IBC Meeting Minutes

November 20, 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	X	Richard Ellison III (alt)		Regino Mercado-Lubo (alt)	X
Kris Giaya	X	Amelia Houghton		Sharone Green (alt)		Casey Moran (alt)	X
Hardy Kornfeld		Eric Rouse	X	Jennifer Wang (alt)		Melissa Scannell	X

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

I. Introductory Remarks

1) The Chair brought to the attention of the Committee the meeting minutes from the previous IBC meeting. **Meeting Decision: Vote to approve the October 16, 2025 Meeting Minutes.**

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

Past incidents that remain open:

1) 5/21/2025: BSL-3 PAPR incident. One person (who later traveled to Africa) had conversion of IGRA test
2) 07/08/2025: Needlestick in BSL-3. Potential TB exposure

Recent Incidents:

3) 10/19/2025: Needlestick while administering Fingolimod to a mouse. Researcher is currently pregnant.
4) 10/24/2025: Broken skin/ cut under glove while researcher was working with HIV infected cells from a plastic or glass shard. Molecular clone of HIV (rNA).
5) 11/05/2025: Issue reported- researcher worked with pseudo-typed lentivirus vectors without gloves last year sometime. This was brought to our attention after a routine HIV screening by PCP and who gave unexpected results. Testing details are not yet available.

III. Protocols Reviewed Administratively

1) Investigator: Chandraker, A
   Title: Renal BioBank
   IBC Registration: 895-24, Amendment
   Training Verification: Acceptable
   Brief Summary: Maintain a repository of biological specimens (blood, tissue, urine, and sputum) and associated clinical data to support kidney disease research. Provide a secure and compliant infrastructure for collecting, coding, and storing human samples for future translational and immunological studies related to renal pathophysiology and transplantation.
   BSL/ABSL: **BSL-2**
   NIH Guidelines: N/A

2) Investigator: Kim, J
Title: Investigating Role of IL-12 Induced Inflammation in Metabolic and Neuronal Disease
IBC Registration: 693-22, Amendment
Training Verification: Acceptable pending completion of PI training
Brief Summary: Using LPS as a positive control for neuroinflammation, we will use an intraperitoneal injection to determine the role of peripheral IL-12 in neuroinflammation
BSL/ABSL: **BSL-2; ABSL-1; Administration to Animals using BSL-2 Precautions/ Sharps Safety**
NIH Guidelines: N/A

3) Investigator: Lee, D
Title: Ocular immunology – autoimmune uveitis
IBC Registration: 848-22, Amendment
Training Verification: Acceptable
Brief Summary: To add: LPS: Subcutaneous route of administration
BSL/ABSL: **BSL-2; ABSL-1; Administration to Animals using BSL-2 Precautions/ Sharps Safety**
NIH Guidelines: N/A

4) Investigator: Mitchell, A
Title: Cell responses to external perturbations and cell evolution in novel environments
IBC Registration: 718-23, Amendment
Training Verification: Acceptable pending completion of PI training
Brief Summary: We will extract DNA from bacterial isolates to sequence their genetic material
BSL/ABSL: **BSL-2**
NIH Guidelines: N/A
Notes: If bacterial isolates are uncharacterized the IBC is unable to assign a RG. RG-2 is assumed but we need more information about what soil bacteria will be of interest (aerobes/ anaerobes, gram positive/ gram negative, AFB positive/ negative, etc.)

IV. Protocols to Discuss

1) Investigator: Atukorale, P
Title: Lentiviral Vectors for Addition of Reporter Genes into Murine Melanoma Cell Line
IBC Registration: 838-22, Amendment
Training Verification: Acceptable pending completion of PI training
Brief Summary: Lentiviral particles will be generated using HEK293T cells; the lentiviral vector will contain a fluorescent protein and a luciferase reporter gene. Packaging plasmids will be co-transfected to produce replication-incompetent viral particles.
Viral supernatant will be collected, filtered, and used to transduce cell lines under BSL-2 conditions. A p24 ELISA will be performed on the supernatant from transduced cells across at least 3 passages. After confirming there is no replication-competent lentivirus, transduced cells will be selected using Fluorescent Activated Cell Sorting. The B16F10 iRFP720 Luc cells will then be delivered subcutaneously into C57BL/6 mice for tumor studies following all guidelines in IACUC protocol 202100093. Mice will be given GFP mRNA-loaded lipid nanoparticles intravenously and then euthanized. Organs will be harvested, imaged, processed, stained, and fixed for analysis of immune cell populations and mRNA uptake from lipid nanoparticles.
Excess lentiviral particles will be flash frozen in liquid nitrogen and stored at -80C.

Materials: lentiviral iRFP720 and luciferase plasmid, packaging plasmid and envelope plasmid
We would like to implement lentiviral transduction for other new tumor cells in the lab and, therefore, would like to include broader approval. These cell lines are: SY5Y, NBT3L, 9464D GD2, CHLA, NGP, SK-N-SH, Kelly, AND PC3.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: Reporter genes will be expressed in murine melanoma cell lines by lentiviral transduction to facilitate analysis of tumor microenvironments.

Experimental Approach: Lentiviral particles will be generated using HEK293T cells; the lentiviral vector will contain a fluorescent protein and a luciferase reporter gene. Packaging plasmids will be co-transfected to produce replication-incompetent viral particles.

IBC Discussion and Vote

Discussion: Reviewer discussed that there is some information needed for the cell lines being used and whether they are using human or murine cell lines. A discussion was raised regarding whether the IBC needs that information to determine what BSL/ABSL this work should be done at. Amendment will be required if other transduced cell lines will be used in vivo.
Meeting Decision: Vote to approve upon completion of action items.
BSL/ABSL: **BSL-2; BSL-2 Enhanced Flow Sorting; ABSL-1; Administration to Animals using BSL-2 Precautions/ Sharps Safety**
NIH Guidelines: III-D, III-F

2) Investigator: Cavacini, L
Title: Expression of Recombinant Monoclonal Antibodies and Protein Antigens
IBC Registration: **642-25, Renewal**
Training Verification: Acceptable
Brief Summary: We are generating and characterizing monoclonal antibodies to a number of target antigens and which will ultimately be used to prevent or treat disease. Specific targets include microbial agents, cytokines and cancer cells. These monoclonal antibodies will be isolated from B cells through either the generation of hybridomas or cloning the antibody genes from sorted B cells. Antibody genes (from hybridomas or B cells) or other human and non-pathogenic bacterial and viral proteins will be cloned into expression vectors that are transfected into either 293 or CHO cells for transient or stable cell line generation in order to generate sufficient material for study.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: To generate and characterizie monoclonal antibodies to a number of target antigens that will ultimately be used to prevent or treat disease. Specific targets include microbial agents, cytokines and cancer cells.

Experimental Approach: Antibody genes will be cloned from antigen specific B cells or from hybridomas by using RT-PCR to generate cDNA. B cells will be from human (PBL?), or from mice engrafted with a human immune system, or from mouse B cells that are transgenic for human immunoglobulin (HuMab mice or huBcl2 transgenic mice).Antibody variable regions are PCR amplified from cDNA and ligated into plasmids expressing either heavy chain (IgG (1-4) or IgA (1-2)) or light chain (kappa or lambda) constant regions. Genes encoding the target antigen will also be PCR amplified from cells. Alternatively, the known gene sequence may be synthesized by an outside vendor. The plasmids are transfected into Expi293 cells (suspension) for transient expression if small quantities of antibody are required or transfected into CHO

cells for stable cell line construction for larger quantities. To generate dimeric IgA or secretory IgA, pcDNA plasmids containing J-chain and/or secretory component are co-transfected with antibody heavy and light chains.

IBC Discussion and Vote

Discussion: Reviewer discussed that flow sorting is covered under a separate registration, so it's not required for this protocol. The reviewer discussed a single action item.
Meeting Decision: Vote to approve upon completion of action items.
BSL/ABSL: **BSL-2; ABSL-1**
NIH Guidelines: III-E, III-F

3) Investigator: Chan, Y
Title: Genome wide evaluation of allelic Effects on In-vitro Phenotypes
IBC Registration: **776-25, Renewal**
Training Verification: Acceptable pending completion of PI training
Brief Summary: We plan to test the genetic effects of a large number of phenotypes in-vitro to determine the extent to which genetic alleles influence these phenotypes in relation to human diseases. The goals are to be able to identify or replicate genetic alleles that could potentially be causative of a variety of human diseases like autism, hearing loss, Alzheimer's disease, viral infections, cell-death and susceptibility to various drugs and chemicals. To achieve this, we require the use of induced pluripotent stem cells as well as other cell culture cell types (fibroblasts, lymphoblastoid cell lines, PBMCs, cancer cell lines, etc) from a variety of different donors with different genotypes and also the ability to make gene edits to make changes to existing alleles in human cells and tissues.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: To test the genetic effects of a large number of phenotypes in-vitro to determine the extent to which genetic alleles influence these phenotypes in relation to human diseases. The goals are to be able to identify or replicate genetic alleles that could potentially be causative of a variety of human diseases like autism, hearing loss, Alzheimer's disease, viral infections, cell-death and susceptibility to various drugs and chemicals.

Experimental Approach: Use a variety of different cells including Human induced pluripotent stem cells (iPSCs), EBV-transformed B-lymphocytes, HEK293(T), A549, HELA, MCF7, AsPC-1, SH-SY5Y, Human Fibroblasts, Human PBMCs, Vero, MDCK. These cell lines are obtained from various biobanks/vendors or obtained from blood-draw or skin-biopsy from patients themselves. Use a variety of different donors with different genotypes.
Gene editing will be used to make changes to existing alleles in human cells and tissues.
A number of assays are then performed on these cell lines. They include,

A. Regular culture and passaging, freeze for storage, thawing out from liquid nitrogen store.
B. Reprogrammed into iPSCs via transfection of cocktail of Yamanaka factors. Delivery of Yamanaka factors will be performed by nucleofection (4D-Nucleofector™) of plasmid cocktail carry the factors.
C. Differentiate iPSCs reprogramed or otherwise into a variety of different other cell/tissue types, including neuronal cells, microglia, astrocytes, lung epithelium, otic progenitors and organoids.
D. Infections of these cell/tissue lines using a variety of viruses. The viruses and where they are obtained are listed below.
E. Treat these cell lines with Cisplatin to determine how different donor lines respond to the drug.
F. Disassociate organoid cells and perform flow cytometry analysis as well as cell sorting by staining for a variety of cell surface and intracellular markers or using the GFP expressed from the virus.

G. Extract DNA from sorted cells, perform next-generation sequencing on these generated libraries.
H. Extract RNA from these cells, sorted or otherwise and perform bulk RNA sequencing on them.
I. Create synthetic variation in these cell/tissue lines via a CRISPR library. Treat with a library of guide RNAs and CRISPR Enzyme, e.g. Cas9 using nucleofection, then make measurements of the infection rates using various established techniques. Examples include (i) plaque assay (ii) immuno-staining (iii) qPCR (iv) FISH (v) Flow cytometry. Use selection assay or cell sorting and perform DNA extraction and sequencing to determine what the genes are and/or what genes were edited.
J. Perform single-cell analysis RNA analysis on these cells, sorted or otherwise, using a variety of single-cell separation devices including 10X genomics, Parse Biosciences, Scale Biosciences, Fluent Biosciences, CS genetics.
K. Extract RNA for testing gene expression changes through qPCR as well as sequencing.
L. Perform flow cytometry, cell sorting to determine the expression of viruses (via antibody staining or detecting GFP expression) as well as staining the cells for cell-type specific markers using antibodies.
M. Detect/measure cytokine markers as well as other protein biological markers, e.g. Abeta and phosphor-Tau via ELISA assays from the media of the cells after infection.
N. Perform high-throughput in vitro spatial, spatial single-cell, RNA-sequencing (e.g. Stereo-seq by Complete Genomics) or proteomics (e.g. Akoya Biosciences) on infected tissue, i.e. cell culture, sliced organoids, etc.

The viruses used include,

1) Sendai Virus (Cantell Strain) - Charles River (https://www.criver.com/)
2) Influenza A/PR/8/34 (H1N1) - Charles River (https://www.criver.com/)
3) Influenza A X-31, A/Aichi/68 (H3N2) - Charles River (https://www.criver.com/)
4) Influenza A/Victoria/3/75 (H3N2) - Charles River (https://www.criver.com/)
5) Influenza A/Hong Kong/8/68 (H3N2) - Charles River (https://www.criver.com/)
6) Human herpesvirus types 6 Herpesvirus (HHV-6A GS) - Zeptometrix (www.zeptometrix.com)
7) HHV-6B (Z29) - Zeptometrix (www.zeptometrix.com)
8) HHV7 - Zeptometrix (www.zeptometrix.com)
9) Herpes simplex type 1 F Macintyre - Zeptometrix (www.zeptometrix.com)
10) Herpes simplex type 1 KOS with GFP inserted into genome. Virus obtained from David Knipe's lab at Harvard Medical School.
11) HCMV from commercial sources, e.g. ATCC.
12) TB40E-GFP: Human cytomegalovirus HCMV strain TB40/E with eGFP gene inserted into its genome.
13) TB40E-delta 128-131-GFP: Human cytomegalovirus HCMV strain TB40/E with the gene segment UL131-UL128 deleted from its genome. It has an eGFP transgene inserted.
14) TR5-GFP: Human cytomegalovirus HCMV strain TR5 with eGFP gene inserted into its genome.
15) TR5-delta 128-131-GFP: Human cytomegalovirus HCMV strain TR5 with the gene segment UL131-UL128 deleted from its genome. It has an eGFP transgene inserted. HCMV from 12-15 are obtained from Tim Kowalik's lab at UMass Chan Medical School.

Core Use:

CRISPR gene editing of human cell lines on genes of interest; BSL-2.

Flow core sorting of fixed and unfixed human cell-lines to isolate cells expressing various intracellular or surface markers; sorting at BSL-2 enhanced.

Imaging fixed organoid tissue sections for neuronal and other cell type markers; BSL-2.

IBC Discussion and Vote

Discussion: Reviewer discussed minimal action items.
Meeting Decision: Vote to approve upon completion of action items.

BSL/ABSL: **BSL-2; BSL-2 Enhanced Flow Sorting**
NIH Guidelines: III-D, III-E, III-F

4) Investigator: Fitzgibbons, T
Title: Hind Limb Ischemia
IBC Registration: **I-846-22, Amendment**
Training Verification: Acceptable pending completion of PI training
Brief Summary: To add: AAV's

Brief Summary and Review by Primary Reviewer

Overview and Objectives: The purpose of this study is to test the hypothesis that treatment with adeno-associated virus (AAV)- based vectors to target revascularization post-injury improves collateral vessel formation in mice with peripheral vascular disease.

Experimental Approach: Using an AAV-based vector to target revascularization post-injury. Intraocular injections of 3.5x10^10 vector particles into C57BL/6J mice will be performed to develop long-term vascular repair/regeneration in a model of hindlimb ischemia. The vector that the investigator will use is an AAV8 packaging the secreted modular calcium-binding protein (hSMOC1) gene driven by the liver-specific thyroxine-binding globulin (TBG) promoter.

IBC Discussion and Vote

Discussion: Reviewer discussed this amendment was straightforward but had some minor recommendations for some clarity. Reviewer discussed the few action items they found.
Meeting Decision: Vote to approve upon completion of action items.
BSL/ABSL: **BSL-1; ABSL-1 with Special Precautions; Administration to Animals using BSL-2 Precautions/ Sharps Safety**
NIH Guidelines: III-D, III-E, III-F

5) Investigator: Hayward, L
Title: A Phase 1/Phase 2 open-label single arm study with dose escalation (Part A), and dose expansion (Part B) parts to evaluate the safety, tolerability, and efficacy of SAR446268, an adeno-associated viral vector-mediated gene therapy in participants 10 to 50 years old with non-congenital myotonic dystrophy type 1
IBC Registration: **928-25, New**
Training Verification: Acceptable
Brief Summary: This is a Phase 1/Phase 2 open-label single arm study with dose escalation (Part A), and dose expansion (Part B) parts to evaluate the safety, tolerability, and efficacy of SAR446268, an adeno-associated viral vector-mediated gene therapy in participants 10 to 50 years old with non-congenital myotonic dystrophy type 1.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: This is a Phase 1/Phase 2 open-label single arm study with dose escalation (Part A), and dose expansion (Part B) parts to evaluate the safety, tolerability, and efficacy of SAR446268, an adeno-associated viral vector-mediated gene therapy in participants 10 to 50 years old with non-congenital myotonic dystrophy type 1 (DM1).

Experimental Approach: DM1 is a rare, progressive genetic disease characterized by a complex range of muscle-related and systemic manifestations that lead to life-threatening complications. Widely considered as the most common form of adult-onset muscular dystrophy caused by the expansion of an unstable CTG trinucleotide repeat in the 3' UTR of the DMPK gene that encodes for myotonin protein kinase in skeletal muscles. Normally, individuals have between 5 and 37 CTG repeats and while repeat lengths >37 are considered abnormal, full penetrance alleles occur with repeats of >50 which are more likely associated with symptomatic disease. The clinical manifestations of DM1 are mostly seen in patients with 50 and up to 4000 CTG repeats and are divided into 4 main subtypes.

There are currently no disease modifying therapies available for DM1 patients. Supportive and symptomatic therapy directed towards the various multi-systemic manifestations and complications such as medications (ie, anti-myotonia medications, anti-arrhythmics) rehabilitative therapy (ie, physiotherapy, speech therapy), surgery (ie, cataract surgery) and medical devices (ie, pacemaker insertion) remains the standard of care and mainstay of treatment. Despite these therapeutic measures, mortality continues to be mostly attributed to pneumonia/respiratory failure, cardiovascular disease, sudden death/arrhythmia, and neoplasms.

Targeting the aberrant expansion of CTG repeats within the DMPK gene that causes the protein splicing abnormalities responsible for the various disease manifestations in DM1 represents a novel disease modifying approach that can potentially alter the course of the disease and provide durable long-term benefits for DM1 patients. DMPK mRNA downregulation (eg, via siRNA and/or ASOs) in particular, appears well-tolerated in animal models of DM1 and has been shown to result in improvements in myotonia and muscle weakness with repeated and regular dose administrations in ongoing early human studies.

Patients will be enrolled in to the study after meeting protocol-specified eligibility criteria. They will receive a one-time treatment of the study drug (SAR446268) via an IV infusion.

AR446268 is expected to deliver micro ribonucleic acid (miRNA) by a non-replicating adeno-associated virus (AAV) carrying a muscle-tropic promoter (engineered Desmin) expressing an engineered miRNA (artificial micro ribonucleic acid [amiR]-DMPK204) to specifically downregulate DMPK mRNA expression. This RNA interference reduces and degrades DMPK mRNA levels which is expected to restore the mis-splicing events, a molecular hallmark in DM1 tissue. Patients will also receive an immunosuppression regimen and subsequently be followed for evaluations of safety and tolerability to the drug

amiR-DMPK. Micro RNA that targets DMPK mRNA for degradation.

DMPK. A protein that regulates muscle contraction and relaxation

The vector genome contains AAV2 inverted terminal repeats (ITR) flanking an expression cassette that is comprised of a desmin (Des) promoter which confers muscle-tropism of the artificial miRNA, amiR-DMPK204, and the bovine growth hormone polyadenylation signal sequence (minBGHpA). The engineered promoter contains elements shown in the literature to be important for high level expression in muscle cells. amiR-DMPK204 is embedded in the stem - loop structure of an optimized mouse miR155 scaffold (miR155-BLOCK-iT; Thermofisher Cat No. K4935-00, K4936-00, K4937-00, K4938-00), where the guide strand, when processed, targets the DMPK mRNA for degradation. The engineered pre-miRNA sequence structure is based on the murine miR-155 sequence. Because intronic expression of miRNAs enhances target knockdown, amiR-DMPK204 is inserted within 5' and 3' arms of the rabbit β-globin intron. Filler sequences (stuffer; S) are upstream and downstream of the expression cassette. The stuffer DNA fragments are composed of sequences from the second intron of the human A1AT gene (Genbank Accession No. K02212.1)

The product will be stored and prepared for infusion at the UMass Research Pharmacy located on the 6th floor of the Ambulatory Care Center. Infusion of the product will occur within the in-patient service, university campus. Blood will be drawn in-patient, but also in the clinical research center where it will also be processed and stored. The research pharmacist who will be preparing the product for infusion will work in an ISO level 5 biocontainment hood. The clinical research center lab has a biosafety cabinet as well as sealed centrifuge containers for the processing of blood.

IBC Discussion and Vote

Discussion: Reviewer discussed that the biohazard section of the protocol should list the same risks that are listed in the informed consent. The vector checklist provided had mentioned a helper virus assay. The reviewer questioned if this really relevant for the manufactuing of this vector using the HeLaS3 procuder cell line.

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

BSL/ABSL: **BSL-2**

NIH Guidelines: III-C, III-E

6) Investigator: Kelliher, M

Title: The role of Rip1 in TNF transduction and Mechanism(s) of Tal1/scl mediated Leukemogenesis

IBC Registration: **267-25, Renewal**

Training Verification: Acceptable pending completion of PI training

Brief Summary: We have engineered mouse models of disease: one examines the process of inflammation in diseases such as bone marrow failure or myeloproliferative disease. We also develop mouse models of leukemia, a cancer of the blood system, to identify oncogenes and tumor suppressor genes that are critical in mediating leukemogenesis and therapy resistance.

Goals: Identify therapeutic targets to prevent relapse in pediatric T-ALL. Determine how inflammatory cell death contributes to bone marrow failure and MDS. Tap Inflammatory cell death to promote anti-tumor immunity.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: This group engineered mouse models of disease to enable the study of inflammatory processes in conditions such as bone marrow failure or myeloproliferative disease. They also developed mouse model of leukemia to identify oncogenes and tumor suppressor genes that are critical in mediating leukemogenesis and therapy resistance. Accordingly, the goals of this project are to:

1. Identify therapeutic targets to prevent relapse in pediatric T-cell acute lymphoblastic Leukemia (T-ALL)
2. Determine how inflammatory cell death contributes to bone marrow failure and Myelodysplastic syndrome (MDS)
3. Leverage Inflammatory cell death pathways to promote anti-tumor immunity

Experimental Approach: The role of RIPK1-mediated inflammatory cell death will be investigated by examining how RIPK1, a central regulator of TNF- and Z-nucleic acid-induced cell death, controls inflammatory responses. Genetically engineered mouse models, including Ripk1 KO, conditional, and kinase-inactive mice, will be utilized. These models have previously demonstrated that TNF- and ZBP1-mediated apoptosis and necroptosis contribute to Systemic Inflammatory Response Syndrome (SIRS), autoimmunity, and bone marrow failure.

To study the role of TAL1 and NOTCH1 as mediators in Leukemogenesis the group has developed mouse models of T cell leukemia induced by the basic helix-loop (bHLH) protein Tal1/Scl. They have established leukemic cell lines from these mice and identified novel and direct Notch1 target genes important in leukemia. They are currently focused on identifying, by single cell RNA sequencing, a subset of leukemic cells important in disease initiation and relapse. They are testing how Btg2 and Klf2 regulate leukemia-initiating cell activity and chemoresistance.

To identify additional genes important in leukemogenesis, they plan to infect primary mouse and human leukemias with retroviral/lentiviral over-expression and shRNA vectors (single and libraries) including the following vectors (Banshee, Block-IT, pGIPZ, pLL3, pSM2c, pLMN, MSCV, pRSMX. LentiCRISPRv2 and pLKO.1) and then transplant transduced cells

into syngeneic mice to determine effects on survival (see Roderick, JE 2013. Blood 123(7):1040-50 and Miller, PG 2013. Cancer Cell 24:45-58). They will then validate these potential genes in vitro using our leukemic cells lines.
To develop new mouse models of leukemia and identify therapeutic vulnerabilities, 5FU-treated bone marrow or primary mouse or human leukemia cells will be infected with mouse CRISPRv2 lentiviruses containing various loss of function tumor suppressors or electroporated with RNP containing Cas9 and gRNAs. These infected bone marrow cells or primary mouse or human leukemia cells will be transplanted into lethally irradiated recipients, syngeneic or immune deficient mice and monitored for disease progression.

IBC Discussion and Vote

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

7) Investigator: McNally, D
Title: Plasmid Manufacturing
IBC Registration: **I-929-25, New**
Training Verification: Acceptable pending completion of PI training
Brief Summary: MassBiologics is establishing the capability to manufacture and test Plasmid DNA for use in further manufacturing.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: MassBiologics is establishing the capability to manufacture and test Plasmid DNA for use in further manufacturing.

Experimental Approach: MassBiologics intends to manufacture plasmid DNA for use as a raw material in the manufacture of gene therapy vectors (covered under IBC protocol I-614-20). MassBiologics will develop processes and procedures to culture e.coli transformed with plasmid DNA at shake flask scale for use in the generation of cells banks, and in Fermenters at up to a 50 L scale for production purposes. The cultured transformed e.coli with then be processed using alkaline lysis and a series of filtration and chromatography steps to generate a purified and concentrated plasmid. Samples will be taken during manufacture and tested at both Mattapan and Fall River sites for yield and purity via spectrophotometry, HPLC and ELISA methods.
To aid development of manufacturing processes samples (<10L) of cultured transformed e.coli will be lysesed, clarified and purified at the Fall River site.
This type of work will occur in two diferent locations, Mattapan and Fall River

IBC Discussion and Vote

Discussion: Reviewer discussed some concerns regarding the list of personnel and the IBC Litmos training and whether our facility is responsible for their training or not. They have all been assigned the training.
Meeting Decision: Vote to approve upon completion of action items.
BSL/ABSL: **BSL-1; BSL-1 Large Scale**
NIH Guidelines: III-D, III-E, III-F

8) Investigator: Yamada, K
Title: Development of RNAi-based Therapeutics
IBC Registration: 927-25 New (TABLED in July)
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, NHP cell lines and primary cells followed by in vivo rodent and large animal models. There are a variety of targets we work with including huntingtin, sFLT1, ApoE etc. RNA-based therapeutics development includes overexpression and purification of recombinant Ago2 for use in *in vitro* experiments as well as study of the impact of siRNA gene modulation on ex vivo perfused organs.

Brief Summary and Review by Primary Reviewer

Overview and Objectives: To develop RNA-based therapeutics for several disease indications.
Testing modified or formulated RNA oligonucleotides (or RNA/protein complexes) developed in the lab in in vitro experiments using mouse, human, NHP cell lines and primary cells followed by in vivo rodent and large animal models. Targets include huntingtin, sFLT1, ApoE and others. RNA-based therapeutics development includes overexpression and purification of recombinant Ago2 for use in vitro as well as study of the impact of siRNA gene modulation on ex vivo perfused organs.
Experimental Approach: Chemically modified oligonucleotide test article description: Oligonucleotides can be administrated by themselves or combined with mRNA/ protein (CAS9 or similar) and virus to enhance distribution. All the compounds are being synthesized in house and are HPLC purified. Full length and identity are confirmed by mass spectrometry. Resulting compounds are considered to be "pharmaceutical grade". In addition, oligonucleotide might have a fluorescent dye for detection in distribution studies.
Project 1: Screening modified RNAs in vitro.
Testing modified RNA oligonucleotides in modified and primary cells of human and mouse origin. The oligonucleotides can either be naked, complexed with mRNA, protein or formulated (liposome, endosome, polymer, small drugs). Use peripheral blood (hPBMCs, serum, and whole blood), fibroblasts etc from patients with and without the conditions of interest (i.e Huntington's disease, ALS, etc.) to test the modified RNA oligonucleotides.
Project 2: Screening modified siRNAs in vivo.
Animal models will be used to test toxicity, tissue distribution, pharmacokinetic/pharmacodynamic (PK/PD) parameters, and efficacy of modified siRNAs. In some experiments co-administration of a drug (i.e guanabenz or dextran sodium sulfate (DSS, etc.) will 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. Oligonucleotides can be naked, in liposomes, and/or injected with a drug.
Project 3: Generation of neurons.
Skin biopsies from mice and patients with and without the condition of interest (i.e Huntington's disease, etc.) will be performed. Fibroblasts will be reprogrammed directly into neurons using lentiviruses.
Project 4: Cell type specific delivery of siRNA in mice and human cells
Mice will be treated with siRNA. Specific organs will be harvested to determine the amount of uptake of a fluorescently labeling siRNA in various cell types. Cytometry to determine knockdown in human cell lines and the mouse. Flow sorting of human cell lines and mouse tissues to quantitate uptake in specific cell types.
Project 5: Distribution and efficacy of siRNA in a larger mammalian brain.
Novel RNA chemical configurations for efficient CNS delivery will be tested in the brain of rodents. and large animals (i.c sheep or non-human primates (cynomolgus macaques and baboons). Oligonucleotide distribution and efficacy are determined by combination of fluoresence microscopy on fixed sections, PNA hybridization assay, QuantiGene, Protein

Simple, Westerns from punches from flash frozen and/or RNA-Later treated sections. The PNA hybridization assay will be used for PK studies using blood and/or CSF. Blood is collected for biochemistry, cell counts and inflammatory markers profiling. Advanced MRI facility will be used to obtain pre- and post-operative images of the brain for surgery planning and toxicity testing respectively. CT imaging with contrast will be used for surgery planning and injection confirmation.
Project 6: Overexpression and purification of recombinant Ago2 for use in in vitro experiments.
Baculovirus containing the mouse Argonaute 2 (Ago2) transgene will be produced in Sf9 cells to overexpress and purify recombinant Ago2 from Sf9 or High Five cells. Baculovirus will be produced by transfecting Sf9 cells with a baculovirus genome containing the Ago2 transgene. Virus will be produced to a titer of ~1e8 pfu/mL in the culture supernatant. This virus-containing supernatant will then be used to infect either Sf9 or High Five cells to induce overexpression of Ago2.
Project 7: Evaluation of marginal organs after ex vivo perfusion with chemically-modified, therapeutic small interfering nucleic acids (siRNAs).
Pig and discarded human hearts and lungs will be perfused at MGH on a normothermic ex vivo machine perfusion circuit. The organs will be perfused with chemically-modified, therapeutic small interfering nucleic acids (siRNAs) developed in the Khvorova lab. Biopsies of the tissue and perfusate will be taken over time as well as at the conclusion of the experiment. Samples will then be transported to UMass Chan for downstream analysis. Histology and flow cytometry (formalin-fixed for histology or stored in Miltenyi/CryoStor preservation solution for flow cytometry), drug distribution levels (RNA-later or flash-frozen samples), and mRNA and protein expression levels (RNA-later or flash-frozen samples). The ex vivo organ perfusions are performed under MGH protocols. Donor serology is documented for all perfused organs (documented HIV, HCV, HBV negative).
Core use:
Flow sorting of unfixed human and mouse cells treated wth fluorophore conjugated oligonucleotides (ex. cy3 or cy5.5, etc.)
Large animal imaging; CT and MRI pre- and post- operative

IBC Discussion and Vote

Discussion:	Tabled again due to inconsistencies and not entirely clear who they are collaborating with.
Meeting Decision:	TABLED
BSL/ABSL:	**N/A**
NIH Guidelines:	N/A

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

1) BT2/BT3; Closed and decontamination/ decommissioning underway?
2) First meeting for new BSL-3 space; Design meeting and preliminary stages.

VI. Information from the field (Senior Biosafety Officer)

1) End of year requirements
2) BSL-2+/BSL-3 agent specific training
3) Drills and exercises
4) Shrewsbury inspection went well and permit renewed.
5) CDC inspection report expected in December now that the government has reopened.
6) Worcester rsNA inspections

VII. Other Business

1) AAALAC site visit: no major biosafety issues identified.

Acknowledgement Items:

1) **Caricchio 878-23**: Kyverna KYV101-001 “A Phase 1 Open-Label, Multicenter Study of KYV-101, an Autologous Fully-Human Anti-CD19 Chimeric Antigen Receptor T-Cell (CD19 CAR T) Therapy, in Subjects with Refractory Lupus Nephritis” Update- IB v8.0
2) **Caricchio 880-23:** CAB-201-001 “A Phase 1/2, Open-label Study to Evaluate the Safety and Efficacy of Autologous CD19-specific Chimeric Antigen Receptor T cells (CABA-201) in Subjects with Active Systemic Lupus Erythematosus” Update- Protocol Am.4 & Model ICF v4.0, Adolescent Assent ICF

Adjourned at 1:15 pm