**IBC Meeting Minutes**
April 16, 2026 (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 | | Eric Rouse | | Regino Mercado-Lubo (alt) | X |
| Kris Giaya | X | Amelia Houghton | X | Sharone Green (alt) | | Casey Moran (alt) | |
| Hardy Kornfeld | | Melissa Scannell | | Jennifer Wang (alt)* | X | Richard Ellison III (alt) | |

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

*JW- Joined 12:05pm

## 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 March 19, 2026 Meeting Minutes**
3) One member can register to attend (and report back to committee any insights/best practices learned) **THE THREE I's VIRTUAL CONFERENCE WILL HELD ON MONDAY, APRIL 27, 2026 - THURSDAY, APRIL 30, 2026, FROM 10:00 AM - 2:30 PM DAILY *****

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

Past incidents that remain open:

1) 11/26/2025: BSL-3. Wet sleeve noted; skin intact. Repeat IGRA screening planned
2) 12/08/2025: Cut noted after cleaning BSC. Low likelihood HIV exposure; PEP; serology planned 060826.

Recent incidents:

N/A

## III. Protocols Reviewed Administratively

1) Investigator: Bucci, V
   Title: Microbiota community modulation via antibiotic and microbial mediated intervention.
   **IBC Registration: I-753-20, Amendment**
   Training Verification: Acceptable
   Brief Summary: We aim to determine how colonization with pathogenic Enterobacteriaceae species (Escherichia coli ATCC 700682 or Citrobacter rodentium) and other pathobionts (Porphyromonas gingivalis) impacts term pregnancy and fetal anthropometry in mice. Our goal is to investigate if colonization with pathogenic species impacts length of pregnancy and pup weight and length in mice, and further, if decolonization during pregnancy prevents changes.

**BSL/ABSL:** **BSL-2; ABSL-2**
**NIH Guidelines:** N/A

2) Investigator: Hu_Y
Title: Pilot Study: Evaluation of Autologous Platelet-Rich Plasma (PRP) and Protein Concentrate (PC) for the Treatment of Hip Osteoarthritis (OA) Using Standard of Care
**IBC Registration:** **I-938-26, New**
Training Verification: Acceptable pending completion of PI training
Brief Summary: Background:Hip Osteoarthritis (OA) is a chronic degenerative joint disease that is a leading cause of pain and disability among adults. Current treatment options include physical therapy, analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), and intra-articular corticosteroid (CS), hyaluronic acid (HA), or platelet-rich plasma (PRP) injections (1-4). Autologous PRP and Protein Concentrate (PC) provide promising treatment options with potential for longer-lasting relief by promoting tissue repair and modulating inflammation, with fewer side effects, however, evidence for PRP and PC to treat hip OA is limited, (2-4) especially as it correlates to platelet dose. Intra-articular PRP injections are safe to perform, especially under image guidance, as these are regularly performed with other injectates for treatment. (1) Currently, there is no data in the literature regarding intra-articular injection of PC for treatment of hip OA, but injection technique and protocols are similar to those of PRP.

**Objectives:**

**Primary Objective:**

To evaluate change in pain intensity and functional improvement at 6 months post-injection utilizing the Visual Analog Scale (VAS) and Western Ontario and McMaster Universities Arthritis Index (WOMAC).

**Secondary Objectives:**

To evaluate change in pain intensity and functional improvement at 6 weeks, 3 months, and durability of response at 12- and 24-months post-injection utilizing VAS, WOMAC; Treatment Satisfaction at 6-, 12-, and 24-months; Adverse Events at all time points.

**BSL/ABSL:** **BSL-2**
**NIH Guidelines:** N/A

3) Investigator: Khvorova, A
Title: Development of RNAi-based Therapeutics
**IBC Registration:** **I-581-24, Amendment**
Training Verification: Acceptable pending completion of PI training
Brief Summary: To Add: Retro Orbital route of administration for proteins or AAV

**BSL/ABSL:** **BSL-2; ABSL-1 with Special Precautions; Administration to Animals using BSL-2 Precautions / Sharps safety**
**NIH Guidelines:** III-D, III-E

4) Investigator: Logan, R
Title: Logan Laboratory General Protocol for Viral Vectors and Humann Tissue and Blood
**IBC Registration:** **I-865-23, Amendment**
Training Verification: Acceptable pending completion of PI training / Acceptable
Brief Summary: For some tests postmortem human brain known to be infected with HIV and human whole blood with unknown contamination will be used for analysis. The overall purpose of this project is to characterize the blood transcriptomic (RNA) profile from human microsample capillary

blood (dried) from donors with brain disorders (e.g., neurological, psychiatric). The advantages of microsample capillary blood is that is allows minimally invasive collection and remote collection.Goals- Better understanding of pathophysiology, identify molecular signatures associated with brain disorders- Identify candidate biological pathways and molecular targets that may be relevant for future therapeutic development - Biomarker discovery, evaluate associations between sets of transcripts and established clinical features or severity categories

**BSL/ABSL:** **BSL-2**
**NIH Guidelines:** N/A

5) Investigator: Tran, K
Title: Adipocyte Biomarkers of Cardiovascular Disease (ABCD)
**IBC Registration:** **I-799-26, Renewal**
Training Verification: Acceptable pending completion of PI training
Brief Summary: We would like to investigate the relationship between adipocytes & adipocyte biomarkers and cardiovascular disease by analyzing heart tissue from deceased patients with and without cardiovascular disease.

**BSL/ABSL:** **BSL-2**
**NIH Guidelines:** N/A

6) Investigator: Xue, W
Title: Identification and validation of cancer genes using mouse models
**IBC Registration:** **I-596-24, Amendment**
Training Verification: Acceptable
Brief Summary: To add: Route of administration ICV injection.

**BSL/ABSL:** **BSL-2; ABSL-1 +BBP with Special Precautions/ Administration to Animals using BSL-2 Precautions/ Sharps Safety**
**NIH Guidelines:** III-D, III-E

## IV. Protocols to Discuss

1) Investigator: Alterman, J
Title: Development of oligonucleotide therapeutics
**IBC Registration:** **I-939-26, New**
Training Verification: Acceptable
Brief Summary: The overall goals of the lab are to develop RNA-based therapeutics for several disease indications. This is done by testing modified or formulated RNA oligonucleotides (or RNA/protein complexes) developed in the lab in in vitro experiments using mouse, human, pig, and NHP cell lines and primary cells followed by, ex vivo tissue and in vivo rodent models. There are variety of targets we work with.

**Brief Summary and Review by Primary Reviewer**

**Overview and Objectives:** The overall goals are to develop RNA-based therapeutics for several disease indications. This is done by testing modified or formulated RNA oligonucleotides (or RNA/protein complexes) developed in the lab in vitro

experiments using mouse, human, pig, and NHP cell lines and primary cells followed by, ex vivo tissue and in vivo rodent models. There are variety of gene targets.
Skin diseases arise from diverse causes, including environmental irritants, chemical allergens, autoimmune responses (e.g., vitiligo), and uncontrolled cell growth (e.g., melanoma). Using autoimmune and immunocompetent animal models to examine how these factors drive cutaneous inflammation. To characterize immune responses to inflammatory stimuli and evaluate novel oligonucleotide therapeutics, with the aim of identifying key signaling pathways and mechanisms of disease. Ultimately, findings from these studies will inform the development of new treatments for autoimmune and inflammatory skin disorders.

**Experimental Approach:** Project 1: Screening modified RNAs in vitro
In vitro work involves testing modified RNA oligonucleotides in modified and primary cells of human, mouse, pig and NHP origin. The oligonucleotides can either be naked, conjugated to an antibody or other ligand, or formulated (liposome, endosome, polymer, small drugs). Peripheral blood (hPBMCs, serum, and whole blood), fibroblasts etc from patients with and without the conditions of interest as a model to test the modified RNA oligonucleotides in a more relevant biological context.
Project 2: Screening modified RNAs in ex vivo tissue
Ex vivo work will include testing modified RNA oligonucleotide in ex vivo tissue from humans, rodents and large animals like pig and sheep (primarily skin, muscle, bone, and synovial tissue) to investigate the functionality of the modified oligonucleotides in a more biologically relevant context.
Project 3: Screening modified oligonucleotides in vivo.
Animal models will be used to test toxicity, tissue distribution, pharmacokinetic/pharmacodynamic (PK/PD) parameters, and phenotypic efficacy in models of disease. In addition, the specific oligonucleotide formulation, or if the co-administration of a drug (i.e small molecule) will enhance various in vivo parameters, will also be tested. In vivo experiments involve injection of mice by several routes (i.e IV, SC, IP, ICV etc.) with the in vitro tested oligonucleotides. Like the in vitro experiments, oligonucleotides can be naked, in liposomes, and/or injected with a drug. Blood samples may be sent to Animal Medicine core for CBC and blood chemistry assessment.
Project 4: Cell type specific delivery of modified oligonucleotides in mice and human cells.
To look at various cell populations to determine the amount of uptake of a fluorescently labeled modified oligonucleotides mice will be injected and specific organs will be harvested. FACS will be used to determine knockdown in human cell lines and the mouse. Flow sorting of human cell lines and mouse tissues will be used to quantitate uptake in specific cell types
Project 5: Generation of chondrocytes from mouse joint tissue.
Chondrocytes will be cultured from mouse tissue to allow for testing of oligonucleotides in relevant cell types.
Project 6: Developing therapeutics for dermatological responses to allergy and inflammation.
To study skin diseases in mice, tools include genetically modified mouse strains, in vivo cell transfers, administering small molecules, antibodies/biologics, light exposure, tumor and other cells/cell lines, modified diets, allergens, irritants, and combination therapies of these categories. Flow cytometry, histology, and molecular biology methods will be used to analyze the mice and their tissues
A mouse model of vitiligo that is based on adoptive transfer of transgenic melanocyte-specific, autoreactive CD8 T cells into hosts. The adoptive transfer is followed by infection of the host with recombinant vaccinia virus, or DCs, that expresse(s) the antigen recognized by the T cells in order to stimulate the cells in vivo. Recombinant vaccinia virus (rVV) vectors, strain Western Reserve, expressing autoantigens (rVV-hgp100, rVV-tyr, rVV-MART1, rVV-trp1). Preclinical studies may include testing novel treatments in mouse models of skin disease for efficacy and mechanism of action, as well as exploring functional contributions of cells or proteins in the pathogenesis of skin diseases. Translational studies may include recruiting subjects with skin diseases and sampling their skin, bodily fluids (such as blister fluid), and blood/PBMCs for analysis of cells and proteins.
Brefeldin A will be used in some experiments to determine which cell populations make cytokines in vivo.

Diphtheria Toxin will be administered either topically, intradermally, or i.p. in order to deplete specific cell types expressing the DTx receptor.
Lipopolysaccharide will be used as an adjuvant in mice immunized by injection of peptides plus adjuvant (LPS and/or poly-IC) or recombinant vaccinia virus either intraperitoneally or through scarification to activate transferred cells in vivo.
Staphylococcus aureus (MSSA; including a GFP expressing strain) and Candida albicans will be used to infect Human skin graft on recipient mice or mouse skin to study the immune cells populating the skin. Skin transplantation: Recipient mice will be transplanted with skin grafts prepared from human donors.
Human cells/tumor cells/PBMCs: In order to study the role of human immune system to control autoimmunity and melanoma, de-identified human primary melanocytes, PBMCs, and excess melanoma specimen obtained from excisional surgery in patients with recurrent melanoma will be transplanted into immunodeficient mice.
Mouse melanoma cells: In order to study the role of immune system to control melanoma, the B16-F10 melanoma cell line will be cultured in sterile condition in our lab, and tumor cells will be injected either subcutaneously or intradermally into the recipient mice.
Chemically modified oligonucleotide test article description:
The complex chemical architecture of these compounds is necessary to support efficient delivery of oligonucleotide throughout the tissues. Oligonucleotides can be conjugated or formulated to enhance distribution. In house production of "pharmaceutical grade" agents. In addition, oligonucleotide might have a fluorescent dye for detection in distribution studies.

Transgenic mouse core: Will be used to breed hTFRC C57BL/6 mice (already generated by Prof. Miguel Esteves.
Flow Core: We will be sorting mouse cells from in vivo experiments or human cells from human blood (in vitro) or skin (ex vivo) after treatment with fluorescently labeled modified oligonucleotides to look at accumulation and silencing in different cell types
Light Microscopy Core: Following injection of modified oligonucleotides we will visualize uptake and localization in different fixed tissue types as well as examining impact on histology (H&E).
Animal Imaging Core: Assessing biodistribution of fluorescently labelled modified oligonucleotides following systemic or local administration by IVIS.
Mouse Metabolic Phenotypic core: In the case where metabolic changes in mice due to oligonucleotide administration are expected (eg. Myostatin treatment for muscle growth) the metabolic phenotyping core may be used.

**IBC Discussion and Vote**

**Discussion:** The reviewer discussed that this is a new protocol, the overall goals of which are to develop RNA based therapeutics for several disease indications with an expansive list of projects and objectives. There's a variety of gene targets, but one issue in the protocol is that the lab doesn't clearly define what the gene targets are. One of the projects planned involves skin diseases similar to those that were described and approved for the Harris Lab. A discussion was raised about the need for specificity in cell lines and vectors, and the importance of accurate descriptions in the protocol.

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

**BSL/ABSL:** **BSL-2; BSL-2 Enhanced Flow Sorting; ABSL-2**

**NIH Guidelines:** III-D, III-E, III-F

2) Investigator: Fang, W
Title: Identification of cellular factors that regulate RNA sensing in innate immunity
**IBC Registration:** **I-785-26, Renewal**

Training Verification: Acceptable
Brief Summary: The goal of this project is to identify mechanisms by which mammalian cells restrict or enhance self-RNA recognition in innate immunity

**Brief Summary and Review by Primary Reviewer**

**Overview and Objectives:** The goal of this project is to identify mechanisms by which mammalian cells restrict or enhance self-RNA recognition in innate immunity

**Experimental Approach:** Cell lines: common established mammalian cell lines such as HEK293T, K562, A549, and keratinocyte line N/TERT2G.
Lentivirus for CRISPR-mediated gene knockout: second generation lentivirus
To generate CRISPR-mediated gene knockouts in mammalian cells, candidate gRNAs are cloned into pLentiCRISPRv1, which are transfected into HEK293T cells together with packaging vectors pCMV-VSV-G and pCMV-dR8.2 dvpr using Lipofectamin 2000. After 72 h, the media is collected, cleared by centrifugation, and added to target cells in 12-well tissue culture plates. Polybrene is supplemented at 8 µg/ml. The plates are centrifuged at 1,200 g for 2 h at room temperature and then returned to the 37°C incubator. After 24 h, cells are washed with PBS and then selected for gRNA expression with puromycin for ~5 days. Genomic DNAs from these polyclonal cell lines are extracted using QuickExtract DNA Extraction Solution. The target locus is PCR amplified, and the products are sequenced by Sanger sequencing to select efficient gRNAs. The polyclonal cells expressing efficient gRNAs are then single-cell sorted to obtain clonal cell lines. Cell lines are screened by Sanger sequencing to find those that contain desired mutations at the target loci.
To generate CRISPR-mediated gene knockdowns, similar workflow will be followed.

**IBC Discussion and Vote**

**Discussion:** The reviewer discussed this protocol is a five-year renewal for the use of human cell lines, lentiviral vectors, and other RNAs, focusing on innate immunity and CRISPR-mediated gene knockout or knockdown. The reviewer discussed confirmation that certain infectious agents and cell lines are no longer in use, and the lab should make updates to the flow addendum if they are still being used.
**Meeting Decision:** Vote to approve upon completion of action items.
**BSL/ABSL:** **BSL-2; BSl-2 Enhanced Flow Sorting**
**NIH Guidelines:** III-D; III-F

3) Investigator: Irazoqui, J
Title: Studies of Innate Immune Responses to Bacterial Infection
**IBC Registration:** **I-675-26, Renewal**
Training Verification: Acceptable pending completion of PI training
Brief Summary: The overall objective of the project is to elucidate pathways of host defense against bacterial infection. There are two specificobjectives: 1) to elucidate genetic pathways using C. elegans, and 2) to perform translational studies using mouse cells and genetic in vivo models.

**Brief Summary and Review by Primary Reviewer**

**Overview and Objectives:** The overall objective of the project is to elucidate pathways of host defense against bacterial infection. There are two specific objectives: 1) to elucidate genetic pathways using C. elegans, and 2) to perform translational studies using mouse cells and genetic in vivo models.

**Experimental Approach:** The experimental approach involves infection of nematodes in Petri plates by direct nematode feeding using solid media cultures of bacterial pathogens Staphylococcus aureus in the USA300 (LAC) background, Pseudomonas aeruginosa in the PA14 background, and Salmonella enterica serovar Typhimurium in the SL1344 background. As controls, we use the nonpathogenic Escherichia coli strains DH5alpha, HT115, and OP50 and C. elegans microbiota. In addition, mouse and human cell line infections are performed in vitro using liquid cultures of Salmonella and Staphylococcus, at MOI ranging from 1 to 100, depending on the application. Additionally, mouse and human cell lines are exposed to LPS in vitro (10-1000 ng/ml). In vivo mouse infections take place by oral gavage with defined density liquid cultures of Salmonella. Mice are housed in the mouse facility during the length of the experiment, after which they are euthanized for pathology and CFU quantification in tissues. The objectives entail the manipulation of recombinant nucleic acids for gene functional studies, in the form of expression constructs for C. elegans genes (e.g. flavin-containing monooxygenase FMO-2) for in vivo expression in nematodes, and expression constructs for mouse and human genes (e.g. TFEB, TFE3, FMO5) for in vitro expression in bacteria and cell lines.

**IBC Discussion and Vote**

**Discussion:** The reviewer discussed that this is a five-year renewal for elucidating host defense against bacterial infection using C. elegans, mouse cells, and human cells. The reviewer described the experimental approach, including infection of nematodes with various pathogens, and in vitro and in vivo experiments using human and mouse cell lines. The reviewer highlighted the use of flow cytometry, light microscopy, and various vectors and plasmids for gene manipulation and expression.

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

**BSL/ABSL:** **BSL-2; BSL-2 Enhanced Flow Sorting; ABSL-2**

**NIH Guidelines:** III-D; III-F

4) Investigator: Mitchell, A
Title: Cell responses to external perturbations and cell evolution in novel environments
**IBC Registration:** **I-718-23, Amendment**
Training Verification: Acceptable pending completion of PI training
Brief Summary: Study the mechanisms of sensitivity of Staphylococcus aureus to toxic compounds (i.e antibiotics, non-antibiotics, natural products, etc)

**Brief Summary and Review by Primary Reviewer**

**Overview and Objectives:** Amendment to Study the mechanisms of sensitivity of Staphylococcus aureus to toxic compounds (i.e antibiotics, non-antibiotics, natural products, etc)

**Experimental Approach:** Adding Staphylococcus aureus (MRSA USA300 strain JE2 and Rosenbach 29213 (Wichita) strain) and mutants in the Nebraska Transposon Mutant Library (NTML) Screening Array (200 Staphylococcus aureus subsp. aureus USA300 JE2 transposon mutants). Also adding Staphylococcus aureus Rosenbach 29213 (Wichita; MSSA) transposon mutant library, and Staphylococcus aureus USA300 JE2 transposon mutant library from Stephen J. Salipante Lab, University of Washington School of Medicine.
No use in animals.

**IBC Discussion and Vote**

**Discussion:** The reviewer discussed this an amendment from Dr. Mitchell on cell responses to external perturbations, focusing on the mechanisms of sensitivity of

Staphylococcus aureus to toxic compounds. The reviewer described the addition of new strains of Staphylococcus aureus and libraries of genetically modified strains for sensitivity testing. The use of transposon mutants and other genetic tools for identifying important genes, and the inclusion of new lab personnel experienced in bacterial work was highlighted.

**Meeting Decision:** Vote to approve upon completion of action items.
**BSL/ABSL:** **BSL-2**
**NIH Guidelines:** III-D

5) Investigator: Reboldi, A
Title: B Cell Response at the Mucosal Interface
**IBC Registration:** **I-666-26, Renewal**
Training Verification: Acceptable
Brief Summary: In mammals, mucosal tissue, such as intestine, lung and skin, are naturally and constantly exposed to such invaders present in the environment. The overall objective of this project is to understand (in mice) how the immune system normally discriminate and react against different type of invaders in order to maintain a balance homeostasis at the mucosal interface. A striking ability of the immune system is to remember a specific invader and generate long-term protection against it after a single encounter: successful vaccines rely on this immunological "memory" in order to induce protection against infectious agents: a second goal of these experiments is to study how these memory cells are generated and maintained at the mucosal interfaces. An additional goal of these experiments is to investigate Human PBMCs Candida parapsilosis

**Brief Summary and Review by Primary Reviewer**

**Overview and Objectives:** The overall objective of this project is to understand how the immune system discriminates and responds to different types of microbial challenges in order to maintain homeostasis at the mucosal interface. Specifically, this project seeks to define the immune, metabolic, and microbial requirements that support the productive and functional induction of immunity in mucosal tissues, both under steady-state conditions and during pathogenic insults.

A second goal is to study how memory cells are generated and maintained at the mucosal interfaces.

**Experimental Approach:** To elucidate the interplay between commensal bacteria and memory cell responses, the anatomical distribution of 16S ribosomal RNA will be analyzed at mucosal surfaces and correlated with dynamic changes in commensal-specific immune cell populations. To investigate B-cell activation, survival, and memory formation, B-cell receptors from B cells reactive to commensal bacteria will be cloned and expressed using retroviral transduction.

To study mucosal B cell response mice will be treated oraly with cholera toxin or with peracetic acid killed salmonella particle. A combination of experimental approaches such as adoptive transfer, fate mapping, immunofluorescence and immunohistochemistry will be performed. In a related set of experiments, to investigate how mucosal memory cells mount a recall response, bone marrow chimeras will be generated to express commensal-specific BCRs through retroviral transduction. A similar experiment will be performed using the enteroinvasive bacterial pathogen Salmonella enterica serovar Typhimurium.

To address metabolic requirements, the relationship between commensal microbiota and oxysterols (important regulators of cholesterol metabolism) will be studied using mice with enzymatic deficiencies. To achieve cell-specific enzymatic deficiency, mice expressing the diphtheria toxin receptor under a cell-specific promoter will be utilized. Treatment with diphtheria toxin will ablate the specific cell population.

Pertussis toxin will be used in vitro to inhibit G protein alpha subunit i and assess its effect on migration.

Mice will be initially orally colonize with Candida parapsilosis (1 × 10 6 -5 × 10 6 CFU) once a week for 4 weeks, B cell and antibody responses will be measured. Following infection, mice will be euthanized, organs will be collected for flow cyometry, ELISA, ELISPOT and CFU measurements. These experiments will be repeated in mice pretreated with broad

spectrum antibiotics (AVNM for one week). Candida parapsilosis is a commensal strain, rapidly cleared by animals after colonization.

**IBC Discussion and Vote**

**Discussion:** Reviewer discussed that this submission is a five-year renewal for understanding immune cell responses to microbial challenges, focusing on mucosal hemostasis and memory cell formation. The reviewer mentioned that B cell receptors from B cells reactive to commensal bacteria, will be cloned and expressed using in vitro retroviral transducer and will be cloned and expressed using retroviral transduction. The lab doesn't specify where these receptors will be expressed or in which cell lines. It's not clear on the experimental approach so a more detailed explanation should be provided in the experimental approach. The reviewer also highlighted the use of mouse models, including bone marrow chimeras and bacterial pathogens.

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

**BSL/ABSL:** **BSL-2; ABSL-2**

**NIH Guidelines:** III-D, III-E, III-F

6) Investigator: Ryder, S
Title: RNA regulation in nematodes
**IBC Registration:** **I-305-26, Renewal**
Training Verification: Acceptable pending completion of PI training
Brief Summary: We wish to understand how information is transferred from mother to progeny through the oocyte cytoplasm. We specifically focus on post-transcriptional regulatory mechanisms where maternally-supplied proteins and RNAs govern early cell fate specification events. We wish to understand the basis of RNA recognition by maternal RNA-binding proteins, identify their most important targets, and describe the regulatory mechanisms. Our work combines in vitro biochemistry, genomics, computational analyses, and functional studies in live invertebrate nematode worms to address this fundamental question in developmental biology.

**Brief Summary and Review by Primary Reviewer**

**Overview and Objectives:** Dr. Ryder's research team wishes to understand how information is transferred from mother to progeny through the oocyte cytoplasm of C. elegans. The team will specifically focus on post-transcriptional regulatory mechanisms where maternally-supplied proteins and RNAs govern early cell fate specification events. The team wishes to understand the basis of RNA recognition by maternal RNA-binding proteins, identify their most important targets, and describe the regulatory mechanisms. The study will combine in vitro biochemistry, genomics, computational analyses, and functional studies in live invertebrate nematode worms to address this fundamental question in developmental biology.

**Experimental Approach:** The biochemical studies will include expressing C. elegans proteins recombinantly in bacteria, purifying them, and then using them in fluorescent binding assays to describe the affinity and specificity in quantitative terms. These experiments will make use of synthetic RNA oligonucleotides or in vitro transcripts that comprise pieces of the 3' untranslated region of maternal RNAs. Proteins will be purified from less than 4L of bacterial culture. Invertebrate animal experiments will include making transgenic animals using single copy integrated GFP reporters. Briefly, plasmids encoding a reporter gene will be produced, propagated in bacteria, and purified. Reporter DNA and plasmids encoding selectable markers are microinjected into C. elegans hermaphrodite germlines. Progeny will be

screened for successful transformation by PCR and by imaging. Transgenic animals will be studied by light and fluorescence microscopy.
The team will also make mutant invertebrate animals by CRISPR-Cas9 genome editing. Recombinant Cas9 in complex with a synthetic guide RNA, or plasmids encoding Cas9 and guide RNAs will be microinjected into adult hermaphrodite germlines. Successful editing is assessed by PCR and sequencing. Animals that harbor mutations will be studied by light and fluorescence microscopy to assess developmental phenotypes, and in reproduction assays to assess reproductive fecundity. Most editing will be done through deletion of a region of a gene of interest using two synthetic guide RNAs. Knock-in of epitope tags, degron tags, and fluorescent protein tags into the endogenous locus using rDNA or sDNA recombination repair donors will also be performed.
Genomics experiments will include whole genome sequencing of mutant worms, RNA-seq of the mRNA content of mutant worms, polysome profiling to define ribosome association, and polyA tail sequencing to assess the distribution of polyA tail lengths. In these experiments, total DNA or RNA will be recovered from bulk worm populations, a sequencing library will be produced, Illumina adaptors added, and the library will sequenced on a next-generation sequencer.
C. elegans will be cultured on plates or in liquid media with E. coli strain HB101, OP50, or Comamonas strain DA1877 as a food source.

**IBC Discussion and Vote**

**Discussion:** The reviewer discussed that this is a 5-year renewal application for using RNAs and CRISPR components in experiments with nematodes. The reviewer describes the use of RNA sequencing, polysome profiling, and poly A tail sequencing, and the addition of new nematode species and genes. The need for additional experiments pertaining to new species and the use of non-K12 E. coli strains for BSL-1 containment was highlighted.
**Meeting Decision:** Vote to approve upon completion of action items.
**BSL/ABSL:** **BSL-1**
**NIH Guidelines:** III-E, III-F

7) Investigator: Wang, J
Title: Receptor-based drug models
**IBC Registration:** **I-303-26, Renewal**
Training Verification: Acceptable
Brief Summary: The global objective of these studies is to define host-pathogen interactions and innate immune responses, with a focus on viruses and bacteria in human and rodent cell culture and rodent models.An example project is to identify host factors and mechanisms of alphavirus (Sindbis virus or SINV, chikungunya, Venezuelan encephalitis virus), arenavirus (LCMV Armstrong, LCMV clone 13, Junin Candid #1), and respiratory virus (influenza A, respiratory syncytial virus) infection in cell culture models. The strains are attenuated and used under BSL-2 conditions, with the exception of LCMV-WE, which is risk group 3 and used under BSL-3/ABSL-3 conditions. GFP-expressing strains of such viruses are used in *in vitro* experiments to rapidly identify infected cells. We are silencing expression of select host factors and host genomes using siRNAs or antisense oligonucleotides or ASOs, assessing the impact on viral infection/replication. *In vivo* experiments are performed in mice with siRNA or ASOs delivery targeting host or viral genomes. For another project, we are testing whether oral commensal organisms (*Prevotella salivae, Veillonella infantium)* can prevent severe manifestations of respiratory viral infections by altering the immune response to infection within the respiratory tract. To elucidate the alterations to the immune pathways during these infections, we are comparing the responses to NOD2-agonist MDP, the TLR2/6 agonist Pam2CSK4, and the TLR2/1 agonist Pam3CSK4 with those to the

commensal bacteria in the context of virus infections. Historically, this registration has included bacteria for *in vivo* studies in mice, SARS-CoV-2 clinical samples for sequencing, and human cell lines for flow sorting. These are inactive projects and have been removed. We have added *Streptococcus pneumoniae* sourced from the clinical laboratory for capsule typing studies. For neuraminidase inhibitor resistant/mutant influenza A is an inactive project, but the protocols are retained under BSL-2+.

## Brief Summary and Review by Primary Reviewer

**Overview and Objectives:** The global objective of these studies is to define host-pathogen interactions and innate immune responses, with a focus on viruses and bacteria in human and rodent cell culture and rodent models.
to identify host factors and mechanisms of alphavirus (Sindbis virus or SINV, chikungunya, Venezuelan encephalitis virus), arenavirus (LCMV Armstrong, LCMV clone 13, Junin Candid #1), and respiratory virus (influenza A, respiratory syncytial virus) infection in cell culture models. The strains are attenuated and used under BSL-2 conditions, with the exception of LCMV-WE, which is risk group 3 and used under BSL-3/ABSL-3 conditions. GFP-expressing strains of such viruses are used in in vitro experiments to rapidly identify infected cells. We are silencing expression of select host factors and host genomes using siRNAs or antisense oligonucleotides or ASOs, assessing the impact on viral infection/replication. In vivo experiments are performed in mice with siRNA or ASOs delivery targeting host or viral genomes.

**Experimental Approach:** No change except in regard to new agents/isolates/genes added - no highlights.
Viruses or bacteria will be used to infect mammalian cell lines (including those of human, NHP, mouse, and hamster origin) in vitro and mice in vivo. The disease progress incurred by the pathogens in various cell types and animals will help determine which molecules are important for pathogenesis. Standard chemotherapeutic agents, soluble protein receptors, or antibodies will also be tested for their effects on pathogenesis. Recombinant viruses expressing fluorescent proteins or luciferase allows us to observe the degree of infection of cells and various organs using fluorescent detection techniques such as fluorescent microscopy and luminescence detection. Retroviral vectors will be used to deliver siRNA or sgRNAs constructs to cell lines and in vivo to determine the effects of decreased expression of specific genes.
siRNA/ASOs as therapeutics: Human cell lines (including A549, U2OS) are pretreated with siRNAs or ASOs targeting the host or virus genome, then infected with SINV, LCMV, Junin Candid #1, or influenza A virus or respiratory syncytial virus at the optimized MOI for 24 h and quantified by plaque assay. siRNAs or ASOs targeting the virus or host are added to the cells prior to infection. In vivo experiments are performed in mice with siRNA delivery targeting host or viral genomes then infection with LCMV or SINV. All are performed at BSL-2/ABLS-2 conditions except for LCMV WE which is BSL-3/ABSL-3
Bacterial commensal and immune agonist experiments: Cell lines (i.e., A549, NP69) are pretreated with commensal bacteria strains alone or with different immune agonists (MDP, Pam2CSK4, Pam3CSK4, LPS) and infected with influenza A virus (H1N1 strain p2009). Mice are inoculated intranasally with sterile PBS, conditioned media from bacterial cultures, resuspended live bacteria, heat-killed resuspended bacteria, MDP, PAM2SK4, PAM3SK4, or LPS. Mice are infected intranasally with virus, euthanized between days 6 and 21, then serum and lungs will be collected for analysis (e.g., RNAseq).
Other: Clinical isolates of Streptococcus pneumoniae will be expanded in media and stored at -80 °. DNA may be isolated from the samples for sequencing.

## IBC Discussion and Vote

**Discussion:** The reviewer discussed that this is a 5-year renewal application for receptor-based drug models, focusing on host-pathogen interactions and innate immune responses. The protocol describes the use of various viruses and bacteria in cell culture and rodent models, and the addition of Streptococcus pneumoniae

clinical isolates for capsule typing studies. The need for a medical SOP for LCMV W E strain, and the importance of accurate descriptions and training for personnel working with BSL-3 agents was highlighted.

**Meeting Decision:** Vote to approve upon completion of action items.
**BSL/ABSL:** **BSL-3; ABSL-3**
**NIH Guidelines:** III-D, III-E, III-F

8) Investigator: Zamore, P
Title: Mechanisms of Small RNA Silencing in Tissue-Specific Mouse Models
**IBC Registration:** **I-664-26, Renewal**
Training Verification: Acceptable
Brief Summary: Our lab investigates mechanisms of targeted gene silencing in mice by either 1. microRNA or 2. piRNA pathways.

Aim #1: Our first approach aims to leverage endogenous mechanisms of microRNA-directed gene silencing to investigate the therapeutic efficacy of synthetic single-stranded oligonucleotide microRNA tethers in specifically silencing mRNA targets in vivo. Since microRNA expression is tissue-specific, we are developing synthetic microRNA tethers to target endogenous gene expression through recruitment of Argonaute-loaded microRNA species. We plan to test this therapeutic approach in vitro using cultured mouse cells, and in vivo in adult mouse skeletal muscle and heart.

These experiments will comprise:

- In vitro transfection of synthetic oligonucleotide tethers into mouse cell lines to determine efficacy of silencing of the Activin A transcript and the impact on myoblast differentiation.
- In vivo injection of synthetic oligonucleotide tethers into mice to determine therapeutic efficacy in a model of age-related sarcopenia.

Aim #2: piRNAs are produced from lncRNAs that are only transcribed in the mouse testes. As verified testicular cell lines are unavailable, biogenesis of these transcripts can only be studied in transgenic mouse models. Specifically, we will investigate post-transcriptional processing of these lncRNAs by splicing and integrator complexes, which determines the fate of these transcripts in piRNA production.

These experiments will comprise:

- Generating transgenic mouse mutants by insertion of the Minx intron sequence, containing either a functional or nonfunctional splice site, into two lncRNA loci *(pi2* and *pi17*). We also plan to insert a flag tag into the coding sequence of Ints11, a component of the Integrator posttranscriptional processing complex. These mutant lines will be produced at the Transgenic Animal Core. Testicular germ cells from these animals will be the material source for all downstream experiments.

## Brief Summary and Review by Primary Reviewer

**Overview and Objectives:** The lab investigates mechanisms of targeted gene silencing in mice by either 1. microRNA or 2. piRNA pathways.

Aim #1: Our first approach aims to leverage endogenous mechanisms of microRNA-directed gene silencing to investigate the therapeutic efficacy of synthetic single-stranded oligonucleotide microRNA tethers in specifically silencing mRNA targets in vivo. Since microRNA expression is tissue-specific, we are developing synthetic microRNA tethers to target endogenous gene expression through recruitment of Argonaute-loaded microRNA species. We plan to test this therapeutic approach in vitro using cultured mouse cells, and in vivo in adult mouse skeletal muscle and heart.

These experiments will comprise:

- In vitro transfection of synthetic oligonucleotide tethers into mouse cell lines to determine efficacy of silencing of the Activin A transcript and the impact on myoblast differentiation.

• In vivo injection of synthetic oligonucleotide tethers into mice to determine therapeutic efficacy in a model of age-related sarcopenia.

Aim #2: piRNAs are produced from lncRNAs that are only transcribed in the mouse testes. As verified testicular cell lines are unavailable, biogenesis of these transcripts can only be studied in transgenic mouse models. Specifically, we will investigate post-transcriptional processing of these lncRNAs by splicing and integrator complexes, which determines the fate of these transcripts in piRNA production.

These experiments will comprise:

• Generating transgenic mouse mutants by insertion of the Minx intron sequence, containing either a functional or nonfunctional splice site, into two lncRNA loci (pi2 and pi17). We also plan to insert a flag tag into the coding sequence of Ints11, a component of the Integrator posttranscriptional processing complex. These mutant lines will be produced at the Transgenic Animal Core. Testicular germ cells from these animals will be the material source for all downstream experiments.

**Experimental Approach:** Aim#1:

a. Synthetic modified microRNA tethers will be tested in vitro by transfection into cultured mouse cell lines C2C12 muscle cells, NIH 3T3 fibroblasts, or mouse HL-1 cardiomyocytes. SBE2-Luciferase plasmid will be co-transfected to determine engagement of downstream activin A signaling factors. Two tethers will be tested: one tether recruits miR1 (specific to skeletal muscle), another tether recruits miR208 (specific to cardiac muscle). As the seed sequences of these tethers are identical in mouse and human, these RNAs can be expected to have biological activity in humans.

b. Synthetic modified tethers will be introduced by intravenous or subcutaneous injection of mice at doses not exceeding 10 mg/kg; injections will be given once to four times per animal. Blood from facial vein puncture will be collected at 3 days, 14 days, 28 days, 42 days, 56 days, 70 days, 84 days, 112 days, and 140 days post-injection for Activin A ELISA and to detect circulating levels of synthetic tether.

Body composition of injected mice will be measured at the Mouse Metabolic Phenotyping Core a maximum of five times/ animal over 138 days. Mice will be sacrificed at day 28, day 84, or day 156; serum and tissues will be processed for RNA sequencing and histology.

Aim #2: Transgenic mice expressing Minx intron in mouse germ cell-specific piRNA genes will be generated in the Transgenic Core. Unfixed germ cells from these mice (as well as WT) will be sorted at the Flow Cytometry Core. Sorted cells will be processed in our lab for all downstream experiments (RNA sequencing, histology, etc.)

**IBC Discussion and Vote**

| | |
|---|---|
| **Discussion:** | Reviewer discussed action items |
| **Meeting Decision:** | Vote to approve upon completion of action items. |
| **BSL/ABSL:** | **BSL-2; BSL-2 Flow Sorting; ABSL-1** |
| **NIH Guidelines:** | III-E, III-F |

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

N/A

## VI. Information from the field (Senior Biosafety Officer)

1) **Large animals moving from Tufts to UConn**

## VII. Other Business

Acknowledgement Items:

1) **Ramanathan 908-24 BB2121-EAP-001** Update-
*Expanded access protocol (EAP) for subjects receiving idecabtagene vicleucel that is nonconforming for commercial release. Idecabtagene vicleucel (ABECMA® [ide-cel; bb2121]), referred to as ide-cel hereafter, is a genetically modified autologous T-cell immunotherapy product consisting of T cells transduced with an anti-BCMA 02 CAR LVV for the treatment of adult patients with MM, including RRMM.* Update-
Updated details of clinical studies (ie, status, cutoff dates), PK summary, efficacy results, available safety data for studies and A detailed summary of changes to the RSI is provided in "Explanation of Changes to the Reference Safety Information" section following the SAR table

2) **Hafer 443-24 & Luzuriaga 864-23: New trial: Merck V116 *A Test-Negative Case-Control Study to Evaluate the Effectiveness of V116, CAPVAXIVETM Against Pneumococcal Pneumonia in Older Adults* |PI: Jennifer Wang** Update-
involves processing/manipulation and/or storage of human samples in a research laboratory covered by a blanket IBC approval

**Adjourned at 1:23 pm**