Quorum: 7

IBC MEETING COMMENTS

June 4, 2025 – Zoom Conference Call

Attendance – IBC Committee			
Present	Name	Expertise	Role
	Paul Gulig	Microbiology	Member
x	Luis Martinez	Microbiology	Member
x	Jason Clements		Member
x	Norman Beatty	Infectious Diseases	Member – arrived 1:20pm
x	Steeve Boulant	Virology	Member
x	Sanford L. Boye	Virology	Member
	Amber Duren		Community Member
x	Mariola Ferraro	Microbiology	Member
x	Dean Gabriel	Plants	Member
x	Gary Heil		Member
	Michael McIntosh	Virology	Member
	John D. McVay	Plants	Community Member
	Mark Moehle	Microbiology	Member
x	Kamal A. Mohammed	Microbiology	Member
x	Christopher Overend		Member
x	[redacted]	Animals	Member
	Elias J. Sayour	Clinical Trials	Member
x	Clay Smith	Virology	Chair
	Daniel R. Swale	Insects	Member
	Amy Vittor	Infectious Diseases	Member

Attendance – Staff and Guests		
Present	Name	Affiliation/Position
X	[redacted]	PI Guest
X	Alek Aranyos	UF EHS
X	Anna Gioseffi	UF EHS
X	Artiom Chacon	UF EHS
X	Asha Rani	UF EHS
X	Craig Moneypenny	UF EHS
X	Jennifer Jackson	UF EHS
X	Kendra Rooks	UF IACUC
X	Kindra Kelly-Quagliana	UF EHS
X	Laura Castillo	UF EHS
X	Laurence Prunetti	UF EHS
X	Pratibha Srivastava	UF EHS
X	Raies Mir	UF EHS
X	Erica Gonzaga	UF EHS Arrived 1:32 pm

Agenda:

	Full Committee Projects	PI
1	ANGPTL4 in cancer	Ryan Kolb
2	Investigation of Determinants of Clostridium difficile Colonization	Gary Wang - Renewal
3	Monitoring mRNA translation fidelity by AAV	[redacted]
4	Optical imaging of rodent brain slice	[redacted]
5	Molecular characterization of Burkholderia species: Genetic, Genomic , and Phenotypic analyses	[redacted]
6	Genetic determinants of Francisella pathogenicity	Aria Eshraghi - Renewal
	Amendments	**PI**
1	Probiotics-mediated oral delivery of Angiotensin-(1-7)/ACE2 as a strategy for retinal neuroprotection	Qiuhong Li BIO6053
2	Effect of neuroinflammation in mouse models of neurodegenerative diseases (it is the renewal/continuation of RD-4529).	[redacted] BIO6317
3	Targeting of biomolecular condensates with synthetic polymers	Amal Narayanan BIO7384

4	Exosome delivery of CRISPR-CAS9	[redacted] BIO5824

Minutes

Meeting was called to order at: 1:01pm

Project Review

Principal Investigator: [redacted]

Project Title: ANGPTL4 in cancer

BIO number not yet assigned.

Vector/Agent(s) to be used: Lentivirus, third-generation (Gag/Pol and Rev are expressed in two separate helper plasmids)

Name and Function of Transcribed Nucleic Acids: ANGPTL4 gRNA; Crispr Cas9 gene editing to delete ANGPTL4. ECSCR gRNA; CRISPR-Cas9 gene editing to delete ECSCR. FGFR1 gRNA; CRISPR-Cas9 gene editing to delete FGFR1. LIPA gRNA; Crispr Cas9 gene editing to delete LIPA.

Host(s) to be used: LentiMax 293T, CAKi-1, 786O, 786P, RCC-GS, RCC-ES cell lines. Immortalized human preadipocytes, rodents.

NIH Guidelines: III-D-3-a for packaging lentivirus in the lab. III-D-4-a for injecting transduced human cell lines into whole animals. III-E-1 for transduction of cell lines with lentivirus

Biosafety Level and Any Additional Requirements: BSL-2+ for lentiviral packaging, work involving human cell culture, and CRISPR mediated cell editing. ABSL-2 for injecting human cell lines into rodents, with subsequent ABSL-1 with precautions for human cells housing. Special considerations include substitution of glassware for plasticware when possible, mucous membrane protection, annual bloodborne pathogens training, the use of safe sharps, and wiping of the injection site.

Training: All training completed

Approval: All approved as recommended (Yes-11, No-0, Abstain-0)

Principal Investigator: Gary Wang - Renewal

Project Title: Investigation of Determinants of *Clostridium difficile* Colonization

BIO number not yet assigned.

Approval: **TABLED and then removed – No longer falls under IBC as recombinant materials are removed**

Principal Investigator: [redacted]

Project Title: Monitoring mRNA translation fidelity by AAV

BIO number not yet assigned.

Vector/Agent(s) to be used: Adeno-associated virus (AAV)

Name and Function of Transcribed Nucleic Acids: Firefly luciferase, Nano luciferase; Monitoring mRNA translation fidelity. GFP; Control for AAV delivery.

Host(s) to be used: Rodent model, Cultured cells (mouse embryonic stem cells, HEK cells, MEF cells)

NIH Guidelines: III-D-4-aExperiments Involving Whole Animals. III-E-1 Experiments Involving the Formation of Recombinant or Synthetic Nucleic Acid Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus (strictly *in vitro*). III-F-1 Experiments involving synthetic nucleic acids that are 1) non-replicative, 2) do not contain an origin of replication, 3) do not contain elements known to interact with either DNA or RNA polymerase, 4) do not integrate in DNA, and 5) do not produce a toxin with LD50 < 100 Nanograms/Kilogram Body Weight. III-F-8 C-I Experiments involving recombinant DNA molecules that do not present a significant risk to health, or the environment as determined by the NIH Director, with advice of the RAC, and following appropriate public notice.

Biosafety Level and Any Additional Requirements: BSL-1 for work with mouse cell lines, established bacterial lines, and plasmid vectors. BSL-2 for work, with human cell lines. ABSL-1 for AAV injections into rodents with subsequent housing at ABSL-1i Bloodborne pathogens training will be required for working with human cell lines.

Training: All training completed

Approval: **Conditional approval based on information of route of administration in mouse work** (Yes-12, No-0, Abstain-0)

Principal Investigator: [redacted]

Project Title: Optical imaging of rodent brain slice

BIO number not yet assigned.

Vector/Agent(s) to be used: Adeno-associated virus (AAV)

Name and Function of Transcribed Nucleic Acids: Cheriff-CFP; Cheriff is a blue-light-activated ion channel, which is tagged with a labeling protein CFP. SomQuasAr6a_EGFP-P2A-somCheRiff_HA; fluorescent voltage sensor, which is tagged with labeling protein EGFP; Cheriff is a blue-light-activated ion channel. SomQuasAr6a_EGFP; fluorescent voltage sensor, which is tagged with labeling protein EGFP. Cre; an enzyme that catalyzes site-specific recombination between DNA sequences, specifically at specific DNA recognition sites called loxP sites.

Host(s) to be used: HEK293T cells, rodents

NIH Guidelines: III-D-4-a for the administration of AAV vectors into whole animals. III-F-8, Appendix C-I for the transduction of HEK293.

Biosafety Level and Any Additional Requirements: For this study, BSL-1/BSL-2/ABSL-1/ABSL-1+ containment and practices are appropriate. BSL-1 containment and practices for handling the AAV. BSL-2 containment and practices are recommended for work involving the Human Cell Lines (HEK 293T). ABSL-1+ (addition of safe engineered sharps, mucous membrane protection substitution of plastic for glassware and wiping of the injection site) for the injection of AAV into rodents with subsequent ABSL-1 for housing.

Training: All training completed

Approval: All approved as recommended (Yes-12, No-0, Abstain-0)

Principal Investigator: Apichai Tuanyok

Project Title: Molecular characterization of *Burkholderia* species: Genetic, Genomic, and Phenotypic analyses

BIO number not yet assigned.

Vector/Agent(s) to be used: *Burkholderia pseudomallei.* Plasmids.

Name and Function of Transcribed Nucleic Acids: antigenic genes: ompA, hcp1, tssM; virulence factor and major antigens. penA; beta-lactamase enzyme.

Host(s) to be used: cloning *E. coli* BL21; *Burkholderia pseudomallei* strains Bp82 and 576mn.

NIH Guidelines:

- Section III-D-1-b for cloning of recombinant/synthetic nucleic acids into *B. pseudomallei* (SA-exempt strains). Note that the NIH has previously given approval to reduce containment for *B. pseudomallei* strains Bp82 and 576mn from BSL-3 to BSL-2 (Documented by attachment of the approval email from NIH to the project registration.
- Section III-D-2-a for cloning of *B. pseudomallei* genes into E. coli BL21.
- Section III-D-4-a for use of *Burkholderia pseudomallei* outer membrane vesicles (OMVs) in *invertebrates*.
- Sections III-F-1/III-F-3/III-F-4/III-F-8 for manipulation of extracted nucleic acids and other extracted cellular derivatives, including OMVs, and phage stocks.

Biosafety Level and Any Additional Requirements:

- BSL-2 practices and containment for all work with risk group 2/3 agents, handling imported soil samples and cultures derived therefrom, cloning of *E. coli* BL21 with *Burkholderia* nucleic acids, and handling of nucleic acids extracted from select agent *Burkholderia* strains.
- ACL-1 practices and containment for *Galleria mellonella* exposed to OMVs.
- BSL-1 practices and containment for all other experimental procedures.
- Bloodborne pathogens training required for handling of human sera.
- *Burkholderia pseudomallei* and *mallei* (regardless of strain) are subject to export control regulations, including nucleic acids.
- Any positive or suspect identification of *Burkholderia pseudomallei* from imported soils must be reported to the Federal Select Agent Program within 24 hours with completion of APHIS/CDC Form 4 within seven days.
- All nucleic acids or other extracts derived from select agent strains of *Burkholderia mallei* or *pseudomallei* must be accompanied by a signed Certificate of Inactivation per facility select agent policies and procedures. Any nucleic acids received from collaborators should also be accompanied by a Certificate of Inactivation.

Training: All training completed

Approval: Conditional approval due to detail corrections needed (no infectious waste, no rodent work, add engineering controls). If rodent work is in scope, project modifications will be required with IBC re-review. (Yes-12, No-0, Abstain-0)

Principal Investigator: [redacted] - Renewal

Project Title: Genetic determinants of Francisella pathogenicity

BIO number not yet assigned.

Vector/Agent(s) to be used: *Francisella novicida; Francisella tularensis* subspecies *holarctica* LVS;

Name and Function of Transcribed Nucleic Acids: opiA, opiB.1, opiB.2, opiB.3, pdpA, pdpB, iglE, vgrG, iglF, iglG, iglH, dotU, iglI, iglJ, pdpC, pdpE, iglD, iglC, iglB, iglA, pdpD, anmK. These genes comprise a secretion system and the proteins that are known to be secreted through it. nuoM; NuoM; a component of the electron transport chain and serves to catalyze the transport of a proton across a membrane with the hydrolysis of one molecule of NADH. OsrR; a transcriptional regulator that plays a role in resistance to oxidative stress. tul4 encodes an uncharacterized lipoprotein in *F. novicida* that has previously been shown to induce protectiion against subsequent challenge by *F. novicida*.

Host(s) to be used: *E. coli* BL21; *Salmonella Typhimurium* (self-lysing/self-limiting PIESV)

NIH Guidelines:

- III-D-1-a for the use of RG-2 agents, Federal Select Agent exempt strains of *Francisella tularensis*, as host-vector systems
- III-D-2-a for cloning DNA from RG-2 agents, Federal Select Agent exempt strains of *Francisella tularensis*, into non-pathogenic prokaryotic or lower eukaryotic host-vector systems)
- III-D-3-a for packaging of lentiviral/
- III-D-4-a for experiments involving whole animals
- III-E-1 for the use of recombinant nucleic acids in non-pathogenic, yet not K-12 lineage, BL21 *E. coli*
- III-F-8 Appendix C-II for plasmid cloning in exempt *E. coli* strains (DH5-alpha).

Biosafety Level and Any Additional Requirements:

- ABSL-2 for infecting rodents with Salmonella vaccines.
- BSL-1 for molecular cloning and BL-21 protein expression analyses
- BSL-2 for experiments involving *Francisella tularensis* subspecies *novicida* or *Francisella tularensis* subspecies *holarctica* strain LVS and mutans strains
- BSL-2+ for experiments involving retroviral/lentiviral vectors. Extra (+) precautions emphasize the use of mucous membrane protection, the use of safe sharps, and the substitution of glassware with plasticware, initial and annual BBP training.

Training: All training completed

Approval: All approved as recommended (Yes-11, No-0, Abstain-0)

Amendments

Principal Investigator: Qiuhong Li

Title: Probiotics-mediated oral delivery of Angiotensin-(1-7)/ACE2 as a strategy for retinal neuroprotection

BIO6053

Summary: Addition of a new plasmid. These changes do not affect the previously approved safety and containment practices: BSL-1 for propagation, storage, and cloning in K-12 *E. coli* and RG1 bacteria (*Lactobacillus paracasei*). ABSL-1-i for oral gavage of modified bacteria with potential for shedding, followed by inactivation of bedding 72 hours post administration and transferring animals to ABSL-1 housing.

These changes also do not affect the applicable NIH guidelines: III-D-4-a for the administration of recombinantly modified bacteria to whole animals. III-F-8, C-II, for propagation and storage of plasmids in K-12 *E. coli* strains

Training: All training completed

Approval: Conditional upon certification of BSC date, no work with biohazards until certification date given (Technical difficulties lead to a “vote by hand”) (Yes-11, No-0, Abstain-0)

Principal Investigator: [redacted]

Title: Effect of neuroinflammation in mouse models of neurodegenerative diseases (it is the renewal/continuation of RD-4529).

BIO6317

Summary: New plasmids and AAV constructs are being added. Due to the number of plasmids and AAVs, they have been added to the registration as attachments. These changes do not affect the previously approved safety and containment practices: BSL-1 for *in vitro* studies involving AAVs and K-12 *E. coli*. BSL-2 for *in vitro* work with human cell cultures (HEK293T). ABSL-1+ for AAV injections in rodents and subsequent ABSL-1 housing.

Special considerations include the use of safe sharps, wiping of the injection site, mucous membrane protection, substitution of plastic for glassware, and annual bloodborne pathogens training. These changes also do not affect previously approved NIH guidelines: III-D-3-e for packaging AAVs in the lab. III-D-4-a for AAV *in vivo* studies in whole animals. III-F-8, C-II for storage and propagation of plasmids in K-12 *E. coli.*

Training: All training completed

Approval: All approved as recommended (Yes-12, No-0, Abstain-0)

Principal Investigator: Amal Narayanan

Title: Targeting of biomolecular condensates with synthetic polymers

BIO7384

Summary: Two new bacterial strains and a new plasmid are added. These changes do not affect the previously approved safety and containment practices: BSL-2 for the generation of Lentiviral vectors and the use of human cell lines. BSL-1 for the work with *E. coli (Migula) Castellani* and *Chalmers, Bacillus subtilis*, and exempt K-12 strains of *E. coli* for maintaining plasmid stocks. Special considerations include annual bloodborne pathogen training, mucous membrane protection, and the use of safe sharps. Please note that the VSV used in producing lentivirus is subject to export control.

These changes also do not affect the previously approved NIH guidelines: III-D-3-a for the production of lentiviral vectors. III-E-1 for the use of lentiviral vectors *in vitro* cell culture (transfection/transduction). III-F-8, appendix C-II for cloning using K-12 lineage *E. coli*. III-E is added for the use of a non-K-12 *E. coli* strain (*E. coli (Migula) Castellani* and *Chalmers*).

Training: All training completed

Approval: All approved as recommended (Yes-12, No-0, Abstain-0)

Principal Investigator: [redacted]

Title: Exosome delivery of CRISPR-CAS9

BIO5824

Summary: Personnel changes and the addition of human stem cells, a new siRNA, and mRNA for *in vitro* production of exosomes. Additionally, the lab is adding *in vivo* experiments in rodents to their work.

These changes do not affect the previously approved safety and containment practices: BSL-1 for in vitro manipulation of CRISPR-Cas9 in rodent cell lines.

The following safety and containment practices are added for the new work being conducted: BSL-2 for culturing human stem cell lines and in vitro manipulation of CRISPR-Cas9 in human cell lines, which requires annual bloodborne pathogens training. ABSL-2+ for the injection of human or mouse exosomes into rodents, with subsequent ABSL-1+Hu housing. The '+' designates the following special considerations: Use of safe sharps, wiping of the injection site, use of BSC for any aerosol generating procedures, mucous membrane protection.

These changes affect the applicable NIH guidelines as follows: III-D-4-a is added for *in vivo* experiments in rodents. III-E for the use of Cas9 technology in mammalian cells remains unchanged.

Training: All training completed

Approval: All approved as recommended (Yes-12, No-0, Abstain-0)

The meeting adjourned at 1:52 pm