

IBC Meeting Minutes
July 17, 2025 (Thursday) at 11:00 A.M.
via Zoom Conference Bridge

IBC members present:

Tom Greenough (Chair)	X	Shaoguang Li		Carol Schrader	X	Edward Jaskolski (alt)	X
Lisa Cavacini	X	Philip Tai	X	Mohan Somasundaran		Timothy Kowalik (alt)	
Colleen Driskill	X	Robert Klugman		Regino Mercado-Lubo	X	Richard Ellison III (alt)	
Kris Giaya	X	Amelia Houghton	X	Sharone Green (alt)		Casey Moran (alt)	X
Hardy Kornfeld		Eric Rouse	X	Jennifer Wang (alt)	X		

Non-members present: Patrice Rando (IACUC/IBC Office)

I. Introductory Remarks

- 1) The Chair brought to the attention of the Committee the action items completed since the previous meeting and those submissions still under review by IBC.
- 2) The Chair brought to the attention of the Committee the meeting minutes from the previous IBC meeting. **Meeting Decision: Vote to approve June 19, 2025 Meeting Minutes**
- 3) Change made to May 22, 2025 Meeting Minutes protocol **Flotte 345-22, Amendment not Gruntman**

II. Report on incidents/accidents from Employee Health Services (EHS)

- 1) PAPR malfunction in BSL-3: two individuals involved in the incident both will be tested again sometime this month
- 2) Needle stick injury: Necropsy of mice in biosafety cabinet. MTB needle stick was immediately attended to with soap and water. Appropriate notifications made. The individual is going to continue to be monitored. Needle holders to be used in the future.

III. Protocols Reviewed Administratively

- 1) Investigator: Binder, R
Title: Zoonotic infectious diseases in the Democratic Republic of the Congo (DRC)
IBC Registration: I-922-25, New
Training Verification: **Acceptable**
Brief Summary: Determine the coronavirus (CoV) and other pathogen epidemiology, genetic diversity, and associated burden of disease among humans and animals in the Democratic Republic of the Congo (DRC). **Aim 1.1:** Leverage the respiratory surveillance efforts to determine the CoV epidemiology, genetic diversity, and associated burden of disease among symptomatic individuals in Kinshasa, DRC. Hypothesis: CoV infections are an underappreciated cause of influenza like illness (ILI) and severe acute respiratory infections (SARIs) in the region. **Aim 1.2:** Map out high-risk CoV human-animal interfaces in Kinshasa. Hypothesis: Cryptic inter- and intra-species CoV transmission is ongoing among animals and humans in non-sylvatic settings, shaping CoV evolution and subsequent outbreaks in the region. No rsNA. BSL-2 proposed

- 2) Investigator: Silverman, N
Title: Skin inflammation, Infectious Colitis and Leishmaniasis Models in Inbred Mouse Strains
IBC Registration: 705-22, Amendment
Training Verification: **Acceptable**
Brief Summary: This amendment is to add animal addendums to reflect our IACUC protocol. Additions for these addendums include: LPS, *Leishmania* species, *Corynebacterium* species, *Clostridium* sp. (segmented filamentous bacteria). No new rsNA. BSL-2; ABSL-2 proposed.

IV. Protocols to Discuss

- 1) Investigator: Baehrecke, E
Title: Genetic Regulation of Autophagy and Cell Death in Drosophila and Cell Lines
IBC Registration: 349-22, Amendment
Training Verification: **Acceptable**
Brief Summary: To add: SLC46A3 knockout will be performed in human epithelial cancer cell line, SNU-387 (ATCC, CRL-2237).

Brief Summary and Review by Primary Reviewer

Overview and Objectives: The objective of this study is to understand genetic regulation and function of autophagy and cell death in the fruit fly *Drosophila melanogaster*, and in cell lines originated from human or other species. It involves the isolation, identification, and characterization of genes that function in these processes. Overall, the goal is to understand the mechanisms that regulate autophagy and cell death during normal development, and under pathological conditions caused by mutations in critical genes that regulate these processes.

Experimental Approach: Molecular and biochemical approaches will be used to identify genes that function in autophagy and cell death in fruit flies. Forward genetic approaches involve screening for mutations that prevent autophagy and cell death. Reverse genetic approaches seek to identify genes or proteins that change the expression associated with autophagy and cell death, and then we test if available mutations in these genes inhibit these processes. All molecular manipulations of these genes are initially conducted in *E. coli*. For example, *Drosophila* genes are cloned and manipulated initially in the *E. coli* plasmid pBluescript, and then transferred into pCaspER or pUAST. These constructs are then introduced into *Drosophila* using these P element transformation vectors that are stable and do not cross into other species (we contract with companies to make transgenics so, no injections are done in the lab). All bacterial and insect vectors are killed by autoclaving prior to disposal. These are standard protocols, do not involve infectious materials, do not involve the use of needles for injection of flies, and do not involve IACUC or IRB approval.

Autophagy function studies will be performed in cell lines originated from humans or other species. CRISPR-Cas9 mediated gene depletion (pLentiCRISPR V1, pLentiCRISPR V2, pALPS) or overexpression (pMXS-IRES-blasticidin) will be done by transduction via replication deficient virus to cell lines. Cells will be selected by transduction using puromycin or blasticidin or sorted using fluorescent markers and subjected to autophagy related studies (immunostaining, western blots) or other cell biology and biochemical assays (live cell imaging, CoIP). siRNA mediated gene knockdown will not require any transfection reagents or viral vector transduction (Accell siRNA). The goal here is to examine the function of the autophagy-related or regulator genes in the autophagosome initiation, formation, maturation and regulation process. EMC1, VMP1 and other autophagy-related genes will be depleted by CRISPR-Cas9 mediated gene knockout. SLC16A11 will be downregulated by siRNA. SLC46A3 knockout will be performed in human epithelial cancer cell line, SNU-387 (ATCC, CRL-2237). Effects of these autophagy genes depletion on autophagosome formation and regulation will

be further examined. In other cases, overexpression of these genes will be used for examination of their expression pattern and identification of their potential interactors.

IBC Discussion and Vote

Discussion: Reviewer felt this was very straightforward and well written. No action items noted.

Meeting Decision: Vote to approve upon completion of action items.

BSL/ABSL: **BSL-2**

NIH Guidelines: III-D

- 2) **Investigator:** Castilla, L
Title: Genetic studies in the development of acute myeloid leukemia
IBC Registration: **145-24, Amendment**
Training Verification: **Acceptable**
Brief Summary: To Add: The induced pluripotent stem cell (iPSC) work: The iPSCs are immortalized cell lines derived from patients with germline or somatic mutations associated with MDS/leukemia. We obtain these cells from colleagues in the field who have generated them and published studies using them. We will differentiate the iPSCs in vitro studies to generate hematopoietic cells and study the role of mutation in differentiation and survival. We will test these processes in cells treated with specific inhibitors to assess if blocking or activating specific pathways can rescue the identified deficiency.
We will sort hematopoietic progenitors to study the molecular markers deregulated by specific mutations.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: The overall goal of this project to study how gene mutations disrupt normal hematopoietic differentiation and induce leukemia using genetic, genomic and pharmacologic approaches. We will use in vitro experiments to elucidate molecular and functional changes by genetic mutations in differentiation, survival and transformation. We will deregulate gene expression by gene editing or with vectors to mechanistically and functionally study leukemia risk in vitro and in vivo. We will use pharmacologic approaches in cells and in mice to test specific gene/pathways function in leukemia progression/risk.

Experimental Approach: The amendment is to add use of induced pluripotent stem cell (iPSC) in vitro. These are immortalized cell lines derived from leukemic patients with germline or somatic mutations associated with MDS/leukemia. We obtain these cells from colleagues in the field who have generated them and published studies using them. We will differentiate the iPSCs in vitro studies to generate hematopoietic cells and study the role of mutation in differentiation and survival. We will test these processes in cells treated with specific inhibitors to assess if blocking or activating specific pathways can rescue the identified deficiency. We will sort hematopoietic progenitors to study the molecular markers deregulated by specific mutations.

IBC Discussion and Vote

Discussion: Reviewer discussed the protocol amendment is straightforward and one action item noted. No cause for concern in terms of a viral vector that might persist. Principal Investigator will get back to the IBC with more specifics about how the iPSC cells were derived.

Meeting Decision: Vote to approve upon completion of action item and more Information from PI

BSL/ABSL:
NIH Guidelines:

BSL-2; BSL-2 Enhanced Flow Sorting
III-F

- 3) Investigator: Fu, A
Title: Molecular pathways in pancreatic beta cell survival, inflammation and interactions with immune cells
- IBC Registration:** **860-22, Amendment**
Training Verification: **Acceptable**
Brief Summary: We would like to immortalize or extend culture lifespan of human primary cells using hTERT lentivirus infection. This is critical to extend the types of experiments we can perform on these cells.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: We would like to immortalize or extend culture lifespan of human primary cells using hTERT lentivirus infection. This is critical to extend the types of experiments we can perform on these cells.

Experimental Approach: We will infect human monocytes purified from PBMCs in non-diabetic and diabetic donors, using lentivirus (packaged commercially) infection. This work will be performed in a Biosafety level 2 hood, and dilutions or manipulation of the virus stocks may also be performed in a chemical fume hood. Any tips, sharps, or plastics in contact with the lentivirus will be placed in 10% bleach for 15 min, then rinsed and placed in standard disposal as appropriate.

IBC Discussion and Vote

Discussion: Reviewer discussed that because they are adding a new lentiviral vector, they may need to amend their vector checklist. Cells are coming for a collaborator lab but the IBC is familiar with that lab so no cause for concern.

Meeting Decision: Vote to approve upon completion of action items.

BSL/ABSL: **BSL-2**
NIH Guidelines: III-D

- 4) Investigator: Gray-Edwards, H
Title: Transgenic Animal Modeling Core
- IBC Registration:** **218-22, Amendment**
Training Verification: **Acceptable**
Brief Summary: To add: anelloviruses, rANV viruses

Brief Summary and Review by Primary Reviewer

Overview and Objectives: To generate genetically modified mice, rats. To use Primary cells from patients' blood or skin biopsy to generate induced pluripotent stem cells (iPSCs) for disease modeling and drug discovery.

Experimental Approach: Adding Anellovirus vectors for gene modification and tg animal development. Sendai viruses for iPSC induction are commercially available from Life Technologies. Adeno-Associated Viruses are obtained from the UMass virus core facility. Genes that are transduced with retroviridae, adenovirus or Adeno-Associated viruses, and anelloviruses will include the Cre recombinase Cas9 nucleases and a variety of reporter genes (β -gal, eGFP) or gRNAs.

Viral core will generate replication deficient non-integrative adeno-associated viruses carrying expression cassettes to express Caspr-Cas9 components or Cre recombinase. AAVs and rANVs will be used as vectors to deliver constructs to pre-implantation embryos in culture, microinjection or in the oviduct or uterus of pregnant females (E0.5 –E3.5). One-cell embryos (zygotes) from mice, rats and hamsters will be injected with recombinant DNA constructs, rAAV viruses, rANV viruses, RNA or proteins to generate transgenic animals.

Pre-implantation embryos (zygote to blastocyst stages) from mice, rats, sheep and pigs will also be incubated with rAAVs to transduce embryos with CRISPR-Cas9 components to generate genetically modified animals. We will use replication deficient rAAVs and rANVs prepared by the UMMS virus core. Recombinant AAVs and rANVs will also be delivered into the oviduct of rodent females. One to three microliters of replication deficient rAAVs and rANVs will be delivered using a hand-held mechanical device. These experiments will be conducted under a hood. The personnel conducting these experiments will be wearing a lab coat, gloves and mouth cover. We may also deliver AAV particles into the oviduct of gilts. These experiments will be done in collaboration with veterinarians at Tufts university

IBC Discussion and Vote

Discussion:	Reviewer discussed more information is required on the packaging systems to ensure that replication isn't possible.
Meeting Decision:	Vote to approve upon completion of action items.
BSL/ABSL:	BSL-2; ABSL-1 with Special Precautions; Administration to Animals Using BSL-2 Precautions/ Sharps Safety
NIH Guidelines:	III-D, III-E

- 5) Investigator: Gray-Edwards, H
 Title: Therapeutic Efficacy Testing in Swine
IBC Registration: **725-24, Amendment**
 Training Verification: **Acceptable**
 Brief Summary: 1) To study the biological behavior and genetic profile of transgenic fetal fibroblasts from Wisconsin Miniature Swine (WMS). 2) To use these cells as a platform for in vitro assays and potential in vivo transplantation studies

Brief Summary and Review by Primary Reviewer

Overview and Objectives: To study the biological behavior and genetic profile of transgenic fetal fibroblasts from Wisconsin Miniature Swine (WMS). To use these cells as a platform for in vitro assays and potential in vivo transplantation studies.

Experimental Approach: Primary fibroblasts derived from fetal skin and fetal muscle of transgenic WMS animals will be cultured and expanded. Downstream applications may include in vitro transfection/transduction studies and evaluation of cellular phenotypes.

IBC Discussion and Vote

Discussion:	Reviewer discussed that there are many inconsistencies in this protocol that require clarification. Reviewer discussed the multiple action items the lab need to be addressed. Chair discussed that when the IBC attaches the approval letter, the IBC should remind the lab that before performing any animal work the lab, they will need to submit an amendment for future in vivo work. Clarification on whether they want to change the title of the protocol or not.
Meeting Decision:	Vote to approve upon completion of action items.
BSL/ABSL:	BSL-2
NIH Guidelines:	III-D, III-E, III-F

- 6) Investigator: Mercurio, A
Title: Mechanisms of Tumor Progression
IBC Registration: **222-25, Amendment**
Training Verification: **Acceptable pending completion of PI training / Acceptable**
Brief Summary: To add: polyinosinic:polycytidylic acid (Poly I:C)

Brief Summary and Review by Primary Reviewer

Overview and Objectives: Amendment to add polyI:C to stimulate mouse NK cells

Experimental Approach: Polyinosinic:polycytidylic acid will be administered to mice via intraperitoneal injection. Spleens of mice will then be harvested from treated mice and activated NK cells will be isolated from spleens and co-cultured with tumor cells.

IBC Discussion and Vote

Discussion: Reviewer discussed that this amendment was well written and low risk. No action items were found.
Meeting Decision: Vote to approve
BSL/ABSL: **BSL-2; ABSL-1**
NIH Guidelines: III-F

- 7) Investigator: Sirianni, R
Title: CFE for Neurological Disorders and Drug Delivery
IBC Registration: **851-22, Amendment**
Training Verification: **Acceptable pending completion of PI training**
Brief Summary: To add: synthetic nucleic acids (DNA/RNA) and Sheep

Brief Summary and Review by Primary Reviewer

Overview and Objectives: This is an amendment to a registration entitled Cerebrospinal Fluid Flow Enhancement for Neurological Disorders and Drug Delivery, to add synthetic nucleic acids to investigate efficacy and tolerability of nucleic acid based nanoparticles for delivery of gene and oligonucleotide based therapeutics in sheep. Nucleic acid based nanoparticles (NANPs) were previously approved in mice. The object is to understand whether NANPs can deliver ASOs functionally in sheep.

Experimental Approach: Adding intrathecal administration of nanoparticles to CSF or brain parenchyma in sheep. MRI is included in IACUC (not IBC). Sheep will then be euthanized, perfused and organs extracted for tissue processing (RNA extraction and Rt-qPCR).

IBC Discussion and Vote

Discussion: Reviewer discussed that there were some action items in relation to the lab following the IBC Coxiella Medical SOP. A larger discussion was brought to the attention of the committee that some the IBC medical SOP's are written in a way that is specific to certain PI's and there is a standard SOP for animal medicine that conflicts with the IBC SOP. The reviewer also mentioned that the PPE being used conflicts with what is used in the MRI suite which raised the question as to which guidelines the lab should be following. The chair discussed that the lab should follow whatever is the highest PPE requirement.

Meeting Decision: Vote to approve upon completion of action items.
BSL/ABSL: **BSL-1; ABSL-1**
NIH Guidelines: III-D, III-E, III-F

- 8) Investigator: Vyas, J
Title: Innate Immune Responses to Fungi
IBC Registration: **923-25, New**
Training Verification: **Acceptable**
Brief Summary: 1. We will characterize the interactions of pulmonary fungal pathogens, namely *A. fumigatus*, with airway epithelium derived from humans. We will further investigate the interactions between fungal-pathogen-stimulated epithelium and the innate immune system. We will also be characterizing the interactions of *Aspergillus* strains directly with macrophages and neutrophils.
2. We will determine the role of type I interferons in innate immune response (*e.g.*, macrophages, neutrophils) to *C. albicans* and *C. auris*. These studies will utilize both *in vivo* and *in vitro* models.
3. We will explore if TNF α and GM-CSF prime neutrophils to respond to invading *Aspergillus* and circumvent Bruton Tyrosine Kinase (BTK) inhibitors by activating downstream molecules.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: To characterize the interactions of pulmonary fungal pathogens, namely *A. fumigatus*, with airway epithelium derived from humans. To investigate the interactions between fungal-pathogen-stimulated epithelium and the innate immune system. To characterize the interactions of *Aspergillus* strains directly with macrophages and neutrophils. To determine the role of type I interferons in innate immune response (*e.g.*, macrophages, neutrophils) to *C. albicans* and *C. auris*. These studies will utilize both *in vivo* and *in vitro* models. To explore if TNF α and GM-CSF prime neutrophils to respond to invading *Aspergillus* and circumvent Bruton Tyrosine Kinase (BTK) inhibitors by activating downstream molecules.

Experimental Approach: 1. *Candida albicans* will be grown overnight fresh for each experiment. *Candida* will be washed and diluted in PBS to create the appropriate *Candida* concentration in PBS that will be injected Intravenously in the mice of interest in the BL2 room or infect *in vitro* cell models. *Candida* will be used in [A] phagocytosis experiments with macrophages [B] *in vivo* murine infection models to measure fungal burden, survival, and immune cell responses; and [D] cytokine and protein measurements by ELISA and Western blots.

2. *Aspergillus fumigatus* will be grown on solid media cultures. Spores (conidia) will be collected from mature cultures by washing the surface with PBS/tween solution to create a suspension of conidia. All work with solid cultures will be performed in a biological safety cabinet. The conidia will be used in the following experimental techniques/procedures:

a. Conidia will be used in phagocytosis experiments with murine macrophages, human airway epithelium, and murine airway epithelium. The resulting interactions will be visualized in fixed and stained macrophages, human airway epithelium and murine airway epithelium using confocal microscopy.

b. Expression of cytokines will be measured after co-incubation with macrophages (mouse) and airway epithelium (human, mouse).

c. *In vivo* airway fungal infection mice model via oropharyngeal method.

3. For the development of melanin ghosts, wildtype *Aspergillus* will be grown as described above (#4). Melanin ghosts are used *in vitro* using airway epithelial lines and primary samples as described in #2a. The following steps are taken in a chemical hood.

[1] The fungal cell walls removed overnight using 30 mg lysing enzymes from *Trichoderma harzianum*

[2] Protein denaturation using guanidinium thiocyanate solution

[3] Protein hydrolysis with proteinase K solution

[4] Folch lipid extraction (rinsed with methanol, transferred to a separatory funnel, chloroform is added to the methanol-saline mixture, which creates three layers, the bottom layer is drained, steps repeated twice, aqueous and the insoluble layer is moved to a round-bottom flask for the next step).

[5] HCl (20 mL of 6M) is added to the flask and heated to ~90C for 1h then cooled to room temperature. Samples are centrifuged and decanted supernatant is placed in a waste container. Pellet is resuspended in PBS.

[6] The sample is centrifuged in a 0.22 µm filter Eppendorf tube, then rinsed with dH₂O, frozen in liquid nitrogen, and lyophilized.

4. For *Candida auris*, [1] all work will be performed in a BSC designated for fungal growth; [2] all materials in contact with the yeast solutions or culture dishes will be one-time use (pipette tips, inoculation loops, etc.); and [3] equipment such as pipettors that are reusable will be decontaminated with germicidal bleach solution for at least 5 minutes.

C. auris strains will be grown in a YPD broth tube from frozen stock. The outer culture tube will be wiped down with germicidal bleach and then labeled. Tubes will be transported to a 30-degree shaker overnight for growth. The shaker and room will be labeled clearly with "*Candida auris*", biohazard, and contact names for questions. The next day, yeast will be collected into plastic tubes for counting. The surface of all plastic materials (tubes, etc.) in contact with *C. auris* will be treated with a germicidal bleach solution before being removed from the BSC for centrifugation or counting. For co-incubation with mammalian cells, *C. auris* yeast and mammalian cells will be co-cultured in a plastic dish. The dish will be incubated in a 37-degree tissue culture incubator designated for pathogen/host experiments only. A sign will be placed on the door, specifically noting the presence of *C. auris* in the incubator, biohazard designation and contact names for questions. After the incubation period is completed, the surface of shelf used will be decontaminated with 10% germicidal bleach solution for at least 5 minutes. Every 6-12 months, all the shelving and components of the incubator will be dismantled and autoclaved.

C. auris will be used in [A] phagocytosis experiments with macrophages; [B] in vivo murine infection models to measure fungal burden, survival, and immune cell responses; and [C] cytokine and protein measurements by ELISA and Western blots.

IBC Discussion and Vote

Discussion:	Action items were already addressed. A member of the committee noticed a typo in the animal addendum that will need to be addressed. <i>Pseudomonas</i> experiments will be described and Section E and H will be revised.
Meeting Decision:	Vote to approve
BSL/ABSL:	BSL-2; ABSL-2
NIH Guidelines:	III-E

- 9) Investigator: Wang, J
Title: In vitro Cap-snatching of Host RNAs by Hantaviruses
IBC Registration: **616-25, Renewal**
Training Verification: **Acceptable**
Brief Summary: To use protocols such as CapSeq (see Reference 1) combined with deep-sequencing to examine the cap-snatching process of three hantavirus strains (Hantaan, Puumala, and Sin Nombre) in infected human cultured cells.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: To use protocols such as CapSeq combined with deep-sequencing to examine the cap-snatching process of three hantavirus strains (Hantaan, Puumala, and Sin Nombre) in infected human cultured cells.

Experimental Approach: the PI proposes to infect a human bronchoepithelial cell line, A549, with different strains of hantavirus in the BSL-3 suite using appropriate precautions and following the hantavirus standard operating procedures (see enclosed document). RNA from infected A549 cells will be harvested using TRIzol at specific time points following infection. Purified RNA will be removed from the BL-3 suite and used for the CapSeq protocol or alternative protocols for selection of capped RNA in the Wang lab. Libraries will be generated from RNA samples for deep sequencing and analysis of hybrid sequences to determine the host RNA used for cap-snatching by hantaviruses. Extracted RNA will also be used for quantitative real-time PCR to examine hybrid human-viral sequences. Different hantaviruses strains will be used to determine if patterns of cap-snatching are strain-specific. Viral stocks will be expanded as needed in Vero cells in the BL-3 suite but only in small volumes (≤ 20 mls).

IBC Discussion and Vote

Discussion:	Reviewer discussed that there is not existing Medical SOP for Hantavirus only an old, archived SOP. The committee discussed developing a Medical SOP for Hantavirus with instructions on what to do if there is an exposure.
Meeting Decision:	Vote to approve upon completion of action items.
BSL/ABSL:	BSL-3
NIH Guidelines:	N/A

V. Report on incidents/accidents/issues involving BSL-3 & ABSL-3 Facilities

- 1) Freezer moves planned.

VI. Information from the field (Senior Biosafety Officer)

N/A

VII. Other Business

- 1) **Harvard University qiagen kit guidelines-** Dissemination and postings discussed. Labeling aspiration flasks with contents.
- 2) **EHS SOP Tuberculosis- 06/13/2025-** Pending Chair review

Acknowledgement Items:

1) Luzuriaga I-631-20 CRC – Update

IMVT-1402 is a fully human immunoglobulin G1 (IgG1) subclass anti-neonatal fragment crystallizable receptor (anti-FcRn) monoclonal antibody (mAb). By competitively binding with the IgG binding site on FcRn, IMVT-1402 blocks FcRn-mediated recycling of IgG, resulting in increased lysosomal degradation and decreased serum levels of IgG. Due to its ability to reduce IgG, IMVT-1402 is an investigational product being developed for the treatment of IgG autoantibody-mediated autoimmune disease. IMVT-1402 has been engineered to reduce both complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) effector function activity.

Adjourned at 1:21pm