disposable gloves, eye protection/ABSL2/Yes

### *Risk Assessment/Discussion:*

Dr. Fields has submitted a renewal of his IBC protocol entitled Molecular Pathogenesis of Chlamydia spp. Dr. Fields' laboratory utilizes pathogenic Chlamydia species to better understand how chlamydial effector proteins facilitate chlamydia survival. To that end, chlamydia spp. are propagated in eukaryotic cells within a Biological Safety Cabinet (BSC). Eukaryotic cells are transfected with plasmid DNA harvested from nonpathogenic, lab-strain E. coli to express tracking genes (GFP, mCherry), mammalian Ahnak, and chlamydial genes CT694 and CT695. Eukaryotic cells are also transfected with siRNA obtained from Origene Technologies targeting human and murine Ahnak. Protein and nucleic acids will be isolated from infected cells for downstream analysis. Once protein is denatured, materials are no longer considered infectious and can be safely handled at BSL1 containment. Cells are cultured and infected using BSL2 containment. Dr. Fields' laboratory also seeks to transform C. trachomatis or C. muridarum via chemical transformation of plasmid DNA. The genes to be overexpressed are all components of the C. trachomatis type III secretion system or are necessary for chlamydial growth. Overexpression of these endogenous genes is not expected to alter pathogenicity or host range of the Chlamydia spp. being transformed. Gene knock outs will also be created. Several chlamydial genes are listed for gene knock out targets and correspond to type III or type II secretion mechanisms. It is expected that knocking out these important genes will result in mutant strains that are less pathogenic or require a higher infectious dose. Infected and uninfected cultures will be viewed via light microscopy. Vessels remain closed during microscopy and are viewed on a microscope located in the same space where they are incubated, negating the need for transport of infectious materials. Alternatively, cultures are fixed via methanol or 4% paraformaldehyde for immunofluorescence microscopy. They will also utilize Listeria monocytogenes (RG2 bacteria) obtained from a UK collaborator to test whether ectopic expression of chlamydial CT695 in HeLa cells interferes with the ability of L. monocytogenes to move by actin-based polymerization. Tissue culture cells will be infected with L. monocytogenes in a BSC, fixed with paraformaldehyde, and processed for immunofluorescence. Dr. Fields' laboratory also utilizes an animal infection model. In this work, mice are infected intravaginally with wild-type C. muridarum or C. trachomatis. Mice are anesthetized during infections, and all infections are carried out inside a BSC under BSL2 containment. Infected animals are housed at ABSL2 housing. At the conclusion of animal experiments, gross pathology will be utilized to look for evidence of infection in reproductive tracts of mice. Carcasses and all biohazardous waste materials will be decontaminated and disposed of according to UK Research Safety guidelines. Chlamydia trachomatis is a RG2 bacterial pathogen and causal agent of sexually transmitted disease and blinding trachoma.

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*Chlamydia pneumoniae*, RG2 bacterial pathogen, is a common cause of respiratory infections. *Chlamydia muridarum* is a RG1 bacterial pathogen of mice and hamsters, and *Chlamydia caviae* is a RG1 bacterial pathogen of guinea pigs. Neither *C. muridarum* or *C. caviae* are known to cause infections in humans. The primary risks associated with this IBC protocol include exposure to the human pathogens *C. trachomatis* or *C. muridarum*, or exposure to human source materials (in this case, cells) that may unknowingly harbor blood borne pathogens. These risks are mitigated using BSL2 containment, including use of BSC, proper PPE including lab coat, disposable gloves, and eye protection, use of anesthesia for animal infections, and appropriate waste handling. Dr. Fields' current IBC protocol will expire on November 9, 2025.

### *IBC Discussion & Vote:*

The protocol IBC-25-128 (version 8.0) was approved pending minor modifications as listed below:

\*

#### ANIMAL RESEARCH:

1. IACUC 2019-3222 states that "Experiments will be performed using intravaginal or trans-cervical infection with *C. muridarum*." However, the transcervical route of administration is not listed in the Animals with Biohazards table. Please update the Animals with Biohazards table for congruency.
2. If *Chlamydia* strains administered to animals are genetically modified, please select "My work involves experiments with RG2 recombinant or synthetic nucleic acid modified microbes in animals (transgenic or otherwise)" and/or "My work involves experiments with recombinant or synthetic nucleic acid modified animal pathogens in animals (transgenic or otherwise)", as applicable.

SCIENTIFIC SUMMARY: The protocol states "all manipulations involving only the bacteria will be handled on open benches" in reference to *Listeria monocytogenes*. *L. monocytogenes* is a RG2 bacterial pathogen, and any activities that may produce aerosols should be conducted within a Biological Safety Cabinet (BSC). Please update accordingly.

NIH GUIDELINES AND OTHER APPLICABLE REGULATIONS: If genetically modified *Chlamydia* is being administered to animals, NIH Guidelines Section III-D-4 will need to be selected.

\*

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

\*

Conflicts of Interest: None

\*

## New Business

NIH Office of Science Policy Update – Modernizing and Strengthening Oversight of Biosafety

<https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy#tab2/>

D. Mazzetti introduced the recent announcement from NIH Office of Science Policy and will update IBC members when the Region 2 listening session has been scheduled.

## Incident Review

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

Nothing to report.

## 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).*

Nelson, Peter, Alzheimer's Disease Research Center Neuropathology and Biomarker research to advance the diagnosis and treatment of Alzheimer's and related dementia's., Amendment, BSO, IBC-25-27 (v.23.0), 10/1/2025

Liu, Xiaoqi, Enhancing the efficacy of prostate cancer therapy, Amendment, BSO, IBC-24-355 (v.45.0), 9/29/2025

Conley, Caitlin, Orthopedic Biomarker Repository, Renewal, BSO, IBC-25-87 (v.6.0), 9/26/2025

Bieberich, Erhard, Function of sphingolipid and associated factors in physiology and disease, Amendment, BSO, IBC-24-346 (v.17.0), 9/25/2025

Zambuto, Samantha, Reproductive tissue engineering, Amendment, BSO, IBC-25-11 (v.20.0), 9/25/2025

Gandhapudi, Siva, Pre-clinical studies of viral and cancer vaccines in mice, Amendment, BSO, IBC-24-443 (v.33.0), 9/24/2025

Lee, Daniel, Modulation of Arginine Metabolism and Polyamines to Mitigate Alzheimer's Disease Pathology, Amendment, BSO, IBC-24-60 (v.21.0), 9/23/2025

Pandya, Chirayukumar, Role of inflammation and oxidative stress in animal and cellular models of ischemic stroke, Amendment, BSO, IBC-24-506 (v.15.0), 9/22/2025

Tsodikov, Oleg, B23-4165: Biochemical and structural studies of enzymes and DNA binding proteins, Amendment, BSO, IBC-24-62 (v.19.0), 9/22/2025

Dutch, Rebecca, Purification of Viral Vectors with Electrodialysis Using Ultrafiltration Membranes, Amendment, BSO, IBC-24-398 (v.31.0), 9/22/2025

Pinto, Amelia, Risk group two virus protocol, Amendment, BSO, IBC-24-83 (v.43.0), 9/22/2025

Weisleder, Noah, Modifying gene expression in vitro and in vivo, Amendment, BSO, IBC-24-499 (v.50.0), 9/22/2025

Iragavarapu, Chait, JNJ-90014496: A Phase 1b Multicenter, Open-label, Study of JNJ-90014496, an Autologous CD19/CD20 Bi-specific CAR-T Cell Therapy in Adult Participants with B-cell Non-Hodgkin Lymphoma, Amendment, BSO, IBC-25-35 (v.14.0), 9/18/2025

Fields, Ken, B22-4074-M3: Molecular Pathogenesis of Chlamydia spp., Amendment, BSO, IBC-24-322 (v.17.0), 9/17/2025

Mishra, Ila, Small peptide hormones in metabolic disorders, Amendment, BSO, IBC-24-89 (v.36.0), 9/12/2025

Chakravarti, Ritu, Immune Mechanisms of Autoimmune Diseases, Amendment, BSO, IBC-24-522 (v.39.0), 9/12/2025

Dupont-Versteegden, Esther, Skeletal muscle atrophy and frailty with aging, Amendment, BSO, IBC-24-497 (v.16.0), 9/11/2025

Zuidema, Jonathan, Synthetic nucleic acid molecules delivery and incorporation into biomaterials, Amendment, BSO, IBC-25-56 (v.18.0), 9/11/2025

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Schardl, Christopher, Alkaloid biosynthesis genes of grass endophytes, Amendment, BSO, IBC-24-392 (v.14.0), 9/11/2025

Long, Alexandra, Mechanisms Underlying Cellular Remodeling Using Chytrid Fungi as an Evolutionary Cell Biology, Amendment, BSO, IBC-25-07 (v.17.0), 9/11/2025

Radka, Christopher, Bacterial lipid immune modulators, Amendment, BSO, IBC-24-56 (v.29.0), 9/11/2025

Tian, Changhai, Intra- and Inter-organ communications by extracellular vesicles in the pathogenesis of cardiovascular and neurodegenerative disorders, Amendment, BSO, IBC-24-402 (v.28.0), 9/11/2025

Zhu, Qingzhang, Adipocyte function in metabolism, inflammation and fibrosis during obesity, Amendment, BSO, IBC-24-493 (v.26.0), 9/11/2025

Spry, Malinda, Thomas Hunt Morgan Biology Department Teaching Labs, Amendment, BSO, IBC-25-65 (v.22.0), 9/10/2025

Spry, Malinda, Biology Teaching Labs-Microbiology BIO209 and BIO309, Amendment, BSO, IBC-25-69 (v.30.0), 9/10/2025

Cormier, Lindsay, Chemotherapeutic development towards cancer, Amendment, BSO, IBC-25-45 (v.16.0), 9/4/2025

### Protocols Meeting Registration Requirements

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

Park, Jonghyuck, Reprogramming Neuroimmune Responses for Functional Regeneration after Spinal Cord Injury; Developing immunotherapeutic nanoparticles for spinal cord injury; Enhancing the Therapeutic Potential of Nanoparticle-Mediated Immunotherapeutics for Spinal Trauma, Renewal, IBC, IBC-25-106 (v.12.0), 9/25/2025

Logeman, Brandon, Sex and physiological State dependent molecular characterization of circuits gov, Amendment, IBC, IBC-25-99 (v.26.0), 9/23/2025

Chattopadhyay, Saurabh, Innate immune responses to virus infection, Amendment, IBC, IBC-24-108 (v.51.0), 9/22/2025

Wang, Wangxia, Mitochondrial Function and microRNA Regulation in Traumatic Brain Injury and Alzheimer's Disease, Amendment, IBC, IBC-24-63 (v.41.0), 9/16/2025

O'Connor, Kathleen, Integrin regulation of carcinoma invasion, Renewal, IBC, IBC-25-90 (v.14.0), 9/11/2025

Gao, Tianyan, Studies of protein phosphatases and mitochondrial metabolism in colorectal cancer, Renewal, IBC, IBC-25-100 (v.14.0), 9/11/2025

Garcia, Erin, Mechanisms of Competition and Cooperation in Burkholderia species, Amendment, IBC, IBC-24-25 (v.25.0), 9/11/2025

Kikani, Chintan, B22-3448: Epigenetic control of muscle stem cell function by PASK-Wdr5 signaling, Amendment, IBC, IBC-24-27 (v.18.0), 9/11/2025

Voss, Stephen Randal, Salamander Genome Project, Amendment, IBC, IBC-24-441 (v.21.0), 9/10/2025

Kempinski, Chase, Yeast engineering for terpene production, Renewal, IBC, IBC-25-101 (v.7.0), 9/8/2025

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## IBC Training

Committee members were reminded to complete IBC Member training on SciShield. D. Mazzetti will send out individual reminders via email to members as needed.

## Adjournment

Douglas Harrison initiated a motion to adjourn the meeting at 1:28pm. Thomas Chambers seconded the motion. All IBC members present (13) voted in favor of the motion.

APPROVED