Quorum: 7

IBC MEETING COMMENTS

July 16, 2025 – Zoom Conference Call

Attendance – IBC Committee			
Present	Name	Expertise	Role
	Paul Gulig	Microbiology	Member
x	Luis Martinez	Microbiology	Member
	Jason Clements		Member
x	Norman Beatty	Infectious Diseases	Member
	Steeve Boulant	Virology	Member
x	[redacted]	Virology	Member
	Amber Duren		Community Member
x	Mariola Ferraro	Microbiology	Member
	Gary Heil		Member
x	Michael McIntosh	Virology	Member
x	John D. McVay	Plants	Community Member
x	Mark Moehle	Plants	Member Left at 1:36pm
	Kamal A. Mohammed	Microbiology	Member
x	Christopher Overend	Microbiology	Member
x	[redacted]	Animals	Member
x	Jeffrey Rollins	Plants	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	Artiom Chacon	UF EHS
x	Kindra Kelly-Quagliana	UF EHS
x	Jennifer Jackson	UF EHS
x	Laura Castillo	UF EHS
x	Savannah Hardiman	UF EHS
x	Asha Rani	UF EHS
x	Dean Gabriel	IBC Guest
x	Craig Moneypenny	UF EHS
x	Raies Mir	UF EHS
x	Pratibha Srivastava	UF EHS
x	Anna Gioseffi	UF EHS
x	[redacted]	PI Guest – left at 1:12pm
x	Kevin Ross	UF EHS

Agenda:

	Full Committee Projects	PI
1	Using transgenics to examine genes that affect traits important to the improvement of sweet corn.	Marcio Resende
2	study of cancer-related genes	Jianrong Lu
3	Use and handling of genetically modified cell lines in non-invasive nanotechnology-enabled imaging	[redacted] (BIO5637 renewal)
4	AAV-mediated retinal gene therapy	[redacted] (BIO5626 renewal)
5	Assessing tau-related changes in vitro	[redacted] (BIO5539 renewal)
	Amendments	**PI**
1	Genomic/Genetic Engineering of Acute Myeloid Leukemia (AML) Cells to Elucidate Pharmacogenomic Impact on Response to Chemotherapeutic and Immunotherapeutic Agents used in AML Therapy	Jatinder Lamba BIO6651
2	Diagnostics and therapeutic/prophylactic vaccines for feline immunodeficiency virus (FIV), HIV-1, feline coronavirus (FCoV), and SARS-CoV-2 (SCoV2)	[redacted] BIO6490
3	Molecular characterization of Burkholderia species: Genetic, Genomic , and Phenotypic analyses	[redacted] BIO7515

4	Cellular therapies for brain tumors	[redacted] BIO6688

Minutes

Meeting was called to order at: 1:03pm

Project Review

Principal Investigator: Marcio Resende

Project Title: Using transgenics to examine genes that affect traits important to the improvement of sweet corn.

Transcription of RD-4775. BIO number not yet assigned

Vector/Agent(s) to be used: Plasmids

Name and Function of Transcribed Nucleic Acids: Various coding sequences, synthetic DNA and maize genes; encode a trehalose metabolic enzyme. GFP from *Aequorea victoria* - green fluorescent protein, an auto-fluorescent protein used for visual selection; fluoresces green to indicate presence of gene.

Host(s) to be used: *E. coli* - PI does not know the strain as the transformations are not carried out in the lab. *Agrobacterium tumefaciens* - PI does not know the strain as the transformations are not carried out in the lab. *Zea mays*- - either B104 or Hi II

NIH Guidelines: III-E-2-a for experiments involving genetically modified plants (Maize).

Biosafety Level and Any Additional Requirements: BSL-1/PBSL-1 practices and containment.

In accordance with the USDA-BRS Permit under 7CFR 340 stipulations, the permitted material must be used in accordance with its condition requirements. Please ensure full compliance with all record-keeping, monitoring, and reporting requirements for the transgenic plants involved in this project. In each area where the regulated material is used or stored, you must clearly post signage stating "Authorized Personnel Only" on the door or an adjacent wall. Prior to removing regulated materials/organisms from the authorized containment facility, all items in direct contact with or exposed to those materials/organisms must be decontaminated according to the permit.

Concerns or Discussions: None

Training: All training completed.

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

Principal Investigator: Jianrong Lu

Project Title: study of cancer-related genes

BIO number not yet assigned

Vector/Agent(s) to be used: Lentivirus, second-generation (Gag/Pol/Rev expressed in a single helper plasmid). Adeno-associated virus (AAV).

Name and Function of Transcribed Nucleic Acids: Various lentiviral vectors expressing shRNAs to deplete expression. YAP; transcriptional coactivator and enhance expression of specific target genes. VSIR; an immune checkpoint protein. VSTM2L encodes a putative immune checkpoint protein. WWC1 is an upstream activator of the Hippo signaling pathway. CTCF is a transcription factor mediating chromatin looping and domain formation.

GSDMD causes cell death (pyroptosis). MLKL (active form) causes cell death (necroptosis). GFP encodes a green fluorescent protein.

Host(s) to be used: bacteria *E.coli* DH5a, BL21 packaging cell line HEK293T (human) human cancer cell lines

NIH Guidelines: III-D-3-a for the packaging and production of Lentiviral vectors using HEK293T cells, III-E-1 for the use of Lentiviral vectors *in vitro* cell culture and also for the use of non-exempt BL21 *E. coli* strain and also III-F-8 Appendix C-II for the molecular cloning using exempt strain of *E. coli* DH5alpha.

Biosafety Level and Any Additional Requirements: BSL-1 and BSL-2 biosafety containment practices for work with *E. coli* strains and human cells/Lentiviral vectors respectively. Bloodborne pathogen training required for all staff.

Concerns or Discussions: None

Training: All training completed.

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

Principal Investigator: [redacted]

Project Title: Use and handling of genetically modified cell lines in non-invasive nanotechnology-enabled imaging

BIO5637 renewal

Vector/Agent(s) to be used: Lentivirus, second-generation (Gag/Pol/Rev expressed in a single helper plasmid). Genetically modified Cells

Name and Function of Transcribed Nucleic Acids: gp1 (optimized), gp100 and MHC H2-Db; Will be translated into target antigen presented on tumor cells. CD70CAR, CXCR2 (IL8 receptor); Help T cell to target CD70 molecule, CXCR2: Increase T cell chemotaxis.

Chicken ovalbumin; facilitate strong immune responses. RNA isolated from tumor lysates; facilitate strong immune responses.

Host(s) to be used: Murine KR158BLuc cell line, rodents.

NIH Guidelines: III-D-4-a for inoculation of rodents with transduced cells. III-F-1 implantation of tumor RNA electroporated dendritic cells into rodents. III-F-8 Appendix C-I for propagation of lentiviral/retroviral vector transduced cells

Biosafety Level and Any Additional Requirements: BSL-1/ABSL-1 for *in vitro* and *in vivo* work using RCV negative retroviral/lentiviral vector transduced B16 KVP, B16 KVP/Db, and KR158B-Luc-gp100 cell lines and subsequent housing of rodent models. Initial and annual bloodborne pathogens training is required for work with human cell lines.

BSL-2/ABSL-2 for *in vitro* and *in vivo* work using retroviral vector transduced 8R70CAR T cell lines and subsequent housing of rodent models.

Concerns or Discussions: None

Training: All training completed.

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

Principal Investigator: [redacted]

Project Title: AAV-mediated retinal gene therapy

BIO5626 renewal

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

Name and Function of Transcribed Nucleic Acids:

- Rhodopsin Kinase promoter- Cas9 endonuclease- guide RNA; Cas9 is a site-specific endonuclease used for gene editing and is guided to the specific cut site using the guide RNA also encoded here. CRISPR associated protein 9 and Tet-ON transactivator protein (rtTA3) is

under the control of a P2A ribosomal skipping-self-cleavage signal between Cas9 and rtTA; The Tet-On transactivator protein and P2A ribosomal skipping signal limit expression to the editing process and subsequent expression of Cas9. CRISPR-associated endonuclease in *Prevotella* and *Francisella* 1 (Cpf1); Cpf1 is an alternative CRISPR system endonuclease to Cas9. It uses a Thymine-rich protospacer adjacent motif (PAM) and cuts on the 5' side of the guide. CRISPR-associated endonuclease in *Prevotella* and *Francisella* 1 (Cpf1) with Tet-ON transactivator protein (rtTA3); Cpf1 is an RNA-guided DNA endonuclease enzyme associated with the CRISPR adaptive immunity system in Streptococcus bacteria. rtTA3 is engineered to be completely inactive in the un-induced state, yet more sensitive and active in the presence of inducer (doxycycline). human Retinoschisin 1; Functional Retinoschesin-1 is a soluble extracellular protein that normally maintains structural integrity of the retina. (e.g. augments defective RS-1 in the mouse knock out model of X-linked recessive retinoschisin).

- Various; Frataxin is a mitochondrial associated protein implicated in iron transport. Defects manifest as a neurodegenerative disease known as Friedrich's Ataxia.
- ATP-binding cassette, sub-family A, member 4 (ABCA4) transports materials across the outer segment membrane of photoreceptors in the retina. Defects manifest as a retinopathy disease known as Stargardt disease.
- Rhodopsin Kinase promoter- Cas9 endonuclease- guide RNA; enhanced humanized green fluorescent protein.
- abbreviated form of chicken beta actin promoter and mCherry fluorescent reporter gene; mCherry is a variant of dsRed where mutations result in a shift of excitation and emission to green and red respectively.
- The combinatorial library of AAV capsid mutations is formed during packaging of reporter gene Td-Tomato and competitively screened for efficient delivery of a given therapeutic vector genome in diverse tissue environments
- human rhodopsin kinase promoter and Cre recombinase, a tyrosine recombinase enzyme derived from the P1 bacteriophage; Site directed recombination.
- chicken beta-actin promoter and Cre recombinase, a tyrosine recombinase enzyme derived from the P1 bacteriophage; Site directed recombination.
- IRBPGNAT2 that includes nucleotide sequence of an interphotoreceptor retinoid-binding protein (IRBP) gene that is positioned upstream of a promoter nucleotide sequence of a cone transducin alpha-subunit (GNAT2) gene. Also CNGB3 (Cyclic nucleotide gated channel beta 3), a protein expressed exclusively in cone photoreceptors. It forms part of an ion channel that takes part in the visual transduction process.
- Multiple; non-conventional myosin that is responsible for photoreceptor structure and protein trafficking in the retina.
- GFP is a green fluorescent reporter gene
- Various others, please see sub form for specific names and functions.

Host(s) to be used: Rodents

NIH Guidelines: III-D-3-e for experiments involving the use of defective DNA viruses in the presence of helper system. III-D-4 for experiments involving use of recombinant nucleic acids in rodent models. III-E-1 for experiments involving the formation of recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus. III-F-8 Appendix C-II for the maintenance of plasmids in *E. coli*.

Application of genome editing in eukaryotic hosts (such as CRISPR/Cas9 technologies).

Biosafety Level and Any Additional Requirements: ABSL-1 for animal studies and subsequent housing. BSL-1 for *E. coli* work and *in vitro* work with AAV without adenovirus. BSL-2/ABSL-2 for work with human cell lines and human/NHP serum/vitreous as well as AAV work with adenovirus. Initial and annual bloodborne pathogens training is required for work with human cell lines.

Concerns or Discussions: None

Training: All training completed.

Approval: [redacted] was in conflict and did not vote. All approved as recommended (Yes-11, No-0, Abstain-0)

Principal Investigator: [redacted]

Project Title: Assessing tau-related changes *in vitro*

BIO5539 renewal

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

Name and Function of Transcribed Nucleic Acids: GCaMP8f; fluorescent calcium indicator. ChR2; optogenetic activation. dLight1, GRAB-DA2m, GRAB-DA1h, GRAB-gDA3m, dLight1.3b; dopamine sensor. eYFP, eGFP, EYFP; fluorescent reporter. Volton2; voltage sensor. hM3D(Gq)-mCherry; excitatory DREADD receptor with a fluorescent tag. hM4D(Gi)-mCherry; inhibitory DREADD receptor with a fluorescent tag. HA-hM3D(Gq)-IRES-mCitrine; Excitatory DREADD receptor with a fluorescent tag and an HA tag. HA-hM4D(Gi)-mCitrine; Inhibitory DREADD receptor with a fluorescent tag and an HA tag. Cre; initiate recombination of any floxed/DIO constructs, i.e. in a transgenic rodent with a floxed fluorescent reporter. jRGECO1b; red-shifted fluorescent calcium sensor. mGFP-2A-Synaptophysin-mRuby; Fluorescent reporters (mGFP and mRuby) expressed along with synaptophysin protein, as a marker for synapses.

Host(s) to be used: Primary culture (brain: neurons, astrocytes, microglia) and organotypic brain slice culture both from rodents. Human-derived iPSC neurons in culture.

NIH Guidelines: III-E-1 for rAAV transduction of cell lines in vitro only. III-F-8, appendix C-I for the use of recombinant/synthetic nucleic acids (plasmids) in tissue culture.

Biosafety Level and Any Additional Requirements: BSL-1 for work with AAV vectors. BSL-2 for *in vitro* experiments with human iPSC-differentiated neurons. ABSL-1 for work involving rodents.

Concerns or Discussions: None

Training: All training completed.

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

Amendments

Principal Investigator: Jatinder Lamba BIO6651

Title: Genomic/Genetic Engineering of Acute Myeloid Leukemia (AML) Cells to Elucidate Pharmacogenomic Impact on Response to Chemotherapeutic and Immunotherapeutic Agents used in AML Therapy

BIO6651

Summary: III-D-3-a for packaging LVV in the presence of a helper system. III-F-8 Appendix C-1 for the maintenance of genetically modified cells in culture Application of genome editing in eukaryotic hosts (such as CRISPR/Cas9 technologies). This work remains approved at: BSL-1 for molecular cloning, BSL-2 for in vitro work with human cell lines, BSL-2+ for work with lentiviruses is approved at containment and practices. The "+" practices require using mucous membrane protection and substituting plastic labware for glassware where possible. Initial and annual bloodborne pathogens training is required for work with human cell lines.

Concerns or Discussions: None

Training: All training completed.

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

Principal Investigator: [redacted] BIO6490

Title: Diagnostics and therapeutic/prophylactic vaccines for feline immunodeficiency virus (FIV), HIV-1, feline coronavirus (FCoV), and SARS-CoV-2 (SCoV2)

BIO6490

Approval: Tabled.

Principal Investigator: [redacted] BIO7515

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

BIO7515

Summary: This amendment adds the work involving using rodent models to test efficacy of OMV, mRNA-LNP, and protein-subunit vaccines. These changes will not alter the previously approved biosafety containment practices nor the NIH guidelines and they remain as stated in the original review.

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 (see email attached to project).
- 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 *Galleria mellonella*.
- Sections III-F-1/III-F-3/III-F-4/III-F-8 C-1 for manipulation of extracted nucleic acids and other extracted cellular derivatives, including OMVs, mRNA-LNP's and phage stocks.

Minimum biocontainment 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.
- ABSL-2 for the inoculation of OMV's from Burkholderia species and mRNA-LNP vaccines into rodents.
- BSL-1 practices and containment for all other experimental procedures.

Note the following:

- *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.

Concerns or Discussions: None

Training: Bloodborne pathogens training required for handling of human sera. All training was completed.

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

Principal Investigator: [redacted] BIO6688

Title: Cellular therapies for brain tumors

BIO6688

Summary: Personnel changes and adding the use of primary human peripheral blood-derived dendritic cells electroporated with RNA, and then implanted into rodents via several means. As a result of these additional changes this will not alter the previously approved biosafety containment practices nor NIH guidelines and they remain as stated.

Biosafety containment practices

- BSL-2 to cover the in vitro work with primary human cells
- ABSL-2+ to cover the *in vivo* studies with 2nd generation LVV-transduced human glioma cells lines, Sendai virus reprogrammed hiPSC, and implantation of primary human cells, tissues, and tumors - patient-derived xenograft models (requires the use of safe sharps and wiping of the injection site) for animal inoculations, ABSL-2 for subsequent housing.
- BSL-1/ABSL-1i for studies with transfected murine cells
- ABSL-1+ to cover *in vivo* experiments with Sendai virus reprogrammed miPSC, 2nd generation LVV-transduced murine cells (negative RCL data provided in Nov. 2019, see original biohazard project registration application), and retroviral vector-transduced murine cells a (requiring the use of safe sharps and wiping of the injection site) and ABSL-1 for subsequent housing.

NIH Guidelines

- III-D-4-a for the work involving rodents implanted with human cells
- III-E-1 for the work utilizing viral vector transfected cell lines.

Concerns or Discussions: None

Training: All Training Completed.

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

Exposure incident discussion. Reportable to the NIH and one requirement is that the IBC is made aware. Staff in Chris Vulpe's lab working with a sample of fibroblasts isolated from patient with Frerichs ataxia. Cells were treated to delete the Fredirich's ataxia. Staff member was using buffer to prepare mixture for plates and used needle in syringe to remove bubbles in the centrifuge tube. The staff missed the tube and stuck finger under nail drawing blood. Findings included using improper tools and improper techniques as well as fatigue at end of day. Modifications have been made to reduce the issue with bubbles. The cell lines that came in contact with staff are not materials currently listed in the registered work. This would be considered unregistered work.

The meeting adjourned at 2:12pm