University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

July 8, 2019 10:00 AM

302 Heiber Building

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			
REDACTED, Vice Chair			
REDACTED, BSO			
REDACTED	absent		
REDACTED	absent		
REDACTED	absent		
REDACTED	absent		
REDACTED	absent		
REDACTED	Late: 10:07		
REDACTED	absent		
REDACTED			
REDACTED	Late: 10:47		
REDACTED			
REDACTED	absent		
REDACTED	absent		
REDACTED			
REDACTED	absent		
REDACTED			
REDACTED			
REDACTED	absent		
REDACTED	absent		
REDACTED	absent		
REDACTED	absent		

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office, Director
REDACTED	IBC Office
REDACTED	EH&S Office Director
REDACTED	EH&S Office
REDACTED	IACUC Office
REDACTED	RCCO Co-Director

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster:	23
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and are able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The June 2019 meeting minutes were reviewed and approved by the committee.

Votes:

For:	7
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

None reported

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocols on the following pages

Protocol:	IBC201900080
Title:	Optogenetics of the PNS
Investigator:	REDACTED
Highest BSL:	BSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: If cells have been harvested from transgenic mice or animals administered recombinant materials, the cells would be considered to be recombinant agents.

2) Animal Gene Transfer, Question 3: YES should be selected, as the NIH considers the plasmids used in modern AAV systems to meet the definition of a helper virus.

3) Waste Management, Question 1b: Clarify that liquid waste will be decontaminated by adding concentrated bleach to a final 1:10 dilution (v:v) bleach in liquid waste. Addition of pre-diluted 10% bleach to liquid wastes further dilutes the disinfectant and results in insufficient concentration of active ingredient for proper decontamination.

Comments:

The goal of these studies is to use multimodal approaches to study the interactions of the peripheral nervous system and the targets of these neurons, that results in organ homeostasis. The investigator will use animal models of common diseases (cancer, IBD, IBS) to determine how these interactions change and contribute to the pathological conditions that are the primary symptoms of these conditions. Experiments will use transgenic mice that express reporter proteins (opsins or GCaMP), some of which will also be injected with either AAV9 or AAV2retro (used for retrograde labeling of neurons). The injections will be made either i.p., s.c. or into the vertebral space. The investigator will use multimodal approaches to study the interactions of the peripheral nervous system (PNS) and the targets of these neurons, that results in organ homeostasis. Moreover, they will use animal models of common diseases (cancer, IBD, IBS) to determine how these interactions change and contribute to the pathological conditions that are the primary symptoms of these conditions. Experiments will use animal models (mouse) that will be injected with varying kinds of AAV. This should be discussed to confirm that animal protocol is approved for Adenoviral injections and is BSL-2 more appropriate. Although AAV vector genomes can persist within cells as episomes, vector integration has been observed in various experimental settings, *Curr Opin Mol Ther. 2009 August ; 11(4): 442–447*

Initial comments:

1) Tissue, blood and body fluids: On the previous page, the investigator did not select the box for primary cells but here the investigator proposes to use mouse cells and tissues.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

Protocol:	IBC201900088
Title:	hiPSC Culture
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• hiPSCS - no need for hSCRO protocol

Determination: Approved

Last day of continuing review period: 7/8/2020

Required modifications: None

Comments:
This protocol has been submitted by a new investigator studying retinal development. *E. coli* will be used for cloning and plasmid amplification. A commercially available hiPSC line will be used for generation of retina organoids via transfection of genes. The investigator has also proposed work with CRISPR/Cas9 and gRNA either by transfection or use of AAV. Clarification is required to determine if CRISPR and gRNA will be expressed via the same viral vector. Viral vectors that encode CRISPR/Cas together with one or more sgRNAs must be generated and used at BSL-2+ containment. If CRISPR/Cas and the sgRNA(s) are expressed using separate viral vectors, BSL-2 is appropriate. The biosafety level will need to be determined based on the investigator's responses. This is a new protocol submitted by a new investigator studying retinal development. In the proposed work the investigator is using an hiPSC line to generate organoids. These organoids will be genetically manipulated by CRISPR/Cas9 technology by use of AAV. The use of AAV and CAS9 technology should be considered for biosafety containment levels and should be clarified.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Approval: No additional comments were provided by the committee; recommendations for approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

Protocol:	IBC201900090
Title:	Chick Retina High Acuity
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 7/9/2020

Required modifications:

1) Viruses, Prions, or Vectors: Rous Sarcoma Virus does not normally infect human cells unless pseudotyped with a different envelope (e.g. VSV-G). Clarify how the Retrovirus infects cells and how it is replication competent.

2) Viruses, Prions, or Vectors: For the source of the viruses, identify if they have been received from a commercial vendor or modified by a collaborator.

3) Risk Group and Containment Practices, Question 1: The Risk Group (RG) for Avian Leukosis/Sarcoma virus is RG1, not RG2 as listed.

4) Risk Group and Containment Practices: As the investigator indicates in other sections of the protocol that the Avian Leukosis/Sarcoma Viruses can infect human cells, in accordance with the recommendations in *Appendix B-V of the NIH Guidelines*, a containment level appropriate for Risk Group 2 (RG2) is recommended. Therefore, the risk group can be designated at RG1 recognizing that the *un*modified Avian Leukosis/Sarcoma Viruses are not infectious to humans, but the biosafety level should remain as BSL-2 to reflect the modification that renders the viral vectors infectious to human cells.

5) Exposure Assessment and Protective Equipment, Question 1: Clarify how Rous Sarcoma Viruses infect human cells. If this incorrect, modify the response.

6) Exposure Assessment and Protective Equipment Question 5: For work with BSL-2 outside of a biosafety cabinet, full face protection is required. Select a face shield or surgical mask (with goggles) to protect the eyes, nose, and mouth.

7) Waste Management, Question 1a: Sharps containers are not provided by EH&S; the investigator must purchase. Revise.

Comments:

Experiments will include RNASeq and ATACSeq assays, explant cultures of chick embryonic retinas transfected with cell-type specific reporters and *in ovo* gene perturbations by both gain and loss of function studies (overexpression and gene knockdown by RNAi and/or CRISPR/Cas9 gene editing strategies, via plasmids vectors (*E.coli* DH5alpha), Tol2 transposon and Avian Retrovirus-RCAS and RIAS) . Work will be performed on chicken embryos, prior to hatching, so no IACUC protocol is needed. One change needs to be made. While the work seems appropriate for BSL-2, the Risk Group should be 1, not 2 as marked. This protocol can be approved with the requested changes/questions answered. This IBC protocol will investigate the molecular mechanisms of chick retina high acuity development. They will use RNASeq and ATACSeq assays, explant

cultures of chick embryonic retinas transfected with cell-type specific reporters and *in ovo* gene perturbations by both gain and loss of function studies (overexpression and gene knockdown by RNAi and/or CRISPR/Cas9 gene editing strategies, via plasmids vectors, Tol2 transposon and Avian Retrovirus). The transgenes to be overexpressed encode fluorescent proteins (RFP, mCherry, eGFP, tagBFP) will be involved, and genes of interest include Vax2, Tbx5, Aldh1a1, Aldh1a3, EphB2, EphB3, ephrin-B2, ephrin-B1, Cyp26a1, Cyp1B1, Cyp26c1 and Fgf8. No cloned toxin genes, oncogenes, and Lentivirus will be used in this protocol. All work is proposed to be performed at level of BSL-2 which is suitable. This protocol describes work in chick embryos to examine mechanisms of retina development. RNASeq and ATACSeq assays will be performed and chick embryonic retina explant cultures will be used in *in vitro* assays. Plasmid vectors, Tol2 transposon, and Avian Retroviruses will be used for overexpression of fluorescent marker genes as well as knockdown using RNA1. CRISPR/Cas9 gene targeting will be performed using plasmid vectors only. The work is proposed at BSL-2 which is appropriate. There are a few minor clarifications needed prior to committee review.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

Protocol:	IBC201900094
Title:	Ketamine on Pain Tolerance and Depression
Investigator:	REDACTED
Highest BSL:	BSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 7/8/2020

Required modifications:
1) Animal Gene Transfer, Question 3: AAV requires a helper virus for infectivity, so the answer should be YES.

Comments:
In this new protocol AAV2 and AAV9 (obtained from *Addgene*) will be used to express reporters (YFP, mCherry), opsins, Calcium indicators (GCaMP), or DREADDs (PSAM) in the mouse nervous system. The AAV will be injected into MOR-cre mice at specific sites to enable infection of specific subpopulations of neurons. The transgenic mice will be bred on site. The protocol is proposed at BSL-1/ABSL-1 which is appropriate as the AAV is obtained from *Addgene* which is considered to be an automatic downgrade.

The application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

Protocol:	IBC201900098
Title:	Calreticulin Acetyltransferase in CHB
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 7/9/2020

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Usage: *NIH Guidelines Section III-D-3* should be checked for use of Lentiviruses in cell culture.

2) Risk Group and Containment Practices, Question 1: Risk Group 3 (RG3) should be marked as HIV is a risk group 3 agent per the *NIH Guidelines*.

3) Exposure Assessment and Protective Equipment, Question 1: Include a statement that the use of human cell lines may harbor endogenous pathogens. The use of Lentivirus may infect human cells, though is replication deficient.

4) Exposure Assessment and Protective Equipment, Question 4: Amend the wording of "BSL-2 hoods" to Biosafety Cabinets (BSCs) to distinguish it from a chemical fume hood.

5) Exposure Assessment and Protective Equipment, Question 5: Include the required Personal Protective Equipment (PPE) for entrance to the animal facility.

6) Waste Management, Question 1a: Amend disposed of as biohazardous waste. liquid waste- does not get collected as chemical waste or disposed of in a waste box. after a 1:10 final (v/v) disinfection for 20 minutes, liquids can be carefully poured in the drain.

7) Waste Management, Question 1c: Animal carcasses should not be placed in the laboratory's biohazard trash/boxes. The carcasses should be returned to the animal facility for disposal according to the specific facility protocols.

Comments:

This IBC protocol will investigate the role of calreticulin acetyltransferase of endoplasmic reticulum in congenital heart block (CHB). Lentiviral vectors (MISSION particles, *Sigma*) will be uses to generate stable knockdowns of calreticulin in human cardiomyocyte derived cell line (AC16 cells) and these cells will be subjected to ER stress. Also, transgenic mice that overexpress calreticulin in the heart will be used to isolate cells from their hearts in the proposed studies. No any known oncogenes or toxins will be used in this protocol. All work was claimed to be performed at level of BSL-2 which is suitable. Lentiviral vectors (MISSION particles, *Sigma*) are used to generate stable knockdowns of calreticulin in AC16 cells (human cardiomyocyte derived cells). Transgenic mice that overexpress calreticulin in the heart, which have been previously generated. These mice will be bred, and 1-3 day old neonatal mice from these breeders or nontransgenic controls will be euthanized, and cells from their hearts will be harvested. These cells will be cultured for less than 7 days and subjected to ER stress. As above, these cells will be subsequently either lysed for protein and RNA analysis, or paraformaldehyde-fixed and stained. Both benchtop and animal work are to be done under BSL/ABSL-2 conditions, which is appropriate.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

Protocol:	MOD201900089
Title:	Amendment for **IBC201600174**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: Cells in which recombinant nucleic acids will be introduced should be designated as recombinant.

2) Primary Cells or Cell Lines: The entry for primary murine cells, skin lists the description of usage as genotyping; however, the application indicates administration to animals. Identify if these cells will be administered to animals or revise the response.

3) Primary Cells or Cell Lines: Provide the source of the murine bone marrow (e.g. collaborator, own colony, vendor).

4) Viruses, Prions, or Vectors: Identify the source of the viruses (e.g. name of vendor, collaborator, etc.).

5) Viruses, Prions, or Vectors: As two different biosafety levels are proposed, provide a separate entry for each MLV (ecotropic BSL-1/ABSL-1+ and amphotropic BSL-2/ABSL-2). The entry for the ecotropic virus should note that a biosafety containment level downgrade *is* being requested.

6) Exposure Assessment and Protective Equipment, Question 1: Include a statement that acknowledges that human cells or cell lines may harbor unknown infectious pathogens.

7) Exposure Assessment and Protective Equipment, Question 4: Include the following engineering controls: Safety-Engineered Sharps Devices are required for any manipulations with biological agents at BSL-2/ABSL-2 or higher.

Comments:

This modification includes the cultivation of commensal strain of bacteria and protist as well as colonization of those commensal organisms to experimental mice to study their influence on immune function. This is an approved BSL-2/ABSL-2 protocol. This modification of an approved BSL-2/ABSL-2 breeding protocol includes the colonization of commensal organisms into a transgenic mouse model to monitor its effect on systemic inflammation. No biosafety issues were noted, and recommendation is for approval.

No review comments were provided to the investigator for response. The application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

Protocol:	MOD201900239
Title:	Amendment for **IBC201700093**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 7/8/2020

Required modifications:

1) Tissues, Blood, or Body Fluids: As tissues will be harvested from genetically modified mice and/or mice in which recombinant nucleic acids have been administered, the tissues should be designated as recombinant.

2) Primary Cells or Cell Lines: As recombinant nucleic acids are introduced into cells, the cells should be designated as recombinant.

3) Primary Cells or Cell Lines: Description of Usage should include AAV use (i.e. virus production, transductions). Revise.

4) Recombinant or Synthetic Nucleic Acid Usage: If plasmids encoding viral vectors will be used in bacteria, then *NIH Guidelines Section III-D-2* should be selected.

5) Animal Gene Transfer: Question 3: AAV requires a helper virus for infectivity, so the response should be YES.

6) Waste Management, Question 1a: Describe the final concentration of bleach that will be used for decontamination. Bleach should be used for decontamination of all viral vectors as well as human cells. Correct the response.

7) Waste Management, Question 1b: Describe the final concentration of bleach that will be used for decontamination. Bleach should be used for decontamination of all viral vectors as well as human cells. Correct the response.

Comments:

This is a modification of an existing protocol requesting to add Adeno-Associated Viral Vector (AAV) into the protocol. The group will use AAV-insulin vector and AAV-GFP vector for evaluation of the therapeutic effect of insulin on blood sugar control in diabetic mice and will be delivered via tail vein injection. Before injection into mice, they will be transferred into human HepG2 liver cells in culture for verification of transgene expression in mRNA and protein levels. All AAV work will be performed at BSL-2/ABSL-2 and they group has created these vectors in their own lab. Approval is recommended after form corrections are made. This modification adds AAV vectors to the existing protocol. BSL-2/ABSL-2 are proposed and appropriate.

Initial comments:

1) Primary cells or cell lines: Under source, please list the institution from which these cell lines were obtained.
2) Exposure Assessment and Protective Equipment: 5.0 - Additional PPE is required for ABSL-2 work (double gloves and shoe covers).

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

Protocol:	MOD201900251
Title:	Amendment for **IBC201700188**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• 0032820.MTA.Glorioso.VA final • NIH approval grant transfer • Amankulor IACUC 00007574 submission 8/2/17 • Amankulor IS00007429-mod3 8/10/17

Determination: Approved

Last day of continuing review period: 7/9/2020

Required modifications:

1) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: *Escherichia coli* K12 or derivative - DH10 beta (NEB10-beta); DH5 alpha (NEB5-alpha); NEB Stable; should be designated as recombinant.

2) Exposure Assessment and Protective Equipment, Question 4: Include the following engineering controls: Safety-Engineered Sharps Devices are required for any manipulations with biological agents at BSL-2/ABSL-2 or higher.

3) Waste Management, Question 3: Absorbent material should be placed over the spill to contain prior to addition of bleach. Revise.

Comments:

This amendment is to add new HSV-1 strains to an existing protocol to study their potential as oncolytic virus vectors. Attenuating mutations, cell targeting features and immune modulating genes will be added if the strain proves useful for oncolytic purposes *in vitro*. BSL-2/ABSL-2 is proposed and appropriate. The WT A21 will not be used in animals, only the attenuated variants. Other than minor form issues recommendation for approval. The investigator proposes to add characterization and use of HSV-1 isolate A21 (from a collaborator, VA San Diego Healthcare System) to the protocol to create novel oncolytic HSV (oHSV) vectors. The A21 isolate will be used as follows: a) Test its ability to infect and destroy glioblastoma cells in tissue culture compared to high-passage laboratory strains of HSV-1 (KOS, F) used to derive current oHSV. b) If the virus proves to be oncolytic, introduce mutations into its genome to render it “safe” for therapeutic use and carry out safety and efficacy testing. These mutations will consist of (i) removal of the joint repeat elements, (ii) introduction of microRNA binding sites into the 3’UTR of essential immediate early genes to prevent virus replication in normal brain tissue, and (iii) retargeting of the virus through modification of envelope glycoproteins D and C to limit infection to tumor cell surface markers. These methods insure vector safety without compromising vector growth in tumors. c) Introduce immune-modulatory genes, as described in this protocol, to enhance anti-tumor immunity.

Initial comments:

1) Biosafety summary: Select the box for genetically engineered animals, as the investigator proposes to use immune compromised mice.
2) Animals: Question 5 should be answered YES as the investigator is proposing in summary to use nude and immune compromised mice for testing the oncolytic activity of oHSV vector.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocols on the following pages

Protocol:	IBC201900092
Title:	HCC 19-031
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2018-01-30 HS-IC0604 OSHA Bloodborne Pathogen Standard Exposure Control Plan_19-031 • 2019-02-25 IP Manual v0.1_19-031 • 2019-05-22 Appendix M_19-031 • 2018-12-19 Protocol_19-031 • 2018-06-29 HS-FM0208PRO Waste Management PROCEDURE_19-031 • 2019-04-26 HS-IC0616 Guidelines for Handling Sharps_19-031 • 2019-02-28 ICF Template_19-031 • 2018-12-13 Investigator Brochure_19-031

Determination: Modifications Required

Required modifications:

1) Human Gene Transfer/Human Clinical Trial: Materials: Question 2: It appears that the Phase 1 clinical data was conducted with new methods or materials with no known/standard safety data. Consider the response to this question to be changed to YES.

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 2a: Describe Phase 1 study ongoing in China with this product.

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 1: Clarify if participants with well-controlled HIV or HBV infection (ie, undetectable HIV RNA levels or HBV DNA levels) while on treatment are eligible for this study.

4) Human Gene Transfer/Human Clinical Trial: Materials: Question 10: Identify the transduction efficiency, see Appendix M, section 1.4.2. - 20-60% ET140202 Receptor-positive transduction efficiency.

5) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: Define "LTI" in the response.

6) Human Gene Transfer/Human Clinical Trial: Question 3a: Transfer of Materials between locations – specify which lab/staff at HCC will receive and handle the study product.

7) Risk Group and Containment Practices: Question 3. Identify individuals who will be handling the study product.

Comments:

This is a phase 1/2 multicenter study designed to determine the safety of autologous ex vivo expanded T-cells [CAR T cells] that are genetically modified using a self-inactivating (SIN) lentiviral vector to express a gamma/delta T-cell receptor (TCR)-based molecule targeting the AFP peptide/HLA-A2 complex and a CD28-based molecule targeting GPC3. It will be administered to patients with AFP-expressing advanced HCC and at least one HLA-A2 allele. Study population consists of persons with advanced liver cancer who are AFP positive/HLA-A2 positive, with few

remaining treatment options. HCC patients overexpress AFP and GPC3(human glypican). AFP is normally present in fetal but not adult cells and is a target for treatment of HCC. The ET140202 transgene consists of 3 parts: a) the AFP158/HLA-A2 Binding Component, an antigen-binding (Fab) fragment derived from ET1402L1, a human antibody against the AFP158-166 peptide/HLA-A2 complex; b) an Effector Component, which consists of portions of the gamma (γ) and delta (δ) chains of an endogenous γδ TCR; and, c) a Co-stimulatory Component, a CD28-based molecule that recognizes and binds human GPC3. The AFP158/HLA-A2 binding component of ET140202 T-cells recognizes and binds to AFP158/HLA-A2 complexes expressed on HCC cells. This binding triggers the effector component of ET140202 T-cells to associate with the endogenous CD3 complex leading to ET140202 T-cell activation and subsequent killing of HCC cells. To optimize T-cell activation and expansion, ET140202 T-cells are equipped with a co-stimulatory component, which consists of a co-stimulatory CD28-based molecule that recognizes and binds human GPC3 expressed on HCC tumor cells. Participants will undergo leukapheresis. Their T-cells are then activated with anti-CD3/CD28 Dynabeads, transduced with a SIN lentiviral vector carrying the ET140202 transgene. The modified T-cells, called ET140202 T-cells, are then expanded *in vitro* and cryopreserved. Participants undergo a lymphodepleting regimen 3-5 days before their first ET140202 T-cell infusion. Starting dose will proceed in a stepwise fashion starting with 200 million cells, in a dose-escalation model with 3 participants enrolled at each dose level. Within each dose level cohort, the administration of the first dose of ET140202 T-cells will be staggered by 14 days between the first 2 participants. The second dose level is 400 million cells; the de-escalation dose is 100 million cells. Each participant will receive subsequent doses every 15 days if safe to do so. The expected number of infusions ranges from 3 to 6 with expected average of 4. When 3 participants within a dose level reach the first 28-day safety evaluation, a Dose Escalation Committee will review safety data and make recommendations for the next dose level. In the expansion phase, up to 50 participants will be given the recommended phase II dose. Participants will remain active on study for 24 months and then followed for another 13 years or until death for safety. If an insufficient number of cells are produced for a participant, they may still qualify to be treated on a compassionate use basis. Preclinical work has been done in mice that showed anti-tumor activity. Mice were administered about 85-fold more ET140202 T-cells than the proposed lowest dose level for this trial. A phase 1 study with this vector and product is underway in China. Participants in that study have received up to 11 infusions at varying doses. No CRS or neurotoxicity reported from that study so far. Secondary objectives include efficacy and PK of the CAR T-cells following infusion.

Biosafety: The delivery vector, pET140202, is a Lentiviral vector based on pCDH backbone that directs the expression of the ET140202 transgene *ex vivo* in autologous T-cells. No helper virus or carrier particles are used. There is minimal DNA sequence homology between the vector and helper plasmids. The Lentivirus delivery protocol is adjusted to achieve 20-60% ET140202 Receptor-positive transduction efficiency. The cell bank used to create ET140202 is tested for adventitious viruses. ET140202 Lentivirus is tested for RCL formation in a highly permissive cell line, according to FDA guidelines. Participants will receive conditioning chemotherapy only after the cells have been successfully manufactured, which is about 4 weeks, under GMP conditions in the Gaithersburg, MD plant of *Lentigen Technology*. Release criteria include sterility, mycoplasma, residual host DNA, RCL, genomic integrity, transducing titer, residual benzonase, pH, appearance, and others. The final study product must pass all QC testing, except a 14-day sterility test, prior to release. The protocol outlines an action plan if the sterility test is positive

and includes blood cultures and empiric antibiotics. ET140202 T-cells will be shipped in LN2 dry shippers and stored at ≤ -130°C. Prior to infusion of product, two staff will verify all the information present on the product infusion bag(s) to ensure that the participant receives only their autologous ET140202 T-cell product. ET140202 T-cell infusion will be administered over a 10-20-minute time per bag, as soon as possible after thawing, and within two hours from thawing. Vital signs will be monitored frequently. Acute infusion reactions will be managed with antihistamines; steroids given only for life-threatening reactions. CRS will be managed per Standard of Care (SOC). Female participants of child-bearing potential, female partners of male participants, and male participants will be asked to follow the contraceptive recommendations.

The study will be paused if: a participant develops uncontrolled T-cell proliferation not responding to management with immunosuppression; a Grade 4 toxicity attributable to ET140202 T-cells; two or more Grade 4 cerebral edema events; respiratory failure requiring mechanical ventilation related to the study treatment; development of detectable Replication Competent Lentivirus during the study; any death that is suspected to be definitely, probably, or possibly related to ET140202 T-cell therapy; or a Grade 5 toxicity of unknown cause.

Initial comments:
1) Clarify if patients with well-controlled HIV or HBV infection (ie, undetectable HIV RNA levels or HBV DNA levels) while on treatment are eligible for this study.
2) Human Gene Transfer: Question 3a, Transfer of Materials between locations: specify which lab/staff at HCC will receive and handle study product.
3) Human Gene Transfer: Question 2a, Describe the Phase 1 study ongoing in China with this product.
4) Risk Group and Containment: Question 3: Identify individuals who will be handling the study product.
5) Human Gene Transfer: Question 10: State the transduction efficiency

The submitted application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	7
Against:	0
Abstained:	0

Protocol:	IBC201900102
Title:	PD-1101
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• AppendixMtemplate VOYcomments • Attachment1VOY • Voyager PD-1101 ICF_Pitt_8-18-15_clean • PD-1101_Protocol_v6.0_12Aug16 • IB_v3.0_full IB w Voyager sig

Determination: Modifications Required

Required modifications:

1) Human Gene Transfer/Human Clinical Trial: Materials: Question 7: Clarify that no well-known side effects or toxicities are expected from delivery of the product in the brain of Parkinson's Disease (PD) participants.

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 8: Describe the solution/buffer that the AAV particles are in.

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: If the agent is determined to be from a virus (a viral vector), the assay(s) used to check whether there is any infectious virus remaining in the agent preparation should be identified.

4) Human Gene Transfer/Human Clinical Trial: Materials: Question 3: Clarify how this is not a new/novel treatment for PD.

5) Human Gene Transfer/Human Clinical Trial: Question 3a: Describe how study product will be transported from IDS to the surgical area.

6) Recombinant or Synthetic Nucleic Acid Work Description: Question 2: If the research includes expression of oncogenes or toxins, then describe how they are expressed. If none, answer "none" or "NA."

7) Viruses, Prions, or Vectors: Clarify how the AAV is not replication-defective. If this is incorrect, change the response.

8) Biosafety Summary: If blood or tissues will be obtained from participants after AAV delivery, "Tissues, Blood, or Body Fluids" should be selected. Note that these tissues may be considered to be recombinant materials.

Comments:

This is a conversion of a legacy IBC protocol that will be transitioning to a new investigator. In the study, AAV2-hAADC is being investigated for the treatment of advanced PD with motor fluctuations. Parkinson's disease is a neurodegenerative disorder involving loss of dopamine producing neurons located in the midbrain. Levodopa is the main and most effective drug used as a therapy to treat Parkinson's disease (PD). A serious problem with this therapy is the inexorable loss of levodopa response overtime. Over the course of a number of years, patients must take higher doses more frequently to maintain therapeutic efficacy, and this escalation in dosing is typically associated with the appearance of motor fluctuations (the so called "ON" "OFF" phenomenon), dyskinesia (involuntary movements), freezing of gait and other complications.

Some of these phenomena are related to the limited conversion of levodopa to dopamine in the brain as well as the inability to store the dopamine produced. The conversion of levodopa to dopamine is a single enzymatic step catalyzed by the enzyme aromatic amino acid decarboxylase (AADC), which is markedly decreased in the brains of patients with PD. The Sponsor is investigating Adeno-Associated Virus serotype 2 encoding human aromatic L-amino acid decarboxylase (VY-hAADC) (i.e., the study drug) for the treatment of advanced PD with motor fluctuations. VY-hAADC is a recombinant gene transfer vector that is derived from AAV and contains the coding information for human AADC. The genetic content consists of an expression cassette containing the CMV promoter, a chimeric CMV/beta-globin intron, the human AADC gene, and the human growth hormone polyadenylation site cloned into the AAV vector plasmid creating the AADC vector plasmid called pAAV2-hAADC. No helper virus is required for manufacture of this AAV construct. No carrier particles are used. The final vector preparation that will be administered to clinical study participants will contain a contrast agent used to visualize the targeting of vector under MRI. AAV2-hAADC is intended to increase dopamine production locally, by delivery of the human AADC gene directly to the putamen in a single surgical setting via SmartFlow cannula connected to Medfusion 3500 infusion pumps. With this approach, transduced striatal cells will express AADC, resulting in local production of dopamine from exogenously administered levodopa. The dose of study drug patients receive depends on the timing of their enrollment into the study, ranging from 1.5×10^{12} vg up to 8.8×10^{12} vg. Study participants will be followed for 36 months post-surgery. The Exposure Assessment and Protective Equipment and Waste Management sections adequately outline risk mitigation. Recommendation for approval. This is an annual renewal, conversion to an electronic submission format, submitted by a new investigator, for a Phase 1b non-randomized, open-label HGT protocol designed to evaluate the safety and tolerability of ascending dose levels of AAV2-hAADC delivered to the putamen of patients with Parkinson's Disease (PD) who have suboptimal responses to levodopa treatment. The study product is a recombinant Adeno-Associated Viral vector that encodes human aromatic L-amino acid decarboxylase (AAV2-hAADC), derived from Adeno-Associated Virus Type 2 (AAV2). Up to 4 dose levels will be studied. The intended target cells are striatal neurons, specifically in the post-commissural putamen. AAV2 is highly specific for neurons with very few glia transduced after direct brain infusion. This study assesses changes in clinical parameters and extent of the infusion coverage of the putamen. AAV2-hAADC is intended to increase dopamine production locally by delivery of the human AADC gene directly to the putamen. Following surgical infusion, it is postulated that transduced striatal cells will express AADC, resulting in local production of dopamine from the L-dopa treatment the patient is receiving. AADC gene transfer is anticipated to lower the therapeutic dose of levodopa and reduce L-dopa-induced side effects including dyskinesia.

Biosafety: AAV2-hAADC consists of recombinant AAV2 carrying the cDNA of the human AADC gene under the control of the CMV immediate early promoter. The viral genes required for propagation (rep and cap) are excised from the viral genome and used to create an AAV helper plasmid. An expression cassette containing the CMV promoter, a chimeric CMV/beta-globin intron, the human AADC gene, and the human growth hormone polyadenylation site is cloned into the AAV vector plasmid between the 2 inverted terminal repeat sequences to create an AADC vector plasmid called pAAV2-hAADC. These plasmids are used to transiently transfect a derivative of HEK293 cells in the presence of an Adenovirus helper plasmid that contains the E2A and E4 and VA ribonucleic acid (RNA) genes from type 2 Adenovirus, which supply the activities necessary for AAV replication. The resulting AAV2- hAADC gene transfer vector structure cannot

further propagate because of the absence of both cis (rep and cap) and trans (E2A, E4 and VA RNAs genes) factors. Product is made under cGMP conditions. Release testing includes testing for safety (sterility testing [bacterial and fungal testing], mycoplasma, adventitious agents testing, replication competent and pyrogenicity and endotoxin testing), identity (genetic and vector genome) appearance, pH, potency and purity (including residual plasmid DNA and residual bovine albumin). Development of neutralizing AAV2 antibodies was seen in nonclinical and clinical studies of AAV2-hAADC. The risk of vector spread outside the brain is considered minimal. Barrier contraception will be required for 6 months after surgery. Vector will be prepared by the U Pitt IDS in containment facilities and will be administered by health care workers training in standard biosafety precautions. Surfaces that may have come in contact with the vector will be sanitized with 10% bleach solution.

Initial comments:
1) Human Gene Transfer: Question 3a: Describe how the study product will be transported from IDS to the surgical area.
2) Human Gene Transfer: Question 7a: Provide a response.

The submitted application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	8
Against:	0
Abstained:	0

Protocol:	IBC201900060
Title:	Brain Development Mechanisms
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Signed cover page • BSL-2+ manual

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: AAV can be used at BSL-2. Clarify if downgrade below BSL-2 is being sought. A Certificate of Analysis (COA) for each virus will need to be provided for downgrade to BSL-1. Some genes to be expressed are oncogenes, which must be used at BSL-2. It is suggested that an amendment/modification is submitted for downgrade of AAVs.

2) Lentivirus and Lentiviral Vectors: Question 2a: 2nd and 3rd generation Lentiviruses are described. Clarify what system will be used in this study (i.e. how many plasmids).

3) Recombinant or Synthetic Nucleic Acid Work Description: Question 2: SOX11 has also been described as an oncogene and should be included in the response.

4) Animal Gene Transfer: Question 1: Clarify what virus will be used that contains more than 50% of the viral genome.

5) Risk Group and Containment Practices: Question 1: As Lentiviruses will be used, risk group (RG-3) should be checked.

Comments:

The investigator is a new assistant professor who is looking at neuron function. The protocol describes the construction of constructs expressed in AAV vectors, Lentiviral vectors, plasmids and genetic modification of mice using the CRE-lox system. The protocol is well-written and clearly lists the genes to be manipulated and those to be administered to animals. One of the genes to be expressed by Lentivirus is Bcl2, a known oncogene (B-cell lymphoma 2 gene). This would require the protocol to be at BSL-2+, per IBC guidance. In addition, a transgenic mouse is planned to express Bcl2-flox, whether the cross will knock it out or generate a tagged version was unclear in the application. The expression of oncogenes with lentiviruses requires that we discuss this protocol. The purpose of this study is to validate and test the function of new markers for these immature neurons using single-nuclei RNA sequencing. In mice, fluorescent reporter proteins (e.g. DCX-mRFP) will be used to observe the dynamics of these cells and measure their growth properties. Crossing these transgenic mice into deficient backgrounds (e.g. DCX-CreER; TBR1flox mice) will allow visualization of differences resulting from manipulation of specific neurodevelopmental pathways. Standard molecular biology techniques for cloning of plasmid DNA in standard plasmids for electroporation and recombinant viral vector plasmids for the generation of recombinant Adeno-Associated Virus vectors and recombinant Lentiviral vectors

will be used. Cells will be recovered in primary neuron cultures from mice and their progenitors will be transplanted to study protracted neuron development in more detail. Downgrade of AAV1, 2 and 8 to BSL-1 is being requested for AAV viral vectors made in the laboratory. Purity will be analyzed by SDS PAGE. This process meets the requirements for downgrade. Approval is recommended pending clarification question/comments.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	8
Against:	0
Abstained:	0

Protocol:	IBC201900074
Title:	Post GWAS Functional Studies
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Reconsidered

Required modifications:

1) Supporting Documents: Question 1: The laboratory listed must have a current BSL-2+ laboratory inspection and an approved Biosafety Operations Manual (BSL-2+ manual) prior to IBC approval.

2) Exposure Assessment and Protective Equipment: Question 4: Work with any BSL-2+ agent is not permitted outside of a biosafety cabinet (BSC); all work with BSL-2+ agents must be conducted in a BSC. Revise the response to Question 4 to reflect this information. In addition, correct the response to indicate that work with Lentiviruses will be performed in an approved BSL-2+ cell culture laboratory. Remove the language regarding work with infected mice in Question 4.

3) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: As it is stated that other genes not listed will be targeted via Lentivirus, add to the response in the application that any tumor suppressors knocked down with Lentivirus or any oncogenes overexpressed via Lentivirus will be handled at BSL-2+. Include this statement to the response to question 1.

4) Viruses, Prions, or Vectors: Use location; this is an open laboratory space and not appropriate for BSL-2+ work. Identify an appropriate tissue culture laboratory that is approved for BSL-2+ work and listed in the Biosafety Operations Manual.

5) Primary Cells or Cell Lines: Include production of Lentiviruses under Description of Usage.

6) Primary Cells or Cell Lines: The information entered in comments for clarification of the cell lines OVCAR-8 and AC16 should be entered into the body of the application.

7) Primary Cells or Cell Lines: Use location; this is an open lab space and not appropriate for BSL-2+ work. Identify an appropriate tissue culture laboratory area that is approved for BSL-2+ work and listed in the Biosafety Operations Manual.

8) Tissues, Blood, or Body Fluids: It is unclear how nervous tissue will be obtained from the Central Blood Bank. Clarify.

Comments:

The investigator will be characterizing disease-associated SNPs in various cell lines using shRNA or CRISPR/Cas9 to knockdown or knockout these genes. *E. coli* will be used to propagate plasmids at BSL-1, which is appropriate. Human blood and nervous tissues will be obtained for isolation of nuclear extracts at BSL-2, although it is unclear where these will come from. Human cell lines will be used for knockdown or knockout studies. shRNAs and CRISPR/Cas9 will be introduced into cells by transduction with Lentiviral vectors (and possibly also mammalian expression plasmids via transfection) at BSL-2. However, one shRNA described as being encoded in a Lentiviral vector

is a tumor suppressor gene, particularly with certain mutations which may be SNPs that the investigator is studying. This would need to be performed at BSL-2+. It is also noted that Cas9 and sgRNAs will be encoded in the same Lentiviral vector. Finally, in the Exposure Assessment section, infected mice are mentioned, yet no animal studies are described. As the protocol contains substantial missing information, it should be sent back to the investigator prior to coming to convened review. The investigator plans to identify and characterize single nucleotide polymorphisms *in vitro*. Studies will be conducted with human primary cells and cell lines. *E. coli* will be used for plasmid amplification and Lentivirus will be used to knockdown various genes. Several oncogenes are noted (FGFR2 and GATA2) but will not be overexpressed. CUX1 and GATA3 (tumor suppressors) will be knocked down; this work should be conducted at BSL-2+. In addition, it appears that RNA and CRISPR/Cas9 will be expressed via the same viral vector. Several form errors were noted and information regarding animals should be removed if this work will not be conducted. It appears that this protocol should be considered at BSL-2+.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Reconsidered: Due to the use of tumor suppressor genes, the study has been requested to come back for review by the committee after the investigator provides additional clarification and revisions on the application.

Supporting documents: None

Votes:

For:	8
Against:	0
Abstained:	0

Protocol:	IBC201900085
Title:	Adult Stem Cells for Tissue Regeneration
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Supporting Documents: The BSM (Biosafety Operations Manual) must be signed (approved) by a member of the Department of Environmental Health and Safety and uploaded in this section of the application prior to final approval by the IBC.

2) Supporting Documents: Investigator must clarify if working under a collaborator or if the investigator has acquired separate laboratory space. The location of use of BSL-2+ agents should be the location (tissue culture room) listed in the Biosafety Operations Manual.

3) Exposure Assessment and Protective Equipment: Question 1: BSL-2+ is required for Lentiviruses and Lentivirally infected cells. Correct the answer.

4) Recombinant or Synthetic Nucleic Acid Work Description: Answer Questions 3a-3e.

5) Recombinant or Synthetic Nucleic Acid Work Description: Question 3: siRNA are mentioned in the application. Thus, the answer to this question appears to be YES.

6) Recombinant or Synthetic Nucleic Acid Work Description: Question 2: Add the oncogenes Sox-2 and Klf4 in the response.

7) Primary Cells or Cell Lines: Clarify why human MSCs will be used at BSL-2+ under the "Description of Usage" question for the entry.

Comments:

The protocol will cover work aimed at improving tissue regeneration for treatment of degenerative skeletal diseases. It will use adult stem cells and the work will aim to improve their growing environment to optimize regeneration for downstream development of scaffolds or factors to improve stem cell treatment. The work will use human bone marrow tissue or cells or skeletal tissue as a source for primary cells (MSC, chondrocytes or osteoblasts). These will be from the University of Washington. Murine skeletal tissue (source: mice from *Jackson Labs*) will be used to assess changes in gene expression and for analysis. Lentiviral vectors (HIV 4-plasmid, from *Invitrogen*) will be used to deliver the usual transcription factors to generate iPS cells from the MSCs and maintain their potency (Oct4, Sox-2, Klf4, cMyc, Nanog), as well as to overexpress genes of interest and markers (Bcl-2, VSVG, eGFP etc.). Because of the use of oncogenes and Lentiviral vectors, the work will be done at BSL-2+. Some small issues had been caught during pre-review screening have been corrected. One small clarification requested regarding tissue source beyond University of Washington. Recommend approval though given the request for approval at BSL-2+, the committee should discuss in detail. The proposed studies will investigate the regenerative activities of human adult stem cells and their use for treatment of degenerative skeletal diseases. Experiments will use human and mouse cell lines transduced with multi-plasmid

Lentivirus vectors. Some of the genes to be expressed are oncogenes. This proposal may be approved at BSL-2+.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	8
Against:	0
Abstained:	0

Protocol:	MOD201900090
Title:	Amendment for **IBC201800031**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Chang_Moore Laboratory Manual.Dec 2018 inspection

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Include Retroviruses in the response.

2) Viruses, Prions, or Vectors: Later in the protocol, it is stated that virally infected cells will be administered to mice. This should be corrected for the appropriate viruses listed below.

3) Primary Cells or Cell Lines: Rat cells: If viruses will be used to transform the cells, the biosafety level should be corrected to BSL-2+.

4) Basic Information: Question 3: Include the description of the new experiments using the new agents in question 3 into the application.

Comments:

The investigator wishes to add a Crispr/Cas13 knockdown system to target backsplice junction sites in host RNAs based upon difficulties with other approaches. This work is a component of broader studies investigating gene function in HHV8. This is apparently to be used to reduce the abundance of specific RNA species in the host, but the description of the proposed studies should be revised to specify the goal. In addition, the description of the proposed studies needs to ad detail specifically with respect to a) the type of vector to be used in the Crispr/Cas system (plasmid or Lentivirus both of which are mentioned) and b) if Lentivirus, whether or not guide RNAs and Cas protein will be on the same vectors. The amendment request seeks to add CRISPR/Cas13-based tools to target backsplice sites to facilitate loss of function studies on viral circular RNAs (circRNAs). These tools rely on recently identified RNA-targeting CRISPR/Cas variants. The tools will be used to target viral circRNA junctions in virus-infected cells lines. Defective CasRx, which only binds RNA and does not cleave, will be used to block backsplicing. Currently the vectors used to deliver the CasRx and defective CasRx-based tools are not described. This needs to be amended before approval.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	8
Against:	0
Abstained:	0

Protocol:	MOD201900176
Title:	Amendment for **IBC201700034**
Investigator:	REDACTED
Highest BSL:	RBL BSL-3
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Hartman IBC Appendix RVF RG system

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Include description of work with CRISPR/Cas9 and SFTSV in the response.

2) Primary Cells or Cell Lines: Define "ADD" under Description of Usage where appropriate.

3) Tissues, Blood, or Body Fluids: Tissues that will be infected with recombinant viruses or from transgenic animals/animals in which recombinant nucleic acids have been introduced should be designated as recombinant.

Comments:

This modification describes addition of a new Risk Group 3 (RG3), non-Select Agent virus (SFTSV), to an existing protocol for an investigator approved for work in the Regional Biocontainment Laboratory (RBL) with both Select Agents and non-Select Agents. Various new reproductive tissues from a variety of species have been added in addition to a cell line that will be modified at another university using a Lentiviral CRISPR/Cas9 library. After transduction in the collaborator's library the cell line will be shipped to the Investigator's laboratory at Pitt for use in *in vitro* infection studies. Work with SFTSV will be performed in the RBL, which is appropriate. The investigator is approved for work with all agents in the protocol, is in good standing with the University's Select Agent Program, and all RBL biosafety manuals and other documentation are current. Recommendation for approval in the RBL. This is a proposal is an amendment of an existing protocol studying Rift Valley Fever Virus in the Regional Biocontainment Laboratory. This amendment would add SFTFV (Severe Fever with Thrombocytopenia Syndrome Virus), a related virus in the Bunyavirus family. The SFTFV being used is *not* recombinant. The amendment will also the use of cells modified at another University with CRSPR/Cas9 to target LRP1 and Lrpap1 genes. Neither of these is an oncogene or tumor suppressor gene. The main issues here are with biosafety, not recombinant DNA issues.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	8
Against:	0
Abstained:	0

Protocol:	MOD201900214
Title:	Amendment for **IBC201800237**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Exempt: Sections III-E or III-F
Additional Documents:	• citiCompletionReport3798651 • EOH-Memo Liu 2019

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Usage: Select *NIH Guidelines Section III-D-1*.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 3: Clarify under question 3 that only plasmids will be nucleofected into cells and the plasmids, which could be used with a packaging cell line to generate viral particles and will NOT be used to generate viral particles. The current language in question 3D "viral vector and nucleofection will be used as the delivery system" is a bit unclear to the committee. Clarify.

Comments:

The purpose of this amendment request is to update a project that has been funded and change the cell lines and plasmids included in the protocol. Nucleic acids will be transfected into cell lines via nucleofection, including for the purpose of siRNA-based studies on cell lines. Several issues need to be addressed including A) the source of the mice needs to be indicated, B) checking the Section III-D-2 box since *E coli* will be used for plasmid amplification, and C) there remains some confusion regarding the use of animals and whether they will be treated with recombinant material in this study and this should be clarified in the summary and in the Live Animals section. The work is proposed at BSL-2/ABSL-1, which appears to be appropriate for the project. Once the IBC-identified issues have been addressed, recommend approval. This is an amendment to add funding and new experimental agents to the protocol. As noted, there is some confusion about whether or not rDNA will be administered to animals (it is written both ways throughout the protocol) or if the confusion lies in the fact that the animals will be receiving agents that are not rDNA in nature. Other than some form issues also that need to be addressed (expansion of animal carcass disposal and spill containment) protocol appears to be BSL-2 in nature and can be approved provided that concerns are appropriately addressed.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	8
Against:	0
Abstained:	0

Protocol:	MOD201900244
Title:	Amendment for **IBC201900012**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Supporting Documents: Upload the final (signed) EH&S approved Biosafety Operations Manual for the laboratory.

2) Exposure Assessment and Protective Equipment: Question 5: Select the boxes for double gloves, hair bonnet, liquid-barrier coverall suit, shoecovers, solid-front wrap around gown, and surgical masks.

Comments:

This amendment is for a protocol investigating anti-retroviral immunity where changes are being made to address the study's biohazard level in response to changes in IBC regulations. The work uses murine models to investigate immunity against MLV and MMTV, a large number of human, cat, hamster, murine, nonhuman primate and human cell lines for plasmid transfection or viral transduction, and *E. coli* for plasmid amplification. A large number of viruses ranging from BSL-1 mouse viruses to replication-competent BSL-2+ Lentiviruses will be used in this project and mice will be infected with viruses up to BSL-2 while viruses up to BSL-2+ will be used in cell lines. Oncogenes are not included in the protocol. The work is proposed at BSL-2+/ABSL-2, which appears to be appropriate for the work. Some forms-related issues have been identified, but once these modifications are complete, approval is recommended. This is a modification in which the investigator has clarified biosafety containment levels for all viruses (Lentiviruses and Retroviruses) used in the study for *in vitro* and *in vivo* use. In addition, the investigator will use replication-competent primate Lentiviruses and replication-defective vectors to express both Cas9 and sgRNAs *in vitro* at BSL-2+. Other than a few incorrect answers to form questions that should be corrected. Recommendation is for approval.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For: 8

Against: 0
Abstained: 0

Protocol:	MOD201900259
Title:	Amendment for **IBC201700175**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED Cover Page • REDACTED BSL-2+ Manual June 2019_KL_BW July 2 2019

Determination: Approved

Last day of continuing review period: 7/8/2020

Required modifications: None

Comments:
Upgrade: The protocol modification has been submitted to update the information on gene editing to ensure compliance with current policy regarding the use of this technology when the Cas-9 and guide RNA are contained within the same vector. The investigator has indicated that the work falls under BSL-2+ containment and has updated the protocol information and included the most recent approved copy of the laboratory's biosafety manual.

Coordinator review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Approval: No additional comments were provided by the committee; the protocol was approved with no comments.

Supporting documents: None

Votes:

For:	9 (late arrival of member)
Against:	0
Abstained:	0

Protocol:	MOD201900285
Title:	Amendment for **IBC201900069**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 7/8/2020

Required modifications: None

Comments:
Downgrade to ABSL-1 may be approved pending IBC discussion. The investigator is injecting small volumes of Canine Adenovirus (CAV-2), AAV, or EnvA pseudotyped replication-defective Rabies Virus into the brains of mice at ABSL-2. The investigator is requesting a downgrade to ABSL-1 after injection of the viruses. It appears that this could be granted after 72 hours post-injection and after the investigator performs a cage change for the following reasons:

a) AAV and Adenoviruses have been shown not be shed after 72 hours *in vivo* (*Reuter et al. Comparative Medicine 62(5):361-370; 2012*). This is the basis of the Lentiviral vector downgrade for some laboratories already.
b) In addition, CAV-2 does not infect human cells efficiently and does not appear to replicate in human cells.
c) The Rabies Virus is replication defective (lacks the glycoprotein for entry) and is pseudotyped by EnvA, which is an Avian Retrovirus envelope that does not bind to mammalian cells. It appears that the investigator expresses Tva (the EnvA receptor) via AAV first and then injects the animals with the pseudotyped Rabies Virus.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Approval: Approval was provided for the requested downgrade to BSL-1/ABSL-1 for activities post-stereotaxic injection after a 72-hour wait period. The investigator should note that personnel working with animals outside of an engineering control (for example a Biosafety Cabinet) may be exposed to allergens and that appropriate PPE should be utilized. The recommended PPE for work at BSL-1 includes a laboratory coat and gloves at a minimum, with the addition of eye and mucous membrane splash protection when performing activities that have a risk of splash, spray, or generation of particulates (e.g. working with chemicals, buffers, pulling glass pipettes outside of an engineering control that provides a protective sash) are performed.

Supporting documents: None

Votes:

For:	8
Against:	0

Abstained: 1 (member stepped out of the room)

Protocol:	IBC201900065
Title:	Production of Adenovirus
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 4: If viruses will be produced to encode heretofore unknown genes and then distributed to other laboratories, then the answer to Question 4 should be YES.

2) Viruses, Prions, or Vectors: If a collaborator requests Cas9 + sgRNAs in an Adenovirus, it would need to be produced and used under BSL-2+. Therefore, both the collaborator and the producing laboratory would need to have approvals for BSL-2+.

3) Exposure Assessment and Protective Equipment, Question 5: Eye and face protection (e.g. safety glasses or goggles and a surgical mask, or a face shield) should be used at BSL-2 for procedures with risk of splash or spray performed outside of a BSC. Revise to add these items to the response.

Comments:

In this protocol the investigator seeks to produce a variety of Adenoviruses with the goal to distribute them to other laboratories. Viruses will express genes and/or proteins that will be determined by the collaborators. Different strains of *E.coli* will be used for plasmid work. Work will be performed with BSL-2 level containment procedures. The investigator should indicate the process that will be in place so that A) no known oncogenes will be used intentionally without modification of this IBC protocol and subsequent approval, B) the other laboratories have their own IBC protocol with the appropriate biosafety level containment in place. This will be a core facility for the production of Adenoviruses synthesized by PCR for distribution to other laboratories. This work will be done at BSL-2 with appropriate PPE being used. The genes being used for cloning/placed into the Adenovirus are determined by the collaborator. The question of what process is in place to determine if a gene of interest from a collaborator is an oncogene needs to be answered. Also, “in house” for Adenovirus sources does not provide the appropriate information of where the viruses were obtained. Due to this being a core facility, this needs to be discussed. If the questions are appropriately answered, then approval is recommended. The investigator will produce a variety of Adenoviruses for distribution to collaborations with other laboratories. These viruses are artificially synthesized by PCR technology. The goal of using different Adenovirus serotypes is to avoid pre-existing immunity in humans against the most common Adenovirus serotypes (example Adenovirus 5). In particular, animal Adenoviruses like the Simian Virus SV11 and frog virus are expected not to have cross reactivity with human serotypes. Animal Adenoviruses are not known to be pathogenic for humans and their handling is similar to the most commonly used serotypes.

Initial comments:
1) First page- Summary: The investigator should describe more clearly the project. Are these Adenovirus are being made with goal of using in humans? Why does the summary mention avoiding the human immune system. Seems unclear.
2) Viruses: The investigator should list the genes that will be inserted. The current description is not appropriate.
3) Recombinant and synthetic nucleic acids: The investigator should list the genes to be inserted. This is also important because the end users or collaborators would be citing this IBC in their IBC to justify the use of Adenovirus containing their gene of interest.

Review comments were provided to the investigator for response. The revised application was placed on the July agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 11:09 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

August 12, 2019 10:00 AM

302 Heiber Building

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			
REDACTED, Vice Chair	absent		
REDACTED, BSO			
REDACTED	absent		
REDACTED			
REDACTED	absent		
REDACTED	absent		
REDACTED			
REDACTED	absent		
REDACTED			
REDACTED	absent		
REDACTED			
REDACTED	absent		
REDACTED	absent		
REDACTED			
REDACTED			
REDACTED			
REDACTED			
REDACTED			
REDACTED			
REDACTED	absent		
REDACTED	absent		
REDACTED			

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office, Director
REDACTED	IBC Office
REDACTED	IACUC Office
REDACTED	RCCO Co-Director

GUEST NAMES
None

QUORUM INFORMATION

Committee members on the roster:	23
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and are able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The July 2019 meeting minutes were reviewed and approved by the committee.

Votes:

For:	12
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

Discussion on scheduled date of the November IBC meeting. It was determined that a poll would be provided to the committee and results will determine the best date for the November meeting.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocols on the following pages

Protocol:	IBC201900105
Title:	Cell Polarity and Cancer
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: MDCK entry: In the virus section the investigator indicates that Lentiviral vectors will be used "for routine expression of GFP markers in mammalian cell lines such as HEK293 and MDCK cells". Revise the entry for this cell line to indicate that it will be recombinant (e.g. transduced with Lentivirus to express GFP).

2) Exposure Assessment and Protective Equipment, Question 5: At BSL-2 use of mucous membrane protection is required when procedures with risk of splash or spray are performed outside of a Biosafety Cabinet (BSC). Indicate that either a surgical mask in combination with the safety glasses already listed, or face shield, will be used when needed.

Comments:

This IBC protocol will investigate the Molecular Control of Cell Polarity in Drosophila and Cancer. Lentiviral vector pLEX-MCS (*System Biosciences*) will be uses to generate the virus: pLEX-MCS-GFP, pLEX-MCS-Lgl and pLEX-MCS-Dlg. These Lentiviruses will be used to infect the cells, including MDCK cells, HEK293 cells, MCF10A cells, MCF7 cells (breast cancer cell), MDA-MB-231 cells (breast cancer cell) for analysis of immunoblotting and immunocytochemistry. No known oncogenes or toxins will be used in this protocol. All work was claimed to be performed at level of BSL-2 which is suitable. The investigator plans to study the role of polarity proteins that determine cell epithelial cell apical-basal polarity in drosophila and mammalian models. Genes for individual proteins will be cloned, modified and expressed in cells and flies using plasmids and Lentiviruses. The investigator states that none of the genes are oncogenes. Minor form issues need to be addressed. Otherwise recommendation for approval.

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900110
Title:	pGLO Bacteria in Plant Organs
Investigator:	REDACTED
Highest BSL:	BSL-1
NIH Guidelines:	• NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED_Certificate • BIO-RAD pGLO Bacterial Transformation Kit • REDACTED_Certificate • Mentor Agreement

Determination: Approved

Last day of continuing review period: 8/12/2020

Required modifications: None

Comments:

This is a proposal from a student mentored at Pitt Bradford who wants to culture non-pathogenic *E. coli* transformed with plasmid pGLO (ampR araC-GFP). Plant seeds will be germinated in the presence of the transformed *E. coli*, grown and then examined for presence of luminescence. No viruses are used. No human or vertebrate genes are used. The investigator has indicated the study uses "Transgenic plants" but this does not appear to be accurate. The application describes growing non-recombinant plants in the presence of a transformed bacteria which is to be taken up by the seedlings. There are some scientific concerns about the project, but that is not the role of the IBC. BSL-1 is appropriate. The IBC should discuss just because the named investigator is a student under mentorship of a faculty member. This is study by a faculty member at Pitt Bradford. The primary contact is a student who apparently will conduct the work. A plasmid encoding a reporter gene (unclear if it is GFP or luciferase) will be grown in *E. coli* with seeds from turnips, onions, and radishes for the purpose of attempting to have the plant express the reporter protein. Other than the confusion of the reporter gene to be expressed and checking the box for Section III-E/III-F, there are no biosafety concerns. This is a study to be performed by a student under the supervision of a Pitt Bradford investigator. A non-pathogenic strain of *E. coli* will be transformed with a plasmid expressing a reporter gene (to be clarified as requested by another reviewer). Turnip, radish, and onion seeds will then be grown in the presence of the modified *E. coli* to determine whether the plants will express the reporter gene. There are several clarifications needed, but in general it appears that this work can be approved at BSL-1. It appears that no work will be done with transgenic plants, exotic plant pathogens, or microbial pathogens of plant-associated insects or animals. Likewise, no toxin genes will be introduced into plants. Therefore, it is recommended that *Section III-D-5* does not apply to the work and that *Section III-E*/*Section-F* should apply instead. Clarification in Exposure Control and Waste Decontamination section has been requested to include the description of the methods used to prevent release of genetically-modified *E. coli* to the environment. Recommendations and suggested work practices and decontamination methods have been added as comments to the appropriate sections of the protocol. Once clarified, recommendation is for approval at BSL-1.

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Approval: No additional comments were provided by the committee; recommendations for approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900117
Title:	Transfected Cells
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-4
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 8/12/2020

Required modifications: None

Comments:

Administrative Review Summary:

The protocol involves the administration of a mouse cancer cell line that was previously recombinantly changed to express red fluorescent protein. The cells are administered to live rodents, which falls under requirements for IBC review. Pre-screening comments have been addressed and revised by the investigator.

The revised application was placed on the August agenda.

Approval: No additional comments were provided by the committee; recommendations for approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900275
Title:	Amendment for **IBC201700038**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• MTA_2478

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Lentivirus: Later the application states that "LVV, Retroviral, and gene editing (Cas9) vectors" will be used. Thus, Cas9 and sgRNAs should be listed under "Inserted Nucleic Acids Information."

2) Viruses, Prions, or Vectors: MMLV: Later the application states that "LVV, Retroviral, and gene editing (Cas9) vectors" will be used. Thus, Cas9 and sgRNAs should be listed under "Inserted Nucleic Acids Information."

4) Recombinant or Synthetic Nucleic Acid Work Description: Since Question 3 has been answered YES you need to provide an appropriate response to Question 3a: Which technologies (e.g., siRNA, shRNA, CRISPR/Cas) will be used to change the gene function or expression.

5) Waste Management, Question 1a: Place the information regarding solid biohazardous waste into the response for question 1a in the actual application section.

Comments:

The investigator would like to use Lentivirus as a read out of AKT and MAPK. The reporter genes are obtained from *Addgene*. No information is given about the vectors, identification, or the way the virus will be made. Perhaps it is better to revise the application to include the details of how the virus will be made. The investigator is asking to add Lentivirus vectors to an existing, approved protocol using fluorescent probes to track gene expression in development of cancer. Some details are lacking; in particular, exactly what these vectors will be expressing. There are a number of other small issues that need to be addressed.

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900284
Title:	Amendment for **IBC201800026**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Clarify how AAV is unable to enter/infect human cells. If this is incorrect, change the response to YES for Question 10.

2) Live Animals, Question 3: Earlier it is stated that murine tissues will be obtained for genotyping and *in vitro* retinal tissue studies. The response should be YES.

3) Animal Gene Transfer, Question 1: Clarify how 50% of the virus genome is present in a single vector. If this is incorrect, change the response to NO.

4) Waste Management, Question 1b: State that the final concentration of bleach will be 10% (1:10 v/v).

Comments:

The request is to amend an approved protocol by adding AAV-2 vectors. The underlying protocol covers studies aimed at understanding the mechanisms of retinal axon regeneration, particularly after trauma (modeled with nerve crush) or disease (glaucoma). The AAV vectors to be added will deliver shRNA targeting expression of FNTA, RabGGTB, Slc6a4 and Integrin-b3 in control and Slc6a4-/- (KO in a serotonin transporter) mice or in a glaucoma model mouse and will be intravitreally injected. These will also deliver markers. AAV delivering Cre and GFP (as markers) will be injected into integrin-B3flox and a young DBA/2J glaucoma mouse before injury and degeneration, respectively. *Vector Biolabs* and *Virovek* will generate the AAV-2 vectors. Work with the vectors will be done at BSL-2 and animal work will be done at ABSL-2. This is an amendment that adds AAV-2 as a tool for targeting retinal ganglion cells to an established protocol for a project investigating the role of the adhesion molecule NrCAM and ligand Sema6D, and serotonin transporter Slc6a4 in retinal degeneration. To address this topic, the investigators will use two transgenic mouse strains that will not receive recombinant nucleic acids or cells, transiently transfected HEK293 cells. In a third project, mice will have AAV-2 injected into their eyes for the purpose of knocking down FNTA (cytoskeletal remodeling), RabGGTB (cell death), Slc6a4, and integrin b3 to examine the role of these proteins in axonal degeneration. Cloning for the project will be done in DH5alpha *E. coli*. The work is proposed at BSL-2/ABLS-2, which appears to be appropriate. Approval is recommended pending minor clarifications.

Initial comments:

1) Recombinant or Synthetic Nucleic Acids Usage: *NIH Guidelines Section III-D-1* is required.

2) Question 3 under "Recombinant or Synthetic Nucleic Acid Work Description" is currently indicated as NO, but since RNAi will be used, it should be changed to YES.

Review comments inserted in the application were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900291
Title:	Amendment for **IBC201900082**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: Clarify how murine tissues are recombinant.

2) Primary Cells or Cell Lines: Clarify how murine colon cells are recombinant.

3) Viruses, Prions, or Vectors: List all non-viral genes (i.e. luciferase) to be expressed from Lentiviruses under Inserted Nucleic Acids Information.

4) Recombinant or Synthetic Nucleic Acid Work Description, Question 3: It is unclear what gene drive technology (e.g. siRNA, shRNA, CRISPR) will be used in the study. Clarify and answer all questions.

5) Lentivirus and Lentiviral Vectors, Question 1: Earlier in the protocol it is stated that 293T cells will be used to package virus. Clarify this discrepancy.

Comments:

The investigator proposes to use a Lentivirus to add luciferase gene into human cell lines (HT-29 can Caco-2) from human colon, and rat cell line (IEC-6) from rat colon. This modification seeks to add the use of a Lentivirus vector to express luciferase in human and rat cell lines. It is not clear from the information provided what the investigator hopes to achieve by doing this. Some clarity is needed. There are a few minor additional details that need to be addressed but otherwise once more information is provided this protocol can be approved.

Initial comments:

1) Recombinant and synthetic nucleic acid: *NIH Guidelines Section III-D-3* should be checked as infectious virus is being used.

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900306
Title:	Amendment for **IBC201800246**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: AAV entry: Earlier in the protocol the investigator indicated that AAV will be made and purified in the investigator's laboratory. Here it appears that AAV will be ordered from the Penn Vector Core. Clarify, and if AAV will be made in the investigator's laboratory, then add the packaging cell line to the cell line section in the application.

2) Recombinant or Synthetic Nucleic Acid Usage: If Lentivirus or AAV vectors will be used for cloning or transformation of *E. coli* in the laboratory then *Section III-D-2* should be selected.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Describe CRISPR/Cas9 vectors mentioned in Question 3d here.

4) Recombinant or Synthetic Nucleic Acid Work Description, Question 3e: Describe how Cas9 and sgRNA will be delivered to iPSCs.

5) Exposure Assessment and Protective Equipment, Question 4: Use of safety-engineered sharps devices is required at ABSL-2 and above. Revise the response to include this information.

6) Waste Management, Question 1b: Clarify that the final concentration of bleach will be 1:10, v:v, bleach: liquid waste.

Comments:

The investigator wants to add AAV to the currently approved protocol. This would be *in vitro* and *in vivo* (mouse) work. The work will utilize AAV to assist CRISPR/Cas9 in mice to target heart development/cell growth related genes. AAV-9 is sourced from Penn Vector Core and is not being requested for a downgrade. The investigator will be doing work at the A/BSL-2 level, which is appropriate. Recommendation for approval. This amendment proposes to use AAV-9 in mice to introduce guide RNA or shRNA for knocking out specific genes that may be important in cardiac development. Human iPSCs will also be transformed with Crispr/CAS9/gRNA for relevant genes using nucleofection. The investigator now classifies the experiments as BSL-2 and does not request a biosafety level downgrade. Approval is recommended. The amendment seeks to modify an existing protocol by adding AAV (serotype 9) to deliver sgRNA for CRISPR/Cas9-mediated knockout of genes in mice. Candidate genes are those in development of the heart. Cas9 transgenic mice will be used and sgRNA will be delivered using AAV vectors, which will be introduced into mice by intraperitoneal or subcutaneous injection. The protocol already has work approved for use of Lentiviral vectors to deliver reporter genes to human ES and iPS cells. BSL-2/ABSL-2 selected

for this work, which is appropriate. Under "Animal Gene Transfer" question 2 should now be YES. Once small revisions are made, approval is recommended.

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900313
Title:	Amendment for **IBC201700188**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• 0032820.MTA.REDACTED.VA final • NIH approval grant transfer • Amankulor IACUC 00007574 submission 8/2/17 • Amankulor IS00007429-mod3 8/10/17

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: Include information on AAV administration to cells in the relevant areas below.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include work with AAV in the response.

Comments:

This is an amendment to add a commercially available AAV-2 vector to the research program. The investigator is currently approved to use HSV vectors at BSL-2/ABSL-2 containment. The AAV is from a recognized vendor, but the investigator is NOT requesting a downgrade. The amendment describes the use of the new viral vector thus has been placed onto the agenda

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocol:	IBC201900052
Title:	HCC#19-024
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• 2016-06-01 Genetic Modification of Oncolytic Newcastle Disease Virus for Cancer Therapy_19-024 • 2018-07 CC-MO-NURS-N-IC02_19-024 • 2019-04-04 Main Consent_19-024 • 2018-07-18 USDA Memo_800_103_19-024 • 2019-02-01 IMP Manual v1_19-024 • 2017-11-01 Toxicity Management Guidelines_19-024 • HSFM0208PRO • CC-MO-NURS-H-PP54 • 2019-02-14 Protocol_19-024 • 2019-01-18 IND18660 Safe to Proceed Letter_19-024 • 2018-12-04 688284_MEDI Permit_Cover_TO exp 11-28-2019_19-024 • CC-MO-PHARM-9.2 Handling of BioSafety Level Products (3-19) • 2019-02-19 Pregnant Partner Consent_19-024 • 2019-05-08 D1160651-MEDI5395 Export License-FBSUK-31MAY23_19-024 • 2018-12-05 Medi5395 IB Ddition 1_19-024 • 2019-02-11 Durvalumab IB Edition-14_19-024

Determination: Modifications Required

Required modifications:

1) Supporting Documents: An updated USDA permit is required in order for the agents to be obtained for use at the UPMC clinical site. The University Biosafety Officer has reached out to the investigator and the clinical coordinator to offer assistance or advice. The study will need to have this permit in place before the work can be started.

2) Risk Group and Containment Practices: Question 2: Remove the check next to BSL-2 containment in the first column, as that research standard is for basic science research, which this study is not proposing.

3) Viruses, Prions, or Vectors: The journal article included in the Supporting Documents (*Cheng et al. J Virol 2016*) clearly shows replication of the virus in human cells (Figure 2) and mice (Figure 7). Thus, the virus is not replication defective (note that attenuation/avirulence and replication-defectiveness are not the same thing). Revise the response to the question to address the information to be correct. The investigator will need to apply for a USDA APHIS permit to authorize transport of the study product, derived from a USDA-regulated animal pathogen, from the study sponsor to the Hillman Cancer Center facilities where it will be used.

Comments:

It is important to note on the IBC correspondence that the investigator will need to apply for a USDA APHIS permit to authorize transport of the study product, derived from a USDA-regulated animal pathogen, from the study sponsor to the Hillman Cancer Center facilities where it will be used.

This is an Phase 1, first-in-human, open-label, dose-escalation, and dose-expansion study to assess the safety, tolerability, pharmacokinetics (PK), pharmacodynamics and preliminary efficacy of MEDI5395 in combination with durvalumab in participants with selected advanced solid tumors. Up to approximately 164 participants may be enrolled in the study across approximately 30 sites globally. MEDI5395 is a fully attenuated (lentogenic) recombinant Newcastle Disease Virus (NDV 73T-R-198), modified from the WT NDV 73T strain expressing the huGM-CSF gene. Newcastle Disease Virus NDV is classified as an Avian Paramyxovirus (genus *Avulavirus*), and has demonstrated strong oncolytic activity against human tumor cells (*Reichard et al, 1992; Amarasinghe et al, 2017*). NDV is an enveloped, negative strand RNA virus, whose infection into cells is predominately mediated through interactions of the hemagglutinin (HN) protein and sialic acid-containing receptors on the malignant and non-malignant host cell. Following this binding, the fusion (F) protein mediates fusion of the virion envelope with the host cell membrane allowing infection and spread to occur (*Lamb et al*, *2006*). Natural NDV is categorized into 3 pathotypes depending on the disease severity it causes in birds (*Dortmans et al, 2011; Alexander, 1988*). Mesogenic (medium virulence) and velogenic (high virulence) strains of NDV can cause significant economic losses to the poultry industry, and thus were classified as select agents in 2008 per Federal Regulation (*Federal Register, 2008*). NOTE: Lentogenic (low virulence) strains cause sub-clinical infections and are not classed as select agents. NDV induces a strong Type I interferon response in infected cells that can lead to activation of natural killer (NK) and T-cells and to the maturation of dendritic cells. These mechanisms can kill virally infected tumor cells, enhance adaptive immune responses, and drive a pro-inflammatory response that leads to immune cell recruitment at the site of infection (*Gujar et al, 2018*). NDV selectively kills tumor cells versus non-tumor cells due to the defects in the interferon signaling pathway that are common among diverse tumor types (*Stoidl et al, 2000*). Differences in sensitivity to the oncolytic effects of specific wild-type NDVs were observed across the cell lines, with non-small cell lung cancer and cervical cancer cells showing the highest sensitivity. Clinically, studies of various NDVs in participants with advanced solid tumors have shown acceptable safety profiles and some antitumor activity (*Hotte et al, 2007; Pecora et al, 2002; Pecora and Lorence, 2007; Freeman et al, 2006; National Cancer Institute, 2018*). Results from 3 Phase 1 clinical studies of PV701 (a mesogenic NDV) in participants with advanced cancers who failed all conventional therapies, showed objective responses in approximately 12% (14/113) of total participants; these responses correlated with total virus dose delivered. Adverse events (AEs) reported across the 3 studies generally included influenza-like symptoms, tumor site-specific events, and infusion-related reactions, and were manageable with standard prophylactic measures such as antipyretics and antidiarrheals, slower infusion, and implementation of a desensitization regimen involving a lower initial dose. A Phase 1/2 study of NDV-HUJ (a lentogenic strain from the naturally attenuated B1 NDV) in 11 participants with recurrent glioblastoma multiforme following repeat IV dosing showed 1 CR (*Freeman et al, 2006*). Grade 1 or Grade 2 fever were the only treatment-related AEs, reported in 5 participants. Additionally, a Phase 2 non-randomized placebo-controlled study of an attenuated NDV strain MTH-68, administered via repeat inhaled dosing in participants with advanced cancers following conventional therapy, reported objective responses in 8 of 33 MTH-68-treated

participants and none of the 26 placebo-treated subjects, with only low grade AEs (*Csatary et al, 1993; Csatary et al, 2004*). Durvalumab is an FDA-approved immunotherapy for cancer, developed by *Medimmune/AstraZeneca*. It is a human immunoglobulin G1 kappa monoclonal antibody that blocks the interaction of programmed cell death ligand 1 with the PD-1. Dose Escalation – the dose escalation phase of the study is designed to evaluate up to 3 planned, sequentially ascending dose levels of MEDI5395. Durvalumab treatment will be started either sequentially or concurrently with MEDI5395. All dose-escalation participants will be required to provide on-treatment biopsies to evaluate viral replication within the tumor. A study specific Dose-escalation Committee (DEC) in accordance with its charter, will be responsible for providing ongoing safety surveillance of the study, with regularly scheduled reviews of safety, PK, and other relevant data. Dose Expansion – expansion cohorts may be opened in parallel or sequentially. Each expansion cohort will enroll one of the solid tumor types evaluated in dose-escalation. The primary hypotheses for this study are the following:
Dose Escalation – MEDI5395 in combination with durvalumab will demonstrate adequate safety and tolerability to support further evaluation in participants with select advanced solid tumors.
Dose Expansion – MEDI5395 in combination with durvalumab will demonstrate adequate safety and tolerability at the selected MEDI5395 dose level and schedule, to support further evaluation in participants with select advanced solid tumors.
The primary objectives of this study are to assess the safety and tolerability, describe the DLTs, and determine the dose and schedule of administration of MEDI5395 in combination with durvalumab. The related endpoints are incidence of AEs, SAEs, DLTs, discontinuation of investigational product(s) due to toxicity, and clinically significant alterations in vital signs, laboratory parameters, ECGs, ECHOs, and ECOG score. MEDI5395 is an oncolytic rNDV (New Castle Disease Virus, Avian Paramyxovirus) that expresses the human GM-CSF gene and has been designed to selectively infect, replicate, and lyse human cancerous cells. MEDI5395 replication and tumor lysis lead to presentation of tumor antigens and induction of local/systemic immune responses. rNDV has been modified to reduce its avian virulence, is fully attenuated, deemed lentogenic (low virulence). The inserted GM-CSF transgene is expected to enhance presentation of tumor antigens and improve antitumor immunity. As a monotherapy, MEDI5395's efficacy may be limited due to rapid emergence of neutralizing antibodies and upregulation of PD-L1 on infected cells. MEDI5395 will be co-administered with the anti-PD-L1 antibody, durvalumab (FDA-approved), to improve clinical outcomes. NDV has been shown to induce a strong Type I interferon response in infected cells that can lead to activation of natural killer (NK) and T-cells and to the maturation of dendritic cells. These mechanisms can kill virally infected tumor cells, enhance adaptive immune responses, and drive a pro-inflammatory response that leads to immune cell recruitment at the site of infection. The sponsor stated that the non-clinical studies conducted as part of the development program for MEDI5395 are considered to be adequate to address the potential adverse effects in humans. The highest non-severely toxic dose of MEDI5395 and NDVmuGM-CSF after repeated dosing to naive BALB/c mice for a total of 6 doses on Days 1, 4, 8, 11, 15, and 19 was the highest dose tested, a nominal dose of 1×10^8 focus forming units (FFU)/dose. This dose provides a 243-fold safety margin for the proposed MEDI5395 human starting dose of 1×10^8 FFU based on body surface area extrapolation to determine human equivalent dose. MEDI5395 has not been administered to humans. Up to about 164 participants may be enrolled across approximately 30 sites globally. The study will have two phases: dose escalation and dose expansion. MEDI5395 will be administered IV, 6 doses over 15-18 days, with a minimum of 3 days between each of the first three doses and at least 2 days between each

subsequent dose. Initially, each participant will receive the planned dose level of MEDI5395 for all doses. At any dose level, a desensitization regimen may be implemented to mitigate first dose infusion related reactions. Durvalumab treatment will be started either sequentially or concurrently with MEDI5395. The study's primary objective is to assess the safety and tolerability, dose-limiting toxicities and determine the dose and schedule of administration of MEDI5395 given in combination with durvalumab. A secondary objective is to determine the presence and duration of viremia from MEDI5395 as measured by viral genome copies in blood.

Biosafety: Wild-type NDV is considered a BSL-2 agent. Human-to-human transmission of NDV has not been reported. Study product is manufactured according to GMP, produced in HeLa-S3 cells, undergoes lot release testing. This rNDV has a modification at the fusion protein (F) cleavage site to reduce the efficiency of F protein cleavage and an insertion of a 198-nucleotide sequence into the HN-L intergenic region, resulting in reduced viral gene expression and replication in avian cells but not in mammalian cells. No genotoxicity, carcinogenicity or reproductive and development toxicity studies completed to date. There is no human clinical data. Potential risks based on its mechanism of action include flu-like symptoms (fever, chills, myalgia, nasal congestion, rhinorrhea, nausea, headache, fatigue); bone marrow toxicity (thrombocytopenia, neutropenia, anemia, leukopenia); and hypersensitivity reactions (immune-complex disease, infusion-related reactions, and inflammation at primary/metastatic tumor sites). Cardiomyopathy was observed in mice in early GLP studies in which the mice received repeat IV doses of 'development process 1' material. Since the putative toxic contaminant remains to be identified, and the human sensitivity to low levels of this contaminant is not known, the Investigator's Brochure lists cardiomyopathy as a potential risk. Other potential risks include hepatotoxicity and severe allergic reactions. Investigators are to monitor (cardiac, heme parameters, LFTs) participants carefully during/following study product administration. From first dose of MEDI5395 through 4 weeks after the last dose of MEDI5395, participants are to avoid all contact with birds, including pets and farm animals, close contact with individuals who have regular contact with birds (for example, bird keepers or poultry workers), and close contact with immunocompromised individuals. The genome copies of MEDI5395 will be measured in saliva and urine pre- and post-MEDI5395 administration.

Initial comments:
1) The investigator is requesting a BSL downgrade. However, it is a RG-2 agent and they propose to conduct the work at BSL-2 with Universal Precautions.
2) Project Summary: Briefly state what MEDI5395 and durvalumab are and why they are given together.
3) Exposure Assessment/PPE: Briefly state the personal protective equipment (PPE) the pharmacy staff will be asked to wear when handling the study product (isolation gown, goggle, glove, mask).

The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900111
Title:	HCC 19-101
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-02-01 Investigators Brochure Ed 1.0_19-101 • 2019-03-06 Main ICF Template v1.0_19-101 • 2019-03-07 PP ICF Template v1.0_19-101 • 2018-01-30 HS-IC0604 OSHA Bloodborne Pathogen Standard Exposure Control Plan_19-101 • 2019-04-04 Pharmacy Manual_19-101 • 2019-02-06 Protocol_19-101 • 2018-06-29 HS-FM0208PRO Waste Management PROCEDURE_19-101 • 2019-04-26 HS-IC0616 Guidelines for Handling Sharps_19-101

Reviewer Summary:

Determination: Deferred/Reconsidered

Required modifications:

The preclinical ASP9801 safety data in mice and macaques (without a tumor burden) revealed a significant safety signal, including risk of death, probably attributable to IL-12, when ASP9801 is dosed repeatedly at the highest feasible doses in those animals. rhIL-12 has been previously associated with a serious toxicity when administered in earlier human clinical trials. This is a serious concern.

1) Human Gene Transfer: Question 3a: Describe how the study product will be transported between the pharmacy and the outpatient clinic at HCC.

2) Human Gene Transfer: Question 7a: Provide details for procedures used to minimize or mitigate anticipated adverse effects.

3) Risk Group and Containment: Question 1: Check Risk Group (RG) 2, as Vaccinia Virus is used.

Comments:

This is a Phase 1 first-in-human open-label study of an oncolytic Vaccinia Virus (ASP9801) given by injection into cancerous lesions in participants with advanced disease and no remaining viable treatment options, with an expected life span of at least 3 months. The attenuated Vaccinia Virus has been modified to express IL-7 and IL-12 and designed to replicate in cancer cells. The virulence genes for VGF and O1L have been functionally inactivated by insertion of the genes IL-12 and IL-7 into these 2 loci, respectively. The B5R membrane protein has been modified for reduced antigenicity. ASP9801 is intended to replicate selectively in tumor tissues resulting in tumor destruction and expression of immunomodulators leading to immune activation in the tumor microenvironment as well as potentially inducing a systemic antitumor response. Modification of the B5R gene should reduce clearance of the virus by the host immune system. ASP9801 may show anticancer activity via direct cell lysis of tumor cells and via immune-mediated cancer cell destruction in solid tumors. There are two parts to the study: Dose Escalation and the Recommended Phase 2 Dose (RP2D) Expansion. Each part will be divided into Group A

(cutaneous/subcutaneous lesions) and Group B (visceral lesions). The study will consist of the following periods: Screening (up to 28 days); Initial Treatment Period (two 28-day cycles); Optional Extended Treatment Period (continued 28-day cycles); and, Follow-up Period. During the initial treatment period, participants will receive ASP9801 via injection to tumors on day 1 and 15 of each of two 28-day cycles. The same tumors will be injected at each time point in cycles 1 and 2. Participants will have baseline and on-treatment biopsies on or before day 1 of cycles 1 and 2, respectively. The baseline biopsies will be taken from 2 sites including 1 tumor selected for IT administration of ASP9801 and another tumor that will not be treated. The on-treatment biopsies will be taken from the same tumors sampled at baseline (non-treated tumor and tumor injected with ASP9801, in that order). After the first administration of ASP9801, participants will be observed for safety for a minimum of 4 hours for Group A and 8 hours for Group B. If adverse events (AEs) greater than or equal to grade 3 are observed during this time, participants will be observed for an additional 8 hours or at the investigator's discretion. Participants who tolerate the treatment and receiving clinical benefit may continue to the extended treatment period. Participants will receive additional IT administration of ASP9801 on days 1 and 15 of each cycle until they meet treatment discontinuation criteria. Enrollment will proceed in a staggered, dose-escalation manner. The starting concentration of ASP9801 in the escalation phase is 1×10^7 pfu/mL. The volume of ASP9801 to be injected per tumor is calculated according to the size of each target tumor to ensure consistent exposure within individual lesions. There is a table in the protocol that indicates the recommended injection volume per tumor; the amount can be reduced if the investigator deems the lesion is flat and has less volume.

Biosafety: Potential risks include pustular rash at injection site; autoinoculation; viral shedding from the injection site/transmission, viral infection, hypersensitivity to ASP9801, flu-like symptoms and immune-related adverse events that can be serious. Starting dose is equivalent to minimum biologically-active dose in nonclinical study. There is no pre-existing clinical data in humans. Preclinical studies in mice and macaques revealed evidence of significant life-threatening toxicity when they received repeated IV doses at the 'maxiumum feasible dose' of 8.5×10^8 pfu/kg and of 3.4×10^9 pfu/kg, respectively. Some macaques became moribund and were euthanized. Pathology findings indicated severe multiorgan inflammatory changes. These changes were thought to be due to induction of IL-12. When the macaques received 3.4×10^7 pfu/kg and 3.4×10^8 pfu/kg, they did not develop severe reactions and had complete recovery. In mice, acute symptoms such as hunched posture, piloerection, hypoactivity, bent head, staggering gait, decreased grasping reflex, loss of balance and dyspnea were seen on days 15 and 22 following their 3rd and 4th doses. These were observed within 15 to 30 minutes after administration and were generally not observed the day after. Viral shedding will be monitored by sampling and plaque assay analysis of saliva, urine and skin samples. Healthcare providers (HCPs) who are pregnant or immunocompromised are not to prepare or administer ASP9801 injections or touch participant injection sites, dressings or bodily fluids. HCPs must wear a protective gown/lab coat, goggles/mask or face shield/mask and gloves, with any wounds covered by occlusive bandages. After administration, the surfaces of the treatment room are to be cleaned with virucidal disinfectant. The injection site(s) of ASP9801 is/are to be covered with gauze and watertight occlusive dressing. Participants will be instructed not to touch or scratch the injection site. If the participant accidentally contacts the injection site, they are to wash their hands thoroughly with soap and water and not touch any other areas of the skin. Participants are to be asked about any close contacts and household members who are infants, pregnant, have a weakened immune

system or have a chronic skin condition such as eczema. These individuals must take care to minimize contact with the participant and avoid direct contact with the injection site or any of the dressings and should *not* assist with subject dressing changes. All household members, caregivers, sex partners or anyone sharing the same bed as the participant must avoid any contact with the injected lesions, dressings, injection site drainage, and body fluids. The participant will be advised not to share towels or clothing. In the pharmacy and clinical areas the study product is to be handled at BSL-2. Preparation of the study product is to occur in a Biosafety Cabinet.

Initial comments:
The preclinical ASP9801 safety data in mice and macaques (without a tumor burden) revealed a significant safety signal, including risk of death, probably attributable to IL-12, when ASP9801 is dosed repeatedly at the highest feasible doses in those animals. rhIL-12 has been previously associated with a serious toxicity when administered in earlier human clinical trials. This is a serious concern.
1) Human Gene Transfer: Question 3a: Describe how the study product will be transported between the pharmacy and the outpatient clinic at HCC.
2) Human Gene Transfer: Question 7a: Provide details for procedures used to minimize or mitigate anticipated adverse effects.
3) Risk Group and Containment: Question 1: Check Risk Group (RG) 2, as Vaccinia Virus is used.

The submitted application was placed on the August agenda.

Deferred/Reconsidered: The Committee voted to obtain external (Ad Hoc) reviews on the safety of administration of the study product to human subjects. Suggested names of individuals were provided by the committee. The Chair agreed to contact them to request their assistance. Additional comments were provided by the committee, which will need to be addressed by the investigator and the revised application will be placed onto the next available meeting agenda.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900075
Title:	Amendment for **IBC201600092**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ Manual REDACTED Laboratory

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: Murine cell entry: In the Description of Use the investigator indicates that murine cells will be transduced with lentiviruses and siRNA. This work should be performed at BSL-2+. Please revise.

2) Exposure Assessment and Protective Equipment, Question 1: For work with lentivirus and siRNA please revise to indicate that work will be performed at BSL-2+ instead of BSL-2 to be consistent with other information throughout the protocol.

3) Exposure Assessment and Protective Equipment, Question 5: Double gloves are required for work at BSL-2+. Also, mucous membrane protection consisting of a face shield or a combination of safety glasses/goggles and surgical mask is required for procedures with risk of splash performed with potentially infectious agents outside of the biosafety cabinet. Please revise this section accordingly.

4) Supporting Documents: Upload the laboratory's most recent BSL-2+ Biosafety Operations Manual that is signed by both the investigator and one of the staff from the Department of Environmental Health and Safety (EH&S) Office. The Department of Environmental Health and Safety (EH&S) Office can be reached at 412-624-9505.

Comments:

May and June meeting determination: Reconsidered

Required modifications from June meeting:

1) Recombinant or Synthetic Nucleic Acid Work Description: Question 2: If the research includes expression of oncogenes or toxins, then describe how they are expressed. This is particularly important if using viral vectors, for consideration of the expression system used with the identified biosafety containment level. If the research does not include any known oncogenes or toxins that will be expressed, then an appropriate response would be “none” or “not applicable”. Note that tumor suppressors which are knocked down (knockout) may be considered to fall under a higher biosafety level due to the potential risks.

2) Viruses, Prions, or Vectors: It should be clarified under "Inserted Nucleic Acids Information" whether genes represent cDNAs expressed from lentiviral vectors or Cas9/sgRNAs targeting genes. Note: IBC Guidance regarding biosafety level containment for Cas9 expression from viral vectors have recently been approved, and the change effects protocols beginning July 1, 2019.

3) Viruses, Prions, or Vectors: Cas9 and sgRNAs should be listed under "Inserted Nucleic Acids Information."

4) Risk Group and Containment Practices: One of the questions here revolves around the gene FOXP1. Some committee members expressed concern that this is an oncogene. Others have referred to it as a potential tumor suppressor gene. See *Banham, 2001. PMID: 11751404*. This paper describes FOXP1 as a tumor suppressor gene. If this proposal wants to use a Lentivirus vector to knock this gene down, then the work will need to be performed at BSL-2+. Other options are open to the investigator to knock this gene down that do not require work at BSL-2+. However, as the proposal stands now, it can only be approved at BSL-2+. Be aware that a laboratory inspection and biosafety operations manual will be required for BSL-2+.

Required modifications from the May meeting:
1) Supporting Documents: If an oncogene will be overexpressed with Lentivirus then BSL-2+ must be selected and the investigator will need to upload the laboratory's BSL-2+ Biosafety Operations Manual.

2) Waste Management: Question 1b: State that the final concentration of bleach will be 10% (1:10 v/v).

3) Waste Management: Question 1b: Clarify that Cavicide will not be used to decontaminate liquid wastes. This EPA registered disinfectant is not appropriate for decontamination of liquid wastes, only surfaces and/or solid wastes.

4) Risk Group and Containment Practices: Question 2: Biosafety level is dependent upon clarification; if an oncogene will be overexpressed with Lentivirus, BSL-2+ must be selected.

5) Recombinant or Synthetic Nucleic Acid Work Description: Clarify if Lentivirus will be used to overexpress FOXP1 as this is an oncogene.

6) Recombinant or Synthetic Nucleic Acid Work Description: Question 1 and Question 3b: Clarify how CRISPR/Cas9 will be delivered to cells.

7) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-3* should also be selected.

8) Viruses, Prions, or Vectors: Clarify if Lentivirus will be used to overexpress FOXP1; FOXP1 is an oncogene and this type of work would require BSL-2+ containment.

Initial comments:
Due to the use of Lentiviral vectors and requiring clarification of tumor suppressor genes, the study has been requested to come back for review by the committee after the investigator provides additional clarification and revisions on the application. This is an amendment to an existing protocol seeking to add Lentiviruses to express and/or knockdown the SPP1 and FBX032 genes. Neither are oncogenes. The work will be performed at BSL-2. There are some form issues that need to be corrected. This modification seeks to add Lentivirus vectors and siRNA to evaluate the role of SPP1 and FBX032 in Systemic Sclerosis. There are a few minor forms issues that need to be corrected, for example that human cells infected with the Lentivirus vectors need to be marked as recombinant materials. Once those changes are made this could be approved as low-risk.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

May meeting decision: Reconsidered: Significant comments were provided by the committee, which needed to be addressed by the investigator. The investigator provided a revised application, and the protocol was placed onto the June agenda.

June meeting decision: Reconsidered (second reconsideration): Due to the use of Lentiviral vectors and requiring clarification of tumor suppressor genes, the study has been requested to come back for review by the committee after the investigator provides additional clarification and revisions on the application.

The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900074
Title:	Post GWAS Functional Studies
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: If viral vectors will *not* be used to express sgRNA and Cas9, then the response to "Inserted Nucleic Acids Information" should be corrected to reflect that change.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 3d: It is stated that vectors will not be used for Cas9/sgRNA, yet four vectors are listed, including a Lentiviral vector (presumably pLKO.1 is meant). Clarify the answers. It is recommended that siRNA, shRNA, and CRISPR/Cas9 each be described separately in the response.

3) Exposure Assessment and Protective Equipment, Question 1: Remove all mention of BSL-2+.

4) Exposure Assessment and Protective Equipment, Question 4: Remove all mention of BSL-2+.

Comments:

The investigator will be characterizing disease-associated SNPs in various cell lines using shRNA or CRISPR/Cas9 to knockdown or knockout these genes. *E. coli* will be used to propagate plasmids at BSL-1, which is appropriate. Human blood and nervous tissues will be obtained for isolation of nuclear extracts at BSL-2, although it is unclear where these will come from. Human cell lines will be used for knockdown/knockout studies. shRNAs and CRISPR/Cas9 will be introduced into cells by transduction with Lentiviral vectors (and possibly also mammalian expression plasmids via transfection) at BSL-2. However, one shRNA described as being encoded in a Lentiviral vector is a tumor suppressor gene, particularly with certain mutations which may be SNPs that the investigator is studying. This would need to be performed under BSL-2+ conditions. It is also noted that Cas9 and sgRNAs will be encoded in the same Lentiviral vector. Finally, in the Exposure Assessment section, infected mice are mentioned, yet no animal studies are described. As the protocol contains substantial missing information, it should be sent back to the investigator prior to coming to convened review. The investigator plans to identify and characterize single nucleotide polymorphisms *in vitro*. Studies will be conducted with human primary cells and cell lines. *E. coli* will be used for plasmid amplification and Lentivirus will be used to knockdown various genes. Several oncogenes are noted (FGFR2 and GATA2), but will not be overexpressed. CUX1 and GATA3 (tumor suppressors) will be knocked down; this work should be conducted under BSL-2+ conditions. In addition, it appears that RNA and CRISPR/Cas9 will be expressed via the same viral vector. Several form errors were noted and information regarding animals should be removed if this work will not be conducted. It appears that this protocol should be considered at BSL-2+.

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900075
Title:	AAV and LV for Production of Genetically-Engineered Marmosets
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Appendix Q • NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Risk Group and Containment Practices: Question 2: BSL-2+ is listed earlier in the protocol but not here. Clarify this discrepancy.

2) Viruses, Prions, or Vectors: It appears that viral vectors will be administered to rodents as well as marmosets. Add rodents to the "Description of Usage" for vectors that will be administered to both marmosets and rodents.

3) Primary Cells or Cell Lines: Describe use/injection of ZFN, TALENs, CRISPR/Cas or BE constructs for gene-editing in marmoset embryos in the "Description of Usage".

4) Primary Cells or Cell Lines: Describe the *in vivo* work under "Description of Usage."

5) Primary Cells or Cell Lines: Clarify if BSL-2 or BSL-2+ will be used.

6) Tissues, Blood, or Body Fluids: Clarify if BSL-2 or BSL-2+ will be used.

7) Supporting Documents: If the work will be performed under BSL-2+ containment conditions, then a Biosafety Operations Manual is required to be uploaded on this page of the application. The manual must be current and signed by a member of EH&S and the investigator. If the investigator plans to work under BSL-2, which would be fine for the work proposed, then the manual is not required. Provide clarification on what biosafety level will be used with the study and ensure it is consistent within the pages of the application.

Comments:

In this protocol, the investigator proposes to use Adeno-Associated viruses (AAV) and 3rd generation Lentiviral vectors (LV) to create transgenic marmosets expressing calcium sensors, optogenetic molecules or fluorescent proteins. Viruses will be injected either into fertilized embryos, or else into the Central Nervous System (CNS) of rodents or marmosets when localized expression is desired. All viruses will be from the Pitt Vector Core Facility or *Addgene*. The investigator also proposes to use Zinc Finger Nucleases, TALENs and CRISPR methods to create transgenic marmosets with Notch3, presinilin-1, or Trem2 genes knocked out. Embryonic and adult stem cells and iPSC cells (fibroblasts) may be cultured *in vitro* and injected with AAV or LV vectors for the purpose of genetic engineering (transgenesis, knock-in or knock-out of genes). All experiments are proposed to be performed at BSL-2+ and ABSL-2 containment. A few clarifications are necessary. In particular, details of experiments in rodents should be provided in the protocol if the investigator intends to use rodents. This protocol describes use of Adeno-Associated viruses and Lentiviral vectors to generate genetically-engineered marmosets.

Lentiviral vectors encoding calcium indicators, optogenetic molecules, or fluorescent protein tags will be microinjected into early-stage marmoset embryos. Healthy embryos will be selected and transferred to recipient female marmosets. ZFN, TALENs, or CRISPR/Cas gene editing techniques will be used via direct injection of RNA or protein complexes into single-cell marmoset embryos. No viral vectors will be used to express gene editing technology and no oncogenes or toxins will be overexpressed. Marmoset tissues, blood and body fluids will be obtained for use to monitor health status, genotype, monitor biomarkers, or for purposes of *in vitro* fertilization or direct injection into oocytes. Primary cells obtained from marmosets in the research colony will be used for *in vitro* studies. The protocol is well-written and the investigator has indicated that work will be performed at BSL-2+ and ABSL-2. There was no information noted in the protocol that would require use of Lentiviral vectors or other r/sNA at BSL-2+ (e.g. 3rd generation Lentiviral vectors used, no overexpression of oncogene or toxin, no silencing of tumor suppressor genes, no use of gene editing techniques in any viral vector) and the protocol could be approved at BSL-2 if the investigator preferred. There are several forms issues and clarifications needed prior to sending to meeting for committee discussion.

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900087
Title:	Amendment for **IBC201800224**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ manual 2019

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: For EBV-infected cells/tissues, clarify if infectious virus will be present or not.

2) Viruses, Prions, or Vectors: EBV can infect human cells and the source for EBV cannot be null. If EBV is not used in the laboratory or if the EBV-infected cells have been lysed such that there is no infectious material, it should not be listed here.

3) Exposure Assessment and Protective Equipment, Question 1: If no infectious EBV will be present in the laboratory, remove the last sentence.

Comments:

This modification adds human tissue samples to a study of necroptosis. B-cells and monocytes will be isolated from blood and bone marrow from humans; IRB protocols numbers were linked. These will be provided by colleagues in the Department of Medicine. These will be used under BSL-2 containment. A variety of cell lines will be used for viral replication and cell death studies at BSL-2. *Escherichia coli* and *Saccharomyces cerevisiae* will be used at BSL-1 for plasmid work and Y2H assays. Retrovirus (MESV) will be used in mouse cells to establish stable cell lines that express a variety of proteins that are involved in the necroptosis pathway and fluorescent proteins (BSL-2). A Lentivirus system will be used to knockdown necroptosis related genes via shRNA. The Lentivirus will be used to introduce "CRISPR pooled libraries" for genetic screening *in vitro*. This knockout library should be managed under BSL-2+ conditions (if guide RNA and CRISPR-Cas9 are on the same Lentivirus). All work was claimed to be performed at level of BSL-2. A few minor corrections are needed to some of the forms.

Review comments were provided to the investigator for response. The revised application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0

Abstained: 0

Protocol:	MOD201900236
Title:	Amendment for **IBC201800289**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: MESV - CAG: Clarify how the virus cannot infect human cells under “Description of Usage” or “Downgrade Justification”.

2) Viruses, Prions, or Vectors: MSCV: Clarify how the virus cannot infect human cells under “Description of Usage” or “Downgrade Justification”.

3) Viruses, Prions, or Vectors: Lentivirus: List Cas9 and sgRNA under Inserted Nucleic Acids Information.

4) Viruses, Prions, or Vectors: Lentiviruses: Clarify if transduced cells will be injected at ABSL-2 or ABSL-1+.

5) Viruses, Prions, or Vectors: Clarify whether murine Retroviruses will be administered to mice at ABSL-1 or ABSL-1+.

6) Exposure Assessment and Protective Equipment, Question 1: Clarify which "viruses may infect human cells."

7) Exposure Assessment and Protective Equipment, Question 4: Clarify the description for experiments with Lentiviral vectors expressing CRISPR/Cas9 to indicate that after five passages transduced human cells will be administered to animals at ABSL-2 and transduced mouse cells will be administered to animals at ABSL-2, and after 72 hours and a cage change by research personnel, the animals can then be transferred to ABSL-1+.

8) Supporting Documents: IBC approval cannot be obtained until the signed and approved laboratory Biosafety Operations Manual and laboratory inspection have been uploaded.

Comments:

This breeding protocol has been modified to include additional agents by a new investigator to the Department of Immunology. The investigator will be conducting *in vitro* and *in vivo* work with human and mouse cells, *E. coli*, and viruses. Work will be conducted with several murine Retroviruses in both human and mouse cells for administration to animals. The investigator is requesting a downgrade to ABSL-1+; however, it is unclear if any of these viruses are ecotropic. In addition, some of the genes express have oncogenic properties (BCL6, IRF4). The investigator is also requesting a downgrade of Lentiviral transduced murine cells after 72 hours. This should be reviewed by the committee as the Lentivirus falls under BSL-2+ due to CRISPR and gRNA expression via the same vector. In addition, it is later noted in the recombinant work that shRNA will be delivered via Lentiviral transduction (one of the genes (FOXO1) has oncogenic/tumor

suppressor properties) and may need to be conducted at BSL-2+. This not described in the protocol. Other form issues and clarifications must be addressed. The buildings and rooms listed are not approved or appropriate for BSL-2+ work; a laboratory inspection and laboratory Biosafey Operations Manual is required prior to approval. In the protocol the investigators wish to have a title change since the investigator is now at Pitt. The investigator would also like to request a downgrade for BSL-2+ CRISPR work with Lentivirus done in murine cells to ABSL-1+ after 72 hours. Investigators will use both human and mouse cell lines, mouse primary cells, Lentivirus vectors, CRISPR Cas9 (in a viral vector), MSCV Retroviruses, and various recombinant plasmids. Oncogenes will be expressed only in a murine Retrovirus; no tumor suppressors will be down regulated. Investigators will work with animal models namely mice and are requesting a downgrade to ABSL-1+ for work with CRISPR Lenti in murine cells after 72 hours with cells being injected into animals. Investigators state this is accepted for work with BSL-2 agents if not in human cells. Downgrade should be discussed. The submitted application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900277
Title:	Amendment for **IBC201800119**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Biosafety Considerations for Research with Lentiviral Vectors

Determination: Modifications Required

Required modifications:
1) Supporting Documents: The IBC will not provide final approval of any BSL-2+ protocol until the Biosafety Operations Manual is signed and uploaded to the application in the "Supporting Documents" section of the application.

Comments:
This is a modification request to add a CRISPR/Cas system in Lentivirus and by transient transfection to an existing protocol. This Lentiviral system will be used to transfer Cas9 and guide RNAs. The guides will target genes that may contribute to the sensitivity or resistance of the leukemia cells to T-cell killing. Work is proposed at BSL-2+ as per recommended CRISPR guidance. A downgrade is being requested for animal work of transduced murine cells to ABSL-1+ after 72 hours post-injection. Work at BSL-2+ is appropriate. Since BSL-2+ work and a downgrade are requested, recommendation for discussion. This modification to an approved application to study graft versus host disease. The investigators correctly addressed a new requirement at the University to perform Lentivirus work where CRISPR gRNA and Cas9 and expressed in the same Lentivirus at BSL-2+. This is a proposed to be a screen so will include oncogenes. This has been clearly modified and explained. In addition, they are requesting downgrade of animals to BSL-1+ after 72 hours post Lentivirus administration as has been approved for other investigators by this committee. The only issue noted is that the investigator did not include a BSL-2+ Biosafety Operations Manual; this should be corrected prior to approval. Recommendation for approval pending the upload of the manual. Recommendation to discuss this application because it seems to be a Lentivirus CRISPR screen and includes BSL-2+ research.

The submitted application was placed on the August agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:54 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

September 9, 2019 10:00 AM

302 Heiber Building

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			
REDACTED, Vice Chair			
REDACTED, BSO			
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED	Late 10:07		
REDACTED	Absent		
REDACTED			
REDACTED	Late 10:05		
REDACTED	absent		
REDACTED			

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office, Director
REDACTED	IBC Office
REDACTED	IACUC Office
REDACTED	RCCO Co-Director

GUEST NAMES
None

QUORUM INFORMATION

Committee members on the roster:	23
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and are able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The August 2019 revised meeting minutes were reviewed and approved by the committee.

Votes:

For:	12
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

1) The IBC Office will be holding monthly IBC Basics workshops starting September 24th. The workshops provide pertinent information regarding the IBC review, including the safety review (environment, personnel, and subjects), the application process, the requirements for review, details on information found on the IBC website, and after the presentation, there is time for participants to work on their protocol applications with help from the office staff. IBC staff will not "write" protocol applications but will be there to answer any questions or help with technical issues.

2) October is "National Biosafety Awareness Month". The IBC Office is asking for suggestions to help promote this awareness and knowledge of the importance of IBCs.

3) The committee voted via poll taken by the IBC Chair that the best date for the November meeting is the 4th due to the Veteran's Day holiday. The meeting date has been updated on the IBC website.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocol:	IBC201900108
Title:	Neurogranin and TBI
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Biosafety Summary: Later in the protocol it is stated that transgenic, knockouts, gene-targeted, or other genetically engineered animals will not be used. If this is correct, then uncheck the box indicating the use of Genetically Engineered Animals.

2) Tissues, Blood, or Body Fluids: Clarify that rat tissues, blood, or fluids will be injected into new animals under "Description of Usage."

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Describe the work to transplant recombinant rat fluids, blood, or tissues to other animals.

4) Live Animals, Question 1: Link the associated ARO/IACUC protocol to the IBC application. The named investigator on the IACUC protocol should be the same as the named investigator on the IBC protocol.

5) Live Animals, Question 3: Earlier in the application it states that fluids and tissues will be isolated from rats. Correct the discrepancy.

6) Live Animals, Question 5: Earlier in the protocol it is stated that genetically engineered animals will be used. Correct the discrepancy.

7) Animal Gene Transfer, Question 4: If the AAV is to be used at BSL-2, animal use will be at ABSL-2 unless a downgrade for animal use is requested.

8) Risk Group and Containment Practices, Question 2: As a BSL-2 agent will be injected into animals, ABSL-2 should be selected.

9) Exposure, Assessment, and Protective Equipment, Question 4: Animals exposed to AAV, listed earlier in this protocol for use at BSL-2, must be housed at ABSL-2 unless the investigator specifically requests a downgrade from the IBC. Correct the language in the response.

10) Waste Management, Question 1b: Revise the answer. Concentrated bleach must be added to collected liquid waste such that the final concentration of bleach is 1:10 v:v bleach: liquid wastes. Adding an already diluted solution of bleach in water to a larger volume of liquid waste will further dilute the active ingredient and result in incomplete decontamination of waste. Clarify.

Comments:

The investigator will inject brain and CSF tissues from rats and commercially acquired AAV vectors expressing neurogranin and GFP stereotactically into the brains of other rats. Tissues will be collected from the injected rats and examined for biomarker levels. BSL-2 and ABSL-1 are proposed which are appropriate without QC information on the AAV vectors from approved

vendors. A few form issues should be corrected before approval. In this proposal, the investigators will inject AAV that express GFP control virus and neurogranin in to the brain and CSF fluids of rats. The vectors are commercially available and will not be made in the laboratory. Following certain time points, tissues and CSF will be collected. Proposed work will occur under BSL-2 and ABSL-1 which is appropriate. Vector maps or commercial product sheets should be provided.

Review comments were provided to the investigator for response. The revised application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	IBC201900119
Title:	Development of Multi-functional Integrated Neural Interface System
Investigator:	REDACTED
Highest BSL:	BSL-1 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 9/9/2020

Required modifications:

1) Funding Sources, Question 1a: Provide the Grant number.

2) Recombinant or Synthetic Nucleic Acid Usage: *NIH Exempt*, *Sections III-E* or *III-F* should be marked as the investigator is using rDNA to express fluorophores in animals as markers.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Remove all references to the UPenn Vector Core if it is *not* being used as a source for vectors.

Comments:

The investigator is proposing to inject AAV (Obtained from *Addgene* or *UNC Vector core*) into the brain of rats or mice to identify specific cell types. Cells (e.g. neurons) will be activated, inhibited or report their activity level at specific time points while electrophysiological recording and/or imaging data will be acquired. BSL-1/ABSL-1 is proposed and appropriate as it meets the criteria for a downgrade.

Pre-screening comments were addressed by the investigator. The revised application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	1 recused for involvement

Protocol:	IBC201900123
Title:	HIV protein and host cellular factors
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Usage: As plasmids will be amplified in *E. coli* and yeast two-hybrid systems will be used, *NIH Section III-D-2* should be selected.

2) Exposure Assessment and Protective Equipment, Question 1: Lentiviral vectors are capable of a single round of infection and integration into the genome of an exposed individual. Potential exposure could result in sero-conversion for exposed individuals. Revise.

3) Waste Management, Question 1b: Revise language to indicate that liquid wastes will be collected in a container containing concentrated bleach such that the final ratio will be 1:10 v:v bleach : liquid waste prior to holding at room temperature for a minimum of 20 minutes followed by disposal via sink.

Comments:

This protocol describes the study of protein-protein interactions relevant to HIV infections using biochemical and biophysical approaches. cDNAs from HIV and SIV and primates will be expressed in *Escherichia coli* and insect cells to obtain purified proteins. Lentivirus will be used with cultured human cell lines for RNAi-based down regulation and overexpression of specific genes. HEK293 cells will also be used for generation of Lentivirus. Reference was made to CRISPR/Cas, but this was not described. RG-3 should be checked. BSL-2 appears to be appropriate, but it is not clear how CRISPR/Cas9 will be delivered; if guide RNA and Cas9 are on the same plasmid, then this research will have to be conducted under BSL-2+. The CRISPR/Cas9 issue needs to be clarified. The investigator proposes to use recombinant plasmids in bacteria, Baculovirus in insect cell lines, and Lentivirus in human cells to overexpress and purify a number of different proteins. The Lentivirus system may also be used to generate cell lines with knocked down expression of select proteins. No oncogenes are used. No animal work is proposed. The work will be conducted at BSL-2.

Review comments were provided to the investigator for response. The revised application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For: 14

Against: 0
Abstained: 0

Protocol:	MOD201900341
Title:	BSL-2 reduction amendment for **IBC201700104**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• List of plasmids potentially used with Lentivirus system

Determination: Modifications Required

Required modifications:

1) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: The *E. coli* should be designated as recombinant.

2) Viruses, Prions, or Vectors: Replication deficient vectors: non-viral vectors (i.e. mammalian expression plasmids) should not be listed in this section. If a plasmid is not used to produce virus, remove it from this section. This information is best placed in Question 1 of the Recombinant and Synthetic Nucleic Acid Work Description later in the protocol.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 3d: What type of vector (e.g., transfection, transduction) and delivery system (e.g., plasmid, RNA, ribonucleoprotein complex, viral vector) will be used for a) siRNA, b) shRNA, c) miRNA, and d) Cas9? There seems to be some confusion from the language; is the laboratory NOT using Lentivirus to infect/express cells but rather Lentiviral plasmids (transfection, not transduction)?

Comments:

This is a modification to an approved protocol defining the role of microRNAs in placental trophoblasts, including antiviral activity. The investigator is requesting a downgrade from BSL-2+ to BSL-2. They state that they are not working on any known oncogenes but do acknowledge that in the past they had silenced two possible tumor suppressor genes, NDRG1 and PPARgamma. Those genes have been removed from the amended application. They are using Lentiviruses with CRISPR/Cas9 editing but they are not expressed on the same vector. The research does not appear to be working with anything else that would require BSL-2+. There is one minor forms issue; they do not have any cell lines marked as recombinant even though they state in the protocol that they will be infecting them with the Lentiviruses or recombinant VSV. Once that is corrected/clarified, this could be approved. The group studies the role of microRNAs in placental development and immune function. The Lentiviral vectors described in the study are a 2nd generation, three-plasmid system and no oncogenes will be expressed. In the past, two potential tumor suppressor genes were targeted for silencing with Lentiviral vectors, but this work has been removed from the protocol. CRISPR/Cas9 will be used for gene editing and two separate vectors will be used to express Cas9 and guide RNAs. The work, as currently described, is proposed for BSL-2, which is appropriate. There are several areas where form issues should be clarified. Upon completion of clarifications, recommendation for approval.

Review comments were provided to the investigator for response. The revised application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	MOD201900348
Title:	Amendment for **IBC201600096**
Investigator:	REDACTED
Highest BSL:	BSL-1 ABSL-1
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 9/9/2020

Required modifications:

1) Tissues, Blood, or Body Fluids: If tissues will be obtained from genetically modified zebrafish or zebrafish in which recombinant materials have been administered, they should be designated as recombinant.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 3c and 3d: Earlier in the project summary and later in the animal gene transfer section, ZFNs are described. Describe the work with ZFNs in the responses.

Comments:

This amended IBC protocol will investigate zebrafish development and regeneration. Genome editing technology including Cas9, TALENS, and ZFNs will be used to generate new mutations in zebrafish. Oncogenes such as FGF8, FGF3, wnt1, Ras, Raf, Etv4, FGFR1, FGFR2, FGFR3, beta catenin, Myc, Twist, Snail, Slug, and Foxm1 will be expressed as mRNA injections in 1-cell stage embryos for transient analysis. No viral vectors will be used in this protocol. All work was claimed to be performed at level of BSL-1 which is suitable. Overall, this is an IBC protocol at low risk. The protocol describes plasmids encoding genes to produce zebrafish transgenic lines. These plasmids will be propagated in *E. coli*. mRNA encoding TALEN or Cas9 with sgRNA will be injected into zebrafish embryos to edit genes *in vivo*. There are several missing pieces of information, such as cell lines/tissues, which should be corrected prior to coming to the committee. The work is BSL-1/ABSL-1 due to lack of infectious materials.

Review comments were provided to the investigator for response. The revised application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocol:	IBC201900111
Title:	HCC 19-101
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-02-01 Investigators Brochure Ed 1.0_19-101 • 2019-03-06 Main ICF Template v1.0_19-101 • 2019-03-07 PP ICF Template v1.0_19-101 • CC-MO-PHARM-9.2 Handling of BioSafety Level Products (3-19) • 2018-01-30 HS-IC0604 OSHA Bloodborne Pathogen Standard Exposure Control Plan_19-101 • 2019-04-04 Pharmacy Manual_19-101 • 2019-02-06 Protocol_19-101 • 2018-06-29 HS-FM0208PRO Waste Management PROCEDURE_19-101 • 2019-04-26 HS-IC0616 Guidelines for Handling Sharps_19-101

Determination: Approved

Last day of continuing review period: 9/9/2020

Required modifications: None

Comments:

Determination: Deferred/Reconsidered from the August 2019 meeting.

The Committee voted to obtain external (Ad Hoc) reviews on the safety of administration of the study product to human subjects. Suggested names of individuals were provided by the committee. Additional comments were provided which were addressed by the investigator on the revised application.

The IBC reviewed and discussed the revised protocol application and noted some concerns about the potential for toxicity for participants. Based on the information, the IBC granted approval with the following conditions:

a) Grade 3 or Grade 4 Adverse Events occurring at any site for this study are to be reported to the IBC as they become known to the investigator.

b) Before initiating to the next higher dose level, the IBC will review the DSMB report.

Initial comments:

Biosafety: Potential risks include pustular rash at injection site; autoinoculation; viral shedding from the injection site/transmission, viral infection, hypersensitivity to ASP9801, flu-like symptoms and immune-related adverse events that can be serious. Starting dose is equivalent to minimum biologically-active dose in nonclinical study. There is no pre-existing clinical data in humans. Preclinical studies in mice and macaques revealed evidence of significant life-threatening toxicity when they received repeated IV doses at the 'maxiumum feasible dose' of 8.5×10^8 pfu/kg

and of 3.4×10^9 pfu/kg, respectively. Some macaques became moribund and were euthanized. Pathology findings indicated severe multiorgan inflammatory changes. These changes were thought to be due to induction of IL-12. When the macaques received 3.4×10^7 pfu/kg and 3.4×10^8 pfu/kg, the did not develop severe reactions and had complete recovery. In mice, acute symptoms such as hunched posture, piloerection, hypoactivity, bent head, staggering gait, decreased grasping reflex, loss of balance and dyspnea were seen on days 15 and 22 following their 3rd and 4th doses. These were observed within 15 to 30 minutes after administration and were generally not observed the day after. Viral shedding will be monitored by sampling and plaque assay analysis of saliva, urine and skin samples. Healthcare providers who are pregnant or immunocompromised are not to prepare or administer ASP9801 injections or touch participant injection sites, dressings or bodily fluids. HCPs must wear a protective gown/lab coat, goggles/mask or face shield/mask and gloves, with any wounds covered by occlusive bandages. After administration, the surfaces of the treatment room are to be cleaned with a virucidal disinfectant. The injection site(s) of ASP9801 is/are to be covered with gauze and watertight occlusive dressing. Participants will be instructed not to touch or scratch the injection site. If the participant accidentally contacts the injection site, they are to wash their hands thoroughly with soap and water and not touch any other areas of the skin. Participants are to be asked about any close contacts and household members who are infants, pregnant, have a weakened immune system or have a chronic skin condition such as eczema. These individuals must take care to minimize contact with the patient and avoid direct contact with the injection site or any of the dressings and should NOT assist with subject dressing changes. All household members, caregivers, sex partners or anyone sharing the same bed as the participant must avoid any contact with the injected lesions, dressings, injection site drainage, and body fluids. The participant will be advised not to share towels or clothing. In the IDS and clinic, the study product is to be handled at BSL-2. Preparation of the study product is to occur in a biosafety cabinet.

Comments sent to the investigator for response in August:
1) The preclinical ASP9801 safety data in mice and macaques (without a tumor burden) revealed a significant safety signal, including risk of death, probably attributable to IL-12, when ASP9801 is dosed repeatedly at the highest feasible doses in those animals. rhIL-12 has been previously associated with serious toxicity when administered in earlier human clinical trials. This is a serious concern.
2) Human Gene Transfer: Question 3a: Describe how the study product will be transported between the pharmacy and the outpatient clinic at HCC
3) Human Gene Transfer: Question 7a: Provide details for procedures used to minimize or mitigate anticipated adverse effects
4) Risk Group and Containment: Question 1: Select Risk Group 2 (RG 2) for the use of the recombinant agents.

August: Deferred: The Committee voted to obtain external (Ad Hoc) reviews on the safety of administration of the study product to human subjects. Suggested names of individuals were provided by the committee. The Chair agreed to contact them to request their assistance. Additional comments were provided by the committee, which will need to be addressed by the investigator.

The revised application was placed onto the September agenda for review and discussion.

Approval: Based on the information provided by the investigator, and the assessment from the Ad Hoc reviewers, the IBC granted approval with the conditions as noted. The committee wishes to be informed of any Adverse Events related to the study agent.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	1; recused for involvement

Protocol:	IBC201900128
Title:	HCC 19-107
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-07-10 Site IP Procedures Manual_19-107 • 2018-10-31 Investigational Product Preparation Procedure_19-107 • 2018-06-29 HS-FM0208-PRO Waste Management PROCEDURE_19-107 • 2019-04-26 HS-IC0616 Guidelines for Handling Sharps_19-107 • 2019-07-31 ICF Sponsor Template_19-107 • 2019-04-18 IB ed2.0_19-107 • 2019-08-19 App M Q&A for Pitt_19-107 • 2018-01-30 HS-IC0604 Bloodborne Pathogen Standard Exposure Control Plan_19-107 • 2019-06-07 Protocol_19-107

Determination: Modifications Required

Required modifications:

1) Human Gene Transfer/Human Clinical Trial: Materials: Question 8: Describe the liquids in which the agent has been resuspended.

2) Human Gene Transfer/Human Clinical Trial: Question 1: Link the IRB protocol number.

3) Human Gene Transfer/Human Clinical Trial: Question 3a: Include a description of transportation of apheresis products to the facility where transduction will occur.

4) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Include a description of *in vivo* research involving recombinant nucleic acids in the response.

5) Viruses, Prions, or Vectors: Source of the Lentivirus cannot be null. Correct the response; identify from where the vector will be obtained.

Comments:

Summary: A phase 2, open-label, multi-cohort study evaluating JNJ-68284528, which is an autologous CAR-T cell therapy that targets B-cell maturation antigen (BCMA), a molecule expressed on the surface of mature B-lymphocytes and malignant plasma cells. The JNJ-68284528 drug product used in this study and the LCAR-B38M CAR-T cell drug product used in the earlier first-in-human Legend-2 study express an identical CAR protein. The manufacturing process of JNJ-68284528 consists of the transduction of autologous T-cells with a SIN Lentiviral vector (lacks a viral derived promoter in the LTR region) cell expansion, and cell harvest. The JNJ-68284528 drug product will be produced using modified manufacturing and scale-up processes. Results from the Phase 1b portion of Study 68284528MMY2001 and the Legend-2 study indicate that JNJ-68284528 and LCAR-B38M CAR-T cells have significant anti-myeloma activity and a safety profile consistent with the known mechanism of action of the product. The primary hypothesis is that JNJ-68284528 will induce a significant response, measured by minimal residual

disease (MRD) negative rate (at least 10%). The study population consists of persons with myeloma with relapsed or refractory disease and having failed multiple rounds of chemotherapy (Cohort A) or early after first round therapy or a stem cell transplant (Cohort B). Following leukapheresis, JNJ-68284528 will be generated from the participant's T-cells selected from the apheresis product. After JNJ-68284528 production and product release, participants will receive a conditioning regimen of cyclophosphamide and fludarabine. JNJ-68284528 will be administered 5 days to 7 days after the start of the conditioning regimen. The target dose is 0.75 x 10^6 CAR-positive viable T-cells/kg (range: 0.5-1.0 x 10^6 CAR-positive viable T-cells/kg). Blood and serum samples will be collected for JNJ-68284528 PK, antibodies to JNJ-68284528, and predictive biomarkers of response or resistance to JNJ-68284528. Safety will be evaluated by: AEs, antibody tests, VS, exams, assessments of cardiac function, Immune Effector Cell-associated Encephalopathy (ICE) score, and ECOG performance status. The primary analysis for each cohort will occur approximately 1 year after the last participant in each cohort has received their initial dose of JNJ-68284528.

Biosafety: LCAR-B38M CAR-T cells were used in the first study of CAR-T cell therapy in humans and target the same protein as this study. In that study done in China, of the 74 participants studied, 91.9% had an Adverse Event of CRS including Grade 1 (n=37); Grade 2 (n=25); Grade 3 (n=5); and Grade 5 (death, n=1). The fatal CRS event occurred in a 40-year-old female who experienced CRS and tumor lysis syndrome and died on Day 13 after receiving the LCAR-B38M CAR-T cell infusion. For most participants, symptoms of CRS were mild and reversible. Grade 1 neurotoxicity was reported for 1 participant. In addition to the woman who died from CRS, 1 male participant had a fatal Adverse Event related to treatment with LCAR-B38M CAR-T cells. A 54-year old man with a history of CAD and extensive anthracycline therapy experienced CRS with a maximum Grade of 2 and died on Day 22 after LCAR-B38M CAR-T infusion due to a potential acute pulmonary embolism and potential acute coronary syndrome. LCAR-B38M CAR-T cell treatment yielded a high overall response rate; 65 participants [87.8%]), including a high proportion of participants with complete response (CR; 64.9%). Among the participants with CR, 87.5% were negative for minimal residual disease (MRD) at some point during the study. As of the clinical cutoff, 12 (18.5%) participants who achieved PR or better subsequently progressed based on the sponsor's assessment. As of the April 29, 2019, 13 participants had received an infusion of JNJ-68284528 in a study. Serious Adverse Events were reported in four participants. These include but were not limited to: cell-related encephalopathy syndrome, CRS, acute kidney injury, thrombocytopenia, mental status changes (Day 40 that was assessed by the investigator as not related to any study treatment). Lists of risks include CRS, neurotoxicity syndrome, tumor lysis syndrome, second primary malignancies, cytopenias, hypogammaglobulinemia, infections, and allergic reactions. Chain of identity procedures will be followed from collection of the leukopak to study product administration. Two study staff must be involved in preparation and verification of the study product. IP preparation and administration will be performed using aseptic techniques. The saline flush preparation is to be performed under a laminar flow hood or biosafety cabinet. All investigational study product that is not used for whatever reason is to be returned to the sponsor. No comments. Moderately high-risk study for myeloma patients with no viable remaining treatment options.

The submitted application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	1; recused for involvement

Protocol:	IBC201900115
Title:	Mycobacterial Aggregation *In Vitro* and *In Situ*
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-2 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 9/9/2020

Required modifications:

1) Recombinant or Synthetic Nucleic Acids Usage: *Mycobacterium avium* and *Mycobacterium fortuitum* are both classified by USDA APHIS as animal pathogens, therefore *NIH Section III-D-1* should be selected.

Comments:

This new protocol studies Mycobacterium strain pathology by creating reporter strains of the bacteria for tracking purposes. Additionally, siRNA will be used to knockout of genes of interest to study mutations. All agents are pathogenic strains to be handled at BSL-2 except *M. smegmatis*, which is BSL-1. There is some concern noted about mutations (stated to be random by the investigator) that may increase pathogenicity. It is unclear how the investigator would test for this, and how to determine if the bacteria warrants a higher biosafety containment. Some descriptive form issues should be addressed. The investigator plans to study mycobacterial physiology, specifically interaction between organisms during biofilm formation as well as interactions between the bacteria and host cells/tissues. All Mycobacterium strains to be studied are BSL-2 organisms. Mycobacterium genes and fluorescent reporters will be cloned into conventional vectors and expressed in other strains. This apparently has the potential to increase virulence of some strains. Transposon mutagenesis of mycobacteria will also be used as at will "recombineering" which involves homologous recombination to create knockout strains. The investigator should state explicitly that it is not anticipated that virulence will be dramatically increased by protein expression since some mycobacteria are BSL-3 organisms. A few minor form issues should also be corrected.

Review comments were provided to the investigator for response. The revised application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	MOD201900311
Title:	Amendment for **IBC201600168**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BI MTA for AAV • MTA00001718 - 2016.01.28 MMRRC - REDACTED KR FEC

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: The investigator indicates that human exosomes will be administered to animals. Describe this under "Description of Usage."

2) Primary Cells or Cell Lines: How are the siRNA and shRNA are being transfected? In the summary the investigator mentions Lentivirus. If Lentivirus is being used, then it would be necessary to mention genes being targeted here. Include this information under "Description of Usage."

3) Viruses, Prions, or Vectors: The Lentivirus work should be done at BSL-2. Amend the BSL in the entry. If the investigator wishes to pursue approval at BSL-2+, a Biosafety Operations Manual and BSL-2+ upgrade inspection will be needed.

4) Viruses, Prions, or Vectors: Clarify how AAV is unable to infect human cells. If this information was provided in error, correct the response.

5) Risk Group and Containment Practices, Question 2: The work can be done at BSL-2. Amend the response. If the investigator wishes to pursue approval at BSL-2+, a Biosafety Operations Manual and BSL-2+ upgrade inspection will be needed.

6) Exposure Assessment and Protective Equipment, Question 1: Replication-deficient Lentiviral vectors can infect human cells in a single round of infection. While this does not result in a productive infection, it can cause sero-conversion in personnel who may be exposed to the Lentiviral vector. Revise the language in the last sentence.

7) Waste Management, Question 1b: Describe "other EPA-registered disinfectants" in the response.

8) Supporting Documents: If work will be performed at BSL-2+, an approved Biosafety Operations Manual is required. However, the Committee does not see any work described that requires BSL-2+. Thus, if the investigator would like to perform the work at BSL-2, all places that describe BSL-2+ should be corrected to BSL-2.

Comments:

This amendment is for an approved protocol investigating the role of klotho protein and mRNA in skeletal muscle recovery after injury. The purpose of this amendment is to add transference of exosomes, some of which may contain an engineered mRNA, from young mice to older mice as part of their protocol. This protocol will use transgenic mice that are injected with a klotho-

expressing AAV and exosomes isolated from human serum. Human mesenchymal stem cells obtained from a commercial vendor and a murine cell lines will be transfected with siRNA (human cell lines) or AAV (murine cell lines) to assess the roles of protein in skeletal muscle regeneration. Plasmids will be amplified in *E. coli*. Lentiviruses will also be used to knockdown protein expression in mice and in cell lines. The list of genes they indicate will be knocked down with a 4-plasmid lentivirus system, Fyn and Src described as proto-oncogenes when overexpressed. Cardiotoxin will be used to cause muscle injury. The work is proposed at BSL-2 and ABSL-2, which appears to be appropriate for the proposed work considering proto-oncogene expression would be knocked down rather than enhanced. No significant issues were identified, and approval is recommended pending any other IBC-recommended changes. The investigator is requesting this modification to add an additional study on circulating exosome/Micro-vesicles transplantation. Injections of naturally occurring young exosomes isolated from young mouse serum will be given to TA muscle of mice. The investigator would also like to use transfected/ engineered exosomes with klotho mRNA inside.

Initial comments:
1) Primary cells: How are siRNA and shRNA being transfected? In the Project Summary the investigator mentions Lentivirus. If Lentivirus is being used, then it would be necessary to mention genes being targeted in this response. If a gene is oncogenic, then work would be needed to upgraded to BSL-2+.
2) Recombinant and synthetic nucleic acid work: Are these exosomes released in mice that carry the Klotho protein and mRNA or is the investigator going to transfect them to carry this cargo? If transfection is involved, then the procedure needs to be explained and how it is planned to be done.
3) Genetically engineered animals: Question 3 should be answered YES, because the investigator is planning to administer exosomes from one mouse (donor) into another mouse (recipient).
4) Animal gene transfer: Question 2 should be YES as the investigator is administering Lentiviral vectors to mice.

Review comments were provided to the investigator for response. The revised application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	MOD201900361
Title:	Amendment for **IBC201700117**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description, Question 3a: Include information regarding shRNA and siRNA.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question3b: Include genes that will be involved in CRISPR/Cas9, shRNA, and siRNA experiments.

3) Animal Gene Transfer, Question 6: As Retroviruses, Lentiviruses, and AAV are infectious to human cells (note that virus infectivity and virus replication are not the same), the following statement should be removed from the response: "No infectious agents are administered to animals."

4) Waste Management, Question 1b: Indicate that potentially contaminated liquid wastes will be treated with bleach at a final concentration of 1:10 v:v bleach:total volume of liquid waste.

Comments:

This is a modification of an existing multi-projects protocol to study the phenotypes associated with modifying proteins encoded by genes that are effectors of the c-Myc Oncoprotein. The investigator seeks to use AAV-8 to express GFP or Cre recombinase to allow knockout of the PDH gene in mouse liver. The investigator is seeking a modification to an existing protocol to use AAV8 to knockout pyruvate dehyrodgenase in the livers of mice. It is not an oncogene or a tumor suppressor gene. Tissues and cells will be isolated for *in vitro* analyses. The investigator is not asking for downgrade. Everything seems to be in order except one minor point, mouse tissues are marked as BSL-2 but everything else (cells/cell lines, viruses) is marked as BSL-2+. If that correction is made this protocol can be approved.

Review comments were provided to the investigator for response. The revised application was placed on the September agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0

Abstained: 0

Protocol:	MOD201900343
Title:	Amendment for **IBC201800278**
Investigator:	REDACTED
Highest BSL:	BSL-3 ABSL-3
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• M. tb DHQ map • rMtb (doxy addicted) • Ops manual RBL Flynn 2019AF • Flynn004 • BCG map

Determination: Approved

Last day of continuing review period: 11/9/2020

Required modifications: None

Comments:
This modification describes addition of a new doxycycline-addicted dual lysin strain of *M. Tb* to an established protocol. The strains will be used for studies in NHPs. The laboratory has extensive experience at BSL-3 and ABSL-3, all personnel have appropriate training, and the investigator has approval for work in the RBL. This is a modification to add a new *mycobacterium Tb* strain to protocol to be used in NHP. The new strain grows in the presence of doxycycline and is killed when doxycycline is removed. The laboratory will be testing how quickly the strain is killed *in vivo* once doxy is removed. They will assess killing of the bacteria at 2 time points (4 and 8 weeks). The laboratory is well-established to work with Tb at BSL-3. There appears to be a simple form omission of the new Tb strain, otherwise recommendation for approval.

Review comments were provided to the investigator for response. The revised application was placed on the September agenda.

Approval: No additional comments were provided by the committee; the protocol was provided approval with no comments.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:37 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

October 14, 2019 10:00 AM
302 Heiber Building

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED , Chair	Absent		
REDACTED , Vice Chair			
REDACTED , BSO			
REDACTED	Absent		
REDACTED	Late 10:07		
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Late: 10:02		
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED			

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office, Director
REDACTED	IBC Office
REDACTED	IACUC Office
REDACTED	RCCO Co-Director

GUEST NAMES	
REDACTED	, Research Clinical Coordinator

QUORUM INFORMATION

Committee members on the roster:	23
Number required for quorum:	5
Meeting start time:	10:00 AM

The Vice-Chair called the meeting to order. A quorum of members was present. The guest was introduced: Ms. REDACTED works in the clinical research setting and wished to observe the IBC proceedings.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The September 2019 revised meeting minutes were reviewed and approved by the committee.

Votes:

For:	11
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

The BSO indicated that there is an upcoming site inspection for AALAC (Animal Care Program) accreditation scheduled for the last week in October. Inspectors will be meeting with animal research investigators and their research staff. In the past, a few site accreditation inspectors have requested information from the IBC Office about collaboration between IBC and IACUC.

IBC OFFICE REPORT

1) The IBC Office reported that there were 7 participants that had signed up for the September protocol building workshop. In addition, the IBC Office participated in the bi-annual (Fall) Orientation to Research Fundamentals (ORF) which is hosted by the Office of Health Sciences and is primarily focused on research needs for clinical studies.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocols on the following pages

Protocol:	IBC201900125
Title:	Retroviral Expression of Gata4
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-3
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Under the "Description of Usage", describe how the Lentivirus is going to be used.

2) Viruses, Prions, or Vectors: In the Exposure Assessment and Protective Equipment section, it is stated that eGFP will be measured. Include eGFP in Inserted Nucleic Acids Information or remove the statement from the other section of the application.

3) Recombinant or Synthetic Nucleic Acid Usage: *Section III-D-1* should be checked for expression of host proteins from a viral vector.

4) Recombinant or Synthetic Nucleic Acid Usage: If viral vectors expressing eGFP will be used in these studies, then *Section III-E/III-F* should also be checked.

5) Lentivirus and Lentiviral Vectors: Question 2: As the virus is replication defective, it likely is produced from multiple plasmids. Correct the response.

6) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells may harbor unknown pathogens.

Comments:

The work will use an esophageal organoid culture system. The laboratory previously used a murine version of the system, which used mouse esophageal cells to generate 3D organoids consisting of squamous cells. Now the laboratory will use similar organoids generated from human fetal esophageal stem/progenitor cells. These have a squamous epithelium and its patterns of expression of keratins 14, 13 and p62 match those of developing esophageal tissue. The laboratory wishes to test the hypothesis that expression of the transcription factor GATA4 in these organoids, where it is not normally expressed, will convert the squamous epithelium to a columnar epithelium. Lentiviral vectors will be used to deliver the GATA4. The work will use human fetal esophageal cells derived from esophageal organoids. It will also use Lentiviral vectors (HIV-1 – 3rd generation, obtained from stocks made by the Medical College of Wisconsin Transgenic Core) to deliver GATA4. This vector will be incubated with primary human esophageal cells, which will then be cultured to form organoids. Cells from the organoids will be subject to analysis by histology and RNA sequence. The investigators will transduce GAT4 (a transcription factor) into primary human esophageal cells. They propose to use Lentiviruses and since GAT4 is not an oncogene, BSL-2 is an appropriate biosafety level. No CRISPR based experiments are proposed. Recommendation for approval.

Initial comments:

1) Lentivirus and Lentiviral Vectors: Question 2: The vector *is* produced from a multi-component system, albeit elsewhere. Amend where necessary.

The application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900127
Title:	Targeting KRAS in Cancer
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: Under the "Description of Usage", clarify that murine tissues will be administered to new animals.

2) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: DH5alpha *E. coli* should be designated as recombinant.

3) Viruses, Prions, or Vectors: As cells transduced with Lentiviruses will be administered to mice, the response to the question asking whether Lentiviruses will be administered to mice should be YES.

4) Recombinant or Synthetic Nucleic Acid Work Description, Question 2: Clarify if oncogenes will be expressed in cells. If YES, then the expression system(s) should be identified.

5) Genetically Engineered Animals, Question 3: Earlier in the protocol it is stated that murine tissues will be administered to animals. Clarify the discrepancy.

6) Waste Management, Question 1b: List the final concentration of bleach and the time for decontamination of biohazardous liquids.

Comments:

This protocol will aim to investigate the role of oncogenic KRAS in human cancers, especially lung and pancreatic cancers. The strategy is to develop therapies that target regulation of KRAS and its downstream effectors. The work uses multiple murine tissues. All will be handled at BSL-2 and all will be obtained from the laboratory breeding colony. Blood and tissues will be obtained from mice treated with cytokines or mice treated with antibodies. LSL-KRAS mutant mouse will be infected with Adenovirus expressing Cre to generate tumors. Several human cell lines (PANC1.0, PANC2.03, BxPC3, PANC2.13, PANC8.13, SW1990, PANC10.03, A549, NCI-H23, NCI-H522, NCI-H1435, NCI-H1563, NCI-H835, NCIH1672, NCI-H720, NCI-H520, 293T, all from *ATCC*) will be used for various purposes. This includes treatment with cytokines or antibodies followed by assaying and plasmid transfection. Some will be transduced with replication incompetent Lentiviral vectors delivering shRNAs to knockdown oncogene expression. Some human cancer cells with knocked-down oncogene expression will then be injected into mice (Nu/Nu and NSG) to study tumorigenesis. Some murine cells (NIH\3T3, mouse lung and pancreatic cancer cells, from *ATCC* or the laboratory's mice) will be used for plasmid transfection and *in vitro* experiments. The HIV 4-plasmid system (from *Addgene*) will be used to deliver shRNA and overexpression constructs, as well as reporters, such as GFP. shRNAs will target oncogenic KRAS, LOF and PKC. Adenoviral vectors (Adv-5, from *ViraQuest Inc*.) will deliver

Cre recombinase. Animal work will be done at ABSL-2. This project will use replication defective Adenovirus, plasmids and 3rd generation Lentivirus to express Wild Type and mutant forms of human proteins regulating oncogenic KRAS. Target genes will be knocked down with transfection plasmids containing shRNA or siRNA. Proteins to be subcloned in bacteria for sequencing. All work is BSL-2/ABSL-2 and no biosafety concerns are noted.

The application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900129
Title:	Brain Tumor Genetics and Therapeutics
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: In the human tissue entry the investigator has indicated that human tissues/cells will be administered to animals. Describe use of these materials in animals in the "Description of Use" response.

2) Viruses, Prions, or Vectors: Retrovirus, Rous Sarcoma Virus, Avian (RSV) - RCAS: If RCAS will be used as an expression vector it should be identified as recombinant and any inserted nucleic acids (Such as any genes or shRNA expressed from this vector) should be described and listed under "Inserted Nucleic Acid Information."

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include a description of work with genetically engineered animals.

4) Exposure Assessment and Protective Equipment, Question 4: Use of Safety Engineered Sharps Devices is required at BSL-2/ABSL-2. Add the use of these devices in the response.

5) Waste Management, Question 1a: Describe the methods used for proper decontamination (for example: specific disinfectant or physical decontamination method used) for solid biohazardous waste.

6) Waste Management, Question 1b: The response should state that the final concentration of bleach will be 1:10 (v/v) bleach. Revise the response.

Comments:

This is a new protocol that will study gliomas in a genetically engineered mouse model along with "reporter mice." Human glioma tissue will be obtained from UPMC and used for *in vitro* assays as well as transplantation into mice at BSL-2/ABSL-2. In addition, human blood will be obtained, but it is unclear how this will be used. An Avian Retrovirus, RCAS, will be obtained from a collaborator at FHCRC, which will be used to apparently express proteins of interest in mice expressing the RCAS receptor (TVA), but these genes are not listed. The virus will be used at BSL-2/ABSL-2, which is appropriate as this virus cannot infect human cells. Lentiviruses will be obtained from commercial sources and used to express shRNA of interest in cell culture, but this is not described in the Primary Cells or Cell Lines section of the application and the virus is described as not being produced from a multi-plasmid system, which would require use at BSL-2+ although the investigator has listed BSL-2 in the protocol. It may be that this is incorrect, especially if the investigator will obtain them commercially. No oncogenes or shRNA targeting tumor suppressor genes will be expressed from Lentiviruses. No gene editing will apparently be performed. As substantial information is missing from the protocol, it is recommended that the investigator correct/clarify the responses. The protocol is to study the role of microenvironment cells within brain tumor formation, development and prognosis. To this end, gliomas will be

induced in mice using the RCAS/tv-a virus system to express PDGF-RCAS, Ras-RCAS, Akt-RCAS, TIMP-1-RCAS, RCAS-p53-shRNA and RCAS-PTEN-shRNA vectors delivered via DF-1 cells. Both murine and primary human cells will be used, subjected to standard RNA/rt PCR applications. Some tumors will be transplanted into mice. No plasmid/bacterial work will be done. Human blood will be used, and cells isolated from it, but no further mention is made. Human glioma cells will be also injected into mice but will not be exposed to rDNA. There are some questions about the use of the Lentivirus to be clarified. It appears that this will be done with a multi-plasmid system, but it is not explained so BSL-2+ is being suggested to encourage the investigator to amend the response about safety features of the virus. The form errors in the application should be addressed prior to approval.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900130
Title:	Genetically Engineered T-cells
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: The second human blood entry and the mouse tissue entry state that cells will be administered to animals. Verify and add the work to the "Description of Usage".

2) Viruses, Prions, or Vectors: As viruses appear to be used to transduce cells that will be administered to animals, correct the response to the question for each entry.

3) Genetically Engineered Animals, Question 3: Earlier in the protocol it is stated that murine blood/tissues will be administered to animals. Correct this discrepancy.

Comments:

EGFR is indeed an oncogene and the researchers indicated they will express this in the Basic Information page of the application. However, in the Virus page they specifiy that the Lentivirus system will be used to expressed EGFRt - a truncated form of EGFR that reportedly lacks the tyrosine kinase domain (PMC5096899) and would therefore not be oncogenic. This is a new protocol in which the investigator will be developing Lentiviral and Retroviral vectors encoding receptors to affect T-cell ability to enhance or target tumor cells. They will transduce PBMC *in vitro* and these then will be introduced to mice bearing tumor-antigen positive tumors. Primary human cells (PBMC from *Vitalant*) and mouse primary cells (harvested organs) will be used. Other human cell lines will be used to generate viral particles and used *in vitro*. Bacterial work will be used for cloning and plasmid amplification. The Lentivirus to be used is a 3-plasmid system and no oncogenes will be expressed, though it is unclear if EGFR would oncogenic or not. The study could be approved at BSL-2 assuming the researchers clarify the EGFR vs EGFRt expression.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900378
Title:	Amendment for **IBC201900074**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Usage: Later in the protocol, it is stated that recombinant or synthetic nucleic acids will not be administered to animals, which is inconsistent with selection of *NIH Guidelines Section III-D-4*. Clarify the discrepancy.

2) Live Animals, Question 6: Later in the application, it is stated that recombinant or synthetic nucleic acids will not be administered to animals, which is inconsistent with the answer. Clarify the discrepancy.

3) Animal Gene Transfer, Question 6: Previously in the application, it is stated that recombinant or synthetic nucleic acids will be administered to animals. Clarify the discrepancy. If recombinant or synthetic nucleic acids will be administered to animals, describe the route of administration for each experimental agent used *in vivo*.

4) Exposure Assessment and Protective Equipment, Question 2: Amend this information if mice are indeed given rDNA materials.

Comments:

This amendment is requesting to add the use of transgenic mice with loxp sites in the CUX1 gene. These mice will be crossbred with Cre mice so that CUX1 can be knocked out in the brain and heart. They propose to perform this work at BSL-2, which is appropriate. This is a request to amend an existing protocol to import conditional knockout mice on gene Cux1 (transferred from a service group in Pitt) for breeding. The mice requested have been knocked-in with two loxp sites in gene CUX1 so that the group can selectively knockout this gene in mice brain and heart by breeding with mice that carry Cre gene in these tissues. No rDNA will be administered to these mice. Genetic modification will only occur via selective breeding. Work will be performed at BSL-2/ABSL-2 which is appropriate. In this protocol, the laboratory wishes to amend the current protocol. Genetic modification will only occur through breeding with Cre mice. Work will be performed at BSL-2/ABSL-2, which is appropriate.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900388
Title:	Amendment for **IBC201800075**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• 180408.NIH Biosketch NEW.REDACTED • copier%40pitt.edu_20180320_125214

Determination: Approved

Last day of continuing review period: 10/15/2020

Required modifications:

1) Tissues, Blood, or Body Fluids: As the mouse work will be performed at ABSL-2, use of tissues/cells/fluids should be performed at BSL-2. Correct the BSL designation.

2) Primary Cells or Cell Lines: Earlier in the project description, and in the Viruses and Viral Vectors section of this modification the investigator indicates that AAV-based viral vectors will be used to manipulate gene expression in cell culture and in mice. Include the use of AAV to the "Description of Usage" for primary cells/cell lines as applicable.

3) Recombinant or Synthetic Nucleic Acid Usage: In this modification the investigator indicates that AAV-based viral vectors will be administered to mice, therefore *NIH Section III-D-4* should be selected.

4) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: For the description of *in vivo* AAV use, include that the work will be done in a biosafety cabinet, not just a BSL-2 laboratory.

5) Animal Gene Transfer, Question 2: While Lentiviral and AAV-based viral vectors are replication-deficient, these vectors can infect cells during a single round of infection. Revise the answer.

Comments:

The amendment is to add the use of AAV vectors (express GFP, cre, CD36, HIF2, HIF1; knockdown CD36, HIF2, HIF1) to a protocol examining the relationship between gene expression and fatty liver disease. Use of the vectors in cell culture and in mice (tail vein injection) is proposed using BSL-2 practices which is appropriate. Genes to be expressed/knocked down are not oncogenes or tumor suppressors. This is an amendment to an existing protocol requesting to add the use of AAV to overexpress some genes (GFP, Cre-recombinase, CD36, HIF2 and HIF1) and knockdown others (CD36, HIF2 and HIF1). None of the genes are oncogenes or tumor suppressors. The work will be performed at BSL-2.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900391
Title:	Amendment for **IBC201600113**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Amend the response to include the update on the transgenic cell experiments proposed in this modification.

2) Waste Management, Question 1b: Clarify that the final concentration of bleach will be 1:10, v:v, bleach:final volume of liquid waste.

Comments:

The amendment has been submitted to add *in vivo* work to this existing IBC protocol. The newly added information describes the use of cancer cells and primary mouse cells used in adoptive transfer experiments in mice.

Several pre-screening comments have been addressed and the revised application was placed on the October agenda for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocols on the following pages

Protocol:	IBC201900114
Title:	MeriaGTX - MGT009 Gene Therapy Study
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-6 (Large Scale) • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Investigator's Brochure v2.0 20 November 2017 • TemplateConsentFormS0_32119097 • Protocol v9.0_12Feb2019_FullySigned • TemplateAssentFormForChildrenAges79S0_32119587 • 114-labelling • General Investigative Plan • TemplateAssentFormForChildrenAges1014S0_32120130

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Include a statement regarding replication capacity of the AAV.

2) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-3* should *not* be selected unless cell culture studies are performed. The protocol does not indicate any cell culture experiments will be performed.

3) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-1* should be checked for use of a viral vector encoding a host protein.

4) Viruses, Prions, or Vectors: University of Pittsburgh IBC guidelines require Adeno-Associated Virus to be used at BSL-2 unless the virus is produced in cells that are not of human origin (HEK293 cells are of human origin) or if each batch is shown to have more than 80% purity. Select BSL-2 or provide additional information requesting downgrade to BSL-1. Remember that BSL-2 is roughly equivalent to "Universal Precautions".

5) Human Gene Transfer/Human Clinical Trial: Materials: Question 4: Include the derivation of the delivery vector system including the source, and any modifications. Derivation examples are viral, bacterial, or plasmid vectors; modification examples are deletions to attenuate or self-inactivate, changes to tropisms, etc. Modify the response.

Comments:

This is an open-label phase I/II dose-escalation trial to determine the safety and efficacy of subretinal administration of AAV2/5-hRKp.RPGR in participants with retinitis pigmentosa caused by mutations in RPGR. Retinitis pigmentosa is a common cause of genetic blindness. The study product (AAV2/5-hRKp.RPGR) is derived from a recombinant, replication incompetent, Adeno-Associated virus (AAV) viral vector platform (linear single strand of DNA packaged in a rAAV protein capsid of serotype 5). The main hypothesis is that in individuals with X-linked retinitis pigmentosa (XLRP) associated with mutations in the retinitis pigmentosa guanosine triphosphatase (RPGR) gene, localized gene augmentation with a human RPGR-ORF15 variant will result in the production of a biochemically active RPGRORF15 protein, facilitating functional and morphological rescue of both rod and cone photoreceptor cells and thereby slow/halt

progressive retinal degeneration. There are no existing treatments for retinitis pigmentosa. The study product will be injected subretinally following a standard surgical vitrectomy. First adults with retinitis pigmentosa will be enrolled, then children with XLRP once the Maximum Tolerated Dose (MTD) is established. The primary endpoint is safety; secondary is efficacy (slowing or halting of vision loss). It will be followed by a randomized trial with a control arm. Up to 18 adult participants will be administered one of 3 different doses in cohorts of 3 participants at a time - a) low dose 1.0 x 10^{11} viral genomes (vg) in 1 mL; b) intermediate dose 2.0 x 10^{11} vg in 1 mL; c) high dose 4.0 x 10^{11}vg in 1 mL. Based on toxicity data, the IDMC will make a recommendation on the dose to administer to the next cohort of 3 participants. Up to 71 participants will be enrolled. Once MTD is established, up to 53 further participants aged 5 years or older will continue to be administered study product, up to the highest dose observed to be tolerated in adults. This includes up to 5 pediatric participants in the confirmation phase and up to 48 in the randomized phase. Efficient transduction of the target rod and cone photoreceptor cells requires the vector to be administered to the sub-retinal space. This is done by intra-ocular sub-retinal injection using a fine cannula introduced through small retinotomies. Participants will be followed on study for 12 months, and in a follow -up study for four more years. The shorter follow up period is reasonable given the expected lack of integration and no potential for latency and reactivation.

Biosafety: The AAV2/5-hRKp.RPGR vector is considered to be Risk Group 1. Safety will be assessed by evidence of reduction in vision, severe inflammation, endophthalmitis, ocular cancer, and grade III or higher SUSAR (Suspected Serious Adverse Reaction). Safety will be enhanced by restricting transgene expression to the target tissue by virtue of the photoreceptor-specific promoter, inherent tropism of the AAV 2/5 vector, direct administration to the sub-retinal delivery space and by restricting the intervention to one eye only in each participant. General risk management will include the detailed review of all participants, appropriate intervals between administration to successive participants, the dose escalation plan, and limiting the risks to children by initially demonstrating an acceptable safety profile in adults. The highest dose intended to be delivered is based on dose-limiting toxicity seen in an earlier trial of AAV2-mediated gene therapy for retinal dystrophy (LCA2), where 1 mL of a study product at 1 x 10^{12} vg/mL was found to be the highest safe dose that could be administered subretinally. However, more recent data from a trial using AAV2/5 indicated that this serotype can elicit an inflammatory reaction to the dose of 1 x 10^{12} vg that could pose a risk. The sponsor elected to use the confirmed safe volume (1 mL) and a titer of 2 x 10^{11} vg/mL. They also stated that AAV2/5- mediated transduction of photoreceptors in dogs and non-human primates is efficient over a wide range of titers, including lower titers than used in this study. The sponsor believes the RPGR transgene can be delivered to the photoreceptors effectively at 2 x 10^{11} vg/mL. Risks include an immune response to the AAV2/5-hRKp.RPGR (mitigated by giving steroids); vector transmission to other organs (photoreceptor-specific promoter used); insertional mutagenesis and oncogenesis (AAV vector genomes integrate into host chromosomes at a very low frequency, limited number of AAV particles will be injected, eye contains predominantly non-dividing cells, therefore ocular tumors are rare, and not reported in rodent studies); germline transmission (using tiny amounts of vector, not high amounts as in hemophilia studies, participants will be asked to use barrier contraception for 12 months); risk of surgical adverse events (using experienced surgeons). There are no completed human clinical studies. The first study in humans with this product is ongoing. AAV2/5-hRKp.RPGR will not be administered to participants with contraindications for transient immune suppression by systemic corticosteroids (history of uncontrolled hypertension, DM, TB,

CKD, osteoporosis, gastric ulceration, severe affective disorder, immunocompromised status), participants with uncontrolled reflux, or who are using NSAIDS on a regular basis at the time of screening. The presence of neutralizing antibodies to rAAV capsid may reduce transduction efficiency following systemic administration, this has not been observed following intra-ocular injection in animal models. The possibility of oncogenic events due to vector-mediated insertional mutagenesis cannot be excluded with certainty, but available evidence suggests it to be unlikely given that rAAV vector genomes integrate into host chromosomes at a very low frequency; a relatively low number of rAAV particles will be administered; and, the eye predominantly contains non-dividing cells and consequently ocular tumors are very rare. Antibody responses to AAV capsid proteins and RPGR by ELISA at baseline and at 4 weeks, 3 months and 6 months will be done. Presence of vector genomes will be assessed by PCR analysis of tears, saliva and serum at 1 day and at 4 weeks following study product administration.

This is an open label, phase I/II dose escalation trial of a recombinant Adeno-Associated Virus vector (AAV2/5-hRKp.RPGR) for gene therapy of adults and children with X-linked retinitis pigmentosa owing to defects in retinitis pigmentosa GTPase regulator (RPGR). Retinitis pigmentosa (RP) is a group of inherited diseases of the retina, characterized by a progressive reduction in vision, initially manifest as night blindness which usually becomes apparent in childhood or early adulthood and is progressive throughout the individual's life-time. There is currently no licensed therapeutic treatment for RPGR XLRP. Among a variety of novel experimental strategies that are currently under investigation, gene therapy is considered the most promising. It is hypothesized that, in those participants with RP associated with mutations in the RPGR gene, localized gene augmentation with a human RPGR-ORF15 variant will result in the production of a biochemically active RPGR-ORF15 protein and thereby facilitate functional and morphological rescue of both rod and cone photoreceptor cells and consequently improved vision. The safety of the proposed approach will be enhanced by restricting transgene expression to the target tissue by virtue of the photoreceptor-specific promoter, the inherent tropism of the AAV 2/5 vector, the direct administration to the sub-retinal delivery space and by restricting the intervention to one eye only in each participant. The AAV is used as a viral vector for the delivery of genes to recapitulate and replace mutated or defective genes. The virus is recombinant, is replication deficient, and generally regarded as the safest viral vector used in the GCT sector. It has a specific therapeutic gene, which will normally only work in the tissue it is exposed to, due to tissue specific promoter. The gene encapsulated into the rAAV vector, is human RPGR (Retinitis Pigmentosa GTPase Regulator). Driving this gene is a fragment of the human rhodopsin kinase specific promoter. Also contained within the vector are 2 palindromic ITR (Inverted terminal repeats) regions which discriminate the AAV therapeutic cassette boundary and are essential for full functionality. A fully validated rcAAV test/assay is performed prior to systematic release of the vector. This test describes a cell-based semi-quantitative assay to detect wild type (wt) AAV species that can replicate in HEK293T cells in the presence of helper Adenovirus (wt Ad5). The assay is designed to show that wt AAV species in final product are below a certain level as detected in a control that has been spiked with known amount of wt AAV. Delivery of vector suspension to the subretinal space will be performed by standard surgical vitrectomy. This will involve a 3-port pars plana vitrectomy followed by injection of vector suspension using a fine cannula through small retinotomies into the subretinal space, resulting in a transient retinal detachment. Risks to visual function will be minimized by controlling the area of ATIMP delivery, and by leaving the contralateral eye untreated. Trial participants will receive 1 of 3 different doses of ATIMP within

the range proven to be safe in the preclinical animal studies: a) low dose 1.0 x 10^{11} vg in 1 mL b) intermediate dose 2.0 x 10^{11} vg in 1 mL c) high dose 4.0 x 10^{11} vg in 1 mL. The ATIMP will be diluted as appropriate for the specific dose intended immediately prior to intraocular administration in Hartmann's solution at the time of administration. Regarding exposure assessment, if accidental release occurs, the viral vector will generally not persist outside a host at ambient temperature for longer than a few days. If exposure does occur, the agent may not actually cause any genetic expression in the site of entry, due to harboring a tissue (ocular) specific promoter which may not solicit a gene expression event in any tissue apart from the intended target (eye). There are a number of procedures outlined in the unlikely event that AAV2-REP1 is released outside of a participant's eye.

Comments:
1) Although the sponsor indicates this is a RG-1 product, AAV vectors are to be considered BSL-2 level agents unless they do not come from cells of human origin. The AAV2/5-hRKp.RPGR is manufactured using a HEK293T cell line, of human origin (potential infectious risk). The Investigator's Brochure (IB) states that the initial clinical batch of AAV2/5-hRKp.hRPGR produced in the HEK293T cell line, encodes the SV40 large T antigen, a known oncogene.
2) It is stated that more than 100 ml will be used; however, the injection will be about 1ml. Clarify.
3) Under Recombinant or Synthetic Nucleic Acid Usage: Tissue culture work is checked (*Section III-D-3*); however, no tissue culture work is planned. This should not be checked.
4) Under Recombinant or Synthetic Nucleic Acid Usage: Experiments involving more than 10 liters of culture at one time should be unchecked (*Section III-D-6*)
5) Under Recombinant or Synthetic Nucleic Acid Usage: Use or cloning of human or animal pathogens as host-vector system should be checked (*Section III-D-1*).

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900131
Title:	Engineering Mammalian Genetic Circuitry
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 10/14/2020

Required modifications: None

Comments:

The proposed project aims to develop control mechanisms for calcium signaling in cells with an emphasis on murine neurons. They will accomplish this objective by electroporation and transfecting cells with gene editing tools including CRE-Lox, siRNA/shRNA, and CRISPR-Cas with a 4-plasmid Lentiviral system. *E coli* will be used for plasmid cloning and tissues will be obtained from mice. The author has meant well by adding numerous citations to their protocol, but this has made it difficult at some points to determine exactly what is being done or used in different sections. There are several comments, mostly forms-related issues, that need to be addressed. The work is proposed at BSL-2, and the revised IBC guidance indicates that Lentiviral-encoded CRISPR-Cas systems need to be performed at BSL-2+; this is the primary issue with this protocol that needs to be addressed before it can be approved. This protocol aims to look at calcium signaling, first in HEK293 cells, then neurons isolated from mice and finally in an cell culture model for pulmonary hypertension. The investigator plans to modify gene expression using a number of systems to introduce genes for calcium signal activators or fluorescent calcium probes. This includes the use of a 4-plasmid Lentivirus vector expressing Crispr/Cas9 and guide RNAs on the same vector. The investigator needs to provide more detail about what they plan to do with the different genes and expression systems they describe using (Cre-Lox is also described). They do not appear to be aware of IBC recommendations that work with Crispr/Cas9 and guide RNA on the same vector requires BSL-2+.

Review comments were provided to the investigator for response, including a copy of the IBC guidance for work with gene editing technology. The revised application was placed on the October agenda.

Approval: No additional comments were provided by the committee; the protocol was approved with no comments.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900141
Title:	HBV Biology
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Deferred/Reconsidered

Required modifications:

1) Protocol Team Members: Add all protocol team members to the application.

2) Supporting Documents: If work will be using Lentivirus and gene editing, then BSL-2+ is required and the signed copy of the laboratory's Biosafety Operations Manual must be uploaded to the application.

3) Primary Cells or Cell Lines: Clarify whether the laboratory is using Lentivirus to transfect CRISPR Cas and guide RNA in cell lines. If YES, then work should be done at BSL-2+.

4) Exposure Assessment and Protective Equipment: Question 1: Lentiviral vector information: This is the first mention of use of a replication-competent Lentiviral vector in the protocol. If the "pCMVdeltaP1deltaenvpA, pSVIIIenv" packing system from NIH AIDS Reagent Program will be used in work on this protocol; then add this vector/packaging system as a separate Lentiviral vector entry in the Viruses section of the application.

5) Exposure Assessment and Protective Equipment: Question 5: In addition to the PPE listed, PPE required at BSL-2+ includes use of a solid-front laboratory gown, and mucous membrane protection consisting of either a face shield or a combination of safety glasses/goggles and a surgical mask for procedures with a risk of splash or spray performed outside a BSC. Revise accordingly.

6) Primary Cells or Cell Lines: If mouse cells/cell lines will be a target for transduction with Lentiviral vectors expressing CRISPR/Cas9 and guide RNA, these cells will need to be handled at BSL-2+. Clarify and change the biosafety level for the entry/entries as needed.

Comments:

In the summary, the researcher is expected to write s brief description of experiments and methods to be performed. However, it seems that the investigator has basically written a statement of the research goals for the laboratory. Remove unnecessary information and just describe what is proposed to be done. If Lentiviral work involves oncogenes, then work with these cell lines will require BSL-2+. The researcher is proposing to use some of these cell lines for Lentivital vector propagation. In the protocol, the investigator seeks to understand Hepatitis Virus replication using cell culture models from multiple species. Viruses will be used for several purposes in these studies, including native Hepatitis Viruses specific to various species (Hepatitis D, Hepatitis B genotypes A-D, Duck Hepatitis B virus, Woodchuck Hepatitis B virus) and for gene expression or knockdown (Lentivirus HIV-1, Adenovirus Adv-5, MMLV type VI, Baculovirus AcNPV). All hepatitis vectors are replication competent. Thus researchers will be vaccinated as a precaution. It is not clear however whether everyone involved in those experiments will be vaccinated or will

be given a chance to be vaccinated. Genes targeted in these studies will include DNA repair, chromatin remodeling and epigenetic regulation, and innate immune signaling. It is not immediately clear whether genes to be targeted with viral vectors will include known oncogenes; recommend that the investigator make this clear. Finally the investigator seems to experience an issue with the setting of the laboratory in BSL-2+ containment, and requests the possibility of having the BSL-2 related protocol reviewed first. If this is the case, the investigator may want to submit a protocol that contains only BSL-2 agents. Once his laboratory is set for BSL-2+ containment, he will then submit an amendment to add the BSL-2+ agents and change the containment level of his protocol to BSL-2+.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Reconsideration: Significant safety concerns were noted in the application which need to be clarified or explained.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900334
Title:	Amendment for **IBC201700132**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Supporting Documents: The Biosafety Operations Manual must be signed and uploaded to the application.

2) Lentivirus and Lentiviral Vectors: Question 2a: Describe the safety features associated with the Lentiviral vector to be used in this study. For example, the investigator indicates that the Lentiviral vector is replication-deficient but does not provide details regarding modifications that render the vector replication-deficient. Are genes needed for packaging of viral particles separated onto multiple vectors? Provide additional information.

3) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: Provide specific strain information for the *E. coli* that will be used in mouse studies. The current entry indicates that non-pathogenic *E. coli* will be used in studies of COPD, which is not in keeping with instillation of *S. aureus* and *P. aeruginosa* (both Risk Group 2 pathogens) also described in this section. The non-pathogenic entry is meant to describe common *E. coli* strains that have been modified to serve as competent cells for plasmid amplification and cloning techniques. Clarify.

4) Risk Group and Containment Practices: Lentiviral vectors are derived from HIV, a Risk Group 3 (RG3) infectious agent. Check the box for RG3 in the application.

Comments:

In the original protocol, the investigator seeks to study the infections, inflammation, COPD and cancers that result from cigarette smoking. Lung tumors will be induced by intranasal delivery of Adenoviruses carrying a Cre construct to transgenic mice expressing the transforming kRas G12D mutation in a lox site. The same Adenovirus is used to drive conditional overexpression (Tfam) or knockout (Tfam, IRS1, IRS2) of target genes after cigarette smoke injury. The investigator also added pathogenic strains of bacteria by intranasal or intratracheal delivery – *E. coli*, *S. aureus*, and *P. aeruginosa* – to induce experimental infections in mouse lungs. In the amendment, the investigator adds Lentivirus and Retrovirus constructs, and plasmids expressing Crispr/Cas9 constructs, to generate cell lines with stable knockdown or overexpression of target genes. These cell lines will be used for *in vitro* studies, to generate organoids, and may be injected into mice to study the tumor microenvironment. The revision also adds three personnel and two mouse cell lines (TRAMP-C2 and E0771). The work will be done at BSL-2+ and ABSL-2. The protocol studies diseases associated with cigarette smoke in mice. The amendment will: a) Add use of Lentiviral and Retroviral vectors. These will be used to generate stable cell lines. The Lentiviral vectors will be generated using the HIV 4-plasmid system and will be used to make stable cell

lines expressing shRNAs (as well as the reporter GFP). Targets are UBR5 and MLKL. Stable cell lines will be injected into mice. Murine embryonic stem cell viral vectors will be used to make stable cell lines that overexpress UCHL1. Resulting cells may also be injected into mice. Viral vectors will be used at BSL-2+. b) Add growth and use of organoids, cultured from murine primary tumors. These will be implanted into recipient mice to permit study of the tumor microenvironment. Add the TRAMP-C2 murine prostate epithelial adenocarcinoma and E0771 murine breast cancer cell lines. These will be introduced into recipient mice to also study the tumor microenvironment. Add CRISPR/Cas9 plasmids for *in vitro* transfection. No viral vectors will be used to express CRISPR/Cas9 or sgRNA. Finally, some personnel changes are recorded. Work will be done at BSL-2+ and ABSL-2. Recommendations for approval after minor changes:

Initial comments:
1) Recombinant or Synthetic Nucleic Acids Usage: Check if Section III-D-2 is needed, if any molecular biology to generate the viral vectors will be done in-house. The answer to question 1 under "Lentivirus and Lentiviral Vectors" indicates the III-D-2 may be needed.
2) Exposure Assessment and Protective Equipment: Amend to include Lentiviral and Retroviral vectors.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900353
Title:	Amendment for **IBC201800214**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ Manual Template Revision REDACTED 2 final • REDACTED BSL-2+ Manual 2019 BSO Signature • Memo REDACTED 2019

Determination: Approved

Last day of continuing review period: 10/14/2020

Required modifications: None

Comments:
Investigators in this protocol use AAV or Lentiviral vectors with CRSPRcas9 and guide RNAs in the same vector. The protocol has been amended to be carried out at BSL-2+ level according to recent University of Pittsburgh IBC recommendations for gene editing technology. EH&S has inspected and approved a BSL-2+ facility in the investigator's laboratory based on attached documents. The is a modification to include Lentiviruses and Adeno-Associated viruses expressing *S. aureus* or *S. pyogenes* Cas9 and sgRNAs. AAV expressing *S. aureus* Cas9 and sgRNAs and Lentiviruses expressing *S. pyogenes* Cas9 and sgRNAs in the same viral vector will be used to knockout host genes or Herpes Virus (HSV or VZV) genomes in differentiated human neurons and human cell lines *in vitro*. In addition, Lentiviruses encoding shRNA libraries targeting human genes will be used to knockdown host proteins to identify genes that affect virus infection. These three viruses and human cells transduced with these viruses will be used *in vitro* only and will be used at BSL-2+, which is appropriate. The investigator recently received BSL-2+ inspection approval for the laboratory areas. There were no issues; recommendation for approval.

The application was placed on the October agenda.

Approval: No additional comments were provided by the committee; the protocol was provided approval with no comments.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900369
Title:	Amendment for **IBC201600188**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• AmbroseBSL-2+

Determination: Approved

Last day of continuing review period: 10/14/2020

Required modifications: None

This amendment describes changes in a mouse model and viruses will be used. The work evaluates antiretroviral inhibitor prophylaxis regimens against intravaginal transmission of HIV-1 including drug resistance mutants using a humanized mouse model. A variety of cells will be infected with HIV-1 used at BSL-2+. Lentivirus, wild-type, mutant, and fluorescent protein and luciferase expressing Lentivirus. *Escherichia coli* will be used at BSL-1. No issues identified the original protocol compares the efficacy of antiretroviral inhibitor prophylaxis regimens against intravaginal transmission of wild-type HIV-1 and drug-resistant mutants in humanized mice. The work will involve replication-competent HIV-1. The current modification is requested to include human cells and tissues and new clinical HIV-1 isolates.

The submitted application was placed on the October agenda.

Approval: No additional comments were provided by the committee; the protocol was provided approval with no comments.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900400
Title:	Amendment for **IBC201700140**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ memo • BSL-2+ operations manual

Determination: Approved

Last day of continuing review period: 10/14/2020

Required modifications:

1) Tissues, Blood, or Body Fluids: If tissues, blood, or body fluids will be used to isolate primary cells and if they have been exposed to viral vectors prior to development of the primary cell culture the biosafety level for these materials should be changed to BSL-2+.

2) Primary Cells or Cell Lines: If any of the cells or cell lines listed will be transduced with viral vectors to modify gene expression the biosafety level should be changed to BSL-2+ to correspond with viral vector section of the application.

3) Exposure Assessment and Protective Equipment, Question 1: Remove the following sentence from the response: "Hence the use of Universal Precautions and the use of BSL-2, ABSL-2 precautions." And correct "BST2+" to "BSL-2+" or "Biosafety Level 2+."

4) Exposure Assessment and Protective Equipment, Question 2: Remove the phrase "and the use of BSL-2 precautions" from the response. And correct "BST2+" to "BSL-2+" or "Biosafety Level 2+."

5) Exposure Assessment and Protective Equipment, Question 5: Minimum PPE at BSL-2+ includes double gloves, dedicated solid-front lab gown, and use of a face shield or a combination of safety glasses or goggles and a surgical mask for procedures with a risk of splash or spray performed outside of a BSC. Revise accordingly.

Comments:

The investigator has modified responses to gene editing questions, such that Lentiviruses will be used to express shRNA targeting tumor suppressor genes and AAV will be used to express Cas9 and sgRNA from the same vector. These procedures require BSL-2+ practices. This is an amendment for a project investigating beta cell neogenesis in the liver. The amendment has been submitted to add additional people to the protocol. Human and murine cells will be included in studies where gene expression is modified, but only the murine tissues have been identified as recombinant materials. Gene overexpression shRNA/siRNA-mediated downregulation or will be performed by viral transduction. As noted, the laboratory appears to be using CRISPR-Cas in conjunction with an infectious viral vector and this work will need to be performed under BSL-2+ conditions as indicated by the updated IBC guidelines. Several of the genes that will be modified via viral transduction-mediated systems include tumor suppressors. There are a couple of minor issues that need to be addressed but because the protocol is proposed at BSL-2 but includes several

elements requiring a higher biosafety level, this protocol will need to be upgraded to BSL-2+ to accommodate these studies before it can be approved.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900373
Title:	Amendment for **IBC201700077**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED BSL-2+ Manual 2017_ April 6 updated

Determination: Deferred/Reconsidered

Required modifications:

1) Supporting Documents: A recent, and signed copy of the laboratory's Biosafety Operations Manual must be uploaded to the application.

2) Waste Management: Question 3: Identify the supplies available and steps to be followed to clean up a spill of biological materials.

3) Waste Management: Question 1b: Liquid waste: Liquid waste must be decontaminated by addition of concentrated bleach to liquid waste such that the final concentration of bleach is 1:10, v:v, bleach:liquid waste. Adding pre-diluted bleach to liquid waste further dilutes the bleach solution and results in insufficient final concentration of active ingredient to achieve effective decontamination. Revise.

4) Exposure Assessment and Protective Equipment: Question 4: Indicate whether a Biosafety Cabinet (BSC) will be used for all manipulations of potentially infectious materials. In addition, at BSL-2/ABSL-2 and above safety engineered sharps devices are required. Revise the section accordingly.

5) Exposure Assessment and Protective Equipment: Question 1: This question asks the investigator to discuss whether any of the materials used in this protocol are potentially infectious for humans. For example, modified AAV vectors and Lentiviral vectors are both capable of a single round of infection in human cells. Replication-competent HIV is infectious for humans. Also, human cells and cell lines, even when purchased from an off-site vendor, may contain undetected adventitious agents that may be infectious to humans. Revise this section accordingly.

6) Animals: Remove or delete linked ARO protocols that have been completed and are no longer active/approved protocols.

7) Recombinant or Synthetic Nucleic Acid Work Description: In question 3d: Describe how CRISPR/Cas9 will be delivered to target cells. For examples, transfection of plasmid DNA, injection of gRNA and Cas9 proteins, using AAV or Lentivirus as a viral vector.

8) Recombinant or Synthetic Nucleic Acid Work Description: How will siRNAs be delivered to rodents? This work is not described elsewhere in the protocol. Will a viral vector (AAV, Lentivirus) be used for delivery? Will siRNAs be delivered directly? Clarify, and if necessary, add siRNA to the description of use section of appropriate viral vector or cell/cell lines entry.

9) Viruses, Prions, or Vectors: In the Lentivirus - HIV-1 entry the description of use indicates that CRISPR Cas 9 will be expressed from this replication-competent virus. If this is the case this entry should be modified to indicate that these materials are recombinant. If the CRISPR Cas9 will be

used with a replication-deficient Lentiviral vector this information should be removed from the current entry and added to an entry for replication-deficient Lentiviral vectors.

10) Viruses, Prions, or Vectors: Lentivirus: HIV-1 entry: In the description of use the investigator indicates that replication-competent HIV will be obtained from a collaborator for use in these studies. However, the investigator has indicated in the additional virus information that the HIV-1 is replication-deficient. Clarify this discrepancy.

11) Primary Cells or Cell Lines: The human stem cells or iPSC entry indicates that these materials will be administered to animals. Add a description of this work to the description of use for this entry.

12) Primary Cells or Cell Lines: For all cell line entries: In the description of use the investigator indicates that cells and cell lines will be transfected with nucleic acids. Therefore, all of these cells and cell lines will contain recombinant material.

13) Tissues, Blood, or Body Fluids: For rat and mouse entries: Will samples isolated from rats and mice be reintroduced or transplanted into other animals? The response indicates that the investigator answered YES to these materials being administered to animals. Clarify.

14) Tissues, Blood, or Body Fluids: For rat and mouse tissues, blood, and body fluids entries: If tissues or other samples are collected from humanized mice infected with HIV these samples must be handled in the laboratory using BSL-2+ precautions. If rat and mouse samples are collected from animals exposed to risk group 2 biological agents they must be handled at a minimum of BSL-2. Revise accordingly.

15) Risk Group and Containment Practices: All personnel listed as handlers on this BSL-2+ protocol must complete the IBC-specific training module and be added in the Protocol Team Members section of the protocol.

Comments:

There were significant clarifications or explanations required for this application. The committee determined that the application should be reconsidered once the investigator provides the responses back to the IBC. This is a modification to add RNAi and CRISPR/Cas to the protocol to alter expression of regulates disease-associated risk gene expression. There are many areas in regard to the CRISPR/Cas that the investigator does not address correctly, mainly the expression system being used for the CRISPR (it appears to be with Lentivirus as its listed at 2+, but that is not stated). Later in the application, it states that the CRISPR and sgRNA will be transduced separately, but that is inconsistent in other areas of the protocol. There are many form issues and inconsistencies in regard to the CRISPR work, as the protocol appears to be at 2+ already, but the CRISPR work as proposed (on separate vectors) can be at 2. It is unclear if this is introduced *in vivo* or is *in vitro* only. Clarification at the Virus page about the CRISPR and sgRNA is needed. In the original project, the investigator seeks to understand the molecular and genetic causes of pulmonary arterial hypertension (PAH) using cell models (primary and transformed human/rodent cell lines, and human iPSCs) and *in vivo* mouse models, including HIV-infected "humanized" mice expressing human immune cells. Cultured cells are infected with Lentivirus (HIV-1) or Adeno-Associated Virus (AAV-6) constructs to induce or knockout expression of genes of interest. Notably, vascular cells are infected with Lentivirus constructs expressing replication-competent HIV. Genetically engineered and "humanized" mice are infected and studied according to related IACUC protocols. Experiments are conducted under BSL-2+ and ABSL-2 conditions. In the modification, the investigator wants to add the use of RNAi and CRISPR/Cas9 to alter the

expression of new genes of interest (some of them oncogenes). The research subjects (transgenic mice?) and the vectors used to infect (HIV?) need to be clearly defined in the protocol. The potential use of a viral vector to alter oncogenes needs to be discussed with the full committee. There are comments for clarification.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Reconsideration: Significant safety concerns were noted in the application which need to be clarified or explained.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900401
Title:	Amendment for **IBC201900012**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ Biosafety Manual • EH&S BSL-2+ Approval

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description: Additional clarification is needed regarding the use of CRISPR/Cas9 in viral vectors. In Question 3d, the investigator indicates that Lentivirus-based systems and AAV-based systems will be used to express CRISPR/Cas9. In question 3e the investigator indicates that CRISPR/CAS9 and gRNA will be expressed from the same vector, however in the response to the initial review comments the investigator indicates that the AAV-based CRISPR/Cas9 system will not express CRISPR/Cas9 and gRNA in the same vector. This is conflicting information and must be clarified in order for the committee to determine the appropriate biosafety level for use of both the Lentivirus and AAV based CRISPR/Cas9 systems.

2) Viruses, Prions, or Vectors: For AAV-based system using recombinant Cas9 in combination with AAV to induce targeted homologous recombination; clarify. Will purified recombinant Cas9 protein be used in conjunction with AAV or will the Cas9 protein be expressed from a viral vector? Additional information is required to allow the committee to complete its risk assessment and assign an appropriate biosafety level.

3) Viruses, Prions, or Vectors: Identify Cas9 and sgRNA under "Inserted Nucleic Acids Information" in the viruses listed below. Note the recent gene editing guidance recommendations:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

Comments:

In this proposal, the laboratory studies antiretroviral immune responses, as well as the molecular details underlying direct inhibition of Retroviral replication by restriction factors. They use plasmids, Retroviral, and Lentivirus transduction for the expression of wild-type and mutant forms of human, non-human primate, feline, and murine proteins and reporter proteins. They will also transfect cells with siRNA or transduce with shRNA or CRISPR-Cas9 for knockdown or knockout of genes/proteins. They will also infect mammalian cells with replication-defective and replication-competent Retroviruses and Lentiviruses. Moreover, they utilize murine models of Retroviral infection. Mice will also be infected with murine Retroviruses and monitored for the development of protective antiviral immune responses. Given the numerous viruses, CRISPR systems and animal systems used this protocol should be discussed. Overall it is well-written with major concerns addressed and BSL-2+ manuals uploaded. Some minor form issues exist. The

proposal will use animal models (mice) and viral particles will be used on the models. This amendment has been submitted in order to request two additions to a previously approved protocol on Retroviral immunity. The amendment requests the addition of a new research technician and the addition of a new helper-free AAV system. This AAV system will be acquired from *Cell Biolabs* and from Rockefeller University. AAV will be used in conjunction with CRISPR/Cas9 to promote homologous recombination at the nucleoporin genes in cell lines. Of the cell lines listed in the main protocol, it was not clear which cell lines would be used with the new AAV system. Can this information be added to the "cell lines" section? Additionally, although the AAV is listed as BSL-2, it will be used with the CRISPR/Cas9 system, which is being used at BSL-2+ in this protocol. Can a note be added to confirm that this will be used at BSL-2+? Otherwise, approval is recommended pending clarifications.

Initial comments:
1) All viruses should be double checked for storage to confirm which ones can be stored as BSL-2 or BSL-2+
2) Although the researcher is requesting BSL-2+, a blanket statement of using Lentivirus-based CRISPR/Cas9 for knockout/tagging is not enough. Specifically, what genes will be targeted or knocked down.
3) Vector maps of backbone viruses should be uploaded and Lentivirus used to transfer sgRNA or CRISPR.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900404
Title:	Amendment for **IBC201900108**
Investigator:	REDACTED
Highest BSL:	BSL-1 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4
Additional Documents:	• COA-lot#190902#58

Determination: Approved

Last day of continuing review period: 10/14/2020

Required modifications: None

Comments:
This modification concerns the downgrade of the use of AAV vectors to BSL-1 due to lack of BSL-2 mouse surgery space. The protocol described the use of AAV vectors to increase neurogranin expression levels (or GFP) in cortical neurons after impact-mediated TBI. Neurogranin is not an oncogene. The AAV vector is commercial (*Vector Biosystems*) and will be injected stereotactically into mouse brains. Vectors are produced without helper virus and purity data provided by *Vector Biosystems* indicates greater than 95% purity. The reason for the downgrade request is that the available surgery facilities are BSL-1 and a BSL-2 requirement would generate significant logistical issues. The underlying protocol examines cortical and hippocampal expression of neurogranin in a controlled model of traumatic brain injury. The work will use cerebrospinal fluid, nervous tissue and blood from rats obtained from DLAR-approved vendors. These fluids, tissues and blood will be used for analysis. The protocol will use AAV vector (AAV9, obtained from *Vector Biosystems Inc*) to deliver neurogranin and GFP, as a marker. The vector will be stereotaxically injected. The nucleic acid cargo delivered by the vector is not an oncogene and the vendor's COA is provided. The vector was produced in HEK293 cells without helper virus and the COA and associated documents note that, by silver stain, the final purity after 2 times CsCl purification, is in excess of 95%. On this basis, recommendation for approval of the downgrade request.

The submitted application was placed on the October agenda.

Approval: No additional comments were provided by the committee; the protocol was provided approval with no comments.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:37 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

November 4, 2019 10:00 AM

302 Heiber Building

VOTING MEMBER ATTENDANCE

Name of Regular/Alternate Member	Status	Substituting For	Present by Teleconference?
REDACTED, Chair			
REDACTED, Vice Chair			
REDACTED, BSO			
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
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REDACTED	Absent		
REDACTED	Absent		
REDACTED			

NON-VOTING MEMBER ATTENDANCE

Name of Regular/Alternate Member	Role of Non-Voting member
REDACTED	IACUC Office
REDACTED	RCCO Co-Director

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster: 23
Number required for quorum: 5

Meeting start time: 10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The October 2019 revised meeting minutes were reviewed and approved by the committee.

Votes:

For:	9
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None

IBC OFFICE REPORT

None

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocol:	IBC201900132
Title:	Breast Cancer Vaccine Strategies
Investigator:	REDACTED
Highest BSL:	BSL-1
NIH Guidelines:	• NIH Section III-D-4
	• NIH Exempt: Sections III-E or III-F
Additional Documents:	• SING KO mice on the FVB
	• Guy Et all

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: Tissue/Blood/Fluids - Mouse (murine) (Bone Marrow Tissue or Cells, Lymphatic Tissue, Other, Plasma, Blood): The investigator has answered YES to the question of whether these tissues/fluids will be administered to animals, yet this is not reflected in the "Description of Usage". Clarify the discrepancy.

2) Tissues, Blood, or Body Fluids: Clarify second tissue/blood/fluids entry. Murine cell lines should be listed in the next section of the protocol application.

Comments:

This project studying tumor vasculature in breast cancer will breed transgenic mice which develop tumors histologically similar to human breast cancers. These mice will be used as tissue donors to create transgenic lines and for laboratory assay. No virus work is proposed. BSL-1/ABSL-1 is appropriate for the work described. The protocol aims to identify breast cancer treatment strategies that can be readily tested in the clinic and identify the best correlates of the treatment's effectiveness based on the clinically relevant mouse model. Mouse tumor cell lines will be used in tumor challenge experiments and to assess the response to therapies *in vivo* and *in vitro*. The work is appropriate at ABSL/BSL-1. There are two sections in the application that need to be addressed prior to approval. Recommend for approval after the changes have been addressed.

Initial comments:
1) Recombinant or Synthetic Nucleic Acid Work Description: The answer to question 3 should be NO.
2) Recombinant or Synthetic Nucleic Acid Work Description: The responses to questions 3a-e should be deleted.
3) Genetically Engineered Animals: Source page, the answer to question 1 should be YES, as mice are being received from Johns Hopkins.

Review comments were provided to the investigator for response. The revised application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900139
Title:	Immune Mechanisms of Asthma and Airway Inflammation
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Include the specific bacterial species in the response.

2) Primary Cells or Cell Lines: Sources: Identify the vendor, sending investigator, or "in house mouse" etc in these areas...not the organ that the cells are derived from.

3) Tissues, Blood, or Body Fluids: Tissue/Blood/Fluids - Mouse (murine): The investigator has answered YES to the question of whether these tissues/fluids will be administered to animals, yet this is not reflected in the “Description of Usage”. Clarify this discrepancy.

Comments:

This is a protocol with the aim of understanding aberrant immune responses in severe asthma, a subset of disease that is poorly responsive to standard therapies. The work will identify targets for the development of new therapeutic approaches. The role of Type 17 cells in asthma will be investigated using genetically altered mouse models. The primary model utilizes mice deficient in T- and B-cells (SCID) which will be adoptively transferred with antigen specific T-cells. In this manner, aberrant immune responses to antigen in the lung can be modeled. The impact of T-cells and various inhibitors on lung function and disease pathology will be determined in addition to viral and bacterial execrations (Influenza Virus, *S. pneumonia*, and other Risk Group 2 pathogens herein) of asthma in immunocompetent mice with allergic airway disease. Replication deficient Adenoviral vectors will be used to deliver proteins that may impact disease in the lung. Adenoviruses will express several interleukin cytokines and other proteins which will be delivered during infection with the goal of improving outcome. In addition to *in vivo* work, a variety of cell types (T-cells, macrophages, fibroblasts, epithelial cells, neutrophils, and dendritic cells) will be harvested for *in vitro* studies. Work will be performed at BSL-2/ABSL-2 which is appropriate. Approval is recommended once clarification of the source of several pathogens is made and IACUC protocol information is included. This protocol describes research to understand aberrant immune responses in a module of severe asthma. Adenoviral vectors will be used to express cytokines or host defense proteins in both *in vitro* studies in human and mouse cells and cell lines and *in vivo* in a mouse model. Bacterial pathogens (*S. Pneumoniae* and *S. Aureus*), as well as a variety of Risk Group 2 influenza A, B, and C strains will be used to challenge mice and to infect cells in culture. A variety of knockout, Cre-LoxP targeted and otherwise genetically modified mice, where proposed host defense pathways have been altered, will be used to examine pathogenesis in allergic airway disease. Naive mouse T-cells derived from transgenic or knockout

mice will be isolated for use *in vitro* and for adoptive transfer to recipient mice. Routine cloning will also be performed. All work with bacterial and viral pathogens as well as replication-defective AdV vectors is described at BSL-2/ABSL-2, which is appropriate. There are a few minor corrections needed.

Review comments were provided to the investigator for response. The revised application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocols on the following pages

Protocol:	IBC201900146
Title:	19-113
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2018-06-29 HSFM0208PRO Waste Management Procedure_19-113 • 2019-08-05 IMA201-101_MainScreening ICF_19-113 • 2018-09-13 Protocol v6.0_19-113 • 2019-09-17 ACTengine_ Cell_Processing_Manual v1.0 final_19-113 • 2017-08-28 IMA201_Pharmacy Manual v1.0_19-113 • 2017-07-18 ACTengine_IMA201 AppM-I-A_19-113 • 2019-03-28 IMA201-101_IB_v4.0_19-113 • 2019-08-05 IMA201-101 Treatment ICF_19-113 • 2019-08-05 IMA201-101_HLA_Screening_ICF_19-113 • 2019-09-18 ACTengine_ Lab Manual_v1.0 final_19-113 • 2018-01-30 HSIC0604 Bloodborne Pathogen Standard Exposure Control Plan_19-113 • 2019-08-05 PBMC Protocol_19-113

Determination: Modifications Required

Required modifications:

1) Risk Group and Containment Practices: The HIV-derived Lentiviral vector is a risk group 3 agent, so Risk Group 3 (RG-3) should be selected.

2) Human Gene Transfer/Human Clinical Trial: Materials: 2a. Indicate there are no prior clinical trials with this product. No relevant animal models.

3) Human Gene Transfer/Human Clinical Trial: Outline the chain of custody procedures for the study product from the pharmacy area to the study participant.

Comments:

Summary: The study product IMA201 consists of autologous T-cells engineered *ex vivo* to express R7P1D5, a T-cell receptor. TCR R7P1D5 has been isolated from a healthy donor and is used without modifications for IMA201. This receptor is introduced into T-cells via transduction using a 3rd generation self-inactivating Lentiviral vector (LV-R73) encoding the alpha and beta chains of the T-cell receptor. The target peptide (A*02-binding, nine amino acid peptide) on the cancer cells is called MAG-003; R7P1D5 is highly specific for this peptide. This is the first study in humans for this product; will be studied in HLA-A*02:01 positive participants with solid cancers (lung, head and neck primarily) that express melanoma-associated antigen 4 and/or melanoma-associated antigen 8 (MAGEA4, MAGEA8), which are MAG-003 source genes. MAGEA genes are frequently co-expressed, with most cancers showing the presence of at least two or more of these antigens. Limited clinical data exists; only one participant has been treated. There is no relevant animal model. The goal of this study is to assess safety and tolerability in participants with solid tumors with few or no remaining treatment options. Participants must be positive for HLAHLAA*02:01 and their tumors must express MAGEA4 and/or MAGEA8. Participants will

receive a single dose of IMA201 T-cells on day 0. Prior to infusion, they receive non-myeloablative chemotherapy for lymphodepletion. The T-cells are then infused and then they receive low-dose IL-2 twice a day for 14 days. The engineered T-cells recognize the antigen on the participant's cancer cells and eliminate them. Secondary endpoints include evaluating the persistence of the engineered T-cells and anti-tumor activity. There is a dose-escalation/de-escalation scheme, stepwise enrollment, and an independent DSMB. Safety procedures are outlined in the protocol. The study will be conducted at MD Anderson Cancer Center and additional study centers may be activated at the discretion of the study sponsor. Up to 16 participants will be included depending on outcome of cell dose-escalation/de-escalation. The plan is to treat a minimum of 10 total participants if safety of a specific dose has been confirmed. A starting dose of 50 x 10^6 cells/m2 CD3+ CD8+ HLA Dextramer+ cells appear to be acceptable for the first cell dose administered.

Biosafety: The product is made in a stem cell research lab at UT Medical School in Houston under GMP conditions. PBMCs are isolated from participants' leukopak and activated. The activated cells are transduced with a GMP grade self-inactivating Lentiviral vector (LV-R73, 4 plasmid) encoding the T-cell receptor R7P1D5 and expanded (IL-15, IL-7). Cells are then harvested, washed, concentrated by centrifugation, and cryopreserved. In-process samples are taken to ensure sterility and quality of the intermediates. Release testing is performed on the final product prior to cryopreservation or post thaw as applicable, to ensure sterility (bacteria, mycoplasma,endotoxin), safety (vector insertion copy number, qPCR) and quality (viability >70%, immunophenotyping (>80%CD3 cells). Sterility and viability testing are performed after initial processing of leukopaks and PBMC isolation. IMA201 T-cell product is supplied at a concentration of 1 x 10^8 cells/mL in one to five cryopreservation bags containing 10- 50 mL/bag, depending on the total number of cells available at the time of harvest. Bags are to be thawed to room temperature just prior to infusion. Blood samples will be collected and banked for RCL testing. Potential side effects/adverse events from IMA201: cytokine release syndrome, off-target toxicities (risk thought to be low), autoimmune toxicities (rx with tocilizumab, steroids), allergic reactions, neurotoxicity, persistence of RCL, infection due to non-sterile product. Low-dose IL-2 side effect include fever, chills, fatigue, nausea, myalgias and local injection site reactions. Chemotherapies used in this study are associated with renal damage, neutropenia, anemia, thrombocytopenia, nausea. Potential benefit is possible response in participants with progressive disease.

Initial comments:
1) Outline the chain of custody procedures for the study product from the pharmacy area to the participant.
2) Question 2a. Indicate there are no prior clinical trials with this product. There is no relevant animal model.

The submitted application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900149
Title:	19-114
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-06-17 Screening ICF1_19-114 • 2018-01-23 Appendix M-I-A_19-114 • 2019-06-17 Treatment ICF2_19-114 • 2019-09-17 ACTengine_ Cell_Processing_Manual v1_19-114 • 2018-01-30 HSIC0604 Bloodborne Pathogen Standard Exposure Control Plan_19-113 • 2018-06-22 Pharmacy Manual_v1.0_19-114 • 2019-04-26 HSIC0616 Guidelines for Handling Sharps_19-113 • 2019-09-18 ACTengine_ Lab Manual_v1.0 final_19-114 • 2019-05-15 Protocol v4.0_19-114 • 2019-05-13 IMA202-101_IB_v2.1_19-114 • 2018-06-29 HSFM0208PRO Waste Management Procedure_19-113

Determination: Modifications Required

Required modifications:

1) Risk Group and Containment Practices: The HIV-derived Lentiviral vectors is a Risk Group 3 agent, so RG-3 should be selected.

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 3: Should be checked YES, this is a first-in-humans study with no prior clinical data. Provide additional information for any follow-on questions.

3) Human Gene Transfer/Human Clinical Trial: Question 3a. Outline the chain of custody for the study product upon arrival from the sponsor.

Comments:

This is a new HGT study evaluating adoptive T-cell therapy with autologous CD8+ T-cells engineered to express R37P1C9, a T-cell receptor (TCR) specific for MAGEA1-003 (tumor antigen). The peptide MAGEA1- 003 is an HLA-A*02-restricted peptide, derived from the melanoma-associated antigen MAGEA1. The participant's T-cells are engineered to express the TCR using a third-generation self-inactivating Lentiviral vector (LV-R37D) that contains both α and β chains of the TCR R37P1C9. The PBMCs are activated, transduced, and expanded *ex vivo*. The final product IMA202 is formulated and cryopreserved until thawed for infusion. This is a first-in-human study of this product. The main objective is to determine safety and tolerability in HLA-A*02:01 positive participants with recurrent or relapsed squamous non-small cell lung cancer (NSCLC) or unresectable hepatocellular carcinoma (HCC) expressing MAGEA1, the source gene of MAGEA1-003. Secondary objectives include assessing the persistence of transferred T-cells, tumor response, and survival. Participants must have failed standard therapies to be eligible for the study and have a life expectancy of at least three months. Study treatment

involves lymphodepletion with fludarabine (40 mg/m2) and cyclophosphamide (500 mg/m2) from Days -6 to -3, infusion of IMA202 T-cells on Day 0, and low-dose IL-2 self-administration for 14 days. On Day 0, participants will receive IV infusion of IMA202. Four (4) dose levels of IMA202-101 will be evaluated. At least 2 participants per cohort will be treated. Participants will be treated per a dose escalation/de-escalation schedule: DL1 50 x 10^6; DL2 150 x 10^6; DL3 2-4 500 x 10^6; and 1-low DL-1 15 x10^6. The cell dose will be based on viable CD3+CD8+HLA-Dextramer cells (which represents the best available correlate to active, R37P1C9 transduced T-cells). An upper limit for the number of transferred total CD3+ cells will be 2 x 10^{10} viable CD3+ T-cells in total. There is no prior clinical data available.

Biosafety: Participants must carry the genetic marker HLA-A*02:01 that is expressed among about 40% of the study population and tumors must express MAGEA1. Among participants with squamous NSCLC and HCC participants, about 33% and 41%, respectively, are expected to express MAGEA1 at sufficient levels to present the target peptide MAGEA1-003 in their HLA molecules. Transduction efficiency ranged from 21.2% to 78.5%. Frozen, autologous participant PBMC, obtained by leukapheresis are thawed and rested for 4-6 hours, and then activated using immobilized antibodies against CD3 and CD28. The following day activated cells are transduced using LV-R37D (a Lentiviral vector encoding the novel TCR R37P1C9) in the presence of IL7 and IL-15 and expanded. The Lentiviral vector LV-R37D is manufactured by *Lentigen*. The components necessary for viral particles' production (gag, pol, rev, VSVg) are split across four different plasmids; regulatory protein tat as well as all the accessory proteins vif, vpr, vpu, nef have been removed. The vector contains a self-inactivating (SIN) LTR that becomes non-functional after integration. There has been no report of replication-competent particles generated using this system. GMP plasmid coding for LV-R37D was produced at *Aldevron*. Samples are taken during the manufacturing process and tested to ensure sterility and quality of the intermediates. The HLA-A*02:MAGEA1-003 restricted TCR R37P1C9 was identified from a healthy, HLA-A*02:01 positive donor. Release testing includes sterility, mycoplasma, endotoxin, viral, cell based RCL testing. From the final IMA202 T-cell product, 1 x 10^8 cells of retained samples will be saved for potential cell-based RCL testing. The end of production samples will be tested for RCL using a qPCR method as a release criterion for the T-cell product. Potential toxicities include allergic reactions, on-target, off-tumor autoimmune effects (high-dose steroids), CRS, T-cell-related encephalopathy, infections, sepsis, persistence of RCL. These toxicities will be managed per SOC. Participants with HIV are permitted to enroll if their viral load is undetectable. Potential participants co-infected with HBV and HCV are not permitted to enroll.

Initial comments:
1) Human Gene Transfer, question 3a: Outline chain of custody for study product upon arrival at HCC from sponsor.
2) Human Gene Transfer, question 3: Should be checked YES, as this is a first-in-humans study with no prior clinical data.

The submitted application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900150
Title:	19-115
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-05-25 IMA203-101_IB_v2.0_19-115 • 2018-07-23 IMA203-101_AppM-I-A_19-115 • 2019-06-13 Treatment ICF2 v2.019-115 • 2019-06-13 Screening ICF1 v2.0_19-115 • 2019-09-18 ACTengine_ Lab Manual_v1.0 final_19-115 • 2018-01-30 HSIC0604 Bloodborne Pathogen Standard Exposure Control Plan_19-113 • 2018-06-29 HSFM0208PRO Waste Management Procedure_19-113 • 2019-04-26 HSIC0616 Guidelines for Handling Sharps_19-113 • 2019-06-07 Protocol v2.0_19-115 • 2019-09-17 ACTengine_ Cell_Processing_Manual v1.0 final_19-115 • 2018-12-31 Pharmacy Manual_v1.0_19-115

Determination: Modifications Required

Required modifications:

1) Risk Group and Containment Practices: The HIV-derived Lentiviral vectors is a Risk Group 3 agent, so RG-3 should be selected.

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 3: Should be checked YES, this is a first-in-humans study.

3) Human Gene Transfer/Human Clinical Trial: Question 3a: Briefly outline the chain of custody procedures once the study product arrives at the study site through infusion into the participant.

Comments:

This new HGT study evaluates an adoptive cellular therapy approach using autologous CD8+ T-cells engineered to express R11P3D3_KE, a T-cell receptor (TCR) specific for PRAME-004. The peptide PRAME-004 is an HLA-A*02- restricted peptide, derived from the preferentially expressed antigen in melanoma (PRAME). The participant's T-cells are engineered to express the TCR using a third-generation, 4-plasmid, self-inactivating Lentiviral vector (LVR11KEA) that contains both α and β chains of the TCR R11P3D3_KE. To improve directed TCR chain pairing and thereby TCR function, single point mutations at the interaction interface of α and β variable domains were introduced. After activation, transduction, and *ex vivo* expansion, the final product is formulated, and cryopreserved until thawed for IV infusion. The parental sequence, TCR R11P3D3, was isolated from a healthy donor. It has been pairing-optimized for IMA203 to promote better expression in T-cells and increased affinity for its target. This study is the first-in-human trial for the IMA203 product in HLA-A*02:01 positive participants with solid cancers whose tumors express PRAME. Participants who are HLA-A*02:01 positive will have their tumor specimens tested for the expression of PRAME by RT-qPCR. The study design consists of a

multicenter, open-label, 3 + 3, dose-escalation/de-escalation approach. It will assess the safety and tolerability in PRAME-positive recurrent and/or refractory solid tumor participants with no remaining treatment options and a life expectancy of at least three months. Participants will be tested for HLA-A*02:01; uterine cancer, melanoma, and ovarian cancer are of special interest because PRAME is frequently expressed in these tumors. Participants with other tumor types that are positive for HLA-A*02:01 and PRAME may also participate. Up to 4 dose levels (DL) will be evaluated (DL-1, DL1, DL2, DL3); with at least 2 treated participants/cohort. Participants will be treated following the dose escalation/de-escalation schedule: low DL-1 15 x 10^6; DL1 50 x 10^6; DL2 150 x 10^6; DL3 500 x 10^6. The cell dose will be based on the number of viable cluster of differentiation (CD)-3+ CD8+ HLA-dextramer+ cells (which represents the best available correlate to the number of active, transduced T-cells) per body surface area (BSA) as defined by the Mosteller formula. A stepwise-treatment approach will be used. Each participant in the DL1 cohort will be staggered by 21 days, as measured from the time of initiation of their lymphodepletion treatment. Each participant in the subsequent cohorts will be staggered by 14 days. A staggered interval of 30 days will be required before starting each new DL cohort, as measured from the time of initiation of lymphodepletion treatment of the last participant in the previous DL cohort. Participants will have a leukapheresis to obtain PBMCs to manufacture the study product. The PBMCs are isolated, pre-activated, and transduced with the Lentiviral vector encoding the target-specific TCR and then expanded. Then participants will undergo non-myeloablative chemotherapy for transient lymphodepletion, followed by infusion of study product (Day 0), prophylaxis for allergic reactions (starting on Day 0 and continuing as clinically indicated), and administration of 1 million IU SC IL-2 starting on Day 0, about 6 hours after T-cell infusion, administered twice daily for 14 days. During the follow-up phase, participants will be monitored quarterly for up to an additional 2 years or until death. Tumor assessments based on routinely taken images and survival data will be collected. Blood samples for replication-competent Lentivirus (RCL) will be collected. PBMCs will be banked for RCL testing if HIV-like symptoms are observed. Participants with detectable levels of TCR-engineered T-cells prior to start of follow-up and/or showing a tumor response or antigen spreading will continue to be monitored quarterly for T-cell persistence. The expected number of treated participants is approximately 15. Because of the dose-escalation/de-escalation study design, the maximum number of treated participants can be up to 24. At the discretion of the sponsor, an extension cohort of an additional 12 participants may be initiated after the completion of the dose-escalation phase. An independent DSMB will closely monitor the safety of the study. Primary objectives are safety and tolerability; secondary, persistence of the TCR-engineered cells and tumor response if any, and its duration. No clinical experience is available for the IMA203 drug product.

Biosafety: Participants must carry the genetic marker HLA-A*02:01 to benefit from the product, it is expressed among about 40%-45% of the study population. The HLA-A*02:01 test will be performed by a CLIA)/ (CAP)-certified laboratory. HLA-A*02:01-positive participants' tumors will be confirmed to express PRAME prior to enrollment. The PRAME biomarker test will be performed at the Immatics CLIA/CAP-certified laboratory. PRAME is a highly tumor-associated, naturally presented target that is virtually absent from relevant normal tissues. There is an absence of unexpected off-target recognition or cross-reactivity towards the tested healthy cells and peptides similar to the target. The overall risk of on-target or off-target toxicities is considered low for IMA203. The CD3+ CD8+ HLA dextramer+ cells are considered the best available correlate for active, target-specific transduced cells. The number of total CD3+ T-cells will be capped at

2.0 x 10^{10} viable CD3+ cells to reduce the risk of CRS caused by too high numbers of infused T-cells. Given the published data from studies with autologous TCR-engineered T-cells, a starting dose of 50 x 10^6 cells/m2 CD3+ CD8+ HLA dextramer+ cells appear to be acceptable for the first cell dose. IMA203 drug products are made in the cGMP suite at the Stem Cell lab at UTHealth in Houston. The manufacturing processes takes about 5-6 days of T-cell culture. The cells are expanded with IL-7 and IL-15. Then undergo two weeks of release testing under GMP conditions that includes viability, sterility, endotoxin, immunophenotyping, copy number, VSV-g (surrogate for RCL). The IMA203 product is supplied at a concentration of 1 x 10^8 viable cells/mL in cryopreservation bags containing 10-50 mL per bag, depending on the total number of cells available at the time of harvest. IV infusion of the first bag of IMA203 will be started at a slow rate (about 1-2 mL/minute). The maximum infusion speed will be limited to 5 mL/minute for the remaining bags. Potential toxicities include but are not limited to allergic reactions, on-target, off-tumor autoimmune toxicities in normal tissues (steroids), CRS (tocilizumab, steroids), T-cell related encephalopathy(high-dose steroids), infections due to non-sterile products, febrile neutropenia, anemia, thrombocytopenia, sepsis, infections, persistence of RCL (using 3rd generation Lentiviral construct). Testing for absence of RCL with a cell-based assay is part of the release testing for the Lentiviral vector and each IMA203 product will be tested for release by a PCR-based surrogate assay for absence of RCL using Vesicular Stomatitis Virus G glycoprotein as readout. At pre-defined intervals during the 3 years following IMA203 infusion, blood will be drawn and banked for RCL testing. The following solid tumors are excluded because of low likelihood of tumor biomarker expression: colon adenocarcinoma, glioblastoma multiforme, kidney chromophobe, lower-grade glioma, mesothelioma, pancreatic adenocarcinoma, pheochromocytoma, paraganglioma, prostate adenocarcinoma, rectum adenocarcinoma, thyroid carcinoma, or stomach adenocarcinoma. Participants with HIV who have undetectable viral loads on ART may join the study as may participants with well-controlled autoimmune diseases off immunosuppressive therapy. An infectious disease monitoring) testing panel will include HBsAg, HBc Ab, HCV, Ab, HIV, HTLV I/II, HIV-1, HCV, HBV, West Nile, and Syphilis.

Initial comments:
1) Human Gene Transfer; question 3a: Briefly outline chain of custody procedures once the study product arrives at the study site through infusion into the participant.
2) Human Gene Transfer; question 7: It appears that the appropriate response should be checked YES, this is a first-in-humans study.

Several pre-screening comments have been addressed and the revised application was placed on the November agenda for review.

The submitted application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0

Abstained: 0

Protocol:	MOD201900403
Title:	Amendment for **IBC201600269**
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	None
Additional Documents:	• SPK-8011-101_Protocol AMD4 V 5_08Nov2018 • 13285_IRB_Determination_RAC_Review_10-05-16[2] • 2017-10-11_SPARK DMC Recommendations form_Dose Escalation_SPK 8011-101 • SPK-8011-101_Master ICF template AMD5_15Nov2018 • Draft Pregnant Partner Consent_v1.0_SPK-8011-101_OK Submit • NIH OD Letter re RAC, OBA review • SPK-8011 IB_Version 4.0_FINAL_19July2018_clean • SPK-8011-101_Summary of Changes AMD3_09Aug2018_AMD4_08Nov2018 • High Wachtel letter 01-19-17

Determination: Modifications Required

Required modifications:

1) Protocol Team Members: Ensure that personnel whom should be able to view and edit this protocol are listed in this section of the application. Review and make appropriate corrections.

2) Basic Information: Amend the response to question 8 to include the correct contact person besides Dr. REDACTED, whom should receive email correspondence from the IBC.

3) Human Gene Transfer/Human Clinical Trial: Link the associated IRB protocol.

Comments:

The study was provided approval at the November IBC meeting, however there are some clerical issues that needed to be clarified prior to issuing the final approval letter. These issues involve identifying the appropriate contact personnel and providing the link to the related IRB protocol. This is an amendment to a phase 1/2 open-label study that evaluates the safety and preliminary effectiveness of an AAV vector transporting a Factor VIII gene (B domain deleted) in males with hemophilia A known as SPK-8011. The primary reasons for the amendment are:

a) introduction of a prophylactic corticosteroid regimen: based on observation and experience from earlier clinical studies of liver-directed AAV gene transfer, including the SJ-UCL trial, the Sponsor's earlier clinical studies, and the Baxalta trial (NCT#01687608), subjects may develop an immune response to the vector capsid, as evidenced by a transient rise in transaminases (AST and/or ALT) and/or a loss in FVIII activity in the peripheral blood, as measured by IFN-gamma ELISPOT. Immunomodulation will be instituted prophylactically rather than reactively in effort to limit the immunologic response in the liver and maintain gene therapy-mediated FVIII expression. The recommended prophylactic oral corticosteroid is based on the AASLD guidelines and includes adjustments in dose depending on body weight (starting at 1mg/kg), similar to published corticosteroid regimens described for the treatment of immune thrombocytopenic purpura (ITP). Additional modifications by the Investigator to the immunomodulatory regimen are

permitted based on the participant's responses to laboratory parameters and/or tolerance of the regimen.

b) extension of the screening period from 12 weeks to 16 weeks (to allow for completion of laboratory testing to determine eligibility).
c) new manufacturing process for SPK-8011: SPK-8011 was initially manufactured using an adherent process; with the implementation of protocol version 5.0, participants will receive SPK-8011 manufactured using a suspension process.
d) addition of emicizumab as a prohibited therapy and inclusion of an emicizumab washout period: emicizumab was approved on 10/04/2018 for prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and pediatric participants with hemophilia A. The mechanism of action and prolonged half-life have not been studied in Spark gene therapy programs.

The modifications to the protocol are for the safety of the study participants. This is an amendment to a Phase 1 /2 open-label study that evaluates the safety and preliminary effectiveness of an AAV vector transporting a Factor VIII gene (B domain deleted) in males with hemophilia A known as SPK-8011. Up to 30 men will be infused with SPK-8011 at up to three different dose levels beginning at a dose of 5 x 10^{11}vg/kg, and subsequently at doses of 1 x10^{12} vg/kg, 2 x10^{12} vg/kg. The study product will be delivered by a single IV infusion over 1 hour in the MUH-CTRC. SPK-8011 is made of three capsid proteins (VP1, 2, and 3) and a single-stranded DNA vector genome molecule encoding the therapeutic gene cassette flanked by AAV2 inverted terminal repeats (ITRs). The AAV capsid that delivers the hFVIII gene is AAV-Spark200, a capsid derived by DNA shuffling of several AAV capsid sequences followed by *in vivo* selection in Fah-/-/Rag2-/-/IL2rg-/- (FRG) mice partially repopulated with human hepatocytes. The vector DNA is codon optimized and packaged into the AAV-Spark200 capsid as a single-stranded genome encoding the FVIII-SQ form of a BDD-hFVIII under the control of a liver specific promoter." Liver-directed AAV gene transfer may cause a dose-dependent immune response to the vector capsid with a transient and asymptomatic increase in liver function tests (ALT and/or AST) and a decline in factor level, and/or by increase in AAV-capsid-specific T-cells in the peripheral blood. This can lead to clearance of vector-transduced hepatocytes and eventual loss of transgene expression (but is not a safety concern). Transient increases of liver function tests can be attenuated by a short course of steroids. Amendments 3 and 4 are included. The amended protocol states that 5 of 7 participants in the 2x10^{12} vg/kg group required steroids, including 2 participants who lost substantial FVIII expression compared to their initial levels. Therefore, a consistent, standardized short course of prophylactic steroids was deemed warranted. The main changes are: introduced a prophylactic corticosteroid regimen; deleted bullet regarding steroid use as corticosteroids are now required, not permitted; updated Schedule of Events to include prophylactic steroid regimen; added Appendix 5 Toxicity Scale; introduced the use of SPK-8011 drug substance produced using a new manufacturing process (SPK-8011 was initially manufactured using an adherent process; SPK-8011 now manufactured using a suspension process); revised inclusion criterion number 6 to provide further clarity; added emicizumab as a prohibited therapy (prevents/reduces the frequency of bleeding episodes in participants with Hemophilia A); clarified washout language to include washout period for participants taking nonfactor replacement products (washout for emicizumab (recent FDA approved medication) was added.

Comments: Two updates were provided in the amendment at the same time.

The submitted application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900122
Title:	The Role of Trisomy 21 in the Development of Pulmonary Hypertension in Participants with Downs Syndrome
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ manual completed and signed 2_8_2019 • REDACTED BSL-2+ Manual 2019

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Although the Lentiviruses used are replication-defective, they can infect human cells. Remove the phrase "therefore, risk of viral infection is further reduced."

2) Exposure Assessment and Protective Equipment: Question 5: PPE for work at BSL-2+ should include double gloves, a dedicated laboratory coat with a disposable apron, or a solid-front wrap around gown, as well as mucous membrane protection to be used for procedures with risk of splash or spray (e.g face shield, or safety glasses/goggles and a surgical mask). Revise.

3) Viruses, Prions, or Vectors: The source cannot be blank. Include source of Lentiviral vectors. From where were the vectors obtained?

4) Primary Cells or Cell Lines: iPS cells are listed, but it appears that endothelial cells will be obtained from humans and later differentiated into stem cells. Clarify and choose the appropriate cell category.

5) Primary Cells or Cell Lines: Add HEK293 cells used to produce Lentiviral vectors as a separate entry in this section as they are not Human stem cells or iPS cells.

Comments:

The Project summary is all about trisomy 21 but the recombinant DNA section will use Lentivirus to knockdown expression of frataxin and ISCU in endothelial cells. These genes are involved in mitochondrial iron-sulfur protein maturation. Neither gene is on chromosome 21. The researchers want to use Lentivirus for "iPSC reprogramming into PBMCs and urine progenitor cells". Why? and what is a urine progenitor cell? And what does any of this have to do with trisomy 21? Clarifications are required. The protocol seeks to understand the role of Trisomy 21 in the development of pulmonary hypertension seen in participants with Down's syndrome. There are some minor forms issues that need to be resolved, and the investigator needs to better explain how the proposed work relates to Trisomy 21 and pulmonary hypertension in Down's syndrome participants. At current, this seems completely disconnected. It is not clear if an IRB protocol is needed to collect the human blood samples that the investigator proposes working with.

Review comments were provided to the investigator for response. The revised application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900138
Title:	Non-amyloid APP function
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-A-1-a or Section III-B-2 • NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Waste Management: Question 1b: Clarify in the response that the final concentration of bleach will be 10% (1:10 v/v) and indicate the time of disinfection will be at least 20 minutes.

2) Exposure Assessment and Protective Equipment: Question 1: Although replication-defective, Lentiviruses can infect human cells. Thus, the response should be modified.

3) Risk Group and Containment Practices: Question 1: HIV-derived Lentiviruses are Risk Group 3, so RG-3 should be selected.

4) Recombinant or Synthetic Nucleic Acids Usage: It is not clear why *Section III-A-1/III-B-2* should be selected. If this is true, then this would require federal board review and should be described in the protocol. Review and correct if appropriate.

5) Recombinant or Synthetic Nucleic Acids Usage: If this work involves the deliberate transfer of drug resistance to any pathogen that does not acquire the resistance naturally, and the drug is used to treat the symptoms of the disease causing pathogen, then the work would require federal board review.

6) Biosafety Summary: If brain tissues will be obtained from live animals, then "Tissues, Blood, or Body Fluids" and "Live animals used in experiments" should be selected. Respond to any additional follow-up questions.

Comments:

Modifications Review, with Member Review after response is received. From the November IBC meeting. The investigator is examining the role of APP and PSEN1 in development of Alzheimer's, using an *in vitro* system in which murine neuronal cells are infected with Lentiviruses expressing wildtype or mutant PSEN1 coupled with mCherry. PSEN1 is a potental oncogene, from at least one report found in *PubMed*, but the investigator says they are not working with oncogenes. It is not clear whether the Lentivirus infection will upregulate or suppress PSEN1 function. If PSEN1 is an oncogene, then this work would need to be done at BSL-2+. The protocol is for a project investigating the effect of stress on neurons. The investigator will isolate neurons from mouse embryos or perinatal mice pups. These cells will be infected with a 3-plasmid Lentiviral vector encoding human PSEN1 or APP with mCherry included as a reporter gene and the fixed samples will be assayed by IHC or western blots. The work is proposed at BSL-2. A journal paper was noted where PSEN1 gene expression was associated with reduced apoptotic activity in a melanoma cell line raising the concern that this could be an oncogene. There was no additional corroborative data suggesting PSEN1 is oncogenic, and it appears that the risk is acceptable under BSL-2 conditions.

Review comments were provided to the investigator for response. The revised application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900147
Title:	Aging and metabolism.
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Deferred/Reconsidered

Required modifications:

1) Supporting Documents: It appears that BSL-2+ will be required for this research, therefore the Biosafety Operations Manual should be uploaded into the application.

2) Exposure Assessment and Protective Equipment: Questions 1 and 4: Language will need to be updated due to expression of shRNA targeting tumor suppressor genes from Lentiviral vectors (BSL-2+).

3) Exposure Assessment and Protective Equipment: Questions 1 and 4: All sections will need to be updated to reflect the BSL-2+ use of viruses encoding CRISPR/Cas and sgRNA in the same vector.

4) Recombinant or Synthetic Nucleic Acid Work Description: Question 3: The expression of shRNA to decrease expression of tumor suppressor genes in Lentiviral vectors requires work to be performed at BSL-2+. Cdkn2a, GRB1, and PPARG, are all identified as Tier 1 tumor suppressor genes in the Cancer Gene Census, a part of the Catalog of Somatic Mutations in Cancer project maintained and updated regularly by the *Wellcome Sanger Institute* (https://cancer.sanger.ac.uk/cosmic).

5) Recombinant or Synthetic Nucleic Acid Work Description: Question 3e: The expression of Cas9 and sgRNA from the same vector requires BSL-2+. Refer to IBC gene editing recommendations at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

6) Viruses, Prions, or Vectors: Retrovirus, Murine Stem Cell Virus (MSCV): The expression of Cas9 and sgRNA from the same vector requires BSL-2+. Refer to IBC gene editing recommendations at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

7) Viruses, Prions, or Vectors: Lentivirus - The expression of shRNA to decrease expression of tumor suppressor genes in Lentiviral vectors requires work to be performed at BSL-2+. Cdkn2a, GRB1, and PPARG, are all identified as Tier 1 tumor suppressor genes in the Cancer Gene Census, a part of the Catalog of Somatic Mutations in Cancer project maintained and updated regularly by the *Wellcome Sanger Institute* (https://cancer.sanger.ac.uk/cosmic).

8) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system): expression of Cas9 and sgRNA from the same vector requires BSL-2+. Refer to IBC gene editing recommendations at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

9) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system): Identify the original source of the viral vector under "Source."

10) Viruses, Prions, or Vectors: Retrovirus, Murine Stem Cell Virus (MSCV): Identify the original source of the viral vector under "Source."

11) Primary Cells or Cell Lines: For appropriate cells, include transduction with viruses under "Description of Usage."

12) Primary Cells or Cell Lines: Include a description of cells used to produce Lentiviruses and Retroviruses. Note: expression of Cas9 and sgRNA from the same vector requires BSL-2+. Refer to IBC gene editing recommendations at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

13) Primary Cells or Cell Lines: Cell/Cell Line - Mouse (murine) (Bone Marrow Tissue or Cells, Lymphatic Tissue, Other, Plasma, Blood): The investigator has answered YES to the question of whether these tissues/fluids will be administered to animals, yet this is not reflected in the "Description of Usage". Clarify the discrepancy.

14) Tissues, Blood, or Body Fluids: Tissue/Blood/Fluids - Mouse (murine): The investigator has answered YES to the question of whether these tissues/fluids will be administered to animals, yet this is not reflected in the "Description of Usage". Clarify the discrepancy.

15) Risk Group and Containment Practices: Question 2; column 1: If BSL-2+ agents will be used, modify the current response.

Comments:

This new study looks at how genetics, environmental exposures, and aging these processes interact in the context of cardiometabolic diseases. Using both primary human tissue and blood as well as primary tissues from transgenic mice, they will use basic laboratory techniques to study proteins and RNA of interest. In addition, human and mouse cell lines will be used for *in vitro* experiments that include plasmid transfection or siRNA work. In the protocol, work with CRISPR/Cas is mentioned via viral transduction, so the protocol needs to be upgraded to 2+, a manual must be uploaded, and the IACUC work will need to be updated as well. Bacteria will be used for basic molecular work. Transgenic animals will be used. There are many areas of sparse information, including the CRISPR work and use of other genes not in the "Description of Usage" sections. This protocol thus will need to be upgraded to BSL-2+, with a downgrade possible for murine cells after 72 hours per IBC recommendations. This seems to be a new investigator, so some guidance on IBC procedures may be helpful. This protocol describes research regarding cardiometabolic diseases particularly with regard to metabolically relevant tissues such as adipose tissue and the liver. A variety of viruses including a 3-plasmid Lentivirus will be used for expression of shRNA, Cre, and CRISPR/Cas9. Potential issue with CRISPR/Cas and guide RNA on the same vector using Lentivirus – should be BSL-2+. Additionally, Cdkn2a is a tumor so should be used at BSL-2+ if using a Lentivirus. Virus will be injected into the tail veins or fat of the mice. Fat may be removed and transplanted directly or expanded *ex vivo* and injected again. Live wild-type and transgenic mice as well as human and mouse tissues/blood and cell lines are proposed to be used

under ABSL-2/BSL-2 guidelines to for a variety of laboratory analyses (protein and RNA analysis and histology). *Escherichia coli* will be used for propagation of plasmid DNA at BSL-1.

Initial comments:
1) Missing information in the project description.
2) Missing information on viruses, prions, and vectors page – Crispr/Cas delivery is not listed, nor is whether any of the shRNAs are for tumor suppressors.
3) Potential issue with CRISPR/Cas and guide RNA on the same vector using Lentivirus – should be BSL-2+. Additionally, Cdkn2a is a tumor so should be used at BSL-2+ if using a Lentivirus.

Review comments were provided to the investigator for response. The revised application was placed on the November agenda.

Reconsideration: Additional comments were provided by the committee, which will need to be addressed by the investigator on a revised application. Once the revised application is submitted, it will be placed onto the next available agenda for reconsideration review.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900414
Title:	Amendment for **IBC201800020**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED BSL_Manual_2018_Signed

Determination: Modifications Required

Required modifications:

1) Risk Group and Containment Practices: Question 2: Use of Lentiviruses expressing oncogenes must be used at BSL-2+. Correct the response.

2) Risk Group and Containment Practices: Question 1: Lentiviruses are Risk Group 3 pathogens. Correct the response.

3) Animals: Question 3: Earlier in the protocol it is stated that teratomas will be harvested for in vitro studies. Thus, the answer should be YES.

4) Recombinant or Synthetic Nucleic Acids Usage: If *E. coli* will be used to propagate plasmids encoding Lentiviral or Sendai-Virus based sequences *Section III-D-2* should be selected.

5) Viruses, Prions, or Vectors: Lentivirus (HIV 4-plasmid system) - HIV-1: Oct3/4, Sox2, KLF4, L-myc and c-Myc are oncogenes and use of Lentiviruses expressing oncogenes must be performed at BSL-2+ per IBC recommendations.

6) Primary Cells or Cell Lines: If Lentiviral vectors expressing oncogenes (Oct3/4, Sox2, KLF4, L-myc and c-Myc) are used to generate iPSCs, this work must be done at BSL-2+.

Comments:

The committee determined that modifications are required which will require Member Review post-revisions. From the November meeting. The proposal seeks to modify an approved protocol. The only requested change is addition of Sendai Virus. The protocol aims to dedifferentiate primary human fibroblasts into undifferentiated iPSCs. The de-differentiation will be performed using plasmid transfection or transduction with Lentiviral vectors. The Sendai Virus will be used as an additional option for de-differentiation. iPSCs will then be differentiated into hepatocytes or transplanted into mice for teratoma formation assays. The Sendai Virus (BSL-2, obtained from *Invitrogen*) will deliver human Oct3/4, Sox2, Klf4, and c-Myc, the usual factors for inducing pluripotency. Animal work will be done at ABSL-2. Human cells will be handled at BSL-2. Once clarification received, recommend approval. In the amendment, the investigator proposes to add Sendai Virus as an option for reprogramming. In the original protocol, the investigator optimizes a protocol for generating differentiated human hepatocytes from fibroblasts through generation of iPSCs. The approaches that the investigator will test include plasmid nucleofection and viral transduction with Lentiviruses. iPSC-generated hepatocytes will be injected into transgenic mice to assay for teratoma tumor formation. The original protocol has been approved at BSL-2 containment, yet a BSL-2+ manual is in place (see section 'Supporting Documents') because of a) the manipulation of unfixed human cells in the facility (All untested human samples are considered potentially infectious for HIV, HBV and other blood borne pathogen) and b) viral vectors (that are

replication defective) will be inserted with some genes that may be oncogenic. If this is the case, the protocol should be reconsidered for BSL-2+ containment instead of BSL-2.

Initial comments:
1) Why is a BSL-2+ protocol appended to the modification request, as the proposed work is BSL-2 or lower. Perhaps this could be clarified or removed, as the laboratory has numerous other active protocols.

Review comments were provided to the investigator for response. The revised application was placed on the November agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900427
Title:	Amendment for **IBC201900026**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Deferred/Reconsideration

Required modifications:

1) Waste Management: Question 1c: As animals will be used in this protocol, "N/A" is not an appropriate response.

2) Waste Management: Question 1b: Indicate that the final concentration of bleach will be 10% (1:10 v/v).

3) Waste Management: Question 1a: The correct time for decontamination should be 20 minutes. Correct the current response.

4) Risk Group and Containment Practices: Depending on use of CRISPR Cas and gRNA usage, the responses to question 2 may need to be revised accordingly.

5) Recombinant or Synthetic Nucleic Acid Work Description: Question 3e: Clarify what vector will be used and how it will be delivered for "transient transfection" of CRISPR/Cas9 and sgRNA. If viruses are produced with both Cas9 and sgRNA in the same vector, then BSL-2+ is required for this work.

Comments:

Significant modifications are required as the application was unclear as to what vectors will be used for Cas9 and sgRNA expression. The investigator has submitted an amendment to their protocol to add a new strain of mice which will be obtained from a collaborator, and then maintain that colony of mice. Several pre-screening comments have been addressed and the revised application was placed on the October agenda for review.

Deferred/Reconsidered: Additional comments were provided by the committee, which will need to be addressed by the investigator on a revised application and then will need to be assigned to the next available meeting agenda.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900454
Title:	Amendment for **IBC201700156**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description: Questions 3a and 3b: The responses state that DNA will be "primarily" from BACs. List other vectors to be used.

Comments:

The amendment is requesting changes in biosafety levels due to associations with other laboratories in the group/division. The change in biosafety level is not something that the IBC Office staff are able to process administratively, thus the study is being placed on the meeting agenda for review consideration for the changes to the biosafety containment for the items identified.

Several pre-screening comments have been addressed and the revised application was placed on the October agenda for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:37 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

December 9, 2019 10:00 AM

302 Heiber Building

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			
REDACTED, Vice Chair			
REDACTED, BSO			
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED			

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	EH&S Office
REDACTED	RCCO Co-Director
REDACTED	EH&S Department

GUEST NAMES
None

QUORUM INFORMATION

Committee members on the roster:	21
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The November 2019 meeting minutes were reviewed and approved by the committee.

Votes:

For:	12
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None

IBC OFFICE REPORT

None

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocols on the following pages

Protocol:	IBC201900138
Title:	Non-amyloid APP function
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Animal Gene Transfer, Questions 2 and 3: Earlier in the protocol it is stated that no recombinant materials will be administered to mice and that no *in vivo* experiments will be performed. The answers to questions regarding animal gene transfer appear to contradict these statements and should be clarified.

2) Risk Group and Containment Practices, Question 2: The biosafety level for research involving animals should be selected.

3) Waste Management, Question 1c: Animal carcasses should be sealed in double bags, labeled, and disposed according to animal facility guidelines. Revise the response.

4) Waste Management, Question 3: Provide additional details describing the process for clean-up and decontamination of a biological spill (for example: clean up materials, contact time for disinfectant, disposal of potentially contaminated clean up materials).

Comments:

The investigator is examining the role of APP and PSEN1 in development of Alzheimer's, using an *in vitro* system in which murine neuronal cells are infected with Lentiviruses expressing wildtype or mutant PSEN1 coupled with mCherry. PSEN1 is a potential oncogene, from at least one report found in *PubMed*, but the investigator says they are not working with oncogenes. It is not clear whether the Lentivirus infection will upregulate or suppress PSEN1 function. If PSEN1 is an oncogene, then it is recommended that this work would need to be conducted under BSL-2+. The protocol is for a project investigating the effect of stress on neurons. The investigator will isolate neurons from mouse embryos or perinatal mice pups. These cells will be infected with a 3-plasmid Lentiviral vector encoding human PSEN1 or APP with mCherry included as a reporter gene and the fixed samples will be assayed by IHC or western blots. The work is proposed at BSL-2. A citation where PSEN1 gene expression was associated with reduced apoptotic activity in a melanoma cell line raises the concern that this could be an oncogene additional corroborative data suggesting PSEN1 is oncogenic was not found, therefore the risk is acceptable under BSL-2 conditions.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900155
Title:	FLT3 SFKs and AML
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: Insect cells are mentioned in the project summary. If work will be conducted with the insect cells, then include an entry for the cells.

2) Viruses, Prions, or Vectors: If cells transduced with Retrovirus will be administered to animals, change the response for animal administration to YES.

3) Recombinant or Synthetic Nucleic Acid Usage: *Section III-D-3* should be selected as cells will be transduced with Retroviruses.

4) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include information regarding administration of recombinant materials to animals.

5) Animal Gene Transfer, Question 2: This should be answered YES if transduced cells are administered.

6) Exposure Assessment and Protective Equipment, Question 5: Include PPE required for entrance to the ABSL-2 animal facility.

Comments:

In this protocol, the investigator will use replication-defective murine stem cell virus (MSCV) to infect human acute myeloid leukemia cell lines for *in vitro* experiments, and for injection into immunocompromised mice. Since the virus transduces a potential oncogene, the biosafety level containment may need to be revised from BSL-2 to BSL-2+. Furthermore, animals will be given human cells that are modified with the MSCV, hence the biological research involving animals should be level 2 instead of level 1. The investigator proposed to use replication-defective murine stem cell virus to infect human AML cell lines and to introduce the cells into mice. The investigator should revise the biosafety indications to Risk Group 2 (RG2) for animals and ABSL-2 for introduction of murine Retrovirus-modified cells. Since an oncogene is expressed and the viruses are capable of infecting human cells, the *in vitro* biosafety should be BSL-2+.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900434
Title:	Amendment for **IBC201800027**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 12/10/2020

Required modifications:

1) Tissues, Blood, or Body Fluids: It was stated later in the protocol that tissues, cells, or organs from animals will be used in *in vitro* experiments. Provide an entry for the use of the harvested materials.

2) Recombinant or Synthetic Nucleic Acid Usage: *Section III-D-3* should be selected as cells will be transduced with Retroviruses.

3) Animal Gene Transfer, Question 2: This should be answered YES.

4) Animal Gene Transfer, Question 4: ABSL-2 should be selected as transduced human cells are administered.

5) Risk Group and Containment Practices, Question 2: ABSL-2 should be selected as human cells are being introduced into animals.

6) Exposure Assessment and Protective Equipment, Question 3: This should be answered YES, as centrifugation may cause airborne transmission.

7) Exposure Assessment and Protective Equipment, Question 4: Safety-engineered sharps devices are required for administration to animals as well as harvest of tissue or blood draws. Revise.

8) Exposure Assessment and Protective Equipment, Question 5: Include all PPE required for entrance to the ABSL-2 animal facility.

9) Waste Management, Question 1: Is the investigator anticipating large volumes (beyond normal tissue culture wastes or bacterial grow ups) to be produced? If so, then other sections of the protocol should reflect that. Animal carcasses are not disposed of in biohazard containers but rather through DLAR after appropriate labeling.

10) Waste Management, Question 3: Describe the EPA disinfectant used.

Comments:

The investigator is modifying an existing protocol to add cloning and expression of a T-cell receptor and genetic elements encoding metabolic modulators, costimulatory molecules and other genes via the MSGV-1 Retroviral vector infection of human T-cells and re-introduction into mice. The modified T-cells will be introduced into mice so it seems that recombinant nucleic acids will be given to animals. The Project Summary should be modified to include the new experiments. There are a number of form issues and questions that should be addressed prior to approval. In the amendment the investigators clone TCR (T-cell receptor, non-oncogene) into a Retroviral

expression vector (MSGV-1), produce Retroviruses with RD114 plasmid, transduce the TCR into the human T-cells and transfer the modified T-cells to mice to study T-cell persistence. There is some confusion about the Retroviral expression system used and whether it is BSL-2+ or BSL-2. It appears that since it is a single round infection virus and does not express oncogenes it is appropriate at BSL-2. Once these issues are clarified, recommendation for approval.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

A revised application was provided to address the committee comments and an approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900461
Title:	Amendment for **IBC201600174**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 12/10/2020

Required modifications:

1) Amendment Request, Question 2: If Pertussis toxin will not be used in this study, it should be removed.

2) Primary Cells or Cell Lines: Both the murine and the primary murine cells, skin should clarify under the “Description of Usage” that these tissues will be obtained from mice housed at ABSL-1 that have not had recombinant materials administered to them.

3) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: *Listeria monocytogenes* should only be manipulated within a biosafety cabinet (BSC). Revise the use location to identify an area with a certified BSC.

4) Toxins: Pertussis toxin is described on the modification page, so an entry for it should be provided.

5) Toxins: Double-mutant heat-labile toxin must be handled at BSL-2; revise.

6) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include a description of all recombinant materials, including *Listeria monocytogenes* and Reovirus.

7) Exposure Assessment and Protective Equipment, Question 1: Remove the statement "The listed bacteria are not known human pathogens". Immunocompromised individuals, pregnant women, and women of childbearing age have a greater risk of infection when working with *L. monocytogenes*.

8) Exposure Assessment and Protective Equipment, Question 4: Immunocompromised individuals, pregnant women, and women of childbearing age have a greater risk of infection when working with *L. monocytogenes* and LCMV. Agents should be handled in a certified biosafety cabinet. Any centrifugation of agents should include the use of rotor lids or individual rotor cups with safety lids. Revise.

Comments:

This modification of an existing protocol to study autoinflammatory diseases and microphage activation syndrome will add Reovirus T1L, T3D-RV, and *Listeria monocytogenes* for inoculation of transgenic mice. This amendment seeks to add two Reovirus strains (T1L and T3D-RV) for use in adult mice and a recombinant strain of *Listeria monocytogenes* that expresses the GP33 peptide from LCMV for IV injection into mice. The experiments will be performed at BSL-2/ABSL-2, which is appropriate for these additional agents.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

A revised application was provided to address the committee comments and an approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900462
Title:	Amendment for **IBC201700012**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Include the use of Adenovirus in the Project Summary. The purpose of this question is to explain to the Institutional Biosafety Committee members about the research that involves recombinant or synthetic nucleic acid molecules. It is useful to also identify any potential risks within the scope of the project. Provide a concise summary of what is proposed in the project. Provide information that specifically relates to the work with the recombinant and/or synthetic nucleic acid molecules.

2) Biosafety Summary: Tissues, Blood, or Body Fluids should be checked since murine tissues will be obtained and used *in vitro*.

3) Primary Cells or Cell Lines: If these cells will also be transduced with the proposed Adenovirus, include this in the "Description of Usage" and what the Adenovirus will express. If using Adenovirus in culture, then the recombinant selection must be YES. Also, in other sections, the application states that the cells may be transfected with plasmids. This would also make these cell lines recombinant. Edit and revise the entry.

4) Animal Gene Transfer, Question 2: The response here should be YES.

5) Animal Gene Transfer, Question 3: Unless a helper virus is used, the answer should be NO.

6) Risk Group and Containment Practices, Question 1: Both human cells and Adenovirus are Risk Group 2. Amend.

7) Exposure Assessment and Protective Equipment, Question 1: Adenovirus should be included in the response.

8) Exposure Assessment and Protective Equipment, Question 4: Safety-Engineered Sharps Devices are required for any manipulations with biological agents at BSL-2/ABSL-2 or higher.

9) Waste Management, Question 1b: Indicate the final concentration of *Cidecon* that will be used to decontaminate liquid waste is 1:128 V:V *Cidecon*:liquid waste and that the liquid waste will be carefully poured down the drain after contact time.

10) Waste Management, Question 3: Provide additional detail describing the process for clean-up and decontamination of a biological spill (for example: clean up materials, contact time for disinfectant, disposal of potentially contaminated clean up materials).

Comments:

This is an amendment requesting to add Adenovirus with the aim of testing a new hypothesis that surface coating of the Adenovirus with a polymer will redirect the virus from liver to tumors to improve its targeted delivery to tumors. Virus will be administered to mice via tail vein injection

to target cancer cells, and this work will be done at BSL-2/ABSL-2 which is appropriate. Approval is recommended once questions have been clarified. This is a modification to add Adenovirus to the protocol. Some clarifications are requested regarding how the viruses will be generated. The application may be approved following the investigators responding to comments.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900464
Title:	Amendment for **IBC201800234**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: Tissues should be handled at BSL-2. Edit the entry and revise the response.

2) Primary Cells or Cell Lines: Cells from animals that have had BSL-2 agents administered should be handled at BSL-2. Revise.

Comments:

An amendment was submitted that updates agents to be administered under BSL-2 containment conditions. Agents appear to be wild type. The new materials include a change in previously determined biosafety containment under *Section III-D-4* that requires review by the committee. Placed onto the December agenda for review by membership.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	1; recused for involvement

Protocol:	MOD201900467
Title:	Amendment for **IBC201600096**
Investigator:	REDACTED
Highest BSL:	BSL-1 ABSL-1
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Waste Management, Question 3: Spills require greater contact time with disinfectant; 60 seconds is not sufficient. Revise.

Comments:

This is an amendment to an existing protocol to generate and breed transgenic zebrafish lines. The amendment requests to add BAC constructions containing zebrafish genomic DA, pRED/ET plasmid, and Tol2 transposase. A transgenic strain of zebrafish was added. The new constructs should be described in question 1 on the Recombinant or Synthetic Nucleic Acid Work Description page. The work is described as RG-1, A/BSL-1 which is appropriate. The amended IBC protocol will investigate Zebrafish Developmental and Regeneration. Genome editing technology including Cas9, TALENS, and ZFNs will be used to generate new mutations in zebrafish. Oncogenes such as FGF8, FGF3, wnt1, Ras, Raf, Etv4, FGFR1, FGFR2, FGFR3, beta catenin, Myc, Twist, Snail, Slug, and Foxm1 will be expressed as mRNA injections in 1-cell stage embryos for transient analysis. Investigators in this protocol use Transient injection of mRNA encoding TALEN constructs or Cas9 with sgRNA to 1-cell stage embryos. No viral vectors will be used in this protocol. All work was claimed to be performed at level of BSL-1. The investigator needs to provide more detail information to indicate if CRISPR/Cas9 and guide RNAs are in the same vector. If so, the protocol should be amended to be carried out under the BSL-2+ level according to recent University of Pittsburgh IBC policy. In addition, the Biosafety Operations Manual for the laboratory needs to be provided in the attached documents section of the application.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900468
Title:	Amendment for **IBC201700301**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 12/10/2020

Required modifications:

1) Primary Cells or Cell Lines: Include cell lines and description of Lentivirus production.

2) Viruses, Prions, or Vectors: Human Immunodeficiency Virus (HIV, Types 1 and2) - Lentivirus: describe Lentivirus experiments involving animals under "Description of Usage."

3) Viruses, Prions, or Vectors: Include shRNA under "Inserted Nucleic Acids Information" where appropriate.

4) Recombinant or Synthetic Nucleic Acid Work Description, Question 3d: Clarify that these viral vectors will express shRNA, not CRISPR/Cas and are used to transduce cells in live mice.

5) Exposure Assessment and Protective Equipment, Question 1: Include a statement that human cells or tissues may contain unknown pathogens.

6) Exposure Assessment and Protective Equipment, Question 1: Include Influenza Viruses in the response.

7) Waste Management, Question 1a and 1b: Include the amount of time for disinfection with bleach.

8) Waste Management, Question 3: Discarding any remaining human tissue should be disposed of as pathological waste. Update the supervisor as the previous supervisor has left the University.

https://www.ehs.pitt.edu/sites/default/files/docs/05-006InfectiousWaste.pdf

Comments:

This amendment is to add frozen embryos that will be used by a core facility to create a knockout line of mice which the investigator will maintain as a colony. Updates to the gene editing section should be reviewed by committee membership.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

A revised application was provided to address the committee comments and an approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900486
Title:	Amendment for **IBC201800225**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 12/10/2020

Required modifications:

1) Amendment Request, Questions 2 and 3: Add in the new CRISPR work in addition to funding and personnel.

2) Primary Cells or Cell Lines: For the Human Cell lines and the Other Human cells or cell lines clarify the *in vitro* and *in vivo* use of the cells under "Description of Usage."

Comments:

This is a simple modification to add personnel, but the gene editing section updates indicates the use of gRNA in a separate vector with the Lentiviral vector.

Review comments were provided to the investigator for response. The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

A revised application was provided to address the committee comments and an approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Follows on the next page

Protocol:	IBC201900152
Title:	SPK-3006-101
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• Opt Muscle Biopsy ICF • Pregnant partner ICF • IB • Main ICF • Protocol

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: The first sentence should be removed. Adeno-Associated Viruses are infectious.

2) Exposure Assessment and Protective Equipment: Question 5: The "other" box is selected. Clarify "other" - what are the PPE used?

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: Describe the assay to detect vector shedding from study participants.

4) Human Gene Transfer/Human Clinical Trial: Question 3a: Address precautions regarding transport of study product from where the product will be stored, thawed and prepared and where the infusions will take place.

Comments:

This is a first-in-humans Phase I/II dose-escalation HGT study that uses an AAV-vector to deliver a gene for the lysosomal enzyme acid α-glucosidase (GAA) in adults with late onset of a glycogen storage disease (late-onset Pompe disease, LOPD). Glycogen deposits accumulate in the skeletal and respiratory muscles leading to muscular weakness and respiratory failure. Available enzyme replacement therapy (ERT) has a limited biodistribution and is highly immunogenic, limiting its long-term efficacy. It must be given every two weeks by IV infusion over 4 hrs. The main purpose of this study is to evaluate the safety, tolerability, and potential efficacy of SPK- 3006 (single strand DNA genome) at different dose levels in LOPD participants. It is not known to what extent the dose levels will raise the activity levels of GAA in humans, though based on pre-clinical experience with SPK-3006, it is anticipated that the SPK-3006 dose levels evaluated may result in clinically meaningful GAA activity levels. SPK-3006 is a recombinant AAV vector that contains a bio-engineered capsid known as AAV-Spark100, and a codon-optimized transgene expression cassette to drive synthesis and secretion of GAA in the liver. The AAV-Spark100 capsid is a variant of the naturally occurring serotype AAV-rh74, which has demonstrated high liver tropism. This capsid is also being studied for the treatment of hemophilia B. Interim results from an ongoing Phase 1/2 study of SPK-9001, in which a single IV dose of $5x10^{11}$ vg/kg of SPK-9001 administered to adult 10 male participants with Hemophilia B, showed good tolerability, with no serious AEs during or after vector infusion through 492 weeks of cumulative follow-up. Two AEs of transient, grade 1 elevations in ALT were reported for two participants. The AEs resolved with short-term tapered prednisone treatment. Vector genomes were shed transiently in bodily fluids and generally cleared within 3 weeks (urine) to 32 weeks (PBMCs) of vector administration. Preclinical studies

have been performed in mice, rats, macaques, and African Green monkeys. Some of the macaques administered SPK-3006 generated antibodies against the human transgene product. So far, anti-transgene antibody development has not been reported in any of the previous or ongoing AAV-mediated studies for Hemophilia B. Because slightly lower expression levels were observed in female vs. male NHPs treated at $2x10^{13}$ vg/kg, the study of multiple ascending SPK-3006 doses in humans will be aimed at identifying a therapeutic vector dose in both male and female participants. The starting dose of $2x10^{12}$ vg/kg is based upon data from *in vivo* efficacy studies of SPK-3006, or its predecessor AAV8-sp7-Δ8-coGAA, in a mouse model of Pompe Disease. Two additional dose levels are a middle dose of 6×10^{12} vg/kg and a high dose of 2×10^{13} vg/kg. The dose levels proposed may not be sufficient to raise the vector-derived sec-GAA levels to therapeutic levels capable of preventing or stabilizing further Pompe Disease progression. To mitigate this risk, this study uses a dose-escalation strategy (independent DMC) designed to minimize the number of participants potentially exposed to sub-therapeutic doses. Participants meeting all study eligibility criteria will receive their standard of care ERT regimen for up to 4 weeks after they receive SPK-3006. Guidance has been provided for investigators to re-introduce ERT if GAA expression levels are not sufficient to effectively manage a participant's LOPD. If a participant resumes ERT, the event will not result in participant discontinuation or withdrawal from the study. The participant will remain on study. Study eligibility will be limited to participants who have received ERT for at least 24 months prior to the administration of the SPK-3006. Participants with high anti-GAA titers (above 1:30,000) will be excluded. Anti-GAA binding and neutralizing antibodies will be closely monitored to detect signs of GAA transgene immunogenicity. The sponsor will also monitor potential cellular immune responses to the GAA transgene. Although the risk of developing an anti-GAA transgene neutralizing antibody responses in ERT-experienced LOPD participants is expected to be low, participants will receive prophylactic sirolimus (2 mg/day) beginning 6 days prior to SPK-3006 infusion and continuing through week 12. This dose is consistent with the maintenance dose in transplant participants.

Biosafety: To ensure safety monitoring, the first two participants are dosed 6 weeks apart in a given cohort. Study substance consists of the AAV vector and empty capsid particles. The AAV vector is produced at the sponsor's facility in Philadelphia. The substance is manufactured using transient triple-plasmid transfection in adherent HEK293 cells with a 3-column purification process. The three-column chromatography purification process purifies the empty capsids and full vector together. The Drug Product is filled at SAFC in California. The final Drug Product lot is 0.2 µm-filtered and aseptically filled in a Grade A hood. Vials are 100% inspected prior to bulk packaging. Stability testing data indicates it is stable, when frozen, up to 18 months. It is thawed just prior to use at room temperature. It is combined with normal saline and human serum albumin (HSA), to a total volume of 250 mL, and infused within six hours of thawing. Participants will be closely monitored during and up to 5 hours after IV administration. To reduce the risk of liver toxicity due to excessive expression of sec-GAA, circulating levels of GAA activity will be monitored. If a participant has a plasma GAA activity level of ≥10,000 nmol/ml/hr, dosing of additional participants will be stopped so the DMC and Sponsor can determine whether dosing will continue at the same dose level or at a different dose level (i.e., lower dose), or to end the study. Potential risks related AAV therapy include severe allergic reactions, elevated liver function tests, lack of efficacy or disease progression, GAA immunogenicity, and side effects associated with steroids and/or sirolimus. Saliva, urine and semen will be collected to check for presence of AAV vector shedding. Sirolimus administration may be associated with an increased susceptibility

to infection and the possible development of lymphoma, hypersensitivity reactions, angioedema, fluid accumulation and impairment of wound healing, hyperlipidemia, proteinuria, latent viral infections, and embryo-fetal toxicity. Participants will not be allowed to receive live virus vaccines while taking sirolimus and for three months afterwards. If liver inflammation is suspected at any time after the administration of SPK-3006, a course of corticosteroids may be started. This is a prospective, multinational, multicenter, open-label, non- randomized, first-in human Phase 1/2, dose-escalation study to evaluate the safety, tolerability, and efficacy of a single intravenous infusion of SPK-3006 in adults with clinically moderate, late-onset Pompe Disease receiving enzyme replacement therapy (ERT). Approximately 20 LOPD participants will be treated in sequential, dose-level cohorts. The planned dose levels for this study are: a) Low dose: 2×10^{12} vg/kg b) Middle dose: 6×10^{12} vg/kg, c) High dose: 2×10^{13} vg/kg. The number of participants in each cohort will be determined by safety, by levels of circulating GAA, and by immunogenicity evaluations. Data from participants treated early in the study will enable potential adaptation of the dosing regimen for an optional additional cohort(s) or for an expanded cohort (or cohorts) at selected dose levels. For an individual participant, the study duration is expected to be approximately 66 weeks including: Screening period of up to 14 weeks (to allow for all screening test results), Dosing with SPK-3006 (one day), Follow-up period of 52 (±2) weeks. SPK-3006 is an Adeno-Associated Viral (AAV) vector encoding a bioengineered form of acid α-glucosidase (GAA) that is more efficiently secreted than the wild-type protein. The single stranded DNA genome consists of an expression cassette encoding a codon-optimized human GAA. The aim of hepatic gene transfer with SPK-3006 is to express and secrete GAA from hepatocytes to achieve stable circulating levels of GAA sufficient to cross-correct enzyme deficiency throughout the entire body. To increase GAA expression and secretion from hepatocytes, novel GAA transgenes were generated and placed under the control of a hepatocyte-specific promoter. SPK-3006 has not been administered to humans. Therefore, relevant clinical and non-clinical information from other AAV-mediated gene therapy programs or data from non-clinical studies with SPK-3006 was considered to describe potential risks, which include: severe allergic reactions or anaphylaxis (Participants will be closely monitored during and up to 5 hours after IV administration of SPK-3006.), elevation of hepatic transaminases (Participants will receive a regimen of sirolimus beginning 6 days prior to SPK-3006 infusion and continuing through week 12 (approximately 91 days total), lack of efficacy/disease progression (To mitigate this risk, this study provides a dose-escalation strategy designed to minimize the number of participants potentially exposed to subtherapeutic doses. Participants meeting all study eligibility criteria will receive their standard of care ERT regimen for up to 4 weeks after the administration of SPK-3006.) Risks associated with GAA transgene immunogenicity (Sirolimus has been shown to be safe when administered concomitantly to AAV vectors and has the beneficial effect of promoting the expansion of regulatory T-cells and promoting transgene-specific hepatic immune tolerance, thus reducing the risk of development of anti-GAA immune responses.) SPK-3006 is derived from recombinant AAV (rAAV) and does not contain biohazards. The GAA gene is not sourced from human material, and SPK-3006 does not involve the use of toxins of biological origin. However, it is recommended that the material be handled as an infectious agent. Personnel performing the dilution and dispensing will use Universal Precautions and appropriate PPE. It is recommended that all preparation steps be performed in a Class 2 biological safety cabinet (BSC) or equivalent suitable for preparation of intravenous infusions (Class 100) in the Investigational Pharmacy or appropriate Dispensing Facility, following aseptic procedures.

Comments:
1) Question 3a: Address precautions regarding transport of study product from where the product will be stored, thawed and prepared and where the infusions will take place.
2) Question 5: Exposure Assessment/Protective Equipment: clarify “other”.

The submitted application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC201900158
Title:	Moderna mRNA-3927-P101
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1
Additional Documents:	• 47.2 Prep of BSL-2 Viruses (10-19) • Moderna_3927-P101_USA_Assent (13-17 years) Dose Escalation_V1.0USA1.0_EN_final_19Sep2019_clean • Moderna_3927-P101_USA_Main ICF Dose Escalation Phase_V1.0USA1.0_EN_Final_09Oct2019_clean • mRNA-3927-P101 Moderna Pharmacy Manual (version 1) • HSIC0609 • Moderna_3927-P101_USA_Assent (6-12 years) Dose Escalation_V1.0USA1.0_EN_Final_19Sep2019_clean.docx • HSFM0208PRO • mRNA-3927-P101 Investigator Brochure V1.0 15Aug2019 • 08OCT2019 - mRNA-3927 P101 - Version 2.0 - Signed Version • Moderna_3927-P101_USA_PP ICF Dose Escalation Phase_V1.0USA1.0_EN_Final_02Oct2019_Clean

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 2: Clarify if there is a risk of needlestick to personnel due to IV administration.

2) Human Gene Transfer/Human Clinical Trial: Question 3a: Describe how the product will be accepted/received at the study site, stored prior to preparation for infusion, and any precautions taken.

3) Human Gene Transfer/Human Clinical Trial: Question 1: Link the appropriate IRB study.

Comments:

This new HGT protocol evaluates a lipid nanoparticle-encapsulated mRNA-based therapy to treat a rare inherited metabolic disorder known as Propionic Academia (PA), caused by a lack of a mitochondrial enzyme called propionyl-coenzyme A (CoA) carboxylase (PCC). The enzyme is a heterodimer that has an equal number of PCC alpha and PPC beta subunits. There may be mutations in the PCCA or PCCB subunits. PA may be more frequent in the Greenland Inuits, Middle East, and Amish and Mennonite communities. There is a buildup of toxic metabolites (hyperammonemia, lactic acidosis, ketoacidosis, and other metabolic disturbances). Mortality rates are high in a predominantly pediatric population. mRNA-3927 consists of 2 mRNA Drug Substances, which encode normal human PCCA (hPCCA) and normal human PCCB (hPCCB). They are formulated at a 1:1 molar ratio in a lipid nanoparticle made up of 4 lipids: SM-86 (a novel proprietary ionizable lipid), OL-56 (a novel proprietary polyethylene glycol-lipid conjugate), 1,2 distearoyl-sn-glycero-3-phosphocholine (DSPC), and cholesterol. mRNA-3927 is provided as a sterile liquid dispersion for injection at a concentration of 2.0 mg/mL in 20 mM Tris, 8% sucrose, and 1 mM diethylenetriaminepentaacetic acid (DTPA). The objective of giving mRNA-3927 is to express functional PCC enzyme in the livers of participants with PA. The mRNA Drug Substances

in mRNA-3927 are chemically identical to naturally occurring mammalian mRNA with the exception that the uridine nucleoside normally present in mammalian mRNA is replaced with N1 -methylpseudouridine, a naturally occurring pyrimidine base present in mammalian transfer RNAs. This nucleoside is included in place of the normal uridine base to minimize the indiscriminate recognition of mRNA-3927 mRNAs by pathogen-associated molecular pattern receptors (eg, toll-like receptors). This is a first-in-humans study. The main objectives are to evaluate the safety and tolerability of mRNA-3927 and characterize the PD responses after single and repeated doses of mRNA-3927. Study design is dose-escalation; evaluates safety, PD, PK of mRNA-3927 in participants 1 year of age and older. To justify giving the product to young children, the sponsor performed long-term repeat dosing studies in a mouse model of PA. In the Dose Escalation Stage of the study, the planned study doses are 0.3, 0.6, and 1.0 mg/kg. The proposed starting dose and clinical dosing regimen of 0.3 mg/kg every 3 weeks was primarily based on results from nonclinical pharmacology studies and further supported by an interspecies population PK/PD model as well as 2 GLP-compliant toxicology studies. Additional cohorts may be enrolled to assess alternate doses (not to exceed 1.5 mg/kg mRNA-3927). For participants in the Dose Expansion Stage, the study drug assigned will be mRNA-3927 at a dose and interval selected by data review in the Dose Escalation Stage. Participants will receive up to 12 doses of study drug before entering the 2-year follow-up period. During the study, participants will attend in-participant, out-participant, and home care visits.

Biosafety: Potential risks identified in nonclinical studies include nonspecific inflammation and development of anti-PEG IgM observed in toxicology studies. mRNA-3927 will be administered to participants in a hospital setting with close observation. mRNA-3927 will be given IV over approximately 1 hour. Participants will be pre-medicated with acetaminophen and H1 and H2 blockers, approximately 60 minutes prior to infusion. The infusion time of mRNA-3927 may be slowed or its duration extended up to 4 hours in the event of an infusion-related reaction. Serum will be collected for testing of antibodies to polyethylene glycol (anti-drug antibody) and to PCCA and PCCB proteins. The study design includes limits on the blood sampling considering the pediatric participants and the risk of anemia in this population. The risk to participants is minimized by compliance with the eligibility criteria, study procedures, close clinical monitoring, and timely escalation of AEs that meet DLT/stopping criteria. Coagulation, chemistries, hematology, inflammatory markers, anti-PEG IgG and IgM, and anti-PCCA and PCCB antibodies will be assessed. Participants may experience symptoms associated with cytokine release syndrome. This study is a Phase 1/2, global, open-label multicenter study in participants with Propionic Acidemia (PA) ≥1 year of age (the first 3 participants enrolled in the Dose Escalation Stage will be ≥8 years of age). Propionic Acidemia is an ultra-rare, serious, pediatric, inherited metabolic disorder with significant morbidity and mortality. There are no approved therapies that address this underlying enzymatic deficiency. This study includes 2 stages: a Dose Escalation Stage followed by a Dose Expansion Stage. The study is designed to evaluate multiple ascending doses of mRNA-3927 and to characterize the safety, tolerability, and pharmacological activity (as assessed by biomarker measurements), of mRNA-3927 in participants with PA. The dose escalation portion of the study will have a 30-day screening period, followed by at least a 4-week observation period. After that, there will be 12 doses of IP followed by a one-year safety follow-up. The 12 doses of the study drug will be done 3 weeks apart. There is a 72-hour in-participant stay for the first dose, and 48-hour in-participant stay for the second and third doses. There will be at least three different cohorts (0.3 mg/kg, 0.6 mg/kg, and 1.0 mg/kg) enrolled with at least three

participants in each cohort. There is an option to add an additional cohort to assess alternate mRNA-3927 dose levels or intervals. The intent is to find the most optimal dose before moving on to the dose expansion portion of the study. mRNA-3927 is a novel lipid nanoparticle (LNP)-encapsulated messenger RNA (mRNA)-based therapy that is intended to treat Propionic Acidemia (PA). mRNA-3927 consists of a single stranded mRNA drug substance that encodes normal human propionyl-coenzyme A carboxylase alpha and beta. The mRNA drug substances in mRNA-3927 are chemically identical to naturally occurring mammalian mRNA with the exception that the uridine nucleoside normally present in mammalian mRNA is fully replaced with N1-methylpseudouridine, a naturally occurring pyrimidine base present in mammalian transfer RNAs. This nucleoside is included in the mRNA-3927 drug substances in place of the normal uridine base to minimize the indiscriminate recognition of mRNA-3927 mRNAs by pathogen-associated molecular pattern receptors (eg, toll-like receptors). mRNA-3927 is a novel LNP-encapsulated mRNA-based therapy that has not yet been administered to humans. Thus, the possible risks and adverse reactions associated with IV administration of mRNA-3927 are currently not known. As with any medicinal product administered intravenously, participants who receive mRNA-3927 may experience signs and symptoms consistent with infusion-related reactions (IRRs). Hypersensitivity reactions and other IRRs are regarded as a potential risk following administration of mRNA-3704. Risk mitigation: a) Participants with a history of hypersensitivity to components of the study drug will be excluded from study participation b) Participants will receive premedication with histamine (H1 and H2) receptor blockers prior to the administration of the investigational product to reduce the possibility of a hypersensitivity reaction c) Study drug will only be administered in hospital settings capable of recognizing and managing IRRs. During the hospital stay, participants will be closely monitored before, during, and after the study drug administration d) Participants will be enrolled in a step-wise fashion during the Dose Escalation phase so that the safety of the study drug can be closely monitored before enrolling the next study participant. The intended target organ for mRNA-3927 is the liver. The mechanism of action for mRNA-3927 is to form functional PCC enzyme in the livers of participants with PA. mRNA-3927 is non-integrating, the dose not involve viral delivery and does not alter the human genome. In addition, mRNA-3927 demonstrates transient expression with short persistence.

Comments:
Human gene transfer:
1) Question 3a: Describe how the product will be accepted at CHP, stored at CHP prior to preparation for infusion, any precautions taken.
2) Exposure Assessment: Question 2: Clarify if there is a risk of needlestick due to IV administration

The submitted application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0

Abstained: 0

Protocol:	IBC201900141
Title:	HBV Biology
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ Manual REDACTED 2019

Determination: Approved

Last day of continuing review period: 12/9/2020

Required modifications:

1) Viruses, Prions, or Vectors: Lentivirus HIV-1 - psPAX2 and pMD2.G: later in the protocol, it is stated that Akt and HBx will be expressed using a Lentiviral or Retroviral vector. Include this under "Inserted Nucleic Acids Information".

2) Viruses, Prions, or Vectors: Retrovirus, Moloney Murine Leukemia Virus (MMLV): later in the protocol, it is stated that Akt will be expressed using a Lentiviral or Retroviral vector. Include this under "Inserted Nucleic Acids Information".

3) Viruses, Prions, or Vectors: Baculovirus - Autographa californica Nuclear Polyhedrosis Virus (AcNPV): The source cannot be null. Identify where virus was obtained or created.

4) Viruses, Prions, or Vectors: Retrovirus, Moloney Murine Leukemia Virus (MMLV) - Type VI: The source cannot be null. Identify where virus was obtained or created.

5) Viruses, Prions, or Vectors: Hepatitis D Virus - D3: The source cannot be null. Identify where virus was obtained or created.

6) Viruses, Prions, or Vectors: Hepatitis B Virus - genotype A-D: The source cannot be null. Identify where virus was obtained or created. Identify the source from which the recombinant materials were obtained.

Comments:

Reconsidered at the October meeting; Required modifications from the October meeting:

1) Protocol Team Members: Add protocol team members to this section of the application.

2) Supporting Documents: If work will be using Lentivirus and gene editing, then BSL-2+ is required and the signed copy of the laboratory's Biosafety Operations Manual must be uploaded to the application.

3) Primary Cells or Cell Lines: Clarify whether the laboratory is using Lentivirus to transfect CRISPR Cas and guide RNA in cell lines. If YES, then work should be done at BSL-2+.

4) Exposure Assessment and Protective Equipment: Question 1: Lentiviral vector information: This is the first mention of use of a replication-competent Lentiviral vector in the protocol. If the "pCMVdeltaP1deltaenvpA, pSVIIIenv" packing system from NIH AIDS Reagent Program will be used in work on this protocol, then add this vector/packaging system as a separate Lentiviral vector entry in the Viruses section of the application.

5) Exposure Assessment and Protective Equipment: Question 5: In addition to the PPE listed, PPE required at BSL-2+ includes use of a solid-front laboratory gown, and mucous membrane protection consisting of either a face shield or a combination of safety glasses/goggles and a surgical mask for procedures with a risk of splash or spray performed outside a BSC. Revise accordingly.

6) Primary Cells or Cell Lines: If mouse cells/cell lines will be a target for transduction with Lentiviral vectors expressing CRISPR/Cas9 and guide RNA, these cells will need to be handled at BSL-2+. Clarify and change the biosafety level for the entry/entries as needed.

Initial comments:
In the summary, the researcher is expected to write s brief description of experiments and methods to be performed. However, it seems that the investigator has basically written a statement of the research goals for the laboratory. Remove unnecessary information and just describe what is proposed to be done. If Lentiviral work involves oncogenes, then work with these cell lines will require BSL-2+. The researcher is proposing to use some of these cell lines for Lentiviral vector propagation. In this protocol, the investigator seeks to understand Hepatitis Virus replication using cell culture models from multiple species. Viruses will be used for several purposes in these studies, including native Hepatitis Viruses specific to various species (Hepatitis D, Hepatitis B genotypes A-D, Duck Hepatitis B virus, Woodchuck Hepatitis B virus) and for gene expression or knockdown (Lentivirus HIV-1, Adenovirus Adv-5, MMLV type VI, Baculovirus AcNPV). All Hepatitis vectors are replication competent. Researchers will be vaccinated as a precaution. It is not clear however whether everyone involved in those experiments will be vaccinated or will be given a chance to be vaccinated. Genes targeted in these studies will include DNA repair, chromatin remodeling and epigenetic regulation, and innate immune signaling. It is not immediately clear whether genes to be targeted with viral vectors will include known oncogenes; recommend the investigator make this clear. Finally, the investigator seems to experience an issue with the setting of his laboratory in BSL-2+ containment and requests the possibility of having the BSL-2 related protocol reviewed first. If this is the case, the investigator may want to submit a protocol that contains only BSL-2 agents. Once his laboratory is set for BSL-2+ containment, he will then submit an amendment to add the BSL-2+ agents and change the containment level of his protocol to BSL-2+.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Reconsideration: Significant safety concerns were noted in the application which need to be clarified or explained.

The revised application was placed on the December agenda for reconsideration review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

A revised application was provided to address the committee comments and an approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900147
Title:	Aging and metabolism.
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 12/10/2020

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description: Question 3: The expression of shRNA to decrease expression of tumor suppressor genes in Lentiviral vectors requires work to be performed at BSL-2+. Cdkn2a, GRB1, and PPARG, are all identified as Tier 1 tumor suppressor genes in the Cancer Gene Census, a part of the Catalog of Somatic Mutations in Cancer project maintained and updated regularly by the Wellcome Sanger Institute.

https://cancer.sanger.ac.uk/cosmic

2) Primary Cells or Cell Lines: Cell/Cell Line - Mouse (murine) (Bone Marrow Tissue or Cells, Lymphatic Tissue, Other, Plasma, Blood): The investigator has answered YES to the question of whether these tissues/fluids will be administered to animals, yet this is not reflected under "Description of Usage."

3) Basic Information: Question 3: The summary states "expression of Cas9 and guide RNA from the same Lentivirus" which requires BSL-2+. If this is not the case, then the wording is inconsistent with the remainder of the application. Review and make appropriate revisions to ensure consistency throughout the application.

Comments:

Determination: *Reconsidered at the November meeting;* Required modifications from the November meeting:

1) Supporting Documents: It appears that BSL-2+ will be required for this research, therefore the Biosafety Operations Manual (signed full copy of approved manual) should be uploaded in this section of the application.

2) Exposure Assessment and Protective Equipment: Questions 1 and 4: Language will need to be updated due to expression of shRNA targeting tumor suppressor genes from Lentiviral vectors (BSL-2+).

3) Exposure Assessment and Protective Equipment: Questions 1 and 4: All sections will need to be updated to reflect the BSL-2+ use of viruses encoding CRISPR/Cas and sgRNA in the same vector.

4) Recombinant or Synthetic Nucleic Acid Work Description: Question 3: The expression of shRNA to decrease expression of tumor suppressor genes in Lentiviral vectors requires work to be performed at BSL-2+. Cdkn2a, GRB1, and PPARG, are all identified as Tier 1 tumor suppressor

genes in the Cancer Gene Census, a part of the Catalog of Somatic Mutations in Cancer project maintained and updated regularly by the *Wellcome Sanger Institute* at:

https://cancer.sanger.ac.uk/cosmic

5) Recombinant or Synthetic Nucleic Acid Work Description: Question 3e: The expression of Cas9 and sgRNA from the same vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

6) Viruses, Prions, or Vectors: Retrovirus, Murine Stem Cell Virus (MSCV): The expression of Cas9 and sgRNA from the same vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

7) Viruses, Prions, or Vectors: Lentivirus - The expression of shRNA to decrease expression of tumor suppressor genes in Lentiviral vectors requires work to be performed at BSL-2+. Cdkn2a, GRB1, and PPARG, are all identified as Tier 1 tumor suppressor genes in the Cancer Gene Census, a part of the Catalog of Somatic Mutations in Cancer project maintained and updated regularly by the *Wellcome Sanger Institute* at*:*

https://cancer.sanger.ac.uk/cosmic

8) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system): expression of Cas9 and sgRNA from the same vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

9) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system): Identify the original source of the viral vector under "Source."

10) Viruses, Prions, or Vectors: Retrovirus, Murine Stem Cell Virus (MSCV): Identify the original source of the viral vector under "Source."

11) Primary Cells or Cell Lines: For appropriate cells, include transduction with viruses under "Description of Usage."

12) Primary Cells or Cell Lines: Include a description of cells used to produce Lentiviruses and Retroviruses. Note: expression of Cas9 and sgRNA from the same vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

13) Primary Cells or Cell Lines: Cell/Cell Line - Mouse (murine) (Bone Marrow Tissue or Cells, Lymphatic Tissue, Other, Plasma, Blood): The investigator has answered YES to the question of whether these tissues/fluids will be administered to animals, yet this is not reflected in the "Description of Usage". Clarify the discrepancy.

14) Tissues, Blood, or Body Fluids: Tissue/Blood/Fluids - Mouse (murine): The investigator has answered YES to the question of whether these tissues/fluids will be administered to animals, yet this is not reflected in the "Description of Usage". Clarify the discrepancy.

15) Risk Group and Containment Practices: Question 2; column 1: If BSL-2+ agents will be used, modify the current response.

Initial comments:

This new study looks at how genetics, environmental exposures, and aging these processes interact in the context of cardiometabolic diseases. Using both primary human tissue and blood as well as primary tissues from transgenic mice, they will use basic laboratory techniques to study proteins and RNA of interest. In addition, human and mouse cell lines will be used for *in vitro* experiments that include plasmid transfection or siRNA work. In the protocol, work with CRISPR/Cas is mentioned via viral transduction, so the protocol needs to be upgraded to 2+, a manual must be uploaded, and the IACUC work will need to be updated as well. Bacteria will be used for basic molecular work. Transgenic animals will be used. There are many areas of sparse information, including the CRISPR work and use of other genes not in the description of usage sections. This protocol thus will need to be upgraded to BSL-2+, with a downgrade possible for murine cells after 72-hours per IBC guidance. The protocol describes research regarding cardiometabolic diseases particularly with regard to metabolically relevant tissues such as adipose tissue and the liver. A variety of viruses including a 3-plasmid Lentivirus will be used for expression of shRNA, Cre, and CRISPR/Cas9. Potential issue with CRISPR/Cas and guide RNA on the same vector using Lentivirus – should be BSL-2+. Additionally, Cdkn2a is a tumor so should be used at BSL-2+ if using a Lentivirus. Virus will be injected into the tail veins or fat of the mice. Fat may be removed and transplanted directly or expanded *ex vivo* and injected again. Live wild-type and transgenic mice as well as human and mouse tissues/blood and cell lines are proposed to be used under ABSL-2/BSL-2 guidelines to for a variety of laboratory analyses (protein and RNA analysis and histology). *Escherichia coli* will be used for propagation of plasmid DNA at BSL-1.

Comments:
1) Missing information in the project description.
2) Missing information on viruses, prions, and vectors page – CRISPR/Cas delivery is not listed, nor is whether any of the shRNAs are for tumor suppressors.
3) Potential issue with CRISPR/Cas and guide RNA on the same vector using Lentivirus – should be BSL-2+. Additionally, Cdkn2a is a tumor so should be used at BSL-2+ if using a Lentivirus.

Review comments were provided to the investigator for response. The revised application was placed on the November agenda.

November reconsideration: Significant safety concerns were noted in the application which needed to be clarified or explained. Once a revised application is provided, it will be placed onto the next available agenda.

The revised application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

A revised application was provided to address the committee comments and an approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:
For: 13

Against: 0
Abstained: 0

Protocol:	MOD201900373
Title:	Amendment for **IBC201700077**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED BSL-2+ Manual 2017_ Aril 6 updated • SIGNED copy of IBC

Determination: Deferred/Reconsidered

Required modifications:

1) Waste Management: Question 1b: Define "other EPA-registered disinfectants" with concentrations to be used and time for disinfection.

2) Risk Group and Containment Practices: Personnel must complete the IBC-specific training and must be added to the protocol personnel page for this BSL-2+ protocol.

3) Animal Gene Transfer: Questions 5 and 6: Finish describing which animals will receive 7C1 and/or AAV siRNA, and the exposure route for these materials.

4) Animal Gene Transfer: Based upon the investigator's draft note left in the answer for question 6 that annotated in ALL CAPS, it appears that humanized lymphoid tissues will be implanted into mice by a collaborator, and then mice will be transferred to investigator's laboratory and infected with HIV-1. If work with replication competent HIV-1 and/or tissues from animals infected with replication competent HIV-1 are used in this study, then add an entry for HIV-1 in the Virus/Viral Vector section.

5) Animal Gene Transfer: Animals obtained from collaborators contain full-length, replication-competent HIV-1.

6) Lentivirus and Lentiviral Vectors: Question 2a: Not all Lentiviruses used by the laboratory is replication-defective. The laboratory also uses full-length replication-competent HIV-1. This should be made clear in the response.

7) Viruses, Prions, or Vectors: Based upon the investigator's draft note left in the Animal Gene Transfer Section, it appears that humanized lymphoid tissues will be implanted into mice by a collaborator, and then mice will be transferred to investigator's laboratory and infected with HIV-1. If work with replication competent HIV-1 and/or tissues from animals infected with replication competent HIV-1 are used in this study, then add an entry for HIV-1 in this section.

8) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: Bacteria should be designated as recombinant. Revise the response to question 6 for this agent as it appears that it may be used to grow/amplify plasmids.

9) Primary Cells or Cell Lines: Mouse cells/cell lines: Provide additional information in the "Description of Use" for these cells (for example: similar to the information provided in the human cells/cell lines entry).

10) Primary Cells or Cell Lines: Human stem cells/iPSCs: Provide additional description of work that will be performed with these cells (e.g. will nucleic acids be delivered solely via transfection or will viral vectors be used for target gene delivery).

11) Protocol Team Members: If personnel will be involved in this work then this individual must complete the IBC-specific training and must be added to the personnel section of the application.

Comments:
Determination: *Reconsidered at the October IBC meeting;* Required modifications from the October IBC meeting:

1) Supporting Documents: A recent, and signed copy of the laboratory's Biosafety Operations Manual must be uploaded to the application.

2) Waste Management: Question 3: Identify the supplies available and steps to be followed to clean up a spill of biological materials.

3) Waste Management: Question 1b: Liquid waste: Liquid waste must be decontaminated by addition of concentrated bleach to liquid waste such that the final concentration of bleach is 1:10, v:v, bleach:liquid waste. Adding pre-diluted bleach to liquid waste further dilutes the bleach solution and results in insufficient final concentration of active ingredient to achieve effective decontamination. Revise.

4) Exposure Assessment and Protective Equipment: Question 4: Indicate whether a Biosafety Cabinet (BSC) will be used for all manipulations of potentially infectious materials. In addition, at BSL-2/ABSL-2 and above safety engineered sharps devices are required. Revise the section accordingly.

5) Exposure Assessment and Protective Equipment: Question 1: This question asks the investigator to discuss whether any of the materials used in this protocol are potentially infectious for humans. For example, modified AAV vectors and Lentiviral vectors are both capable of a single round of infection in human cells. Replication-competent HIV is infectious for humans. Also, human cells and cell lines, even when purchased from an off-site vendor, may contain undetected adventitious agents that may be infectious to humans. Revise this section accordingly.

6) Animals: Remove or delete linked ARO protocols that have been completed and are no longer active/approved protocols.

7) Recombinant or Synthetic Nucleic Acid Work Description: In question 3d: Describe how CRISPR/Cas9 will be delivered to target cells. For examples, transfection of plasmid DNA, injection of gRNA and Cas9 proteins, using AAV or Lentivirus as a viral vector.

8) Recombinant or Synthetic Nucleic Acid Work Description: How will siRNAs be delivered to rodents? This work is not described elsewhere in the protocol. Will a viral vector (AAV, Lentivirus) be used for delivery? Will siRNAs be delivered directly? Clarify, and if necessary, add siRNA to the description of use section of appropriate viral vector or cell/cell lines entry.

9) Viruses, Prions, or Vectors: In the Lentivirus - HIV-1 entry the description of use indicates that CRISPR Cas 9 will be expressed from this replication-competent virus. If this is the case this entry should be modified to indicate that these materials are recombinant. If the CRISPR Cas9 will be used with a replication-deficient Lentiviral vector this information should be removed from the current entry and added to an entry for replication-deficient Lentiviral vectors.

10) Viruses, Prions, or Vectors: Lentivirus: HIV-1 entry: In the description of use the investigator indicates that replication-competent HIV will be obtained from a collaborator for use in these

studies. However, the investigator has indicated in the additional virus information that the HIV-1 is replication-deficient. Clarify this discrepancy.

11) Primary Cells or Cell Lines: The human stem cells or iPSC entry indicates that these materials will be administered to animals. Add a description of this work to the description of use for this entry.

12) Primary Cells or Cell Lines: For all cell line entries: In the description of use the investigator indicates that cells and cell lines will be transfected with nucleic acids. Therefore, all of these cells and cell lines will contain recombinant material.

13) Tissues, Blood, or Body Fluids: For rat and mouse entries: Will samples isolated from rats and mice be reintroduced or transplanted into other animals? The response indicates that the investigator answered YES to these materials being administered to animals. Clarify.

14) Tissues, Blood, or Body Fluids: For rat and mouse tissues, blood, and body fluids entries: If tissues or other samples are collected from humanized mice infected with HIV these samples must be handled in the laboratory using BSL-2+ precautions. If rat and mouse samples are collected from animals exposed to risk group 2 biological agents they must be handled at a minimum of BSL-2. Revise accordingly.

15) Risk Group and Containment Practices: All personnel listed as handlers on this BSL-2+ protocol must complete the IBC-specific training module and be added in the Protocol Team Members section of the protocol.

Initial comments:
There were significant clarifications or explanations required for this application. The committee determined that the application should be reconsidered once the investigator provides the responses back to the IBC. There were significant clarifications or explanations required for this application. The committee determined that the application should be reconsidered once the investigator provides the responses back to the IBC. This is a modification to add RNAi and CRISPR/Cas to the protocol to alter expression of regulates disease-associated risk gene expression. There are many areas in regard to the CRISPR/Cas that the investigator does not address correctly, mainly the expression system being used for the CRISPR (it appears to be with Lentivirus as its listed at 2+, but that is not stated). Later in the application, it states that the CRISPR and sgRNA will be transduced separately, but that is inconsistent in other areas of the protocol. There are many form issues and inconsistencies in regard to the CRISPR work, as the protocol appears to be at 2+ already, but the CRISPR work as proposed (on separate vectors) can be at 2. It is unclear if this is introduced *in vivo* or is *in vitro* only. Clarification at the Virus page about the CRISPR and sgRNA is needed. In the original project, the investigator seeks to understand the molecular and genetic causes of pulmonary arterial hypertension (PAH) using cell models (primary and transformed human/rodent cell lines, and human iPSCs) and *in vivo* mouse models, including HIV-infected “humanized” mice expressing human immune cells. Cultured cells are infected with Lentivirus (HIV-1) or Adeno-Associated Virus (AAV-6) constructs to induce or knockout expression of genes of interest. Notably, vascular cells are infected with Lentivirus constructs expressing replication-competent HIV. Genetically engineered and “humanized” mice are infected and studied according to related IACUC protocols. Experiments are conducted under BSL-2+ and ABSL-2 conditions. In the modification, the investigator wants to add the use of RNAi and CRISPR/Cas9 to alter the expression of new genes of interest (some of them oncogenes). The research subjects (transgenic mice?) and the vectors used to infect

(HIV?) need to be clearly defined in the protocol. The potential use of a viral vector to alter oncogenes needs to be discussed with the full committee. There were comments for clarification.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Reconsideration: Significant safety concerns were noted in the revised application which need to be clarified or explained. The investigator was requested to meet with the IBC Chair and Biosafety Officer prior to submitting a revised application for reconsideration review.

The revised application was placed on the December agenda.

Reconsideration: Significant safety concerns were noted in the application which need to be clarified or explained. The investigator must provide a revised application for reconsideration review.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900427
Title:	Amendment for **IBC201900026**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Animals: Question 3: It is not clear how animals will be used in the study. Clarify if tissues, cells, or organs from animals will be used in *in vitro* experiments. For example, if tissues are harvested for tissue culture experiments, organs harvested for electrophysiology (clamp patch) experiments, or for biometric-analysis.

2) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Describe how the laboratory plans to create TMEM39A whole-body knock-out mice using CRISPR/Cas9 technology and use of these mice thereafter. This information must be stated within the protocol.

3) Biosafety Summary: If tissues, blood, or body fluids will be obtained from genetically modified mice, that box should be selected.

4) Basic Information: Question 3: The description of the source of KO mice and type of CRISPR systems used should be stated in the protocol application itself, not in the comment section.

Comments:

Determination: *Reconsidered at the November meeting;*

1) Waste Management: Question 1c: As animals will be used in this protocol, "N/A" is not an appropriate response.

2) Waste Management: Question 1b: Indicate that the final concentration of bleach will be 10% (1:10 v/v).

3) Waste Management: Question 1a: The correct time for decontamination should be 20 minutes. Correct the current response.

4) Risk Group and Containment Practices: Depending on use of CRISPR Cas and gRNA usage, the responses to question 2 may need to be revised accordingly.

5) Recombinant or Synthetic Nucleic Acid Work Description: Question 3e: Clarify what vector will be used and how it will be delivered for "transient transfection" of CRISPR/Cas9 and sgRNA. If viruses are produced with both Cas9 and sgRNA in the same vector, then BSL-2+ is required for this work.

Initial comments:

Reconsideration is required, as the application was unclear as to which vectors will be used for Cas9 and sgRNA expression. The investigator submitted an amendment to their protocol application to add a new strain of mice which will be obtained from a collaborator, and then

maintain that colony of mice. Several pre-screening comments were sent to the investigator and the revised application was placed onto the November agenda.

The revised application was placed on the December agenda for reconsideration review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900107
Title:	Biological Validation of p62ZZIs
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2 Plus Inspection Report 2019 • 036-16 Legacy Protocol for REDACTED - 07-05-16 • BSL-2 Plus Operation Manual - Approved March 2019

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: For the Chinese Hamster Ovary cell line entry: Provide more detail regarding the delivery of si/shRNA to knockdown p62 expression. Will the CRISPR/Cas9 system be used for this purpose? If si/shRNA will be used in addition to CRISPR/Cas9, this information must be added to the Recombinant and Synthetic Nucleic Acids Work Description page and other pages of the form as needed.

2) Viruses, Prions, or Vectors: Baculovirus - pFastBacâ„¢/NT-TOPOÂ®vector: list genes that will be encoded in the Baculovirus vectors under "Inserted Nucleic Acids Information."

3) Recombinant or Synthetic Nucleic Acid Work Description: If si/shRNA will be used to silence expression of p62 in CHO cells then add this information to this section.

4) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include the use of recombinant Baculoviruses in the response.

5) Exposure Assessment and Protective Equipment, Question 1: Question 1: As Lentivirus will be produced and will be used to transduce cells as stated earlier in the protocol, then the first sentence does not make sense and should be revised.

6) Exposure Assessment and Protective Equipment, Question 5: Proper PPE at BSL-2+ consists of either a disposable, solid-front lab gown or a combination of a BSL-2+ dedicated standard laboratory coat with a disposable, solid-front apron. Revise.

Comments:

The investigator seeks to understand the role of the p62 gene by knocking it out in human cancer cells using Lentiviral vectors or plasmid constructs. The protein knockout will be done using either pFastBack vector or Lentiviral constructs obtained from a collaborator at the Loma Linda University. The Lentiviral vector is replication-defective but can infect human cells. Due to its potentially oncogenic nature, all work with the Lentivirus will be done at BSL-2+, while all other work will be done at BSL-2. This renewal protocol will study the impact of nucleoporin p62 ZZ domain inhibitors in mammalian cells, for what ultimate purpose, it is not clear. Lentiviral vectors will be used at BSL-2+ to knockout the p62 gene in human cell lines. The p62 protein and mutant derivatives will be overexpressed in CHO cells (not clear how this will be done).

Initial comments:

Missing information in the primary cells or cell lines section. Off-entry in the bacteria, yeast, fungi section where a vector is listed as another microorganism, should include the sf9 insect cell line. An entry for Baculovirus should be entered. Overall, several instances where clarifications are needed.

The submitted application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900153
Title:	Negative strand RNA virus: attenuation and pathogenesis (BSL-3)
Investigator:	REDACTED
Highest BSL:	RBL
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• IBC Recombinant viruses (Tables)

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Morbilliviruses and "MV" are described later in the protocol and should be listed in the application.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Define "MV" and if this is a virus to be used in this research, it should be listed on the Viruses page.

3) Supporting Documents: Once the final Laboratory Safety Operations Manual has been reviewed and approved then it should be uploaded in the application.

Comments:

This is a well-written, detailed protocol involving work with negative-stranded RNA viruses, specifically Severe Fever and Thrombocytopenia Syndrome virus (SFTSV) and Heartland Virus (HV). Wild-type, laboratory-adapted, and/or attenuated strains will be studied as well as clinical isolates will be used in the studies. Viruses may be recombinantly modified to express reporter proteins (e.g. ECFP, mCherry, etc.) and/or genes from other viruses, or to delete specific genes of interest. Site-directed mutagenesis will be used to alter structural and non-structural genes and non-translated sequences. Natural and synthetic defective interfering minigenomes will be generated and used to test feasibility of use as therapeutic interfering particles. Reverse genetic systems for SFTSV and HV will be generated with standard molecular procedures performed at BSL-2. All work with live SFTSV and recovery of live virus from reverse genetic system will be performed at BSL-3. Work to develop reverse genetic systems for HV will be performed at BSL-2, but for convenience work with live virus and recovery of virus from reverse genetic systems will be done at BSL-3. In addition to the *in vitro* studies, ferrets will be exposed to wild-type and recombinant strains of SFTSV in the RBL at ABSL-3, which is appropriate. At this time HV will not be used in ferrets. The investigator has started development of the investigator-specific Biosafety Operations Manual and the laboratory personnel have started and/or substantially completed the training process. This is a lengthy and well-documented protocol for project investigating the biology of negative stranded RNA viruses. The investigators propose to use ferrets for their *in vivo* work and a large number of human, nonhuman primate, dog, and hamster cell lines in this project. Two viruses (SFTSV and Heartland Virus) will be the primary agents of interest and a wide variety of procedures will be performed to modify these viruses to investigate their biology. Several issues, most of which are likely to be easily addressed, need clarification.

Initial comments:
1) Is any cloning going to be done in *E. coli*? If so, these bacteria need to be included in the appropriate places on the application.
2) The investigator indicates that more than 100 mL of virus will be prepared. Is this accurate, and if so, it will need to be indicated on the subsequent form by checking *NIH Guidelines Section III-D-6*.
3) The correct PPE is indicated for but is listed as 'other' rather than in the checkboxes. It should be indicated in the checkboxes.

The submitted application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900154
Title:	Synthetic Biology and Tissue Reprogramming
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Deferred/Reconsidered

Required modifications:

1) Supporting Documents: The signed laboratory Biosafety Operations Manual should be uploaded into the application.

2) Exposure Assessment and Protective Equipment: Question 1: If the work will be conducted under BSL-2+, and not BSL-2, this should be stated in the answer.

3) Exposure Assessment and Protective Equipment: Question 5: A laboratory coat is required for all work at BSL-1 and above. Revise.

4) Animal Gene Transfer: Question 3: As AAV requires a helper virus, the answer should be YES.

5) Animals: Question1: Link the submitted IACUC protocol.

6) Risk Group and Containment Practices: Question 2: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

7) Recombinant or Synthetic Nucleic Acid Work Description: Question 3e: Describe what type of vectors will be used. Note, expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

8) Viruses, Prions, or Vectors: CRISPR, ZFNs, TALENs, and any other genes that will be expressed in viral vectors should be listed under "Inserted Nucleic Acids Information."

9) Viruses, Prions, or Vectors: Adeno-Associated Viral Vector (AAV) - 1,2,6,8, DJ, KP01: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

10) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system) - third generation lentivirus: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

11) Primary Cells or Cell Lines: For Other Human cells/cell lines and Mouse cells/cell lines entries: Provide more detail in the "Description of Use" to allow the committee to determine the specific "synthetic biology tools" that will be tested in these cell lines (similar to information in Human Stem Cells/iPSC entry).

12) Primary Cells or Cell Lines: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing guidance at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

13) Tissues, Blood, or Body Fluids: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing guidance at: http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

14) Tissues, Blood, or Body Fluids: Tissue/Blood/Fluids - Humans: As human tissues will be added to mice, the question describing administration to animals should be answered YES.

Comments:

This is a new protocol to study tissue regeneration in mouse models and develop technologies to study human regenerative process *in vitro*. They propose to use both primary and cell lines from human and mouse and do *in vivo* and *in vitro* work. Viruses (Lenti and AAV), bacteria (for molecular work) and transgenic animals will be used. This protocol is written as BSL-2 but the investigator indicates that CRISPR and TALEN work will be done in a viral delivery system on the same vector in some experiments and on separate vectors in others. It is unclear which will be done where. Regardless, because of this, the protocol should be at BSL-2+ for this work and consequently ABSL-2 for the work done with viral CRISPR/Cas+sgRNA into animals. Some animals will express Cas9, but again, it is unclear if only those animals will be receiving the sgRNA separately. In addition, use of oncogenes is also noted, but again it is not clear with what delivery system (viral versus plasmid or direct injection) will be used. Some may be used in CRISPR from the protocol. This is the over-reaching issue of the protocol which will require a biosafety manual, inspection, plus areas of clarification for CRISPR use in the protocol. There are several areas of missing or vague information to address as well. Mostly the cell line sections need to have expanded descriptions of use, the entire risk assessment will need to be updated to reflect the work, and more explanation to what will be delivered to animals exactly, and how, needs to be described. Protocol should be discussed at convened meeting due to the higher status needed. In this protocol, the investigator will study tissue re-generation in animal models (mouse). Given the use of CRISPR and TALEN, this protocol should be amended for BSL-2+ as guide RNA and CAS9 will be used in the same backbone. A more detailed description of the protocol and vector maps would be helpful to clarify some issues. If animals are expressing CAS9, will those animals only receive sgRNA and not the combined Cas9/sgRNA vector that they will use in culture. This protocol should be discussed. The investigator states that in cases where Cas9 nuclease will be used, oncogenes will be avoided as much as possible. Given that statement, what potential oncogenes would be used?

The submitted application was placed on the December agenda.

Reconsideration: Significant safety concerns were noted in the application which need to be clarified or explained. The investigator must provide a revised application for reconsideration review.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900422
Title:	Amendment for **IBC201700323**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTEDLab_BSL-2+ 2019 Safety Manual

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system) - HIV-1: CRISPR/Cas9 and gRNA should be listed under "Inserted Nucleic Acids Information."

2) Animal Gene Transfer, Question 1: Clarify what viruses with >50% of their genome will be used in animals, as this is not described anywhere else in the protocol.

3) Exposure Assessment and Protective Equipment, Question 5: PPE at BSL-2+ includes either a disposable, solid-front lab gown or a combination of a BSL-2+ dedicated laboratory coat with a disposable, solid-front apron. Revise.

4) Waste Management, Question 1b: Clarify that concentrated disinfectant will be added to liquid waste so that the final concentration of active ingredient will be that required on the product label. For bleach this means that concentrated bleach should be added to liquid waste such that the final concentration is 1:10 v:v bleach:liquid waste. Define what is meant by "appropriate EPA registered disinfectant" Revise.

Comments:

The investigator is adding additional personnel to the protocol, adding additional mouse cancer cell lines, annual updating of the laboratory biosafety manual and proposing to study a new gene, that encodes the EZH2 protein. The existing protocol specifies BSL-2+/ABSL-2 work and is appropriate for the additional studies. The modification to an existing protocol will add personnel to the research team and includes an upgrade of biosafety level to BSL-2+ which provides for use of a 2nd generation Lentiviral vector.

The submitted application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900449
Title:	Amendment for **IBC201800153**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED BSM

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Include an entry for HSV-1 R3616 recombinant virus. *In vivo* work should be described under "Description of Usage."

2) Viruses, Prions, or Vectors: Zika: *In vivo* work should be described under "Description of Usage".

3) Animal Gene Transfer, Question 1: As replication-competent ZIKV will be administered to mice, the answer should be YES.

4) Animal Gene Transfer, Question 6: Include HSV and ZIKV in the answer.

5) Exposure Assessment and Protective Equipment, Question 1: *Campylobacter jejuni, Acinetobacter baumannii*, and *Helicobacter pylori* can all cause infection in healthy adults and this information should be added to the response.

6) Exposure Assessment and Protective Equipment, Question 5: At BSL-2+ full mucous membrane protection is required for procedures with a potential for splash or spray performed outside of a Biosafety Cabinet (BSC). Indicate that either safety glasses or safety goggles will be used in combination with a surgical mask when needed.

Comments:

The modification is proposed to add several strains of wild type Zika Virus and two strains of Herpes Simplex Virus to a previously approved protocol. The strains of Zika Virus are correctly identified as being non-recombinant and will be handled at BSL-2, which is appropriate. However, one of the viral strains described in the HSV entry (strains: R3616 mutant and wild type F), the R3616 mutant strain, appears to have been generated using recombinant techniques and should therefore be marked as recombinant. The investigator should clarify whether the HSV strain to be added to this protocol is derived from the R3616 strain described in the two attached references, and if so, this modification should be assigned to the IBC for review of the new recombinant materials.

The submitted application was placed on the December agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900463
Title:	Amendment for **IBC201600046**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Vaccinia Signage • Sharps exemption • Safety Manual

Determination: Modifications Required

Required modifications:

1) Funding Sources: Internal funding is indicated but NIH grants are included. Is this protocol in support of the NIH grants and if so, it seems like that option should be selected. If the grants are no longer active, then they should be removed.

2) Tissues, Blood, or Body Fluids: Murine Tissue/Blood/Body Fluids entry: Revise the biosafety level as the information in this entry indicates that samples will be obtained from mice housed at ABSL-1 and ABSL-2. Samples obtained from mice housed at ABSL-2 must be handled at BSL-2 until materials have been inactivated.

3) Viruses, Prions, or Vectors: MSCV entry: Generation of Retrovirus via transfection in HEK293T and use of Retroviral particles to transduce murine cells/bone marrow must be performed at BSL-2 in the laboratory. Transduced cells/bone marrow may be administered to animals at ABSL-1+. Revise biosafety level to BSL-2 to represent work in laboratory and revise downgrade justification language to indicate that production or MSCV particles and transduction will be performed at BSL-2 and transduced cells will be administered at ABSL-1+.

4) Recombinant or Synthetic Nucleic Acid Usage: Selected *Section III-D-2*, as plasmids containing pathogen nucleic acids will be propagated in *E. coli*.

5) Live Animals, Question 1: Question 1: Remove the expired/closed IACUC protocol and update the question with a new or other active protocol.

6) Animal Gene Transfer, Question 1: The answer should be YES for use of replication-competent Vaccinia Virus administration to mice.

7) Exposure Assessment and Protective Equipment, Question 1: The investigator needs to add a statement to address that the use of human cells or cell lines may include unknown infectious agents.

Comments:

Although the Retrovirus is replication-deficient, the transgene expresses an oncogenic protein such as caspase 9. Therefore, the transduced cells *in vitro* and the recipient mice are still suitable to be kept at a BSL-2 level. This is a modification for a previously approved protocol that now incorporates a request for a downgrade to BSL-1 for MSCV use, addition of HEK293 T-cells. The protocol is for a project investigating DC subsets in mice after infection with a variety of pathogenic microbes. Several of the microorganisms, the Vaccinia Virus, and the human cell lines need to be used at BSL-2 conditions while the mice, most of the bacteria, and the MSCV can be

used under BSL-1 conditions. Diphtheria toxin (1 mg) will also be used. The overall protocol is proposed for BSL-2 conditions, which is appropriate. Some minor issues need to be addressed but approval is recommended pending these changes.

Review comments were unable to be provided to the investigator for response prior to the meeting. The application was placed on the December agenda.

Modifications Required: Reviewer and additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	1; recused for involvement

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 11:03 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

January 13, 2020 10:00 AM

302 Heiber Building

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			
REDACTED, Vice Chair			
REDACTED		REDACTED	
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED	Late: 10:30		
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	RCCO Co-Director
REDACTED	EH&S Department

GUEST NAMES
REDACTED, REDACTED, REDACTED, REDACTED, and REDACTED

QUORUM INFORMATION

Committee members on the roster:	20
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present. Introductions for the guests were made. The guests will be observing the proceedings and will be attending the IBC Membership Orientation in the upcoming month.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The December 2019 meeting minutes were reviewed and approved by the committee.

Votes:

For:	9
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None

IBC OFFICE REPORT

The IBC Office will be holding the IBC 101: Basics of IBC Review Workshop in January and continuing to repeat for Spring term months.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Follows on the next page

Protocol:	IBC201900140
Title:	Neurorestorative effect of NAMPT
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 1/13/2021

Required modifications:

1) Viruses, Prions, or Vectors: Identify the source from which the viral vector was originally obtained.

2) Viruses, Prions, or Vectors: Clarify that AAV will only be administered to mice, not rats.

3) Recombinant or Synthetic Nucleic Acid Usage: *Section III-D-2* should be selected if AAV plasmids will be grown in *E. coli*.

4) Exposure Assessment and Protective Equipment, Question 4: Hamilton syringes will be used for intracranial injection; however, safety-engineered sharps devices are still required for blood draws, tissue harvest, etc. Revise.

5) Exposure Assessment and Protective Equipment, Question 5: Hair bonnet and coverall suit are required for entrance to ABSL-2 animal facilities; revise.

Comments:

This is a protocol with the aim of developing a recombinant NAMPT protein as a therapeutic strategy to recovery after stroke. AAV-9 will be propagated in the laboratory to express NAMPT, shRNAs and GFP and will be used in-vitro and injected intracranially into mice. Work is requested at BSL-2/ABSL-2 which is appropriate. Recommend approval once revisions are addressed. This is a new protocol to replace an expired one by this investigator. This study will develop a recombinant NAMPT protein as a therapeutic strategy to recovery after stroke. Animal work (transgenic mouse) and in vitro work is proposed. AAV will be used and propagated in the laboratory to express NAMPT, shRNAs and GFP and will be used *in vitro* and *in vivo*. No CRISPR work and no tumor suppressor work will be done. Bacteria will be used for molecular work. BSL-2 is appropriate for the work. The investigator should provide a bit more elaboration on the use of rDNA in the summary and correct some form issues, but approval at BSL-2 is recommended.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900159
Title:	Neural control of vocalization
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Biosafety Guidelines for Dr. REDACTED's Laboratory • References

Determination: Approved

Last day of continuing review period: 1/14/2021

Required modifications:

1) Live Animals, Question 3: Earlier in the protocol it is stated that tissues will be obtained from animals for *in vitro* studies. Thus, the answer should be YES.

2) Exposure Assessment and Protective Equipment, Question 4: Any use of disposable syringe needle units should be Safety-engineered sharps devices; revise. In addition, in the section 4 paragraph, indicate that 70% alcohol will not be used as the primary disinfectant for equipment as it is not an EPA-registered disinfectant. Remove the reference to the hospital medical waste stream as solid wastes should be disposed of via the biological waste stream.

3) Waste Management, Question 1a: 70% alcohol is not an EPA-registered disinfectant; revise. Disposable syringes and needles should be placed directly into the Sharps container after use; revise. Remove reference to hospital medical waste stream.

Comments:

In this protocol, the investigator seeks to characterize the cortical control of muscles involved in vocalization and breathing. For this, a recombinant of the N2c strain of Rabies Virus will be injected into muscles of macaque monkeys and retrograde cortical neurons will be identified. The investigator has been trained in the virus center and is using a well-characterized virus made by a colleague at the Thomas Jefferson University, Philadelphia PA. Experiments will be performed under BSL-2 and ABSL-2 containment, which is appropriate. There are a few comments and clarification requests before approval. The goal of this proposal is to characterize the pathways underlying cortical control of muscles of vocalization. A recombinant Rabies Virus will be injected into various tissues of monkeys and then a short time later the monkeys will be euthanized, and tissues harvested for histological analysis. This protocol is very straight forward; other than a few minor points it can be approved.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900162
Title:	*C. perfringens* Type F NanH sialidase
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 1/14/2021

Required modifications:

1) Toxins: Provide additional information regarding the enterotoxin: How will the toxin be purified and from what bacteria? This should be included under "Description of Usage."

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Place the information regarding the recombinant work with cell lines in the body of the protocol, not just in the comments.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Provide justification that the proposed modifications will not increase pathogenicity.

Comments:

The proposed study will examine the role of NanH Sialidase in *Clostridium perfringens* type F enteric diseases. Recombinant proteins will be produced in *E. coli*. Cell lines will be treated with *C. perfringens* and enterotoxin, sialidase, and mutants to test CPE binding, cytotoxicity, cell permeability, bacterial growth and binding. Clarification is required regarding purification of toxin. BSL-2 containment is appropriate. This protocol is for a project investigating NanH sialidase in type F isolates of *Clostridium perfringens*. The project summary was limited in detail, but it appears they will treat cells with recombinant NanH or NanJ sialidase and culture supernatants from different *C. perfringens* strains. They will also be treating cells with CPE toxins that are expressed in *E. coli* but there are a significant number of unknown details regarding these procedures. The work is proposed at BSL-2, which may be appropriate, but considering some of unknowns that need to be addressed before the work can be fully assessed, recommending revisions to be made before approval.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900171
Title:	Rat Diabetic Model for Urogynecologic Mesh Implantation
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: If tissues will be isolated from animals kept at ABSL-2, they should be used at BSL-2. Clarify this discrepancy.

2) Viruses, Prions, or Vectors: If the investigator is requesting a downgrade, then BSL-1 should be selected as the appropriately identified biosafety level.

3) Animal Gene Transfer, Question 4: If tissues will be isolated from animals kept at ABSL-2, they should be used at BSL-2. Clarify this discrepancy.

4) Risk Group and Containment Practices, Question 2: It is unclear why BSL-2 and ABSL-2 is selected if the investigator is requesting a downgrade to BSL-1. Select the correct biosafety level.

5) Exposure Assessment and Protective Equipment, Question 4: Safety-Engineered Sharps Devices are required for any manipulations with biological agents at BSL-2/ABSL-2 or higher; revise.

6) Waste Management, Question 1b: Clarify that liquid waste will be decontaminated by adding concentrated bleach to a final 1:10 dilution (v:v) bleach in liquid waste. Addition of pre-diluted 10% bleach to liquid wastes further dilutes the disinfectant and results in insufficient concentration of active ingredient for proper decontamination.

7) Waste Management, Question 1c: Pathological waste is for disposal of human material. Animal carcasses should be sealed in double bags, labeled, and disposed according to animal facility guidelines. Revise.

8) Waste Management, Question 3: Provide additional detail describing the process for clean-up and decontamination of a biological spill (e.g. clean up materials, containment of spill, disposal of potentially contaminated clean up materials).

Comments:

This protocol is for a project investigating the role of macrophages in the urogenital mesh-related dysfunction in females with diabetes. The project uses Wistar rats as a model, and experiments will use cells and tissues isolated rat cells, and in some experiments, these rats and cells will be transduced with AAV to selectively knock down macrophage HDAC3 expression. The work is proposed at BSL-2, which is appropriate. There are minor forms-related issues that need to be addressed, but once that is completed, this work could be approved. This is a protocol to study the role of diabetes in urinary incontinence or pelvic organ prolapse in women, as diabetic women are predisposed to these disorders, resulting in the need for mesh implants and the resulting complications associated with that procedure. The investigator will use a rat diabetic model to test

immune responses to mesh implantation and to test a cell therapy based on AAV to improve outcomes. Primary cells harvested from WT rats will be (BM and monocytes) will be injected into recipient rats to modulate immune response. The AAV is purchased and not generated in the laboratory and will express siRNA to downregulate HDAC3 and will be injected directly into the rats. Blood and other tissues will be harvested from rats and used in downstream analysis such as mRNA studies. No Lentivirus is used, BSL-2 is appropriate for the work. There are some minor form issues, including adding in the associated IACUC protocol, but recommendation is for approval at BSL-2. The disposal of the animal carcasses appears to be incorrect, and should be revised.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900173
Title:	SA-AKI and its Recovery
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 1/13/2021

Required modifications: None

Comments:
This study of infection-associated acute kidney injury will involve breeding of transgenic mice as well as murine macrophage transplants to mice whose macrophages have been depleted via administration of Diphtheria Toxin A (DTA). DTA will be handled at BSL-2 which is appropriate. Modifications are required. This application is to explore the roles and mechanism of immune dysfunction in sepsis-associated acute kidney injury. 100ng solution of DTA will be administered IP daily for 3 or 7 days to iCx3Cr1 Knockout (KO) mice in order to induce the depletion of macrophages for immune dysfunction. No known oncogenes or other toxins will be applied. No viral vectors will be used in this protocol. All work is proposed to be performed at level of BSL-2 which is suitable.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Approval recommended: No additional comments were provided by the committee prior to the meeting. The committee granted approval, and a letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900466
Title:	Amendment for **IBC201600152**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: CRISPR/Cas9 and guide RNA should be included under "Inserted Nucleic Acids Information". Clarify whether the *Origene* Lentiviral vector encoding CRISPR/Cas9 also encodes guide RNA (gRNA).

2) Recombinant or Synthetic Nucleic Acid Usage: If Lentiviral vector plasmids will be grown in bacteria, then *Section III-D-2* should be selected.

3) Recombinant or Synthetic Nucleic Acid Work Description, Section 3e: Clarify whether the guide RNA is on a separate plasmid from the CRISPR/Cas9. Currently the response states that there are "no separate vectors." If they are not separated, then the Lentivirus must be used at BSL-2+.

Comments:

This is an amendment to update the plasmid and virus listings as well as update external funding. The investigator's research is focused on the molecular and genetics basis of human lung and related diseases. The research includes genetic and biomarker studies for several advanced lung diseases, and researchers are currently investigating genes and their associated pathways and their roles in the pathogenesis of lung and related diseases. The investigator will use commercial and primary cells to perform molecular biology works to elucidate the molecular mechanisms related to diseases. The investigator will also generate Lentiviral particles using plasmids designed to express specific target genes of interests, and clone specific DNA segments using total DNA isolated from blood cells for the study of targeted genes functions. This protocol will study the molecular and genetics basis of human lung and related diseases using primary cells and cell lines, expressing or knocking down genes the investigator believes may be involved in disease. A few minor corrections are needed. Of most importance, the investigator may not be aware of the May 2019 IBC Guidance document requiring BSL-2+ if Lentivirus vectors are used to introduce CRISPR/Cas and guide RNA on the same virus.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900484
Title:	Amendment for **IBC201600230**
Investigator:	REDACTED
Highest BSL:	BSL-1 ABSL-1
NIH Guidelines:	• NIH Section III-D-4
	• NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:
1) Waste Management, Question 1c: Pathological waste is for human specimens. Animal carcasses should be sealed in double bags, labeled, and disposed according to animal facility guidelines.

Comments:
The modification is being submitted to update the protocol and identifies that there are recombinant cells being administered to live animals, which falls under *Section III-D-4* of the *NIH Guidelines*. Initially the study only described breeding/cross-breeding, but these new updates, moves the study into the IBC review classification of experiments.

Administrative review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Approval recommended: No additional comments were provided by the committee prior to the meeting.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900501
Title:	Amendment for **IBC201700003**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 1/13/2021

Required modifications: None

Comments:
The investigator proposes to add new reagents and microorganisms to protocol. Specifically, Plasmids p616.1, pWR3.26, pC15-Rz, wild-type Reoviruses, and *Pseudomonas aeruginosa* Bacteriophages. The investigator plans to begin using DNA plasmids to produce Rhinovirus strains and to use Reoviruses. Both will be used to infect mammalian cells *in vitro*. Pseudomonas Bacteriophages will be used to infect *P. aeruginosa* bacteria to grow and expand the phages. Other than adding the specific virus strains that are going to be used, the amendment is suitable for approval.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Approval recommended: No additional comments were provided by the committee prior to the meeting. The committee granted approval, and a letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900505
Title:	Amendment for **IBC201600216**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 1/13/2021

Required modifications: None

Comments:

This modification to an existing protocol requests the use of recombinant *Pseudomonas aeruginosa* strains PA01 and PA14, which have been stably transfected with recombinant DNA expressing tdTomato. This will allow the research group to track the agents in infected mouse corneas. These strains will be obtained from a collaborator. Work is requested at BSl-2/ABSL-2. The modification seeks to add the common *Pseudomonas aeruginosa* strains PA01 (the natural, chloramphenicol-resistant reference strain) and PA14 (a human isolate with increased host range) to an approved protocol. After stably transfecting with tdTomato (fluorescent marker), the strains will be used to infect mouse corneas. The tdTomato will allow the *P. aeruginosa* cells to be easily monitored following infection. The *P. aeruginosa* strains will be obtained from a collaborator and will be handled at BSL-2. Then 200,000 cfus (colony forming units) will be placed onto the corneas of wild type or various conditional mouse (iKlf4CN and iKlf5CN) strains. Differences in colonization and corneal ulcer formation will be assessed. The underlying protocol is approved to use Adenoviral vectors to infect murine corneas and express SLURP-1, as well as to use HSV-1 to induce keratitis. Use of Cholera Toxin on Human Corneal Limbal Epithelial cells is also approved in the parental protocol.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Approval recommended: No additional comments were provided by the committee prior to the meeting. The committee granted approval, and a letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900512
Title:	Amendment for **IBC201700368**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 1/14/2021

Required modifications:

1) Viruses, Prions, or Vectors: Include the Lentivirus, as well as identifying the vector name (pLL and pQE) and state in the "Description of Usage" that no viral particles will be used or generated in the laboratory, only plasmids.

2) Recombinant or Synthetic Nucleic Acid Usage: *Section-III-D-2* may apply for the use of bacteria to clone plasmids as referred to elsewhere in the protocol.

Comments:

This is a protocol modification in which the investigator has included *E. coli* to propagate plasmid DNA and to include Lentiviral vectors in which genes of interest are cloned from the vectors into non-Lentiviral expression plasmids for presumably transfection of human and/or Xenopus cells (although this is not clear). Production of virus will not be conducted. Further clarification on introduction of recombinant materials into cells and animals should be sought from the investigator prior. It is uncertain if this study qualifies for NIH Exemption from IBC review.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900525
Title:	Amendment for **IBC201700365**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 1/13/2021

Required modifications:

1) Exposure Assessment and Protective Equipment, Question 1: Include a statement that human cells may harbor unknown infectious agents.

2) Exposure Assessment and Protective Equipment, Question 4: Safety-engineered sharps devices are required at ABSL-2; include the selection of the devices on the application.

Comments:

In the original protocol, the investigator seeks to find treatments for cystic by targeting how Interleukin IL-17 regulates secretion of CXCR ligands. Bronchial epithelial cells will be transfected with plasmids or infected with Adv-5 Adenovirus to over-express HDAC5. The same virus is also delivered to wild type (WT) or transgenic mice intratracheally to evaluate the effect of HDAC5 overexpression on the progress of tracheal infection by *Pseudomonas aeruginosa*. All work is performed in BSL-2 and ABSL-2 conditions. In this amendment, the investigator proposes to isolate fibroblastic reticular cells from transgenic mice, grow them in culture and transplant them into WT mice by intraperitoneal injection. A few minor corrections are needed before approval. The investigator proposes to modify the "Genetically Engineered Animals: Source" and "Primary Cells or Cell Lines" section. The investigator proposes to isolate fibroblastic reticular cells (FRC) from gene knockout mice and transfer them into wildtype mice.

Comments:

1) Amendment page: Add the new strains of mice and new cell lines that are proposed to be added. Information could not be found in the main protocol.
2) Animal gene transfer: Add the new TLR2,4, 9 KO that the investigator is proposing to add as a part of this modification.
3) Animal gene transfer: In question 6, add the FRCs from different TLR Kos to be administered IP in WT mice.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

See following pages

Protocol:	IBC201900165
Title:	BioMarin 307-201
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• HSIC0609 • BMN 307-201 Adult ICF_V3_15NOV2019_US V1 • BioMarin 307-201 Risk Assessment, updated 12-02-2019 • BMRN 307-201_Pharmacy ManualVersion 1.0 FINAL 25SEP2019_Signed • BMN 307-201 ParentGuardian ICF_V3_15NOV2019_US V1 • 47.2 Prep of BSL2 Viruses (10-19) • HSFM0208PRO • HSIC0604 • BMN 307 IB v2.1_06DEC2019 • Protocol 307-201 A1 • PTCMDIV820d

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: The response is incorrect as AAV can infect human cells. In addition, include a statement that human cells (i.e. blood, fluids collected from the study subjects) may harbor unknown infectious agents.

2) Exposure Assessment and Protective Equipment: Question1: Indicate that AAV vectors like BMN 307 may be transmitted by spills, splashes, aerosols, or needle stick exposures.

3) Viruses, Prions, or Vectors: AAV can infect human cells. Modify the response to indicate that this is true.

4) Viruses, Prions, or Vectors: In the virus entry, indicate that the vector is replication-defective. “Description of Usage” section.

5) Tissues, Blood, or Body Fluids: The source of materials is not from the company, but rather from participants enrolled in the study. The materials are manipulated by the company but the original source of the materials is the study participant.

6) Tissues, Blood, or Body Fluids: As body fluids may contain recombinant AAV, they should be designated as recombinant.

7) Human Gene Transfer/Human Clinical Trial: Materials: Question 10: Describe the intended *ex vivo* or *in vivo* target cells (tissues, or organs) and the transduction efficiency. Transduction is the process by which genetic material is inserted into a cell by a virus. Transduction efficiency is a measure that indicates how well a gene is incorporated into cells by the vector. Some vectors will transduce cells at a higher efficiency than others.

8) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: The current response is incorrect. Describe the procedures that will be used to test vector shedding.

9) Human Gene Transfer/Human Clinical Trial: Materials: Question 8: Describe the buffer in which the viral particles will be suspended.

10) Human Gene Transfer/Human Clinical Trial: Materials: Question 7a: Indicate that participants will receive prophylactic steroids.

11) Human Gene Transfer/Human Clinical Trial: Materials: Question 7: This is a first in humans Phase 1 study for this product. Indicate there could be possible toxicities that are not yet widely known. Check YES.

Comments:

This is an open-label HGT study of BMN 307, which is an Adeno-Associated Virus vector-mediated gene transfer of human phenylalanine hydroxylase (PAH) in persons with phenylketonuria (PKU - inability to metabolize phenylalanine (Phe) into tyrosine) with Phe levels exceeding 600 umol/L. Mutations in PAH lead to deficient metabolism of Phe and increased plasma Phe levels and neurocognitive and neuropsychiatric disorders in patients. BMN 307 carries a codon-optimized version of the human PAH gene with a liver-targeted promotor to hepatocytes, which may result in active human PAH enzyme production in the liver. *In vivo* primary PD studies demonstrated that a single dose IV administration of BMN 307 in ENU2 mice normalized plasma and brain Phe levels long-term. There is existing data regarding the safety and biodistribution of AAV5 gene therapy and experience with an AAV5-gene therapy for hemophilia A currently in Phase 3 development. There is no previous clinical experience with BMN 307. The goal of the study is to achieve Phe reduction with consistent normal or near normal plasma Phe levels and a long-term sustained response. The study has a dose escalation phase (Part A, 18 years of age and older), followed by a dose expansion phase (Part B, 15 years of age and older, about 30 participants). Guidance on dose selection (dose cohort expansion, dose escalation) will be based on Data Review Board (DRB) evaluation of safety, tolerability, and preliminary PD data. During Part A (dose finding), three dose cohorts are proposed (2E13 vg/kg, 6E13 vg/kg, 2E14 vg/kg), with enrollment of up to 6 participants per cohort. Based on DRB review, more than 6 participants and up to 10 participants may be enrolled in each dose cohort. At least 1 participant in each of the expanded dose cohorts will have a mean plasma Phe more than 1200 µmol/L during screening. Participants will receive a single IV dose of BMN 307 and followed for 5 years. Two participants will enroll in dose cohort 1 of Part A and receive a single IV administration of BMN 307 at 2E13 vg/kg. The 2 participants will be dosed at least 1 week apart. After 24 weeks, participants whose plasma Phe levels are less than or equal to 360 µmol/L at two consecutive visits will be allowed to increase intact protein intake per an algorithm. Up to 100 participants will be enrolled and participate in the study assuming 4 doses are studied in Part A and cohorts are maximally expanded (n = 10) and 2 doses are included in Part B (n = 30 for each dose).

Biosafety: BMN 307 consists of a recombinant, replication-defective vector based on Adeno-Associated virus (AAV) serotype 5. The two AAV coding genes rep and cap have been deleted and the viral Inverted Terminal Repeats (ITRs) responsible for genome packaging and episomal retention retained. The therapeutic expression cassette encodes a codon-optimized cDNA for human PAH and includes a liver-specific enhancer from the human gene for ApoE, a liver-specific promoter from the human gene AAT, a hybrid intron, and a polyadenylation sequence from the bovine growth hormone gene. BMN 307 viral lots are packaged using insect Sf9 cells and Baculovirus transfer vectors. Replication-competent AAV has not been seen with this system. No participant developed neutralizing antibodies against Factor VIII in BioMarin's hemophilia gene therapy program using the same AAV5 capsid. Immunogenicity of BMN 307 will be monitored and include assessments of anti-AAV5 capsid and anti-hPAH total binding antibodies as well as hPAH- and capsid-specific cellular immunity. Participants with detectable antibodies to the AAV5

capsid at screening will be excluded. Pre-clinical studies in a PKU disease mouse model and in non-human primates demonstrated evidence of efficacy and safety. The nonhuman primate studies demonstrated safety at a dose of 2 $x10^{14}$ vg/kg, the highest dose proposed for this trial, with dose-dependent mild to moderate liver inflammation demonstrated by elevated transaminase levels. BMN 307 is manufactured centrally and formulated in 10 mL vials with a fill volume of at least 8 mL. The vector is suspended in buffered saline with surfactant at a concentration of 6 x 10^{13} vg/mL. Vials are shipped to sites on dry ice and stored at less than or equal to -60ºC. BMN207 must be administered within 8 hours of removal from the refrigerator. The participant will be observed for at least 8 hours following infusion

The risk for germline transmission is thought to be low. To minimize risks, sexually active male participants must use effective contraception for at least 12 weeks post-BMN 307 administration. After 12 weeks, male participants may stop contraception use if they have had 3 consecutive semen samples with no detectable viral vector DNA. Women of childbearing potential must have a negative serum pregnancy test prior to entry and agree to use effective contraception throughout the study. Participants will receive prophylactic oral prednisolone to reduce the risk of liver inflammation associated with systemic AAV vector administration. Blood, saliva, semen, urine, and stool samples will be collected for vector shedding analysis. The risk from these shedding vector genomes is considered to be very low, because the vector is replication-defective and vector genomes alone are not expected to be capable of efficient gene transfer. AAV vectors like BMN 307 may be transmitted by spills, splashes, aerosols, or needle stick exposures. The use of concentrated high titer vector stocks and the procedures associated with formulation prior to infusion increases these risks. BMN 307 will be prepared inside a biological safety cabinet to minimize these risks and PPE will be worn during preparation and dosing. Intact AAV is environmentally stable, necessitating care when decontaminating work surfaces. BMN 307 is based on a recombinant Risk Group 1 AAV virus, requiring the use of BSL-1 containment under *NIH Guidelines*. Staff are to avoid droplet and aerosol exposures and adequately decontaminate work surfaces.

Initial comments:
1) Viruses, Prions, Vectors: Indicate that the vector is replication-defective.
2) Human Gene Therapy/Human Clinical Trial: Materials, Question 7: This is a first in humans Phase 1 study for this product. Indicate there could be possible toxicities that are not yet widely known. Check YES.

3) Human Gene Therapy/Human Clinical Trial: Materials, Question 7a: Indicate that participants will receive prophylactic steroids.

4) Exposure Assessment, PPE Question 1: Indicate that AAV vectors like BMN 307 may be transmitted by spills, splashes, aerosols, or needle stick exposures.

The submitted application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:
For: 9

Against: 0
Abstained: 0

Protocol:	MOD201900530
Title:	Amendment for **IBC201900092**
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-05-22 Appendix M_19-031 • 2019-04-26 HS-IC0616 Guidelines for Handling Sharps_19-031 • 2019-06-19 Prescreen ICF Template_V 2.0_19-031 • 2019-08-01 Protocol v3 tracked changes_19-031 • 2019-08-01 Protocol v3.0_19-031 • 2019-10-23 Administration Memo - Clarification of Product Administration_19-031 • 2018-06-29 HS-FM0208PRO Waste Management PROCEDURE_19-031 • 2019-08-01 Summary of Changes-PA3_19-031 • 2018-12-13 Investigator Brochure_19-031 • 2019-02-25 IP Manual v0.1_19-031 • 2018-01-30 HS-IC0604 OSHA Bloodborne Pathogen Standard Exposure Control Plan_19-031 • 2019-06-19 Main ICF v3.0_19-031

Determination: Approved

Last day of continuing review period: 1/13/2021

Required modifications:
No changes required. Expiration date has been updated, starting another full year.

Comments:
This is an amendment to a previously approved IBC study. It is phase 1/2 multicenter study designed to determine the safety of autologous *ex vivo* expanded T-cells [CAR T-cells] that are genetically modified using a self-inactivating (SIN) Lentiviral vector to express a gamma/delta T-cell receptor (TCR)-based molecule targeting the AFP peptide/HLA-A2 complex and a CD28-based molecule targeting GPC3. It will be administered to participants with AFP-expressing advanced HCC and at least one HLA-A2 allele. Study population consists of participants with advanced liver cancer who are AFP positive/HLA-A2 positive, with few remaining treatment options. HCC participants overexpress AFP and GPC3 (human glypican). AFP is normally present in fetal but not adult cells and is a target for treatment of HCC. The ET140202 transgene consists of 3 parts: i) the AFP158/HLA-A2 Binding Component, an antigen-binding (Fab) fragment derived from ET1402L1, a human antibody against the AFP158-166 peptide/HLA-A2 complex; ii) an Effector Component, which consists of portions of the gamma (γ) and delta (δ) chains of an endogenous γδ TCR; and, iii) a Co-stimulatory Component, a CD28-based molecule that recognizes and binds human GPC3.

Modifications to the study do not affect biosafety parameters. Modifications include: changes to the study title, inclusion and exclusion criteria, dose escalation processes, clarifying what is and is

not a dose-limiting toxicity, windows for study product infusions, and the adverse event and safety sections (AEs that occur during the Screening Period will be recorded in EDC on the Medical History case report forms; and, AEs that begin or worsen after enrollment eligibility will be recorded in EDC on AE case report forms). Other modifications include: PET scans are not required during the screening period; a CBC with differential should be done on the day of leukapheresis; HLA class I allele typing may be completed during pre-screening with an approved prescreening consent form; clarified which tubes should be used to collect study samples in the Schedule of Assessments; systemic steroids must be stopped 14 days prior to infusion; and, the first and second participant at each dose level will receive their 1st (on day 0) and 2nd (on day 15) infusion in the in-patient setting. The required length of stay in the hospital after each of these infusions is approximately 2 days (depending on the investigator's medical judgment). All other participants (including 3rd participant at each dose level) may receive their initial infusion and subsequent infusion(s) in the out-patient setting.

The modified application was placed on the January 2020 agenda.

Approval recommended: No additional comments were provided by the committee prior to the meeting. The committee granted approval, and a letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900154
Title:	Synthetic Biology and Tissue Reprogramming
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED_Pitt SOP

Determination: Modifications Required

Required modifications:

1) Supporting Documents: Contact Pitt EH&S to discuss getting the laboratory areas approved for BSL-2+ work and submitting the Biosafety Operations Manual for EH&S, IBC, and DLAR review. The SOP attached is not the required document.

2) Exposure Assessment and Protective Equipment: Question 4: Safety-engineered sharps are required for work at BSL-2/ABSL-2 and above. Indicate that safety-engineered sharps devices will be used in these studies.

3) Exposure Assessment and Protective Equipment: Questions 1-2: All work with Lentivirus or AAV should be handled as infectious, regardless of expression of CRISPR, as they are human derived viruses.

4) Exposure Assessment and Protective Equipment: Question 2: If needles will not be used with 2+ agents, what will be used instead to deliver the agents to the animals?

5) Exposure Assessment and Protective Equipment: Question 3: Centrifugation increases the risk for airborne transmission and all BSL-2+ agents should be centrifuged in sealed rotor buckets and opened in a biosafety cabinet.

6) Exposure Assessment and Protective Equipment: Question 4: It is stated that "handling mouse with needles that carry CRISPR, special instructions will be followed" but in the response to question 3, it is stated that no needles will be used. This is inconsistent, and needs to be revised.

7) Risk Group and Containment Practices: Note that Lentivirus, even replication incompetent, is still HIV derived and thus is considered to fall under the *NIH Guidelines* Risk Group 3 (RG3).

8) Lentivirus and Lentiviral Vectors: Provide a bit more detail about the safety features and what each of those separate plasmids encodes.

9) Viruses, Prions, or Vectors: Identify the vector being used (pLOC, for example), not just "third generation Lentivirus" What is the source of the vector originally- this "source" pertains to source of the vector itself, not the working stocks.

10) Primary Cells or Cell Lines: Human stem cells/iPSC entry: Delivery of CRISPR/Cas 9 systems using Lentiviral vectors must be performed at BSL-2+. Revise the biosafety level or remove language regarding use of CRISPR for this entry as appropriate.

Determination: ***Reconsidered at the December 2019 IBC meeting***
Required modifications from the December meeting:

1) Supporting Documents: The signed EHS-approved BSL-2+ biosafety manual should be uploaded here.

2) Exposure Assessment and Protective Equipment: Question 1: If the work will be conducted under BSL-2+, and not BSL-2, this should be stated in the response.

3) Exposure Assessment and Protective Equipment: Question 5: A laboratory coat is required for all work at BSL-1 and above. Revise.

4) Animal Gene Transfer: Question 3: As AAV requires a helper virus, the answer should be YES.

5) Animals: Question1: Link the submitted IACUC protocol.

6) Risk Group and Containment Practices: Question 2: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing policy at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

7) Recombinant or Synthetic Nucleic Acid Work Description: Question 3e: Describe what type of vectors will be used. Note, expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing policy at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

8) Viruses, Prions, or Vectors: CRISPR, ZFNs, TALENs, and any other genes that will be expressed in viral vectors should be listed under "Inserted Nucleic Acids Information."

9) Viruses, Prions, or Vectors: Adeno-Associated Viral Vector (AAV) - 1,2,6,8, DJ, KP01: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing policy at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

10) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system) - third generation lentivirus: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing policy at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

11) Primary Cells or Cell Lines: For Other Human cells/cell lines and Mouse cells/cell lines entries: Provide more detail in the description of use to allow the committee to determine the specific “synthetic biology tools” that will be tested in these cell lines (similar to information in Human Stem Cells/iPSC entry).

12) Primary Cells or Cell Lines: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing policy at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

13) Tissues, Blood, or Body Fluids: Expression of Cas9 and sgRNA from the same viral vector requires BSL-2+. Refer to IBC gene editing policy at:

http://www.ibc.pitt.edu/sites/default/files/documents/IBC%20Gene%20Editing%20Policy%2013May19.pdf

14) Tissues, Blood, or Body Fluids: Tissue/Blood/Fluids - Humans: As human tissues will be added to mice, the question describing administration to animals should be answered YES.

Comments:
This is a new protocol to study tissue regeneration in mouse models and develop technologies to study human regenerative process *in vitro*. They propose to use both primary and cell lines from human and mouse and do *in vivo* and *in vitro* work. Viruses (Lentivirus and AAV), bacteria (for molecular work) and transgenic animals will be used. This protocol is written as BSL-2 but the investigator indicates that CRISPR and TALEN work will be done in a viral delivery system on the same vector in some experiments and on separate vectors in others. It is unclear which will be done where. Regardless, because of this, the protocol should be conducted under BSL-2+ for this work and consequently ABSL-2 for the work done with viral CRISPR/Cas+sgRNA into animals. Some animals will express Cas9, but again, it is unclear if only those animals will be receiving the sgRNA separately. In addition, use of oncogenes is also noted, but again it is not clear with what delivery system (viral versus plasmid or direct injection) will be used. Some may be used in CRISPR from the protocol. This is the over-reaching issue of the protocol which will require a biosafety manual, inspection, plus areas of clarification for CRISPR use in the protocol. There are several areas of missing or vague information to address as well. Mostly the cell line sections need to have expanded descriptions of use, the entire risk assessment will need to be updated to reflect the work, and more explanation to what will be delivered to animals exactly, and how, needs to be described. Protocol should be discussed at convened meeting due to the higher status needed. In this protocol, the investigator will study tissue re-generation in animal models (mouse). Given the use of CRISPR and TALEN, this protocol should be amended for BSL-2+ as guide RNA and CAS9 will be used in the same backbone. A more detailed description of the protocol and vector maps would be helpful to clarify some issues. If animals are expressing CAS9, will those animals only receive sgRNA and not the combined Cas9/sgRNA vector that they will use in culture. This protocol should be discussed at the next meeting. The investigator states that in cases where Cas9 nuclease will be used, oncogenes will be avoided as much as possible. Given that statement, what potential oncogenes would be used?

The submitted application was placed on the December agenda.

Reconsideration: Significant safety concerns were noted in the application which need to be clarified or explained.

Review comments from the December meeting were provided to the investigator for response. The revised application was placed on the January 2020 agenda for Reconsideration review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900144
Title:	Bioactive fatty acid induced cell signaling
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Reconsidered

Required modifications:

1) Supporting Documents: If the protocol will be approved with BSL-2+ containment, then a Biosafety Operations Manual and laboratory inspection upgrade will need to be completed.

2) Waste Management: Question 3: Contact time for spill should be 20 minutes. In addition, clarify that the disinfectant used to clean equipment will be EPA-registered.

3) Waste Management: Question 1b: Clarify that liquid waste will be decontaminated by adding concentrated bleach to a final 1:10 dilution (v:v) bleach in liquid waste. Addition of pre-diluted 10% bleach to liquid wastes further dilutes the disinfectant and results in insufficient concentration of active ingredient for proper decontamination.

4) Exposure Assessment and Protective Equipment: Question 5: University Guidelines for work at BSL-2+ also requires the use of double gloves and a solid-front wrap around gown (additional information is available at the website:

(https://www.ehs.pitt.edu/sites/default/files/docs/05-016Lentivirus.pdf). Revise the application accordingly.

5) Risk Group and Containment Practices: Question 2: BSL-2+ should be selected if TSG will be knocked down using a Lentivirus/Lentiviral vector.

6) Viruses, Prions, or Vectors: Clarify if the tumor suppressor gene, LKB1 (STK11), will be knocked down via Lentivirus. This work may need to be conducted at BSL-2+.

7) Viruses, Prions, or Vectors: "Stbl3 *E.coli* strain" should be removed from the viral vector designation, as this is a bacterial strain.

8) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: The application identifies pFN18A Halo Tag T7 flexi vector elsewhere in the protocol for protein expression, but KRX is described here. Clarify this discrepancy.

9) Primary Cells or Cell Lines: The project summary states that shRNA and siRNA studies will be performed in HepG2 cells. Here, mouse cell lines are used for knockdown studies. Clarify.

10) Primary Cells or Cell Lines: Nonhuman primate cells (COS-7) should be used at BSL-2.

Comments:

The goal of this protocol is to address how bioactive fatty acids lead to post-translational modifications of proteins and subsequent changes in cell function. Mouse and human hepatocyte cell lines will be used for gain/loss of function studies. siRNA for LKB1 will be delivered via lipofectamine transfections and shRNA studies will be performed with a 4-plasmid Lentiviral system. Human HepG2 cells will be used for siRNA and shRNA knockdown as stated in the project summary; however, mouse cell lines are also listed for knockdown studies in the protocol.

Monkey kidney cells (COS7) will also be used for overexpression studies, and these cells are currently listed as BSL-1. As noted, the cell line experiments should be performed at BSL-2. No animal experiments are proposed. If revisions are made, the protocol could be suitable for approval. The investigator is studying proteins involved in bioactive fatty acids in human, nonhuman primate, and mouse cell lines. Cell lines will be obtained from *ATCC* and will be studied *in vitro*, including expression of siRNA or shRNA targeting LKB1. shRNA will be expressed in cells using a 4-plasmid Lentiviral vector purchased from *Addgene*. siRNA and plasmids will be transfected into cells. All cell work will be performed at BSL-2. The exception in the protocol is nonhuman primate cells, which is listed at BSL-1 but should be performed at BSL-2. *E. coli* will be used to propagate plasmids and for protein expression studies at BSL-1. No animals will be used in the protocol. A few clarifications are required prior to approval at low risk.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda. Additional comments were provided regarding the use of gene editing and a tumor suppressor gene. The information will be discussed at the meeting.

Action: Reconsideration: The committee discussed whether the investigator should be at BSL-2 or use BSL-2+ and it was determined that the investigator needs to revise the application and be clear upon which biosafety level is going to be used with this research study. IBC recommends BSL-2+ if there is qualifying work with Lentiviral vectors. Reconsideration review at the next available IBC meeting once the revisions have been completed.

Supporting documents: None

Votes:

For:	10
Against:	0
Abstained:	0

Protocol:	IBC201900160
Title:	Mucosal Pathogen Colonization and Invasive Disease
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Waste Management: Question 1a: Ethanol solutions are not an EPA-registered disinfectant and should not be used for primary decontamination. List another EPA-registered disinfectant such as bleach, Vesphene, etc. An ethanol rinse may be used after sufficient contact time with an EPA-registered disinfectant.

2) Waste Management: Question 1a: Define the "EPA-registered disinfectant" to be used and at what concentration.

3) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells or cell lines may harbor unknown infectious agents

4) Exposure Assessment and Protective Equipment: Question 5: List all PPE required for entrance to the ABSL-2 animal facility. Full face protection (goggles and surgical mask or surgical mask and full-face shield) is required.

5) Exposure Assessment and Protective Equipment: Question 4: Ethanol solutions are not an EPA-registered disinfectant and should not be used for primary decontamination. Identify another EPA-registered disinfectant such as bleach, Vesphene, etc.

6) Exposure Assessment and Protective Equipment: Question 1: All MRSA exposure incidences must be reported to Employee Health Services immediately. MRSA can be spread from person-to-person, and for this reason it is considered a threat to both laboratory personnel and the surrounding community if exposure occurs. When reporting the exposure, be sure to specify that the agent is an antibiotic resistant strain of *Staphylococcus aureus*. Revise.

7) Exposure Assessment and Protective Equipment: Question 2: Add the centrifugation of human cell lines and Lentivirus (since the laboratory is generating it) as a risk.

8) Exposure Assessment and Protective Equipment: Question 4: As Lentiviruses and human cells must be handled within a biosafety cabinet, modify the statement "Human pathogens will be handled in a biosafety cabinet when possible" in the response.

9) Risk Group and Containment Practices: Question 1: Risk Group 3 (RG-3) should be selected for work with Lentivirus.

10) Animals: Question 6: YES should be selected if recombinant bacteria will be administered to animals.

11) Viruses, Prions, or Vectors: List non-viral nucleic acids to be expressed from Lentiviral vectors under "Inserted Nucleic Acids Information" (e.g. CRISPR/Cas9, gRNA).

12) Viruses, Prions, or Vectors: The source for the Lentivirus cannot be left blank.

13) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: Identify the Strep B that will be used to isolate the BHC toxin from. If the laboratory is purchasing the toxin already purified, then list the vendor on the "toxins" page of the application.

14) Primary Cells or Cell Lines: Identify the cells used for production of Lentiviruses and describe this information under "Description of Usage."

15) Biosafety Summary: Question 1: It appears that the animals listed in the associated IACUC protocol are transgenic; if this is correct, then select the box for Genetically Engineered Animals, and provide responses to follow-up questions within the application.

16) Basic Information: The IBC strongly recommends that the investigator report and findings of increased virulence to the Department of Environmental Health and Safety (EH&S) and the IBC.

Comments:
This proposal will evaluate bacterial colonization in early development. It is unclear what genes and specific bacterial strains will be manipulated in this study and a plan to minimize potential virulence that can arise from bacterial modifications. A meeting review would be necessary to understand if this BSL-2 or BSL-2+. The goal of this protocol is to evaluate bacterial infection and colonization in pregnancy and early infancy. Gene expression in both mammalian cells and bacteria will be altered and studied both *in vitro* and *in vivo*. There are a few minor questions that need to be addressed. Study personnel are not listed. The principal investigator needs to be more specific about what genes are being modified, particularly in the bacteria. There is a concern that the genetic changes in the bacteria could increase virulence, and the investigator has said they would use less pathogenic strains to mitigate this concern. Accordingly, the investigator should list what specific strains are to be used. Considering some of these bacteria are human pathogens that can cause pneumonic disease, is BSL-2 sufficient for this work?

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Additional comments were provided by the committee the protocol application was recommended for discussion.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900168
Title:	Synthetic Biology and Tissue Reprogramming
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Biosafety manual

Determination: Modifications Required

Required modifications:

1) Supporting Documents: A laboratory Biosafety Operations Manual signed by EH&S and the investigator must be uploaded for review.

2) Exposure Assessment and Protective Equipment: Question 5: University Guidelines for work at BSL-2+ also require use of double gloves and a solid-front wrap around gown (additional information is available on the website:

https://www.ehs.pitt.edu/sites/default/files/docs/05-016Lentivirus.pdf . Revise accordingly.

3) Exposure Assessment and Protective Equipment: Question 4: All human cells must be used in a biosafety cabinet, regardless of whether viruses are used.

4) Exposure Assessment and Protective Equipment: Question 1: As AAV is listed at BSL-2+ earlier, reference to BSL-2 should be removed or revised.

5) Exposure Assessment and Protective Equipment: Question 4: Safety engineered-sharps devices are required for work at BSL-2/ABSL-2 and above. Indicate that safety engineered-sharps devices will be used in these studies.

6) Lentivirus and Lentiviral Vectors: Question 2a: Provide specifics on the safety features of the Lentiviruses.

7) Viruses, Prions, or Vectors: Question 9: Clarify if more than 100 mL of the Lentivirus will be generated at one time. If the volume of Lentivirus will be less than 100 mL during one time of production, then the response should be NO. The volume of more than 100 ml over the course of the experiments would not fall under the criteria, since the total volume is less than 100 mL at one time.

8) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: Clarify if more than 10L of *E. coli* will be produced at one time. If not producing more than 10 Liters of bacterial culture at one time, but this is the expected entire volume, then revise the current response to be NO.

9) Primary Cells or Cell Lines: Update the level of any cells used with the Lentivirus (with guide RNA on the same vector as the Cas gene) as BSL-2+.

Comments:

In this protocol, the investigator seeks to study liver regeneration in living mouse models and isolated human tissue. Mice or mouse-derived immune cells will be genetically modified using CRISPR-Cas9 delivered by plasmids, nanoparticles, or viral vectors. Human induced pluripotent

stem cells will be used to make complex liver organoids, and these will also be modified using CRISPR-Cas9. Viral vectors will include type 3 Lentivirus and Adeno-Associated Virus strains 1, 2, 6, 8, 9, DJ, and KP01. Work is proposed to be done at BSL-2 and ABSL-2. Some clarifications are necessary, and work should be done under BSL-2+ conditions. This protocol describes a study involved in using synthetic biology to program cells including hematopoietic stem cells from human cord blood for improved tissue regeneration and repair. CRISPR/Cas and Zinc Finger approaches will be used to modify gene expression in the liver and other organs and human organoids and a "liver chip" 3D tissue model. Modified cells may be expanded in liver organoids and introduced into immunocompromised mice. *Escherichia coli* will be used for plasmid amplification at BSL-2, it is not clear why the 10L large size samples are chosen. Human tissue, mouse tissue, and various cell lines including embryonic stem cells will be modified using CRISPR using AAV, Lentivirus, or nanoparticles, to target A1AT, LGR5, VEG, Factor VIII, IX, and CFTR genes. These will be, in some cases, introduced into immune compromised NSG or FRGN mice. This is in collaboration with a group at the University of Arizona, and it is not abundantly clear where the recombinant DNA work will be performed.

Initial comments:
1) The study should be changed to BSL-2+ throughout the application.
2) Animal gene transfer question 3 should be YES.
3) Recombinant Usage: Question 3e: If viral vectors are used to express CRISPR/Cas9 and guide RNA are expressed on the same virus, then the work must be performed at BSL-2+ according to IBC recommendations.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	10
Against:	0
Abstained:	0

Protocol:	MOD201900386
Title:	Amendment for **IBC201700014**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED_BSL-2+manual_5152019

Determination: Modifications Required

Required modifications:
1) Primary Cells or Cell Lines: Human cells: As Lentiviruses requiring BSL-2+ will be used to infect cells, the cells should also be listed at BSL-2+.

Comments:
Personnel were added, along with the following additional Lentiviral vectors and genes. This protocol was already at the appropriate BSL/ABSL level for the work, Lentivirus/genes were already being used at the same level, this modification was just additional ones being added. Due to the BSL-2+ level, convened review required for the recommend approval of this protocol. The investigator proposes to add the following: LRT2B; Lentiviral vector for constitutive expression of sgRNAs which includes tdTomato fluorescent marker, LRT2B-Blast V2; Lentiviral vector for constitutive expression of sgRNAs, which includes tdTomato and BlasticidinS resistance markers, FLNS-NG; Lentiviral vector for constitutive expression of the codon-optimized base editor FNLS in mammalian cells, and L3C9PIR; Lentiviral vector that expresses doxycycline-inducible Cas9 and sgRNAs for CRISPR-mediated knockout. The investigator also proposes to add the following genes: ELF1, USP39, BNC2, NT5E,, NFIC, NFIA, NFIX, TFAM, cux1, TEAD1, TEAD3, PARP2, GATA2, GATA3, MEF2 family genes, apobec3c, IRF2, MSH6, LEF, FGFR2, RBPJ, SATB1, SATB2, DHX9, p14, p16, p15, P53, p73, TEAD4, LEKR1, TIPARP, PP1H, CNTLN, CSTF1, FUBP3, NUP153, IGF2BP2, TMA16, MFAP1, HNRNPD, HNRNPU, ELAVL1, KHDRBS1, MAP3K1, and BAMMA1. The investigator also proposes to add new personnel.

Initial comments:
1) Primary cells and cell lines: Describe genes that will be transfected. Especially the ones using Lentivirus.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	MOD201900492
Title:	Amendment for **IBC201600223**
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Biological exposure procedure • Lentivirus guidelines • Biological Spill procedure • REDACTED - BSL-2+ manual November 2019

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: Cells transfected or infected with recombinant nucleic acid materials should be designated as recombinant.

2) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: *E. coli* should be designated as recombinant.

3) Exposure Assessment and Protective Equipment, Question 1: Because some Lentiviruses will be used at BSL-2 (as described earlier in the protocol), the second to last sentence of the response should be modified/clarified.

Comments:

The investigator is requesting a modification to their existing protocol to be compliant with the University's requirements for BSL-2+ for CRISPR/Cas9 work if the guide RNA is on the same vector. There are a few form errors that need to be corrected, after which the protocol can be approved. The investigator will use 4-plasmid Lentiviral vectors to express Cas9 and gRNA in the same vector in cell culture. These will be used at BSL-2+. The laboratory uploaded an EHS-approved Biosafety Operations Manual. Several form errors should be corrected prior to approval.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	0

Protocol:	IBC201900133
Title:	Understanding and Engineering T-Cell Mediated Immunity
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Approved BSL-2+ Manual

Determination: Approved

Last day of continuing review period: 1/14/2021

Required modifications:
1) Waste Management, Question 2: *Wescodyne* should be used at a ratio of 3 ounces *Wescodyne* to 5 gallons liquid waste in order to comply with the manufacturer's EPA-registered product label. Revise the response to indicate that bleach will be 1:10 v:v bleach:liquid waste and *Wescodyne* will be diluted according to the product label.

Comments:
The investigator proposes to use cell-line based assays to identify the antigens of T-cells from mouse tumor models and human samples. This includes single cell high throughput sequencing, isolating, cloning, and testing T-cell receptors, screening T-cell receptors against libraries of antigen targets, and validating targets in cell-based assays. The investigator also proposes to generate novel chimeric antigen receptors to modulate T-cell immunity. This includes designing and cloning new receptors, expressing them in primary human hematopoeitic cells and cell lines, and performing cell-based assays to test their function, and adoptive transfer of modified cells in mouse models of autoimmunity. All work proposed at BSL-2+. Using an antigen expressing library and cell-based validation assays. Further the investigators will engineer human cells to express novel TCR it is unclear whether these modified cells will be passive transferred into mice. They describe production of Lentivirus libraries expressing oncogenes and/or CRISPR/Cas9 to knockdown host miRNA and or tumors suppressor proteins and this work is listed as BSL-2+. However, no Biosafety Operations Manual is provided for review.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:
For: 9

Against: 0
Abstained: 1; recused for involvement

Protocol:	MOD201900490
Title:	Targeting extracellular signaling-regulated kinase 5 (ERK5) in brain tumors and their microenvironment. Amendment for **IBC201700191**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Vector maps • Vector in retroviral backbone • Lentiviral packaging vector 1 • Lentiviral packaging vector 2 • Vector maps • 1. shRNA Lentiviral amendment package 11.19.19 • Vector in retroviral backbone • Lentiviral packaging vector 3 • Vector maps • Vector maps • Vector in retroviral backbone

Determination: Modifications Required

Required modifications:
1) Risk Group and Containment Practices, Question 2: It is unclear why BSL-2+ is selected. Nowhere in the protocol are BSL-2+ reagents used. Select BSL-2 only.

Comments:
This amendment is to use a Lentiviral vector to knockdown ERK5, CARM1, PFKFB3 and MAT2A in the following brain tumor lines: DIPG 7 DIPG 4 DIPG 12 SFSF8628FL U87 GS8-18 KNS42 SJG2. ERK5 is considered an oncogene and as a Lentiviral vector is used, the research should be worked with at BSL-2+, not BSL-2 as listed. The laboratory would then need a Biosafety Operations Manual approved from EH&S. Additionally, question 3 in the animal gene transfer section should be YES, based on information in a previous section. Recommend this to be approved pending changes/revisions. In this amendment, the investigator wants to add the use of a 3rd generation Lentivirus for transducing an inducible shRNA construct in cultured cells. The shRNA will target suspected oncogenes, namely ERK5, CARM1, MAT2A and PFKB3. Cultured human and mouse brain tumor cell lines infected with the Lentivirus will be studied *in vitro* or injected into immunocompromised mice to study tumor growth. The investigator considers this BSL-2 but recognizes the potential need for precautionary measures including an upgrade to BSL-2+. The question to consider is whether knocking down one or more of these genes could potentially be tumorigenic.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	10
Against:	0
Abstained:	0

Protocol:	MOD201900516
Title:	Amendment for **IBC201600043**
Investigator:	REDACTED
Highest BSL:	RBL ABSL-3
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• An Improved Tobacco Mosaic Virus (TMV)-Conjugated Multiantigen Subunit Vaccine Against Respiratory Tularemia • REDACTED 2019 Biosafety Manual

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Define "TMV."

2) Tissues, Blood, or Body Fluids: Tissues from animals that have been administered with recombinant viruses and/or bacteria should be designated as recombinant.

3) Primary Cells or Cell Lines: Cells in which recombinant nucleic acids have been administered should be designated as recombinant.

Comments:

This is a modification of an existing protocol. The MOD seeks to add personnel and include a TMV subunit vaccine protocol. The modifications are minor. The work involves a pathogenic strain of *Francicella tularensis*. The recombinant DNA aspects (KAN resistance and GFP expression) do not involve any oncogenes or CRISPER technology. This modification can be approved at RBL and ABSL-3. This modification describes addition of personnel and the use of Tobacco Mosaic Virus expressing *F. tularensis* proteins as a potential vaccine in mouse and rabbit models of disease. Recombinant TMV will be sent from a collaborator at the University of California to a collaborator at New York Medical Center where the virus will be conjugated with *F. tularensis* proteins. Mice will be vaccinated with the conjugated TMV vaccine candidate and then shipped to the University of Pittsburgh. Conjugated TMV vaccine will also be shipped to the University of Pittsburgh where the personnel will use the experimental candidate to vaccinate rabbits. After vaccination, mice and rabbits will be challenged with virulent *F. tularensis* in the RBL. TMV and vaccinated animals will be handled at BSL-1, which is appropriate. All animals will be exposed to virulent *F. tularensis* in the RBL aerobiology suite and will be housed/samples will be handled in the RBL. After appropriate Biohazards Committee-approved inactivation methods have been completed and an inactivation certificate generated and signed by the investigator, samples may be removed for further processing at a lower biosafety level. There are a few minor form issues that should be corrected. Recommendation for approval after minor form issues have been corrected.

Review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	9
Against:	0
Abstained:	1; recused for involvement

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:54 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

February 10, 2020 10:00 AM

302 Heiber Building

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			
REDACTED, Vice Chair			
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED			
REDACTED			
REDACTED	Late: 10:25		
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED	Absent		

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	RCCO Co-Director
REDACTED	EH&S Department

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The January 2020 meeting minutes were reviewed and approved by the committee.

Votes:

For:	12
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None

IBC OFFICE REPORT

None

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocols on the following pages

Protocol:	IBC201900174
Title:	A study of Vascularized Bone Marrow Transplant Induce and Maintain CTATolerance
Investigator:	REDACTED
Highest BSL:	BSL-1 ABSL-1
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment, Question 4: Explain why syringes will be disposed as chemical hazards and what chemicals are being used. If the syringes will just be exposed to the mouse cells, they should be disposed of via Sharps container.

2) Waste Management, Question 1b: Confirm if liquid waste will be treated with bleach or *Peroxal 70 Bio*; remove *Peroxal* if bleach will be used. If using *Peroxal*, then provide the concentration for liquid waste listed on the product label and that the disinfectant can be diluted.

3) Waste Management, Question 1c: Animal carcasses should be sealed in double bags, labeled, and disposed according to animal facility guidelines.

4) Waste Management, Question 3: Provide additional detail describing the process for clean-up and decontamination of a biological spill (e.g. clean up materials, contact time for disinfectant, disposal of potentially contaminated clean up materials).

Comments:

The aim of this protocol is to investigate composite tissue allograft (CTA) tolerance. In this study, GFP transgenic mice will be used as bone marrow transplant donors and the donor-derived cells will be tracked using flow cytometry or immunofluorescence. Recipient wild type mice will receive immunosuppressant treatment and bone marrow cells IV. Additionally, the strain will be bred in-house for availability purposes. *In vitro* work will be performed using rat anti-mouse lymphocytes serum (ACCURATE). Work is proposed at BSL-1/ABSL-1 which is appropriate. The investigator plans to investigate mechanisms of tolerance in composite tissue allografts (CTA) involving the presence of the donor tissue bone marrow component. They plan to obtain bone marrow form GFP transgenic mice for transplant to recipient mice (recombinant nucleic acid delivery) and breed the transgenic animals. They will also use rat anti-mouse serum to detect cells. BSL1/ABSL-1 is proposed and is appropriate.

No review comments were provided. The application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900523
Title:	Amendment for **IBC201700284**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: Cell/CellLine - Rat: clarify "we wish to alter gene expression" in Description of Usage. If recombinant or synthetic nucleic acids will be administered to the cells, then the cells should be designated as recombinant agents.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: If recombinant materials, including viruses, will be administered to animals, then this *in vivo* work should be included.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 3: The response should be YES.

4) Live Animals, Question 5: The answer should be NO. Correct the response.

5) Live Animals, Question 6: The current response is NO to this question. Clarify if this is correct.

6) Animal Gene Transfer, Question 3: The application states YES to the question of whether animal experiments involve use of a replication-defective human or animal virus in the presence of a helper virus. Yet it is stated earlier that recombinant materials will not be administered to animals. Clarify this discrepancy and correct all relevant sections of the protocol to reflect the work that is planned.

7) Exposure Assessment and Protective Equipment, Question 2: It was noted that needles would be used for administration of human cells; add this information to the response.

8) Exposure Assessment and Protective Equipment, Question 4: Safety-engineered sharps devices are required for work with BSL-2 agents; add information.

9) Exposure Assessment and Protective Equipment, Question 5: Also indicate appropriate PPE that will be used in animal facilities. Minimum required PPE at ABSL-2 includes use of a hair bonnet, liquid-barrier coverall suit, gloves, shoe covers, surgical mask, and face shield. Revise accordingly.

Comments:

The amendment request identifies changes to personnel and seeks to add human mesenchymal stem cells to the approved protocol. These will be used in preclinical models of stroke and will be delivered intravenously into “wild type” (C57BL6) mice and their effects on conferring neuroprotection will be assessed. The hMSCs will be injected systemically at various timepoints after ischemic injury. The hMSCs will be isolated/harvested from bone marrow (obtained from *Lonza Biochemicals*). The aim of the overall project is to examine the effects of modulation of

gene expression on ischemic stroke. The underlying project is already approved to use HEK293s, various mouse and rat primary neural cells, Lentiviral vectors (HIV 3- and 4-plasmid) and AAV-2 vectors. Work will be conducted under BSL-2/ABSL-2. Once questions are addressed, recommendation for approval. This amendment describes the addition of human mesenchymal stem cells to the protocol and a change in personnel. The cells will be used for neuroprotection experiments in a preclinical stroke model. The hMSCs will apparently not be genetically modified and undifferentiated cells will be injected intravenously into mice. Additional work will focus on the mechanisms by which Prx4 and Rho-associated coiled-coil containing kinases and myosin light-chain contribute to the integrity of the blood brain barrier following stroke. A variety of human and rodent cells will be used at BSL-2. Lentivirus and AAV will be used for expression of wild-type, dominant negative, and other mutant alleles of Prx4, ROCK, GFP related genes. Recommend approval pending modifications.

Initial comments:
1) Viruses, Prions, or Vectors: The 4-plasmid system still states TBD as a source. Revise.
2) Live animals: Clarify: Question 5 is answered YES, but elsewhere the application repeatedly states that only C56BL6 mice will be used. The answers to "Genetically Engineered Animals: Source" also are a little confusing relating to the response to question 5.
3) Animal gene transfer: Question 3 should be YES due to AAV use. No major issues noted.

Review comments were provided to the investigator for response. The revised application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD201900528
Title:	Amendment for **IBC201600089**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• MSDSAAV_UPenn vector core • Certificate of Analysis - *Addgene* • COA_Vigene DA sensor and ACh sensor • *Addgene*_raav_sds • *Addgene* order 521286_COT • CAV2_SDS_PAGE • Vigene DA sensor silver stain

Determination: Approved

Last day of continuing review period: 2/13/2021

Required modifications:

1) Tissues, Blood, or Body Fluids: It appears that the application indicates the administration to animals for these materials. If the materials will be administered to animals, then describe these *in vivo* experiments in the "Description of Usage".

2) Primary Cells or Cell Lines: The icons for administration to animals has been selected; if the material will be administered to animals, then describe this work in the "Description of Usage".

3) Viruses, Prions, or Vectors: Adeno-Associated Viral Vector (AAV) - AAV1, 2, 5, 6, 8, 9, DJ, DJ/8: as a downgrade is being requested, BSL-1 should be selected. Any AAVs used at BSL-2 should be listed separately from AAVs used at BSL-1.

4) Live Animals, Question 1: Provide an updated IACUC number as the one provided has expired and is no longer active.

5) Exposure Assessment and Protective Equipment, Question 5: Also select coverall suit and surgical mask for entry to the ABSL-2 animal facility.

6) Waste Management, Question 3: Include the surgical mask to the list of PPE for spill cleanup. Also, appropriate contact time for spills is 20 minutes; revise the three places contact time is discussed on the application. In addition, the biohazard material should be disposed of via the normal biohazard waste stream. EH&S does not collect the waste; revise the statement.

Comments:

This amendment is requesting a downgrade to use AAV from *Addgene* at BSL-1. This investigator and protocol are already approved to use AAV at BSL-1 from other sources (UPenn, UNC, U Pitt) with the appropriate testing documents provided to the committee. This new AAV does not contain oncogenes and the Certificate of Analysis (COA) is provided. Recommend approval for this downgrade per Pitt AAV testing guidelines. There are comments on the application that should be corrected prior to approval. This is an amendment to a previously-approved IBC protocol seeking to downgrade AAV use to BSL-1. The AAV in question is purchased from *Addgene* and, according

to the certificate of testing results, is 100% pure. The experimental protocol involves the viral vector/construct into the brains of mice and rats to identify the effects of overexpression or knockdown of neuron associated genes. It appears the animals will be injected with Canine Adenovirus-2 under BSL-2 conditions and then transferred to BSL-1 conditions the next day. The protocol also includes *E. coli* for propagating the vectors, several rat, mouse, and human cell lines, and Lentiviral vectors for transfecting cell lines and injection into animals. A number of supporting documents are included as support and justification for the downgrade request. This work does not include any work on oncogenes or tumor suppressors. It appears that this protocol should be approved pending some minor forms-related issues.

Review comments were provided to the investigator for response. The revised application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

See following pages

Protocol:	IBC201900170
Title:	MRT5005-101
Investigator:	REDACTED
Highest BSL:	BSL-2 BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1
Additional Documents:	• ICF - MRT5005-101 Pregnant Partner Consent_12.20.2019 • ICF – ConsentFormPartB Expansion_ CLEAN CHP template 12.20.2019 • IB - MRT5005 Investigator Brochure version 2.0_10Oct2019_CLEAN_FINAL • Protocol - MRT5005-101 SAD MAD Combined Study_Amendment3_29July2019_Final

Determination: Approved

Last day of continuing review period: 2/12/2021

Required modifications:

1) Waste Management: How will unused study product be disposed of at the end of the study?

2) Waste Management: Question 3: The Investigational Product (IP) is provided in multiple vials to be poured into medication chambers of one or several nebulizers in a dose-dependent fashion. Describe the spill clean-up procedures in case of vial breakage, medication chamber leak, inadvertently dropped nebulizer, etc.

3) Human Gene Transfer/Human Clinical Trial : Question 3a: Human Gene Transfer/Clinical Trial: How will the product be prepared for dispensing? Where will it be administered?

Comments:

This multicenter phase 1/2 first in human study evaluates a mRNA therapy that encodes for wild-type cystic fibrosis transmembrane conductance regulator (CFTR) protein in the cells of participants with cystic fibrosis. Study product (MRT5005) is delivered via an aerosol to the lungs of persons with CF. MRT5005 delivery is facilitated by formulating it within lipid-based nanoparticles. Once inside the cell, CFTR mRNA in the cytoplasm is translated into native human CFTR protein and helps to restore CFTR chloride channel activity in the lungs. CO-hCFTR mRNA is the active drug substance of MRT5005. The LNP consists of the cationic lipid imidazole cholesterol ester (ICE), which is mixed with two other non-cationic lipids, dioleoylphosphatidylethanolamine (DOPE) and pegylated dimyristoylglycerol (DMG-PEG-2K). The study has 3 parts including single and multiple ascending dosing phases. In the expansion phase, included in this submission, participants receive multiple doses at a tolerated dosing level. MRT5005 was administered at 5 dose levels: 8, 12, 16, 20, and 24 mg of CO-hCFTR mRNA in Part A. The highest dose level that was tested in Part A, 24 mg, will not be tested in Part B. An intermediate dose level that was not tested in Part A, 12 mg, will be tested in Part B. Part B will evaluate 5 doses of MRT5005. Based on the results of Part B, 8 more people will be enrolled in Part B Expansion of the study and treated with 5 doses of MRT5005 at dose level(s) selected for further testing. An *InnoSpire Go* nebulizer will be used to administer MRT5005 by nebulization at a flow rate of approximately 0.3 mL/minute.

Biosafety: The Investigator Brochure (IB) states that gene therapy through mRNA delivery involves no risk of random genome integration and can introduce protein into the cytoplasm

without the need for nuclear entry. Following inhalation administration in rats and NHP, CO-hCFTR mRNA is minimally distributed beyond the respiratory tract, and mRNA concentrations in the blood are negligible. In the early parts of the study, enrollment was staggered for safety reasons. The first enrollees complained of headache and cough. Some experienced fever and chills and other flu-like symptoms. MRT5005 safety and tolerability is being assessed based on the number of pulmonary exacerbations; concomitant medication use; and changes from baseline in physical exam, weight, vital signs, oxygen saturation (pulse oximetry), ECG, clinical laboratory tests, chest x-ray, and spirometry. Assays to detect CO-hCFTR mRNA and ICE in the blood post-treatment are being performed along with assays to detect antibodies and T-cell immune responses to CFTR protein and anti-PEG (a component of MRT5005) antibodies. Interim analysis results from single doses of MRT5005 showed no evidence of T-cell sensitization to CFTR, nor were anti-CFTR anti-drug antibodies detected. Blood levels of CO-hCFTR mRNA and ICE were assessed to look for potential systemic absorption of MRT5005. Levels were BLQ in all placebo participants. Low and transient levels of mRNA and/or ICE were detected in only five participants, mainly at the 8-hour post dose and Day 2 timepoints. These five participants were the same participants that experienced febrile reactions, raising the possibility that the appearance of mRNA and/or lipid in the blood may trigger an inflammatory response. The primary objective of the study is to evaluate the safety and tolerability of single and multiple escalating doses of MRT5005 administered by nebulization to adult participants with CF. The secondary objective of the study is to evaluate the effect on percent predicted forced expiratory volume in 1 second (ppFEV1) and other spirometry parameters after single and multiple escalating doses of MRT5005 administered by nebulization to adult participants with CF. Cystic fibrosis is an autosomal recessive genetic disorder that affects most critically the lungs, but also the pancreas, liver, and intestine. Cystic fibrosis is caused by a mutation in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which functions as a channel for transporting chloride across the plasma membrane. The CFTR protein is an important regulator of salt and water balance, and absent or decreased function of CFTR in the airway epithelium of CF participants results in thick, viscous secretions, which clog the airways and impair mucociliary clearance and other host defense mechanisms. This leads to chronic bacterial infection and inflammation of the lungs resulting in extensive damage and eventually respiratory failure. Lung disease is the major cause of morbidity and mortality in CF participants. Messenger RNA (mRNA) therapy represents a new and novel approach for treating CF lung disease. In this therapeutic application, mRNA encoding for the wild-type CFTR protein is delivered directly by aerosol to the respiratory tract of CF participants resulting in the expression of normally functioning CFTR protein in the lungs. The efficient delivery and uptake of mRNA by bronchial epithelial cells is facilitated by formulation of the mRNA within cationic lipid-based nanoparticles. Following cellular uptake, translation of CFTR mRNA in the cytoplasm leads to the production of normal CFTR protein and restoration of CFTR chloride channel activity in the lungs. This strategy was used to develop MRT5005, which utilizes an imidazole cholesterol ester lipid nanoparticle (ICE LNP) as the vehicle to deliver codon-optimized human cystic fibrosis transmembrane conductance regulator mRNA (CO-hCFTR mRNA) to the lungs. CO-hCFTR mRNA is the active drug substance of MRT5005. The primary component of the ICE LNP is the cationic lipid imidazole cholesterol ester (ICE), which is mixed with two other non-cationic lipids, dioleoylphosphatidylethanolamine (DOPE) and pegylated dimyristoylglycerol (DMG-PEG-2K), to form the lipid nanoparticle (LNP). Complexation within the ICE LNP protects CO-hCFTR mRNA from degradation and facilitates its aerosol delivery and uptake by the target bronchial epithelial cells in the lungs of CF participants. Study MRT5005-101

represents an important first step in the evaluation of mRNA therapy as an approach to restoring CFTR function in the lungs of CF participants. The goal was to design a single study that could achieve both Phase 1 and 2 objectives while ensuring participant safety. This Phase 1/2, first-in-human study will evaluate the safety and tolerability of single and multiple escalating doses of MRT5005 administered by nebulization to the respiratory tract of adult participants with CF. There were no noted concerns.

Pre-screening comments were sent to the investigator for response. The revised application was sent to the reviewers and then placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC202000005
Title:	HCC 19-161
Investigator:	REDACTED
Highest BSL:	BSL-2 BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-10-04 Pharmacy Manual v2_19-161 • 20019-09-30 HSIC0604 -OSHA Bloodborne Pathogen Standard Exposure Control Plan • 20019-04-26 HSIC0616 Guidelines for Handling Sharps • 2019-06-27 Protocol v1_19-161 • 2019-07-01 IB-Edition 1_19-161 • 2018-06-29 HSFM0208PRO Waste Management PROCEDURE • 2019-10-10 Master ICF Template_v2.0_19-161

Determination: Approved

Last day of continuing review period: 2/12/2021

Required modifications:

1) Human Gene Transfer/Human Clinical Trial: Materials: Question 7a: Include description of evaluation of SYNB1891 pharmacokinetics.

2) Human Gene Transfer/Human Clinical Trial: Question 1: Identify the related IRB submission.

3) Human Gene Transfer/Human Clinical Trial: Question 3a: How will study product be transported from the HCC Pharmacy to the patient? Where will the injections take place?

4) Protocol Team Members: AK is noted on the first page (question 8) to receive communications from the system, but is not listed in this section, and therefore may not be able to view or edit the application.

Comments:

This is a phase 1, two arm study. Arm 1 will evaluate the safety and tolerability of SYNB1891 in a dose-escalating manner and establish the dose to be utilized in Arm 2 when it is combined with atezolizumab (PD-L1 inhibitor), in participants advanced solid tumors and lymphoma. SYNB1891 is a strain of modified live probiotic bacterium (*Escherichia coli* [*E. coli*] Nissle 1917) (EcN) programmed as a live immuno-biotherapeutic agent. It is a genetically modified strain of EcN that, under hypoxic conditions, is designed to produce cyclic-di-AMP, which functions as a STING agonist. A STING agonist stimulates the type 1 interferon pathway and proinflammatory cytokines to promote T-cell infiltration into tumor cells. Modifications include: a) deletion of the thyA and dapA genes renders the bacteria incapable of growth in environments lacking thymidine or diaminopimelic acid; b) placement of the dacA gene (responsible for production of the STING agonist CDA) under the control of an anaerobic promoter to enable selective expression in hypoxic environments, like those found in the tumor. The modifications do not add any virulence factors or antibiotic resistance genes. Studies in mice with tumors showed that SYNB1891 produced cyclic-di-AMP in the tumor, with increased levels of IFN beta and cytokine and regression of the tumors. A GLP toxicology study in mice indicated that a dose of 4.7×10^7 live cells/dose given once a week was tolerated. This is the first study in humans. The starting dose in humans will be

1 x 10^6 live cells per dose, given by injection into the tumor, to be escalated in 3-fold increments depending on tolerability. Participants enrolled to Arm 1 may receive up to four 21-day cycles of SYNB1891 monotherapy. On Days 1, 8, and 15 of Cycle 1 and Day 1 of Cycles 2 through 4, participants will receive an i.t. injection of SYNB1891 into an eligible lesion. If the initial eligible lesion undergoes complete regression and is no longer injectable (at the discretion of the investigator), a subsequent eligible lesion (until no more eligible lesions remain) may be injected. At the end of Cycle 4, participants in Arm 1 who do not have progressive disease (i.e., those who achieve and sustain complete response [CR], partial response [PR], or stable disease [SD]) may receive additional cycles of SYNB1891 administered by i.t. injection on Day 1 of each cycle for up to 24 months after the initial dose of study treatment until documentation of progressive disease or other discontinuation criteria, satisfaction of a predefined study stopping rule, or no eligible lesions remain. Once the MTD is established, participants in Arm 2 will receive SYNB1891, starting at a 10-fold lower dose than the MTD, given in combination with atezolizumab (1200 mg IV every 3 weeks). Participants may receive up to four 21-day cycles with SYNB1891 administered by i.t. injection into an eligible lesion on Days 1, 8, and 15 of Cycle 1 and Day 1 of Cycles 2 through 4. Atezolizumab will be administered on Day 1 of each of the 4 planned cycles. On days when atezolizumab and SYNB1891 are both administered, SYNB1891 will be administered first, followed by at least 1 hour of observation prior to the atezolizumab infusion. Those in Arm 2 who do not have progressive disease at the end of Cycle 4 may receive additional cycles of SYNB1891 and atezolizumab for up to 24 months until documentation of progressive disease or other discontinuation criteria, satisfaction of a predefined study stopping rule, or no eligible lesions remain. Up to 70 participants will be enrolled, with a life expectancy of at least three months, who have injectable lesions.

Biosafety: SYNB1891 is a strain of a genetically modified live probiotic bacterium called EcN. Modifications include insertion of an anaerobically inducible gene whose enzymatic product generates the STING-agonist cyclic di-AMP within the hypoxic tumor area, and deletion of two critical genes which result in auxotrophies that prevent bacterial proliferation upon injection of the tumor. EcN (parent strain) is a non-pathogenic member of the *E. coli* family originally isolated by A Nissle in 1917 because of its antagonistic activity against some pathogenic enterobacteria. While not FDA-approved, EcN is commercially available in Europe, Australia, and Canada at a recommended dose of 2.5 to 25 × 10^9 CFU per capsule administered once or twice daily (*Mutaflor* package insert). EcN has been used as a probiotic for over 90 years. SYNB1861 was tested for antibiotic susceptibility and is susceptible to 11 of 16 antibiotics tested. In both study arms, each cohort will initially consist of three participants. Dose-limiting toxicities are clearly outlined in the protocol. Potential adverse events for SYNB1861 include cytokine release syndrome (premedication with antipyretics), infections, injection site reactions including abscesses, ulcers, infection. Atezolizumab (FDA-approved) is associated with known adverse reactions including immune-related hepatitis, pneumonitis, endocrine events, others. SYNB1891 has a concentration of 1× 10^{11} live cells/mL. SYNB1891 doses will be prepared in a biosafety cabinet by staff wearing sterile sleeves, sterile gloves, and a facemask to minimize contamination. Unused product will be returned to the sponsor at the end of the study. The overall objective is to evaluate the safety and tolerability of escalating doses of intratumoral (i.t.) injections of SYNB1891 to determine the single-agent maximum tolerated dose (MTD) as monotherapy and the recommended Phase 2 dose (RP2D) in combination with atezolizumab. SYNB1891 is a strain of modified live probiotic bacterium (*Escherichia coli Nissle* 1917) (EcN) programmed as a live immuno-biotherapeutic

agent designed to treat participants with cancer. It is a genetically modified strain of EcN that, under hypoxic conditions, is designed to produce cyclic di-Adenosine monophosphate (CDA), which functions as a STimulator of INterferon Genes (STING) agonist (Section 2). Upon intratumoral (i.t.) administration, SYNB1891 is taken up by antigen-presenting cells (APCs) (dendritic cells and macrophages) leading to STING activation, the release of interferon beta (IFNβ) and the initiation of STING-mediated antitumor immune responses (*Corrales, 2015*). Moreover, the bacterial chassis itself provides additional immune stimulation within the tumor microenvironment (TME) via activation of pattern recognition receptors (PRRs: pathogen-associated molecular pattern [PAMP] and damage-associated molecular pattern [DAMP] receptors) mediated by lipopolysaccharide (LPS)/endotoxin. SYNB1891 contains dual thymidine and diaminopimelic acid auxotrophies, which inhibit its growth. SYNB1891 contains no antibiotic resistance genes and has demonstrated susceptibility to a wide range of antibiotics. The maximum time of study participation for a participant is up to 26 months, including the screening period (up to 28 days), treatment administration period (up to 24 months), and safety follow-up period (30 ± 5 days after the last dose). Participants in Arm 1 will receive an initial 4 cycles of SYNB1891. Participants who do not have progressive disease at the end of Cycle 4 may receive additional cycles of SYNB1891 for up to 24 months after the initial dose of study treatment until documentation of progressive disease or other discontinuation criteria, satisfaction of a predefined study stopping rule, or no eligible lesions remain. Participants in Arm 2 will receive an initial 4 cycles of SYNB1891 and atezolizumab. Arm 2 participants who do not have progressive disease at the end of Cycle 4 on combination therapy may receive additional cycles of SYNB1891 and atezolizumab for up to 24 months after the initial dose of study treatment until documentation of progressive disease or other discontinuation criteria, satisfaction of a predefined study stopping rule, or no eligible lesions remain. There were no noted concerns.

The application was placed on the February 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	IBC202000008
Title:	AVROBIO, Inc. AVRO-RD-01-201 Fabry
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 47.2 Prep of BSL2 Viruses (10-19) • HSFM0208PRO • AVRO-RD-01-201 Amendment 3.1_ 14Feb2019 • AVRORD01201_USA_ICF_Optional Genetic_Am3__V1.0_24Jul2019 • AVRO-RD-01-201 Laboratory Manual v6_Approved_19 10 03 • HSIC0609 • PTCMDIV820d • AVRORD01201_USA_ICF_Am3_V1.0_24Jul2019 • AVRO-RD-01-201 Treatment Manual_USA_V1.0_06Aug19_final • AVR-RD-01 IB Edition 3 31January2019 final

Determination: Modifications Required

Required modifications:

1) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: The Investigator's Brochure states "Testing for RCL during production is also an integral part of the release criteria of the GMP-grade Lentiviral vector. Prior to release, clinical-grade (GMP) Lentiviral vector includes testing to ensure there is no evidence of RCL." As such, describe methods used for replication-competent virus (RCV) testing.

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 10: Describe the transduction efficiency of the Lentiviral vector. Transduction is the process by which genetic material is inserted into a cell by a virus. Tranduction efficiency is a measure that indicates how well a gene is incorporated into cells by the vector. Some vectors will transduce cells at a higher efficiency than others.

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 4: Include the derivation of the delivery vector system including the source, and any modifications. Derivation examples are viral, bacterial, or plasmid vectors; modification examples are deletions to attenuate or self-inactivate, changes to tropisms, etc.

4) Human Gene Transfer/Human Clinical Trial: Materials: Question 8: Describe any other material used in preparing the agent (vector and transgene) administered to subjects. Examples would include the liquids that will be used to re-suspend the agent. What other materials are included with the agent that is administered to participants?

5) Human Gene Transfer/Human Clinical Trial: Materials: Question 7a: Briefly mention the possibility of immunogenicity related to study product and testing for AGA antibodies.

6) Human Gene Transfer/Human Clinical Trial: Question 3a: Describe the chain of custody for the study product from the time of arrival at CHP to infusion into study participant(s).

7) Viruses, Prions, or Vectors: The 3-plasmid Lentiviral vector IS replication-defective. Correct the response.

8) Viruses, Prions, or Vectors: The source of the Lentiviral vector is not study participants, but rather the pharmaceutical company sponsoring the study. Correct the response.

9) Viruses, Prions, or Vectors: List the specific cDNA (i.e. gene name) under "Inserted Nucleic Acids Information."

10) Viruses, Prions, or Vectors: Lentiviruses CAN infect human cells. Correct the response.

11) Tissues, Blood, or Body Fluids: As transduced human CD34+ cells will be administered into study subjects, the response to whether cells will be administered to humans should be YES.

12) Tissues, Blood, or Body Fluids: As subject CD34+ cells will be transduced with recombinant Lentivirus, human cells should be designated as recombinant.

13) Basic Information: Question 3: Briefly describe the study product, major study procedures required (apheresis, myeloablation, etc.)

Comments:
This is a multinational, open-label study to assess the efficacy and safety of AVR-RD-01 in about 8 to 12 male participants 18-50 years of age with classic Fabry disease. Fabry disease is a rare lysosomal storage disorder caused by alpha-galactosidase A (AGA) deficiency, due to mutations in the GLA gene. This deficiency in enzyme activity leads to accumulation of glycosphingolipids, resulting in multi-organ damage (heart, kidney) and early mortality. Available enzyme replacement therapy (ERT) requires IV infusions every two weeks and only slows disease progression. The study will enroll males who have not received ERT within the past 10 years and/or previous chaperone therapy. AVR-RD-01 is autologous CD34+-enriched cell fraction transduced with a 3rd generation Lentiviral vector (LV)/alpha galactosidase A (AGA) that encodes for the human GLA complementary deoxyribonucleic acid (cDNA) sequence. Mobilized autologous CD34+ hematopoietic stem cells (HSCs) obtained from the participant's peripheral blood are genetically modified *ex vivo* with an LV encoding the human GLA cDNA sequence. After the participant has undergone a myeloablative conditioning regimen, the genetically-modified cells are returned to the participant by IV infusion (one time, 3- 20 x 10^6 /kg). The infusion is intended to permanently engraft into the bone marrow, divide, and differentiate, effecting 'metabolic cooperativity' (also known as 'cross correction'), so that functional hydrolase AGA is secreted from augmented cells, circulated, and taken up through mannose-6-phosphate receptors and trafficked to the lysosomes of bystander cells. Once inside the lysosome, the functional enzyme reduces glycosphingolipid storage, allowing reconstitution of lysosomal function, cell survival, and avoidance of end-organ damage. The primary objectives are to evaluate the effect of AVR-RD-01 on substrate (globotriaosylceramide [Gb3]) in kidney biopsies; safety and tolerability (AEs, SAEs); immunogenicity; presence of replication competent Lentivirus (RCL); and integration site analysis (ISA) for detection of insertional mutagenesis. The IB indicates there are two ongoing clinical trials of AVR-RD-01 for males with Fabry disease. The study in Canada is a multi-center, non-randomized, open-label prospective pilot study intended to enroll up to six adult male participants with classic Fabry disease on ERT. Preliminary results from three participants indicate presence of vector and a trend in increased AGA enzyme activity post-AVR-RD-01 transplant, providing early evidence that the LV encoding human GLA cDNA may be effective in delivering and integrating the GLA transgene into the human genome and subsequently enabling expression of functional AGA. Preliminary safety data indicates AVR-RD-

01 was generally well-tolerated. Information for a single participant who enrolled in the other study who received AVR-RD -01 is available and it appears that the study product was generally well-tolerated. Participants will undergo conditioning with IV busulfan once daily for four days prior to infusion of study product.

Biosafety: The manufacturing process is comprised of Lentiviral vector production and AVR-RD-01 (LV-transduced enriched CD34+ cells), production. The sponsor states LV vector production and AVR-RD-01 production processes comply with Good Manufacturing Practice guidelines. Prior to study product cryopreservation, a portion of the cell product is put aside for QC testing. Release testing includes determination of total viable cell count (acceptance criterion of $\geq 3 \times 10^6$ autologous CD34+ cell-enriched population cells/kg of participant body weight). Safety testing includes sterility, mycoplasma, and endotoxin. The AGA enzyme activity assay is done to assess potency of AVR-RD01. AGA enzyme activity is measured in the media from cultured-transduced cells as part of release testing. Vector copy number and percent transduction are also analyzed. Testing for RCL during production is part of the release criteria. For LV vector production, a triple or quadruple transfection method using 3 or 4 plasmids is done according to manufacturer SOP. The 4 HIV-1 accessory genes (vif, vpr, vpu, nef), and the structural protein, env, are not included in the 3-plasmid set. In the 4-plasmid set, HIV tat, is removed. Participants will be routinely tested for AGA antibodies. The potential long-term impact of the conditioning regimen with busulfan is not fully known. Participants will be offered sperm cryopreservation. Participants' reproductive potential will be assessed by evaluating the change from baseline in sperm count, volume, sperm concentration, total motility, progressive motility, and morphology. Participants with a female partner of childbearing potential will be required to use contraception or remain sexually abstinent and are prohibited from donating sperm after receiving busulfan or donating blood, organs, tissues, or cells for transplantation. Measures of engraftment will be evaluated by determining the average vector copy number (VCN) per diploid genome using qPCR and presence of chimerism in bone marrow aspirates. In case there is a transplant failure or prolonged BM aplasia after AVR-RD-01 treatment, a CD34+ stem cell back-up of un-transduced cells will be collected at apheresis and cryopreserved for use as rescue treatment. Participants will be routinely tested for AGA antibodies to assess immunogenicity of AVRRD-01. Staggered enrollment with interim DMC reviews are planned. Participants will be followed for 15 years after AVR-RD-01 treatment. AVR-RD-01 is an *ex vivo* LV-mediated gene modified cell therapy for Fabry disease. AVR-RD-01 aims to perform autologous transplantation of a mobilized CD34+ cell-enriched population procured from the participant's peripheral blood that is genetically modified, *ex vivo*, with an LV encoding the human AGA cDNA sequence. After the participant has undergone out-patient conditioning, the genetically-modified cells are returned to the participant by IV infusion. The infused genetically-modified cells are intended to engraft into the BM, divide, and differentiate, effecting cross-correction wherein functional hydrolase AGA is secreted from the genetically-modified cells and taken up by other cells via mannose-6-phosphate receptors. Once in AGA activity-deficient cells, the replacement enzyme is trafficked to the lysosomes, where it reduces the level of glycosphingolipid storage, thereby enabling cell survival and the prevention of end-organ manifestations that lead to the early morbidity and mortality associated with Fabry disease. No noted biosafety concerns.

The application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13 (late arrival)
Against:	0
Abstained:	0

Protocol:	IBC202000009
Title:	AVROBIO AVRO-RD-02-201 Gaucher
Investigator:	REDACTED
Highest BSL:	BSL-2 BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• AVRO-RD-02-201_Laboratory Manual_19 05 09_ NOT FOR IRB-REB SUBMISSION • AVRO-RD-02-201 Adult PISF_clean_FINAL_04Oct2019.doc • HSIC0609 • AVRO-RD-02-201_Parent Guardian PISF_clean_FINAL_15Oct2019 • 47.2 Prep of BSL2 Viruses (10-19) • AVRO-RD-02-201 Treatment Manual_24May2019CAN • HSFM0208PRO • AVRO-RD-02-201 protocol Amendment 2_Summary of Changes_04Oct2019 • AVRO-RD-02-201 Protocol Amendment 2_Signed • AVR-RD-02 IB Edition 3_04Oct2019_Final Draft_clean • PTCMDIV820d

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 5: Check safety goggles

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 10: Describe the transduction efficiency of the Lentiviral vector. Transduction is the process by which genetic material is inserted into a cell by a virus. Tranduction efficiency is a measure that indicates how well a gene is incorporated into cells by the vector. Some vectors will transduce cells at a higher efficiency than others.

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: The investigator's brochure states, "In addition, testing for RCL during vector production is an integral part of the release criteria of the Good Manufacturing Practice (GMP)-grade vector. Screening of participants for RCL will also be periodically performed as specified in the protocol." Describe the methods used for replication-competent virus (RCV) testing.

4) Human Gene Transfer/Human Clinical Trial: Materials: Question 4: Include the derivation of the delivery vector system including the source, and any modifications. Derivation examples are viral, bacterial, or plasmid vectors; modification examples are deletions to attenuate or self-inactivate, changes to tropisms, etc.

5) Human Gene Transfer/Human Clinical Trial: Question 3a: Describe chain of custody for the study product from the time it arrives at CHP to infusion into study participants.

6) Lentivirus and Lentiviral Vectors: Earlier in the protocol, the Lentiviral vector is described as a 3-plasmid system. Clarify the discrepancy and be sure the response is consistent throughout the application.

7) Viruses, Prions, or Vectors: The source of the Lentiviral vector is not the study participants, but rather the pharmaceutical company sponsoring the study. Correct the response.

8) Viruses, Prions, or Vectors: Later in the protocol, the Lentiviral vector is described as a 4-plasmid system. Clarify this discrepancy - is it a 3-plasmid or a 4-plasmid system? Be consistent throughout the application.

9) Viruses, Prions, or Vectors: The 3-plasmid Lentiviral vector IS replication-defective. Correct the response.

10) Viruses, Prions, or Vectors: List the specific cDNA (i.e. gene name) under "Inserted Nucleic Acids Information."

11) Tissues, Blood, or Body Fluids: As transduced human CD34+ cells will be administered into study participants, the appropriate response to whether cells will be administered to humans should be YES. Question 6 in the Pop-up box for the agent.

12) Tissues, Blood, or Body Fluids: As participant CD34+ cells will be transduced with recombinant Lentivirus, human cells should be designated as recombinant. See question 4 in the Pop-up box for the agent.

13) Basic Information: Question 3: Project Summary: Briefly describe the study product, apheresis, myeloablative conditioning requirement.

Comments:

This protocol evaluates AVR-RD-02, an *ex vivo*, Lentiviral vector (LV)-mediated (3rd generation, 4-plasmid system), autologous cell therapy that consists of CD34+ stem cells that are genetically modified to express functional glucocerebrosidase (GCase) for the treatment of Gaucher disease (a lysosomal storage disease). Autologous CD34+ HSC obtained from the participant will be modified *ex vivo* with an LV to contain a codon-optimized complementary DNA (cDNA) sequence that encodes for functional human GCase. After the participant undergoes a myeloablative conditioning regimen with busulfan, the genetically-modified cells will be given back to the participant by a single IV infusion (between 3 and 10×10^6 cells/kg). After infusion, it is anticipated that some of the most primitive stem cells (long-term engrafting cells) engraft in the bone marrow. It is expected that progeny of these genetically modified cells will produce functional, human, wild-type GCase and restore the cell's deficient enzymatic activity. This GCase is expected to break down/prevent the accumulation of glucosylceramide (GluCer) and its deacetylated form, glucosylsphingosine (GlcSph), primarily in the lysosomes of cells of the reticuloendothelial system, especially monocytes and macrophages. In a mouse model of Gaucher disease, replacing the faulty macrophages (Gaucher cells) with functional macrophages prevents, and reverses, the progressive damage caused by the long-term build-up of Gaucher cells in the various organs impacted by Gaucher Disease. The primary objectives are safety, tolerability, immunogenicity, GCase activity, and need for enzyme replacement therapy (ERT) following treatment with AVR-RD-02. This study will enroll 8 to 16 participants (male or female) who are ≥16 and ≤35 years of age with Type 1 Gaucher disease. There will be two arms: a) participants who have been receiving ERT for a minimum of 24 months immediately preceding screening, have demonstrated clinical stability during the 6 months prior to screening, and have not been treated with substrate reduction therapy (SRT) during the 24 months before screening (ie, switch-stable); b) participants who have either never received ERT or SRT, or have not received ERT or SRT within 12 months of screening

(treatment-naïve). Enrollment will occur in a staggered manner with review by an independent DMC.

Biosafety: AVR-RD-02 is an autologously-derived product; it is not tested for transmissible infectious agents but should be handled as though potentially infectious. Stem cell infusion can cause tightness in chest, hypotension, coughing, chest pain, decreased urine output, weakness, hypersensitivity reactions, electrolyte disturbances, and rarely, engraftment syndrome (fever, rash, non-cardiogenic pulmonary edema). Blood samples are obtained to detect neutralizing antibodies (immunogenicity). Testing for RCL will be done at pre-specified time points to determine infection of non-target cells. Participants with an average vector copy number will be tested for vector insertional site analysis. One theoretical LV-related risk is generation of a RCL caused by recombination of vector plasmids during the vector production process or by mobilization of proviral DNA *in vivo* by infectious retroviruses (ie, HIV). The LV2/GBA structure is derived from a 3rd generation, 4- plasmid system (1 transfer plasmid, 2 packaging plasmids and 1 envelope plasmid) containing a SIN element. The packaging plasmids consist of a plasmid expressing the structural and enzymatic gag/pol genes and a plasmid expressing Rev protein that facilitates export of genomic RNA from the nucleus to the cytoplasm. The envelope plasmid expresses Vesicular Sstomatitis Virus G protein (VSV-G) in order to pseudotype the LV for improved CD34+ transduction. None of the 4 HIV accessory genes (vif, vpr, vpu, nef) essential for HIV-1 replication *in vivo* are expressed during production of LV2/GBA since they have been deleted from the 4-plasmid set. The HIV gene Tat, a regulatory protein, has also been removed. In an earlier study in which participants received a product consisting of peripheral blood or bone marrow CD34+ cells from persons with Gaucher disease transduced with a Retroviral vector and containing the human GBA-based cDNA expressed from the retroviral LTR promoter (G1Gc), no long-term adverse effects of peripheral blood mobilization, bone marrow collection, or transduced cell infusions were found. Participants in this study did not receive myeloablative therapy prior to infusion. Busulfan conditioning regimens may lead to nausea, stomatitis, vomiting, anorexia, diarrhea, insomnia, fever, hypomagnesemia, abdominal pain, anxiety, headache, hyperglycemia and hypokalemia. A back-up unit of mobilized peripheral blood will be collected at apheresis and cryopreserved for rescue treatment in the unlikely event that there is prolonged bone marrow aplasia after AVR-RD-02 treatment. The use of LV for transduction of HSCs raises the concern of possible insertional mutagenesis, potentially leading to cell transformation and eventual malignant changes. Participants will be monitored over the long-term. This is an adaptive, multinational, open-label study to assess the safety and efficacy of AVR-RD-02 in approximately 8 to 16 participants (male or female) who are ≥16 and ≤35 years of age and post-pubertal at Screening with a confirmed diagnosis of Type 1 Gaucher disease (based on clinical phenotype, genotyping, and deficient GCase enzyme activity in plasma and PBLs). The planned study will consist of two arms:

• One study arm will include participants who have been receiving ERT for a minimum of 24 months immediately preceding Screening, have demonstrated clinical stability during the 6 months immediately preceding Screening, and have not been treated with substrate reduction therapy (SRT) during the 24 months immediately preceding Screening (ie, switch-stable participants). Switch-stable participants must discontinue ERT at least 2 weeks before the scheduled transplant day.

• A second arm will include participants who have either never received ERT or SRT or have not received ERT or SRT within 12 months of screening (treatment-naïve participants).

Neither switch-stable participants nor treatment-naïve participants will receive ERT following gene therapy, unless clinically warranted (see criteria for ERT below)

AVR-RD-02 is an *ex vivo*, Lentiviral vector (LV)-mediated, gene modified, autologous cell therapy composed of CD34+ hematopoietic stem cells (HSC) that have been genetically modified to express functional glucocerebrosidase (GCase) for the treatment of Gaucher disease. Autologous CD34+ HSC obtained from the participant's mobilized peripheral blood will be genetically modified *ex vivo* with an LV to contain a codon-optimized complementary deoxyribonucleic acid (cDNA) sequence that encodes for functional human GCase. After the participant has undergone a conditioning regimen, the genetically-modified cells will be returned to the participant by intravenous (IV) infusion. Following infusion, a proportion of the most primitive stem cells (long-term engrafting cells) engraft in the bone marrow niche. Progeny of these genetically modified cells are expected to produce functional, human, wild-type GCase, thus restoring the cell's deficient enzymatic activity. This replacement GCase is expected to physiologically break down, and prevent, the accumulation of glucosylceramide (GluCer) and its deacetylated form, glucosylsphingosine (GlcSph), primarily in the lysosomes of cells of the reticuloendothelial system, especially monocytes and macrophages. In a mouse model of Gaucher disease, replacing the faulty macrophages (Gaucher cells) with functional macrophages prevents, and reverses, the progressive damage caused by the long-term build-up of Gaucher cells in the various organs impacted by the disease, principally the bone marrow, liver, and spleen (*Dahl et al., 2015*). Given the close similarities between mammalian lysosomal systems, it is reasonable to expect a similar outcome in human Gaucher participants.

The application was placed on the February 2020 agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC202000013
Title:	LogicBio LB001-001
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• LB001-001_IB_v1.0_20Dec2019 • LB001-001_hLB-001 Pharmacy Manual_v1.0_17Jan2020 • HSIC0604 • PTCMDIV820d • LB001-001_Assent 5-12 yr_v1.0_04Dec2019_FINAL • LB001-001_ICF_v1.0_04Dec2019_FINAL • LB001-001 Protocol V1.0_11Dec2019 • 47.2 Prep of BSL2 Viruses (10-19) • HSFM0208PRO • HSIC0609

Reviewer Summary:

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: The application states that "the gene remains episomal." Yet, the uploaded supporting documents state that " hLB-001 uses the natural cellular process of homologous recombination to integrate a copy of the human mutase gene into the albumin locus." Clarify the discrepancy.

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 10: Describe the intended *ex vivo* or *in vivo* target cells (tissues, or organs) and the transduction efficiency. Transduction is the process by which genetic material is inserted into a cell by a virus. Transduction efficiency is a measure that indicates how well a gene is incorporated into cells by the vector. Some vectors will transduce cells at a higher efficiency than others.

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: Earlier in the protocol, the investigator has stated, "Blood, saliva, feces, and urine will be collected to evaluate viral shedding." As such, correct the response to align with the protocol.

4) Human Gene Transfer/Human Clinical Trial: Materials: Question 4: Describe the dose of virus that will be administered to humans.

5) Human Gene Transfer/Human Clinical Trial: Identify the IRB protocol that should be linked to the IBC protocol application.

6) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Include a statement that the AAV vector will be administered to human study subjects.

7) Basic Information: Question 3: Basic Information: Define the study product hLB-001, mode of delivery, and target (hepatocytes).

Comments:

LogicBio is developing hLB-001 for the treatment of participants with isolated methylmalonic acidemia (MMA) associated with mutations in the gene encoding methylmalonyl-CoA mutase (MMUT) gene (SNOMED CT: 42393006|Methylmalonic acidemia [disorder]). MMA is a

metabolic disorder in which the body is unable to process certain amino acids and fats properly. Infants considered to have severe MMA are diagnosed in the first days to weeks of life either based on newborn screening results or following presentation with clinical signs including vomiting, lethargy, respiratory distress, hypotonia, hypothermia, hepatomegaly, and progressive encephalopathy. Key laboratory findings to support diagnosis include hyperammonemia, elevated serum lactate, abnormally high methylmalonic acid in the blood and urine (*Hörster et al, 2007*), pancytopenia, and elevated urine ketone bodies (*Baumgartner et al, 2014*). Infants with the severe form of MMA are at risk for neurologic injury including basal ganglia stroke, failure to thrive, and poor feeding. Subsequently, they may progress to impaired cognition, severe infections, and renal failure with devastating impact on participant quality of life and survival (*Manoli et al, 2016*). MMA has an autosomal recessive pattern of inheritance with an incidence in the United States and European Union of approximately 1 in 50,000 to 100,000 live births (*Therell et al, 2014*) (*Almási et al, 2019*). It is caused most commonly (60%) by mutations in the gene encoding the mitochondrial enzyme methylmalonyl-CoA mutase (MMUT), resulting in a failure of conversion of methylmalonyl-CoA to succinyl-CoA and a subsequent accumulation of methylmalonic acid in tissues and blood. Participants with MMA caused by MMUT gene mutations are typically non-B12 responsive, present at a younger age, and have a more severe phenotype (*Manoli et al, 2016*). Severity of symptoms within the MMUT mutation participant population is correlated with the mutation type and the level of residual enzyme activity. Based on *in vitro* fibroblast assessments and/or gene allele correlations, MMA participants may have a complete loss of enzyme activity, designated as mut0, or a partial reduction, designated as mut-. The mut0 subtype is more prevalent and associated with higher morbidity and mortality at an early age (*Manoli et al, 2016*). While rapid MMA diagnosis via universal newborn screening can lead to early institution of medical and dietary management, the natural history of MMA remains characterized by periods of relative health alternating with intermittent and potentially life-threatening metabolic decompensations. Thus MMA continues to be associated with substantial morbidity despite improved survival (*de Baulny et al, 2005*) (*Dionisi-Vici et al, 2006*) (*Kölker et al, 2015*). Currently, MMA has no curative therapies. Participants with mutase-deficient MMA are treated lifelong with a demanding dietary regimen of protein restriction in addition to ammonia scavenger therapy in an attempt to mitigate acute illnesses that contributes to metabolic brain injury and progression to renal failure. Liver transplantation is an emerging intervention in MMA participants, increasingly performed at an early age to suppress hyperammonemia episodes, stabilize neurocognitive function, and improve long-term survival (Niemi et al, 2015). However, donor liver availability is limited; and transplantation has significant acute and long-term risks associated with the surgical procedure as well as prolonged immunosuppression. Study LB001-001 is a FIH phase 1/2 open-label interventional study to evaluate the safety, tolerability, biologic activity, and clinical efficacy of hLB-001 in pediatric participants with MMA. Participants to be enrolled will have a severe form of MMA associated with deficiency of methylmalonyl-CoA mutase (referred to commonly as MMUT) as defined by the following criteria:

a) any MMA participant with confirmed mut0 genotype (null mutation)

b) any MMA participant with confirmed mut- genotype (partial deficiency) who has had a documented peak methylmalonic acid serum or plasma level ≥ 100 μmol/L

Approximately 8 participants will be enrolled at approximately 6 centers in the United States.

Participants will undergo an initial screening, which will include general health and disease complications, MMA diagnosis confirmation, dietary management, concomitant medications, Adeno-associated viral vector (AAV) neutralizing antibody titers, and liver and renal function

testing. Participants will be required to be in stable condition for the 2 months prior to the start of screening, defined as no changes in chronic treatment except for adjustments to medications and diet, for weight gain and plasma amino acid profile as required for optimal care. The screening assessments may take place over multiple days. Participants who are receiving MMA-related medications should continue these medications as per the standard of care at the clinical site. Participants meeting all initial screening criteria will undergo a run-in period of at least 1 month from start of screening to hLB-001 dosing. During this period, participants will establish a baseline for methylmalonic acid levels and demonstrate ongoing clinical stability. During the week prior to dosing, it will be confirmed that the participant continues to meet all inclusion/exclusion criteria; and the participant will be admitted to the study site for study drug administration. AAV administration to pediatric participants has been associated with clinically significant liver enzyme elevations, resulting in a recommendation of prophylactic steroid administration for a minimum duration of 1 month (ZOLGENSMA prescribing information, 2019). A standardized regimen of oral prednisolone 1 mg/kg/day or its equivalent will be initiated 24 hours prior to starting the hLB-001 infusion. Daily corticosteroid dosing will continue for a planned 30-day course, potentially longer if evidence of ongoing immune response, followed by a 30-day taper. Prior to steroid discontinuation, normalized adrenal function will be confirmed by AM cortisol levels. The anticipated period of study participant hospitalization is 4 days, from day -1 to 48 hours post-dose. For a period of 1 week following initiation of steroid dosing, participants will be asked to remain within a 1-hour access of the study site due to the possible risk for corticosteroid-associated catabolism. Post-hLB-001 dosing, participants will return to the study site for weekly visits during month 1, every other week for months 2 and 3, and again at months 6, 9, and 12. Home healthcare visits may be conducted at weeks 2, 3, 6, and 10; additional home visits may be discussed with the study physician. During this 12-month (52-week) period, management regarding medications and dietary adjustment will follow the institutional standard of care. Following completion of their EOS visit, study participants will be encouraged to enroll in a long-term follow-up study. The hLB-001 mechanism of action is to provide a corrected copy of the mutated MMUT that is integrated into the genome of hepatocytes and subsequently expresses functional MMUT. The technology relies on site- and sequence-specific homologous recombination to achieve genomic integration. The clinical capsid, rAAV-LK03, does not transduce murine hepatocytes efficiently and the albumin homology arm sequences differ by species. Therefore, while the clinical vector hLB-001 could be tested *in vitro* with human hepatocytes, all *in vivo* mouse and NHP nonclinical studies to support pharmacology and safety were conducted using surrogate products. Four mouse pharmacology studies were conducted by the Sponsor or designated contract research organization to evaluate 2 mouse-specific surrogates of hLB-001 in MMA mice with transgenic Mmut skeletal expression via muscle creatine kinase (MCK) (Mut-/-/MCK-Mut+; severe MMA phenotype). The initial mouse surrogate construct, mLB-001a, encoded the human MMUT transgene identical to that in hLB-001. However, based on published results that document suboptimal mitochondrial import and processing for a human sequence in mouse, the mouse surrogate mLB-001b was developed to test the same capsid and homology arms as mLB-001a but now encoding mouse Mmut complementary DNA (cDNA). This first-in-humans protocol will enroll pediatric participants with severe methylmalonic acidemia (MMA) who will receive hLB-001. MMA is an autosomal recessive metabolic disorder in which infants cannot metabolize some amino acids and fats properly. This company is developing gene therapy products using recombinant Adeno-Associated Vvirus (rAAV) vectors that encode a transgene preceded by a 2A-peptide coding sequence and flanked by homology guide arms to enhance site-specific homologous integration.

The study product is a liver-targeted rAAV vector using the LK03 capsid (rAAV-LK03), designed to integrate the human methylmalonyl-CoA mutase gene (MMUT, also MUT). The goal is to provide durable hepatocyte expression of the MMUT gene without the use of exogenous promoters or nucleases. hLB-001 uses the natural cellular process of homologous recombination to integrate a copy of the human mutase gene into the human albumin locus. Following integration, expression of the transgene is driven by the endogenous ALB promoter. The transgene coding sequence is fused to ALB at the DNA and RNA levels but translated as a separate protein as the result of ribosomal skipping facilitated by the 2A-peptide sequence. The albumin target site was selected to permit high level expression of MMUT protein in transduced hepatocytes, which is anticipated to restore mitochondrial function, reduce serum MMA levels, and ameliorate the MMA disease state. A single administration of 1×10^{14} vg/kg hLB-001, expected to be biologically active, will be given. The primary objectives are safety and tolerability; secondary objectives include change from baseline in serum MMA levels, serum fibroblast levels, propionate oxidation rate, and clinical efficacy outcomes (hospitalizations, transplantations, growth parameters). The study will consist of 2 cohorts, each comprising approximately 4 participants for a total sample size of about 8, who have not had a liver transplant. The first cohort will enroll children ages 3-12 years; second cohort, 6 mos-3 years. The sample size is based on practical considerations considering the ultra-orphan participant population. Per the Investigator's Brochure (IB), non-clinical studies showed that hLB-001 surrogate vectors delivered IV to newborn or juvenile animals were safe, well tolerated, and led to transduction of hepatocytes, genomic integration, and liver transgene expression.

Biosafety: This is the first GeneRide vector developed for clinical use. Enrollment will be staggered; a DSMB will oversee the study. Children who test positive for anti-rAAV-LK03-neutralizing antibodies with titers above protocol-specified threshold of 1:10 may not enter the study. Oral prednisolone 1 mg/kg/day (or equivalent) will be initiated 24 hours prior to starting the hLB-001 infusion. Steroid dosing will continue for a planned 30-day course, longer if evidence of ongoing immune response, followed by a 30-day taper. Viral shedding (PCR) will be monitored in urine, saliva, and stool samples obtained over the first 4 weeks post-dosing. Children will be followed on a long-term basis under another protocol. No childhood vaccinations will be given within a 14-day window either prior to or following hLB-001 administration. Live vaccines are not to be administered for at least 3 months after stopping high-dose steroids. Possible adverse events include abnormal liver function tests, allergic reactions, long-term risk of hepatic cancers, and infections.

The application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900144
Title:	Bioactive fatty acid induced cell signaling
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 2/10/2023

Required modification:
1) Recombinant or Synthetic Nucleic Acids Usage: Uncheck *Section III-D-1* if no pathogens will be used as host-vector systems.

Comments:
Determination: Modification required for approval; *Reconsidered at the January 2020 meeting*
Required modifications from the January meeting:
1) Supporting Documents: If the protocol will be approved with BSL-2+ containment, then a Biosafety Operations Manual and laboratory inspection upgrade will need to be completed.

2) Waste Management: Question 3: Contact time for spill should be 20 minutes. In addition, clarify that the disinfectant used to clean equipment will be EPA-registered.

3) Waste Management: Question 1b: Clarify that liquid waste will be decontaminated by adding concentrated bleach to a final 1:10 dilution (v:v) bleach in liquid waste. Addition of pre-diluted 10% bleach to liquid wastes further dilutes the disinfectant and results in insufficient concentration of active ingredient for proper decontamination.

4) Exposure Assessment and Protective Equipment: Question 5: University guidance for work at BSL-2+ also requires the use of double gloves and a solid-front wrap around gown (additional information is available at the website:

(https://www.ehs.pitt.edu/sites/default/files/docs/05-016Lentivirus.pdf). Revise the application accordingly.

5) Risk Group and Containment Practices: Question 2: BSL-2+ should be selected if TSG will be knocked down using a Lentivirus/Lentiviral vector.

6) Viruses, Prions, or Vectors: Clarify if the tumor suppressor gene, LKB1 (STK11), will be knocked down via Lentivirus. This work may need to be conducted at BSL-2+.

7) Viruses, Prions, or Vectors: "Stbl3 *E.coli* strain" should be removed from the vector designation, as this is a bacterial strain.

8) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: The application identifies pFN18A Halo Tag T7 flexi vector elsewhere in the protocol for protein expression, but KRX is described here. Clarify this discrepancy.

9) Primary Cells or Cell Lines: The project summary states that shRNA and siRNA studies will be performed in HepG2 cells. The response indicates that mouse cell lines are used for knockdown studies. Clarify.

10) Primary Cells or Cell Lines: Nonhuman primate cells (COS-7) should be used under BSL-2.

Initial comments:
The goal of this protocol is to address how bioactive fatty acids lead to post-translational modifications of proteins and subsequent changes in cell function. Mouse and human hepatocyte cell lines will be used for gain/loss of function studies. siRNA for LKB1 will be delivered via lipofectamine transfections and shRNA studies will be performed with a 4-plasmid Lentiviral system. Human HepG2 cells will be used for siRNA and shRNA knockdown as stated in the project summary; however, mouse cell lines are also listed for knockdown studies in the protocol. Monkey kidney cells (COS7) will also be used for overexpression studies, and these cells are currently listed as BSL-1. As noted, the cell line experiments should be performed at BSL-2. No animal experiments are proposed. If revisions are made, the protocol could be suitable for approval. The investigator is studying proteins involved in bioactive fatty acids in human, nonhuman primate, and mouse cell lines. Cell lines will be obtained from ATCC and will be studied *in vitro*, including expression of siRNA or shRNA targeting LKB1. shRNA will be expressed in cells using a 4-plasmid Lentiviral vector purchased from *Addgene*. siRNA and plasmids will be transfected into cells. All cell work will be performed at BSL-2. The exception in the protocol is nonhuman primate cells, which is listed at BSL-1 but should be performed at BSL-2. *E. coli* will be used to propagate plasmids and for protein expression studies at BSL-1. No animals will be used in the protocol. A few clarifications are required prior to approval at low risk.

Initial review comments were provided to the investigator for response. The revised application was placed on the January 2020 agenda. Additional comments were provided regarding the use of gene editing and a tumor suppressor gene. The information will be discussed at the meeting.

January Action: Reconsideration: The committee discussed whether the investigator should be at BSL-2 or use BSL-2+ and it was determined that the investigator needs to revise the application and be clear upon which biosafety level is going to be used with this research study. IBC recommends BSL-2+ if there is qualifying work with Lentiviral vectors. Reconsideration review at the next available IBC meeting once the revisions have been completed.

January IBC meeting review comments were provided to the investigator for response. The revised application was placed on the February agenda for Reconsideration Review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD201900373
Title:	Amendment for **IBC201700077**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED BSL-2+ Manual 2017_ April 6 updated • SIGNED copy of IBC

Determination: *Disapproved/Approval Withheld*

Required modifications: None

Comments:

The Institutional Biosafety Committee reviewed the amendment and found that it does not meet the criteria for approval. The IBC determined that there are remaining numerous inconsistencies and discrepancies within the protocol modification. The Modification application has been disapproved, as it has come to the committee three times, without improvement. If work is still planned, then the investigator will need to submit a new modification application that clearly and accurately describes the proposed changes without inconsistencies. The original (parental) protocol (**IBC20170077**) continues to have approval until the expiration date. Work with the agents described in the Modification cannot be initiated until IBC approval is obtained.

Review the following comments for more information as to why the committee determined the disapproval of the Modification:

1) Animal Gene Transfer: Questions 5 and 6: In the Recombinant and Nucleic Acid Work Description there is language that indicates planned work with "delivery of siRNA to recipient mammalian cells and to rodents using AAV or 7C1 nanoparticles". However, in Questions 5 and 6 it is indicated that *no* siRNA will be administered directly to animals. The language in these two sections is conflicting. This is a discrepancy.

2) Animal Gene Transfer: Questions 5 and 6: In the Recombinant and Synthetic Nucleic Acids Work Description the investigator indicates that AAV will be used to deliver siRNA to rodents.

3) Recombinant or Synthetic Nucleic Acid Work Description: Question 3e: The information provided by the investigator in the second set of answers to this question "Use of AAV has a size limitation to package gRNA and Cas9 in a single backbone, so that we will have two different AAV vectors, one expresses Cas9, another one expresses gRNA. Use of Lenti-vector has no size limitation, so both Cas9 and gRNA are packaged into a single Lenti-particle. We also use plasmid DNA for transfection of targeted cells" conflicts with the information provided immediately above in answer to the same question. The committee could NOT determine whether Lentivector used in these studies will express both Cas9 and gRNA in a single Lenti-particle, as the responses contain conflicting information. In addition, it was unclear from the application if both AAV and Lentiviral vectors will be used to deliver CRISPR/Cas9 or if only Lentiviral vectors will be used. If AAV will be used to deliver CRISPR/Cas9 it would also need to be added under inserted nucleic acids in the Viruses, Prions, or Vectors section.

4) Viruses, Prions, or Vectors: AAV entry: AAV entry: In the Recombinant and Synthetic Nucleic Acids Work Description section the investigator indicates that AAV will be used to deliver siRNA to rodents. If this is the case, siRNA should have been added to the Inserted Nucleic Acids Information for this entry.
5) HIV entry: Both replication-competent and replication-deficient HIV/HIV-based viral vectors are described in the Description of Usage, but the Additional Virus Information section indicates that replication-deficient virus/viral vectors will be used. If both replication-competent ***and*** replication-deficient vectors will be used, then separate these agents into 2 different entries, as there is differences between the risks and biosafety concerns for replication-competent and replication-deficient vectors. If the intent of the 2 different entries listed in this section was to clarify infection of humanized mice with replication-competent HIV in a collaborator's laboratory and transfer of the mice to the investigator for tissue harvest then both entries would need to be revised to clearly indicate that that one describes the collaborative work only and the other entry describes the use of Lentiviral vectors to deliver genes or RNAs to cells in culture.
6) Primary Cells or Cell Lines: Mouse cell/cell line entry: In the description of usage for this section it is indicated that "Animals will not be treated with siRNA directly." However, in the Recombinant and Synthetic Nucleic Acids Work Description section the investigator indicates that siRNAs will be delivered to rodents using AAV or 7C1 nanoparticles. This is an inconsistency in the content of the application.
7) Primary Cells or Cell Lines: Human stem cells or iPS cells entry: Clarify how human cells are sourced from animal facilities. Are these human iPS cells that have been administered to animals (e.g. human-izing mice) and that have then been re-isolated from animals for *in vitro* use? It is not clear from the responses in the application that this is what is planned. The committee needs to know clearly this information.
8) Tissues, Blood, or Body Fluids: Second Mouse Entry: Clarify. The source and description of use for these materials are identical to the first mouse entry that is designated at BSL-2+. If these tissues are also from humanized mice infected with replication-competent HIV, the tissues must be used at BSL-2+. This is an inconsistency in the application.
9) Protocol Team Members: If Wei Sun will be involved with procedures described in this protocol the individual must take the *NIH Guidelines* training and be listed in the personnel section of the protocol application.
10) Amendment Introduction: Wei Sun is not listed in the personnel section of the protocol. Add this individual to the protocol if he or she will be handling any of the materials described in the protocol.

December meeting comments:

Determination: *Reconsidered from the December 2019 IBC meeting (second reconsideration)*

Required modifications:
1) Waste Management: Question 1b: Define "other EPA-registered disinfectants" with concentrations to be used and time for disinfection.

2) Risk Group and Containment Practices: Protocol personnel must complete the required *NIH Guidelines* training and must be added to the personnel page for this protocol.

3) Animal Gene Transfer: Questions 5 and 6: Finish describing which animals will receive 7C1 and/or AAV siRNA, and the exposure route for these materials.

4) Animal Gene Transfer: Based upon the investigator's draft note left in the response for question 6 that is in capital letters, it appears that humanized lymphoid tissues will be implanted into mice

by a collaborator, and then mice will be transferred to investigator's laboratory and infected with HIV-1. If work with replication competent HIV-1 and/or tissues from animals infected with replication competent HIV-1 are used in this study, then add an entry for HIV-1 in the Virus/Viral Vector section.

5) Animal Gene Transfer: Animals from collaborators contain full-length, replication-competent HIV-1.

6) Lentivirus and Lentiviral Vectors: Question 2a: Not all Lentiviruses used by the laboratory are replication-defective. The laboratory also uses full-length replication-competent HIV-1. This should be made clear in the response.

7) Viruses, Prions, or Vectors: Based upon the investigator's draft note left in the Animal Gene Transfer Section, it appears that humanized lymphoid tissues will be implanted into mice by a collaborator, and then mice will be transferred to investigator's laboratory and infected with HIV-1. If work with replication competent HIV-1 and/or tissues from animals infected with replication competent HIV-1 are used in this study, then add an entry for HIV-1 in this section.

8) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: Bacteria should be designated as recombinant. Revise the response to question 6 for this agent as it appears that it may be used to grow/amplify plasmids.

9) Primary Cells or Cell Lines: Mouse cells/cell lines: Provide additional information in the description of use for these cells (e.g. similar to the information provided in the human cells/cell lines entry).

10) Primary Cells or Cell Lines: Human stem cells/iPSCs: Provide additional description of work that will be performed with these cells (e.g. will nucleic acids be delivered solely via transfection or will viral vectors be used for target gene delivery).

11) Protocol Team Members: If personnel will be involved in this work then the individual(s) must complete the *NIH Guidelines* training and must be added to the application.

October meeting comments:

Determination: *Reconsidered at the October 2019 IBC meeting;* Required modifications from the October IBC meeting:

1) Supporting Documents: A recent, and signed copy of the laboratory's Biosafety Operations Manual must be uploaded to the application. The current manual is from 2017 and is not signed by a staff member of EH&S and the investigator.

2) Waste Management: Question 3: Identify the supplies available and steps to be followed to clean up a spill of biological materials. This information should be included in the investigator's BSL-2+ biosafety manual and is available on the EH&S website for reference: http://www.ehs.pitt.edu/assets/docs/bio-spill.pdf

3) Waste Management: Question 1b: Liquid waste: Liquid waste must be decontaminated by addition of concentrated bleach to liquid waste such that the final concentration of bleach is 1:10, v:v, bleach:liquid waste. Adding pre-diluted bleach to liquid waste further dilutes the bleach solution and results in insufficient final concentration of active ingredient to achieve effective decontamination. Revise.

4) Exposure Assessment and Protective Equipment: Question 4: Indicate whether a Biosafety Cabinet (BSC) will be used for all manipulations of potentially infectious materials. In addition,

at BSL-2/ABSL-2 and above safety engineered sharps devices are required. Revise the section accordingly.

5) Exposure Assessment and Protective Equipment: Question 1: This question asks the investigator to discuss whether any of the materials used in this protocol are potentially infectious for humans. For example, modified AAV vectors and Lentiviral vectors are both capable of a single round of infection in human cells. Replication-competent HIV is infectious for humans. Also, human cells and cell lines, even when purchased from an off-site vendor, may contain undetected adventitious agents that may be infectious to humans. Revise this section accordingly.

6) Animals: Remove or delete linked ARO protocols that have been completed and are no longer active/approved protocols.

7) Recombinant or Synthetic Nucleic Acid Work Description: In question 3d: Describe how CRISPR/Cas9 will be delivered to target cells. For examples, transfection of plasmid DNA, injection of gRNA and Cas9 proteins, using AAV or Lentivirus as a viral vector.

8) Recombinant or Synthetic Nucleic Acid Work Description: How will siRNAs be delivered to rodents? This work is not described elsewhere in the protocol. Will a viral vector (AAV, Lentivirus) be used for delivery? Will siRNAs be delivered directly? Clarify, and if necessary, add siRNA to the description of use section of appropriate viral vector or cell/cell lines entry.

9) Viruses, Prions, or Vectors: In the Lentivirus - HIV-1 entry the description of use indicates that CRISPR Cas 9 will be expressed from this replication-competent virus. If this is the case this entry should be modified to indicate that these materials are recombinant. If the CRISPR Cas9 will be used with a replication-deficient Lentiviral vector this information should be removed from the current entry and added to an entry for replication-deficient Lentiviral vectors.

10) Viruses, Prions, or Vectors: Lentivirus: HIV-1 entry: In the description of use the investigator indicates that replication-competent HIV will be obtained from a collaborator for use in these studies. However, the investigator has indicated in the additional virus information that the HIV-1 is replication-deficient. Clarify this discrepancy.

11) Primary Cells or Cell Lines: The human stem cells or iPSC entry indicates that these materials will be administered to animals. Add a description of this work to the description of use for this entry.

12) Primary Cells or Cell Lines: For all cell line entries: In the description of use the investigator indicates that cells and cell lines will be transfected with nucleic acids. Therefore, all of the cells and cell lines will contain recombinant material.

13) Tissues, Blood, or Body Fluids: For rat and mouse entries: Will samples isolated from rats and mice be reintroduced or transplanted into other animals? The response indicates that the investigator answered YES to these materials being administered to animals. Clarify.

14) Tissues, Blood, or Body Fluids: For rat and mouse tissues, blood, and body fluids entries: If tissues or other samples are collected from humanized mice infected with HIV these samples must be handled in the laboratory using BSL-2+ precautions. If rat and mouse samples are collected from animals exposed to risk group 2 biological agents, then they must be handled at a minimum of BSL-2. Revise accordingly.

15) Risk Group and Containment Practices: All personnel listed as handlers on this BSL-2+ protocol must complete the NIH Guidelines training module and be added in the Protocol Team Members section of the protocol. It may take up to 72 hours after completion of the training for the team member to appear in the system to be added to the Protocol Team Members section.

Initial comments:
There were significant clarifications or explanations required for this application. The committee determined that the application should be reconsidered once the investigator provides the responses back to the IBC. This is a modification to add RNAi and CRISPR/Cas to the protocol to alter expression of regulates disease-associated risk gene expression. There are many areas in regard to the CRISPR/Cas that the investigator does not address correctly, mainly the expression system being used for the CRISPR (it appears to be with Lentivirus as its listed at 2+, but that is not stated). Later in the application, it states that the CRISPR and sgRNA will be transduced separately, but that is inconsistent in other areas of the protocol. There are many form issues and inconsistencies in regard to the CRISPR work, as the protocol appears to be at 2+ already, but the CRISPR work as proposed (on separate vectors) can be at 2. It is unclear if this is introduced *in vivo* or is *in vitro* only. Clarification at the Virus page about the CRISPR and sgRNA is needed. In the original project, the investigator seeks to understand the molecular and genetic causes of pulmonary arterial hypertension (PAH) using cell models (primary and transformed human/rodent cell lines, and human iPSCs) and *in vivo* mouse models, including HIV-infected "humanized" mice expressing human immune cells. In addition, the investigator has an ongoing collaboration with a collaborator at the University of Georgia who studies a macaque model of SIV (simian immunodeficiency virus)-induced PAH. Cultured cells are infected with Lentivirus (HIV-1) or Adeno-Associated Virus (AAV-6) constructs to induce or knockout expression of genes of interest. Notably, vascular cells are infected with Lentivirus constructs expressing replication-competent HIV. Genetically engineered and "humanized" mice are infected and studied according to related IACUC protocols. Experiments are conducted under BSL-2+ and ABSL-2 conditions. In this modification, the investigator wants to add the use of RNAi and CRISPR/Cas9 to alter the expression of new genes of interest (some of them oncogenes). The research participants (transgenic mice?) and the vectors used to infect (HIV?) need to be clearly defined in the protocol. The potential use of a viral vector to alter oncogenes needs to be discussed with the full committee. There are comments for clarification.

Review comments were provided to the investigator for response. The revised application was placed on the October agenda.

Reconsideration: Significant safety concerns were noted in the application which need to be clarified or explained. The investigator was requested to meet with the IBC Chair and Biosafety Officer prior to submitting a revised application for reconsideration review.

The investigator provided a revised application which was placed on the December agenda for Reconsideration Review.

Reconsideration: Significant safety concerns were noted in the application which need to be clarified or explained. The investigator must provide a revised application for reconsideration review.

Second reconsideration review comments were provided to the investigator for response. The revised application was placed on the February agenda for Reconsideration Review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	11
Against:	1; member felt reconsideration would be appropriate
Abstained:	1; recused for involvement

Protocol:	IBC201900109
Title:	TERT Delivery to Human fibroblasts
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ MANUAL • IRB protocol • BSL-2+ Signature from EH&S

Determination: Approved

Last day of continuing review period: 2/12/2021

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 5: PPE at BSL-2+ includes double gloves. Revise.

2) Exposure Assessment and Protective Equipment: Question 4: correct "BSL-2" to "BSL-2+"

Comments:

Fibroblast immortalization can be affected by overexpression of telomerase reverse transcriptase (TERT). In this work, hTERT will be transduced into participant fibroblasts using replication-incompetent viral vectors, to prevent fibroblast senescence. The work will use human fibroblasts, derived from participants. These will be transduced with hTERT and then assayed. This work will be done at BSL-2+. A K12-derivative of *E. coli* (*Thermo Fisher Scientific*) will be used to propagate viral vector plasmids. Lentiviral vector assembled using the HIV 3-plasmid system (Magee Virus Core), and VSV-G pseudotyped, will be used to deliver hTERT. The transfer plasmid encoding hTERT will be obtained from *Addgene*, amplified in K12 *E. coli* and then sent to the Magee Virus Core for assembly of the viral vector. Participant-derived fibroblasts will be immortalized by transduction with hTERT-delivering Lentiviral vector. This work will be done at BSL-2+. Overall recommend approval after some clarifications are made. But due to proposed safety level and to receive confirmation that the laboratory Biosafety Operations Manual is current and in place and laboratory inspections are completed. This protocol describes the generation of immortalized human fibroblasts through Lentiviral-based expression of telomerase reverse transcriptase (TERT). A 4-plasmid Lentivirus will be used to transform primary human fibroblasts obtained from participants (an IRB protocol was identified) and used at BSL-2+, since TERT is an oncogene. *Escherichia coli* will be used for amplification of the viral vector plasmid at BSL-1. The virus is supposed to be supplied by the Magee Viral core, but the group is amplifying it in

E. coli. It is not clear why the group would be doing this with *E. coli* unless it is for storage of the plasmid or to provide DNA for transfection to the core.

Initial comments:
1) Funding Sources: Identify the NIH Institute and give a grant identifier rather than an accounting code
2) Primary Cells or Cell Lines: Elaborate the original source of the cells; clarify when the cells will be made "non-living".

Review comments were provided to the investigator for response. The revised application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900172
Title:	Strategies to Induce Tolerance to CTA
Investigator:	REDACTED
Highest BSL:	BSL-1 ABSL-1
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 4: The YES response indicates that this application will also produce materials that will be produced and distributed to other investigators/researchers. If this is true, then question 3 should be modified to include a description of the activities the "core" will be performing for other investigators. If the laboratory is not distributing materials to other investigators as a function of the work, then the response to question 4 should be changed to NO.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Earlier in the protocol, it is indicated that this is a core facility. Describe actions of core facility and how animals/samples will be disseminated to other investigators.

3) Waste Management, Question 1b: Liquid waste disposal: EH&S does NOT recommend use of *Peroxal 70 Bio* for decontamination of liquid or solid waste. This product is a 70% hydrogen peroxide solution that is toxic to aquatic life and poses a high risk of chemical burn to personnel if splashed on skin or in eyes. Contact EH&S (biosafe@ehs.pitt.edu) for instructions for proper disposal of unused *Peroxal 70 Bio* as chemical waste and for recommendations for alternative EPA registered disinfectants.

Comments:

SD eGFP rats will be sacrificed. Bone marrow (BM) cells will be harvested from femurs and tibias. Following red blood cells depletion, remaining BM cells will be washed in complete medium. Dendritic Cells (DC) will be isolated and injected either into the footpads or in the penile vein of wild-type SD rats. Rabbit anti-rat lymphocyte serum will be used to study T- and B-cell lymphocytes depletion. There are a few issues between the IBC and the IACUC protocol that need to be corrected or clarified prior to approval. The A/BSL level (1), is appropriate for the work and recommendations are for approval with convened review due to this being identified as a Core Facility. This project will study the role of donor bone in establishing micro-chimerism and inducing allograft tolerance in SD rats. Bone marrow cells from eGFP tagged rats will be injected into wild type rats. BSL-1/ABSL-1 is proposed. Recommendation for approval.

Review comments were provided to the investigator for response. The revised application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	IBC201900178
Title:	Nef Function in Humanized Mice
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL-2+ Manual for REDACTED 533 and 545

Determination: Approved

Last day of continuing review period: 2/10/2021

Required modifications: None

Comments:

This protocol will investigate the function of the HIV regulatory factor Nef using humanized mouse models of HIV infection. The work will also examine the effects of small molecule Nef inhibitors on HIV-1 replication in these mouse models. This work will use human thymus and liver (obtained from Magee and CHP). Cells will be isolated and implanted in NOD/SCID/gamma null (NSG) mice (obtained from DLAR). PBMCs isolated from human blood (from *Vitalant*) will be used for *in vitro* replication of HIV-1 and for implantation. CD4 and CD8 cells will be isolated from blood for *in vitro* infection. Lymphatic, blood and immune tissue will be isolated from infected mice for various assays. 293T cells will be used to generate replication-competent HIV. Other cell lines (HeLa, CEM) will be infected. All will be obtained from *ATCC* or the NIH AIDS Reagent Repository. Plasmids will be used to generate replication-competent HIV-1 used will be strain NL4-3 (obtained from NIH AIDS Reagent Repository). This strain generates replication-competent virus particles in many cell types on infection. The plasmids will also occasionally carry a reporter or will be mutated. Work will be done at BSL-2+ and ABSL-2. Hazard assessments and waste disposal plans are appropriate and detailed. Once queries are addressed, recommend approval. This protocol describes research to examine the role of the Nef protein, an HIV regulatory factor, and effects of small molecule inhibitors of Nef in humanized mouse models of HIV infection. Human thymus and liver as well as PBMCs from human blood obtained from a blood bank will be transplanted into immunodeficient mice. Mice will be infected with replication-competent HIV-1 after transplant. Tissues will be isolated from infected mice for use in *in vitro* assays and human cells and cell lines with be used to generate HIV and for *in vitro* assays. Replication-competent HIV will be generated in the investigator's laboratory from plasmid DNA and some strains may be recombinantly-modified to express reporter genes (e.g. luciferase/GFP). All work will be performed at BSL-2+ and ABSL-2, which is appropriate. There are several minor

clarifications required. Recommend approval once all requested minor changes to protocol have been completed.

Initial comments:
1) Primary Cells or Cell Lines: Check: These cells are not stem or iPS
2) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-3* may be needed
3) Genetically Engineered Animals: Source: It appears that Question 2a should be answered.

Review comments were provided to the investigator for response. The revised application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	1; recused for involvement

Protocol:	MOD201900487
Title:	Amendment for **IBC201700034**
Investigator:	REDACTED
Highest BSL:	RBL ABSL-3
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED IBC Appendix RVF RG system

Determination: Approved

Last day of continuing review period: 2/10/2021

Required modifications: None

Comments:

This amendment adds the use of Adenoviral vectors expressing Cre recombinase for the generation of transgenic mice, in a protocol in which the work involves RG-3 classified agents and is conducted at RBL and ABSL-3. In the original protocol, the investigator seeks to develop a system to genetically modify Rift Valley Fever Virus (RVFV) to study its pathogenic mechanisms. These experiments use wild type and recombinant live RVFV to infect donated reproductive tissues from human, sheep, goat, cow, and hamster. Cell lines used for *in vitro* infection include iPSC-derived human neurons and other human-derived cells, CT-1 bovine cells, BV2 mouse cells, sheep oTr-LTa trophobalasts, pig iTR trophoblasts, and canine MDCK cells for transcript and protein expression studies. Wild type and transgenic mice are infected with RVFV through various means (aerosol, intranasal, subcutaneous, intratracheal, intragastric). Hek293T human cells are used to generate viruses from transfected plasmids, and Vero E6 primate cells are used to propagate the virus. A Lentiviral system is used at another university to create a Crispr/Cas9 library in BV2 and Huh7 cells, which is then transferred to Pitt. Furthermore, Influenza Viruses (IFV A/California/07/2009) is used as a process control for the reverse genetics system. Vesicular Stomatitis Virus (VSV) Indiana or San Juan are also used to express or inhibit expression of putative target receptors (lipid receptors LRP1 and Lrpap1) during *in vitro* studies. Finally, Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV), a product of the reverse genetics system, is used in the same types of experiment as RVFV (how exactly they plan to use SFTSV is not clear). IFV, RVFV, and SFTSV can enter human cells and are not replication-defective. AdV-5 and VSV can enter human cells and are replication-defective. This amendment describes the addition of Adenoviral vectors that express Cre recombinase for use in murine cells and tissues from genetically modified mice. The overall project is to study the biology of Rift Valley Fever Virus, a Risk Group 3 organism. Work will be done in the RBL, so the BSL is more than adequate. Minor information is requested in the Tissues, blood, and body fluids section.

Review comments were provided to the investigator for response. The revised application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD202000035
Title:	Amendment for **IBC201700337** Pseudovirus for 2019 n-CoV
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• psPAX2 • psectag2_man • pMD2 • pcdna3_1_man • pLVX-IRES-Puro Vector Information • pNL4-3.luc.r-e- • pNL4-3.luc.R-E- sequence and map

Determination: Approved

Last day of continuing review period: 2/10/2021

Required modifications:

1) Primary Cells or Cell Lines: For Other Human cells or cell lines remove the information regarding administration to animals, as this will not be performed in this laboratory.

2) Viruses, Prions, or Vectors: Lentivirus HIV-1 - HIV-1 NL4-3 ΔEnv Vpr Luciferase Reporter Vector (pNL4-3.Luc.R-E-): This vector should be designated as recombinant. List all non-viral genes (e.g. luciferase) under "Inserted Nucleic Acids Information."

3) Viruses, Prions, or Vectors: Vesicular Stomatitus Virus (VSV) - pMD2.G: It appears that the researchers are not producing Vesicular Stomatitis Virus, this entry should be removed. This information can be included the Recombinant and Synthetic Nucleic Acid Work Description section.

4) Viruses, Prions, or Vectors: Other - pSecTag2A: This section is for viruses, viral vectors, prions. As this is not a viral vector, it should be removed from this section. This information can be included the Recombinant and Synthetic Nucleic Acid Work Description section.

5) Viruses, Prions, or Vectors: Other - pcDNA3.1: This section is for viruses, viral vectors, prions. As this is not a viral vector, it should be removed from this section. This information can be included the Recombinant and Synthetic Nucleic Acid Work Description section.

6) Exposure Assessment and Protective Equipment, Question 1: Based on information earlier in the protocol, it appears that CoV glycoprotein will be expressed from non-viral vectors. Thus, the following statement is incorrect: "Proteins related to human pathogens (e.g. Coronavirus) will be expressed using Lentiviral vectors." Remove or clarify this statement.

7) Exposure Assessment and Protective Equipment, Question 1: The following statement should be removed, as it appears that CoV will not be used in this study: "Work with the n2019-CoV (Coronavirus) will include work with only the S-gene of the virus. Therefore this, and the luciferase gene will be the only ones expressed."

8) Exposure Assessment and Protective Equipment, Question 4: All appropriate PPE needed to perform laboratory procedures must be accessible to personnel in the room/area where work will

be performed. Personnel should not have to transit between several spaces in order to collect needed PPE. Clarify.

Comments:
This modification seeks to add work with replication-deficient Lentiviral vectors pseudotyped with an envelope protein from 2019n-Cov. There is significant detail missing regarding the new Lentiviral vector to be obtained from a collaborator and how the viral particles will be pseudotyped. Additional clarification is required. If comments can be addressed prior to the next scheduled meeting this reviewer agrees that low risk review may be appropriate. If clarifications cannot be made, this modification should probably come to convened review and discussion of whether member review of pending clarifications would be appropriate. This is a modification to an existing protocol to add "a pseudovirus with the envelope glycoprotein from the new 2019-nCoV." Presumably, this means pseudotyping HIV with Novel Coronavirus envelope. However, no details are provided on the new Lentiviral vector system obtained from the collaborator's laboratory, how pseudotyping will performed, and what will be done with such viruses. Animal studies are referred to in the protocol, but it does not appear that animals will be used in this study. All work is proposed at BSL-2, which is probably acceptable, but without specific details, it is hard to assess.

Review comments were provided to the investigator for response. The revised application was placed on the February agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	2; recused for involvement

Protocol:	MOD202000041
Title:	Amendment for **IBC201700059**
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• xxLAI structure#2 • pNL4.Luc.R-E- HIV backbone

Determination: Approved

Last day of continuing review period: 2/12/2021

Required modifications:

1) Basic Information, Question 3: Add a brief description of the new planned work to the project summary.

2) Primary Cells or Cell Lines: The description of usage does not describe the planned work with HEK293T-hACE2 cells as described in the amendment cover sheet. Revise.

3) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: *E. coli* should be designated as recombinant in the drop-down menu.

4) Viruses, Prions, or Vectors: Description of nCoV pseudotyped viruses is not clear. Replication competent and replication-defective viruses should be separated into 2 different entries.

5) Viruses, Prions, or Vectors: Inserted Nucleic Acids Information is for any non-viral genes expressed from a Lentiviral vector. Information about other plasmids should not be entered here.

6) Lentivirus and Lentiviral Vectors, Questions 2 and 2a: Lentiviruses produced from multiple plasmids should be described here.

Comments:

This is a modification to an existing protocol to add new personnel, new plasmids expressing viral envelopes, and addition of a new cell line. Personnel from a collaborator's laboratory will work in the REDACTED laboratory space to pseudotype HIV with nCoV and VSV-G glycoproteins. 293T-hACE2 cells will be used to test the ability of potential anti-nCoV antibodies to neutralize the pseudotyped viruses. Several forms issues and clarifications need to be corrected prior to approval. This modification to an existing protocol describes work with a Lentiviral vector pseudotyped with envelope proteins from 2019-nCoV and VSV-G glycoproteins. Pseudovirus will be used to produce antibodies *in vitro* and a new HEK293T-Ace2 cell line will be used to test the neutralizing capability of any antibodies developed. There are several forms issues and clarifications needed.

Due to the meeting deadline, initial review comments were not able to be provided to the investigator in advance of the meeting. The application was placed on the February agenda, as it was received by the office.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval. There was no described use of full virus, therefore BSL-2 is appropriate.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD202000042
Title:	Amendment for **IBC201800241**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• IBC Recombinant viruses (Tables) 200131 • REDACTED USDA permit • REDACTED BU Approved IBC

Determination: Approved

Last day of continuing review period: 2/10/2021

Required modifications:
1) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Define "N95s" and clarify what recombinant materials are currently administered (or planned for administration) to animals.

Comments:
This is a modification to an existing protocol to generate recombinant Measles Virus and Vesicular Stomatitis Virus to express Coronavirus glycoproteins, including the novel 2019 human CoV. There are no biosafety concerns, clarification of what recombinant materials will be administered to animals is needed. This is a modification to an existing protocol describing use of Measles Vaccine Virus backbones and VSV-based vaccine vectors to develop vaccines for high-consequence pathogens. The modification specifically adds expression of single Coronavirus glycoproteins, including 2019-nCoV. There are no biosafety concerns as the investigator is experienced and has approval to generate candidate vaccines via expression of glycoproteins from other RG3 agents.

Due to the meeting deadline, initial review comments were not able to be provided to the investigator in advance of the meeting. The application was placed on the February agenda, as it was received by the office.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval. There was no described use of full virus, therefore BSL-2 is appropriate.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	2; recused for involvement

Protocol:	MOD202000043
Title:	Amendment for **IBC201600015**
Investigator:	REDACTED
Highest BSL:	RBL ABSL-3
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Appendix 1C • Appendix 5 • Appendix 4 • Appendix 2 • Appendix 3A3B • Appendix 1A • Appendix 1B • SINV chimeric viruses safety test • Appendix 1D

Determination: Approved

Last day of continuing review period: 2/12/2021

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description, Question 3e: The response in question 1 states, "Use of CRISPR/Cas9 DNA plasmid vectors purchased from commercial sources that contain both the Cas9 and guide RNA on the same vector." Here it is stated that CRISPR/Cas9 and guide RNA will be on separate vectors. Clarify the discrepancy.

2) Exposure Assessment and Protective Equipment, Question 4: Include Coronavirus in the response.

3) Supporting Documents: The laboratory's Biosafety Operations Manual needs to be uploaded for IBC review.

4) Supporting Documents: Attached documents do not display properly; upload as PDFs.

5) Supporting Documents: Appendix 3: Section 3B - Propose an appropriate biosafety level for work with “pathogen immunogens”.

Comments:

This is a modification of an existing protocol to update personnel, change the title, and add the following: a) RNA viruses expressing Coronavirus (SARS, MERS, 2019 nCoV) spike proteins; b) 2019 nCoV (not recombinant) to be used for challenge experiments in mice c) rabbits and rats that will be immunized with the RNA viruses expressing Coronavirus spike proteins. The attachments do not open and should be uploaded as PDFs. A few minor form issues should be corrected. Recommend approval pending modifications. The modification describes changes of personnel, addition of RNA viral vaccine candidates expressing proteins from Corona Viruses (MERS, SARS, and 2019-nCoV) as well as challenge experiments to expose vaccinated animals to 2019-nCoV and MERS. The investigator is well-established, approved for work with MERS CoV at BSL-3/ABSL-3 in the RBL and will work with infectious 2019-nCoV in the RBL using all

practices approved for work with MERS. The attachments were reviewed and there was a comment provided regarding the attachments.

Due to the meeting deadline, initial review comments were not able to be provided to the investigator in advance of the meeting. The application was placed on the February agenda, as it was received by the office.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	2; recused for involvement

Protocol:	MOD202000050
Title:	Amendment for **IBC201600202**
Investigator:	REDACTED
Highest BSL:	RBL ABSL-3
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Appendix 6 • Appendix 1D • PI biosafety manual approval • Appendix 3 • Appendix 1B • Appendix4A4B • REDACTED Sindbis EEE VEE WEE Chimeras RA 12-2014 Final (1) • Appendix 1A • PI biosafety manual • Appendix 2 • Appendix 5 • Appendix 1C

Determination: Approved

Last day of continuing review period: 2/12/2021

Required modifications:

1) Basic Information, Question 3: The recombinant nucleic acid work to be added to the protocol in this modification should be described in the response.

2) Tissues, Blood, or Body Fluids: Rabbit: BSL use is listed as BSL-2. Yet, the "Description of Usage" describes BSL-3. Clarify the discrepancy.

3) Tissues, Blood, or Body Fluids: Rat: BSL use is listed as BSL-2. Yet, the "Description of Usage" describes BSL-3. Clarify the discrepancy.

4) Tissues, Blood, or Body Fluids: Rabbit: The Source cannot be empty. Provide a response.

5) Tissues, Blood, or Body Fluids: Rat: The Source cannot be empty. Provide a response.

6) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: *E. coli* should be designated as recombinant.

7) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include a description of recombinant work to be performed in rats and rabbits.

8) Recombinant or Synthetic Nucleic Acid Work Description, Question 2: Identify the expression system(s) used for expression of SV40 Large T antigen.

9) Animal Gene Transfer, Question 4: Identify the highest biosafety level that will be used in these studies for consistency.

Comments:

This is a modification to include expression of Coronavirus Spike proteins and T-cell epitomes in Alpha- and/or Flavivirus-based viral vectors to be used as vaccine candidates. Mice, rats, and rabbits will be immunized. The investigator is experienced and has approval to do similar work at BSL-2 and BSL-3 as appropriate. There are several minor clarifications that should be made. Recommendation for approval pending minor clarifications. This is a modification of an existing protocol to include expression of Coronavirus Spike proteins and T-cell epitopes from Alphavirus DNA and RNA vectors for immunization of mice, rats, and rabbits. Personnel and IACUC protocols were also managed. Several inconsistencies and missing new information should be corrected prior to approval.

Due to the meeting deadline, initial review comments were not able to be provided to the investigator in advance of the meeting. The application was placed on the February agenda, as it was received by the office.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	2; recused for involvement

Protocol:	MOD202000046
Title:	Amendment for **IBC201800164**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 2/10/2021

Required modifications:

1) Basic Information, Question 3: Include nCoV work in the project summary.

2) Funding Sources: Include an active funding source, the NIH funding that is listed has expired.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Describe the work to be done with CoVID 19.

4) Live Animals, Question 1: Identify an active IACUC/ARO protocol number.

5) Exposure Assessment and Protective Equipment, Question 4: Since no BSL-2+ work is proposed in this protocol, remove all references to BSL-2+.

Comments:

This is a protocol modification to produce Adenoviruses expressing nCoV antigens that will be used to infect mice to generate an immune response. All work will be performed at BSL-2/ABSL-2, which is appropriate. Several forms issues must be corrected prior to approval. This is a modification of an existing protocol to express a glycoprotein from the 2019-nCoV in an Adenoviral vector. The vaccine candidate will be administered to mice to examine potential immune response. The work is proposed at BSL-2/ABSL-2, which is appropriate.

Due to the meeting deadline, initial review comments were not able to be provided to the investigator in advance of the meeting. The application was placed on the February agenda, as it was received by the office.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval. There was no described use of full virus, therefore BSL-2 is appropriate.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	2; recused for involvement

Protocol:	MOD202000051
Title:	Amendment for **IBC201900008**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 2/10/2021

Required modifications:

1) Basic Information, Question 1: The title does not reflect the work in this study. Revise.

2) Basic Information, Question 3: Include 2019-nCoV Spike 1 Vaccinia Virus in the project summary.

3) Primary Cells or Cell Lines: Mouse (murine) (2F8): Provide information on how these cells are recombinant in the Description of Usage.

4) Primary Cells or Cell Lines: Other Human cells or cell lines (HeLa): Provide information on how these cells are recombinant in the "Description of Usage".

5) Viruses, Prions, or Vectors: Vaccinia Virus entry: Confirm the information in "Description of Usage". Will Vaccinia Virus expressing Spike protein from 2019-nCoV be administered to mice to reverse tumor formation or to determine whether mice develop immune response to the Spike protein?

6) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Provide a description of work with 2019-nCoV Spike 1 Vaccinia Virus and what recombinant materials will be administered to animals.

7) Live Animals, Question 1: Remove the expired IACUC protocol.

Comments:

The investigator is requesting the use of WR strain Vaccinia Virus to the protocol at BSL-2 for *in vivo* work. It is unclear if the work has been discussed with EH&S and if the staff have been adequately counseled as to the use of VV. Recommend approval once the laboratory is registered with EH&S and all staff members are enrolled in employee health counseling/vaccination with employee health. This is a modification to an existing protocol to add Vaccinia Virus encoding the Spike protein from the 2019 Novel Coronavirus for vaccine development. The modification is not complete as new work has not been updated in all sections as necessary. Once modifications and clarifications are provided, it may be approved.

Due to the meeting deadline, initial review comments were not able to be provided to the investigator in advance of the meeting. The application was placed on the February agenda, as it was received by the office.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval. There was no described use of full virus, therefore BSL-2 is appropriate.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	2; recused for involvement

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 11:37 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

March 9, 2020 10:00 AM

302 Heiber Building

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			
REDACTED, Vice Chair			
REDACTED		REDACTED	
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED	Absent		
REDACTED			
REDACTED			
REDACTED			
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REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED			
REDACTED	Absent		

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office

GUEST NAMES

REDACTED, Export Control Office

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present. The guest was introduced and is observing the meeting as she is working with the EH&S team in the capacity of export control issues.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The February 2020 meeting minutes were reviewed and approved by the committee.

Votes:

For:	16
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

The Biosafety Officer was in attendance at a meeting of the Pandemic Preparedness Working Group for the University and has made the committee aware of a Preparedness Guide for Researchers.

IBC OFFICE REPORT

A new method of notifying committee members of the lower risk studies being placed onto the next available agenda for review. The members should look at the status bar (blue rectangle at the top left side of the workspace) to determine whether the review is under “Committee Review” or if the study is under “Member Review” and waiting upon individual assessment and evaluation.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocols on the following pages

Protocol:	IBC202000002
Title:	Cancer pain mediators
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• GeneCopoeia-ORF-cDNA-Clone-Collection

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: The name of the virus should be modified, as "*E coli*" is not a strain or type of Lentiviral vector.

2) Recombinant or Synthetic Nucleic Acid Usage: It is unclear how cloning will be done with the Lentiviral vectors as elsewhere it states that no bacteria will be used and viral particles will be purchased, not generated. Uncheck *NIH Section III-D-2* if no cloning will be performed.

3) Lentivirus and Lentiviral Vectors, Question 2a: Describe the safety features of each different Lentivirus or Lentiviral vector system that is used in this research.

4) Animal Gene Transfer, Question 2: This should be marked YES for use of Lentivirus.

5) Exposure Assessment and Protective Equipment, Question 4: Safety engineered sharps devices are required for work with BSL-2/ABSL-2 agents; revise.

Comments:

This is a new protocol from an investigator who will study cancer pain and therapies in cell culture and in mice. Lentiviruses (and perhaps Retroviruses?) will be cloned and used to express cDNAs and shRNAs in cells. These viruses will apparently be used to transduce human cells and these cells will be injected in mice. All work will be performed under BSL-2/ABSL-2 containment. There is a significant amount of missing information in the application that should be corrected by the investigator prior to coming to the committee. Based on response to comments, the investigator may need to revise additional sections in the application. This is a new protocol that seeks to better understand how oral cancers induce pain and to identify new strategies for managing cancer pain. Cell culture and immune suppressed mouse models will be utilized in this protocol in addition to Lentivirus and Retrovirus. Transduced human cell lines will be injected into the tongues of mice; however, details of this remain unclear. Work is proposed at BSL-2/ABSL-2. There is a significant amount of information missing from the application that will need to be added before approval.

Review comments were provided to the investigator for response. The revised application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	IBC202000006
Title:	Immune Cell Tracking and T-cell Transfer Mediated Colitis
Investigator:	REDACTED
Highest BSL:	BSL-1
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 3/9/2021

Required modifications: None

Comments:
The investigator will be studying inflammation and will be using adoptive transfer and bone marrow transplants. T-cells, Tregs, and bone marrow from donor mice will be adoptively transferred into recipient mice. Wild-type and genetically engineered mice will be used in the studies as well as bred. The are no biosafety concerns and no use of viruses or human cell lines. BSL-1/ABSL-1 has been proposed and is appropriate.

Administrative Review comments were provided to the investigator for response. The revised application was placed on the March agenda.

Approval recommended: No additional comments were provided by the committee.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	IBC202000017
Title:	Gram-positive Bacterial Protein Secretion
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: Note that interstate transport of *L. monocytogenes* and *S. pneumoniae* will require a transport permit from USDA/APHIS. A copy of the permit should be provided to EH&S. The University of Pittsburgh requires all transport of biological agents to be performed by an approved commercial carrier (e.g. *FedEx*, *World Courier*, etc.). Hand carry or transport of infectious materials in personal vehicles is not permitted. If large quantities of biological material need to be transported with temperature control, the University can assist with making arrangements with a commercial vendor to move entire freezers, etc.

2) Recombinant or Synthetic Nucleic Acid Usage: *NIH Sections III-D-1* for use or cloning of human or animal pathogens used as host-vector systems, and *Section III-D-2* for use of cloning of pathogen DNA or RNA in a non-pathogenic prokaryote or lower eukaryote should be checked. *S. pneumoniae* and *L. monocytogenes* are human and animal pathogens (*Section III-D-1*) and propagation of plasmids in *E. coli* meets the requirement for *Section III-D-2*.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include information regarding protein expression and purification. If protein will be expressed in a specific bacteria, insect or other cell line as part of the growth and purification process; add the strain and/or cell line to the appropriate portion of the protocol.

4) Exposure Assessment and Protective Equipment, Question 1: Include a statement that immunocompromised individuals, pregnant women, and women of childbearing age have a greater risk of infection when working with *L. monocytogenes*.

5) Exposure Assessment and Protective Equipment, Question 4: Immunocompromised individuals, pregnant women, and women of childbearing age have a greater risk of infection when working with *L. monocytogenes*. Agents should be handled in a certified biosafety cabinet. Any centrifugation of agent should include the use of rotor lids or individual rotor cups with safety lids. Revise. Safety-Engineered Sharps Devices are required for any manipulations with biological agents at BSL-2/ABSL-2 or higher (e.g. needles, scalpels); revise. In addition, revise the statement "benches and workspaces are cleaned with ethanol daily". Ethanol solutions are not EPA-registered disinfectants and should not be used as a primary means of chemical disinfectant; identify an EPA-registered disinfectant.

6) Exposure Assessment and Protective Equipment, Question 5: Indicate appropriate PPE that will be used in animal facilities. Minimum required PPE at ABSL-2 includes use of a hair bonnet, liquid-barrier coverall suit, gloves, shoe covers, surgical mask, and face shield. Revise accordingly.

7) Waste Management, Question 3: The spill should be contained prior to addition of bleach by placing absorbent material over the spill; revise.

Comments:
Plasmids will be used for cloning and expression of gram-positive bacterial proteins. Recombinant *Listeria monocytogenes* and *Streptococcus pneumoniae* will be administered to mice while lungs, livers, and spleens will be collected from the infected animals for bacterial cell counts. Human, murine, and Ptk2 from Adult Male Rat Kangaroo (*Potorous tridactylus*) are used for *in vitro* experiments to access for bacterial adhesion and intra-cellular growth. Work is proposed at BSL-2 which is appropriate.

Administrative review comments were provided to the investigator for response. The revised application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	IBC202000022
Title:	Mechanisms of lung infection and inflammation
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: DH5a for the propagation of DNA plasmids should only require BSL-1. Revise the BSL.

2) Recombinant or Synthetic Nucleic Acid Usage: *Section III-D-3* should be selected for use of viruses in cell culture.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Describe work with transgenic animals and administration of recombinant materials to animals. The Viruses, Prions, or Vectors section of this protocol lists administration of recombinant Influenza and Lentivirus to animals. If these agents will not be administered to animals, then revise the entries and correct the inconsistencies within the application.

4) Live Animals, Question 6: Lentivirus to be administered to animals was mentioned in earlier section of the protocol. This is inconsistent with other comments provided by the investigator. Correct all inconsistencies.

5) Animal Gene Transfer, Question 6: Confirm that Lentivirus will be administered. Include administration of Influenza A.

6) Exposure Assessment and Protective Equipment, Question 4: The use of needles is listed in Question 2. Safety-engineered sharps (e.g. needles, scalpels) are required at BSL-2/ABSL-2 for blood draws, tissue harvest, tissue disaggregation.

Comments:

This protocol seeks to understand the relationship between inflammation and infection in the lungs as it pertains to asthma and related respiratory diseases. The investigator is using both cell culture and a transgenic mouse model to look at the role of the IL-22 receptor in this pathogenesis. Mice will be infected with a Lentivirus to generate IL-22 receptor expression. Several strains of bacteria are listed as well as a GFP-expressing Influenza Virus. The investigator does need to provide more details and information on exactly what they are planning to do in the mice as well as cell culture and how the Influenza and bacteria identified in the application fit into the study. This immunological study will use several infectious and inflammatory agents to analyze the role of IL-22 and related proteins in lung ailments such as asthma, respiratory distress syndrome, and infections. Transgenic mice and human and murine cell culture/cell lines will be used along with various bacteria including *Haemophilus influenza*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Influenza Virus. Additionally, 4-plasmid Lentivirus will be used to silence expression of IL-22R in mice and cell culture and to express ARRBI and ARRB2 in cell culture

only. Wild-type and recombinant Influenza (GFP expressing) will be used to infect mice. Work is to be performed at BSL-2/ABSL-2, which is appropriate.

Review comments were unable to be provided to the investigator for response prior to the meeting due to the meeting deadline. The application was placed on the March agenda; reviewer comments and any additional comments from the committee will need to be addressed by the investigator.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	MOD202000018
Title:	Amendment for **IBC201800277**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Lentivirus packaging agent COA • *Addgene* Lentivirus vector information • *Takara Bio* Lentivirus vector information

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: Cell/CellLine - Other Human cells or cell lines (THP-1 (*ATCC*® TIB-202™), U-937 (*ATCC*® CRL-1593.2™), K-562 (*ATCC*® CCL-243™), and T2 (174 x CEM.T2) (*ATCC*® CRL-1992™)): as T-cells will be transduced with recombinant viruses, this entry should be designated as recombinant.

2) Risk Group and Containment Practices, Question 1: Select Risk Group 3 (RG-3) for use of Lentiviral vectors.

Comments:

The aim of this modification to is to transduce human cell lines (*Jurkat and 293T*) and primary T-cells with leukemia-reacting T-cell receptors via a 4th generation Lentivirus system. The transduced cells will be incubated with four additional leukemia cell lines to test target killing function *in vitro*. The work will be conducted under BSL-2 conditions. Approval is recommended.

Review comments were provided to the investigator for response. The revised application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	MOD202000063
Title:	Amendment for **IBC201600106**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: AAV can infect human cells. Correct the responses.

2) Viruses, Prions, or Vectors: The source cannot be null. Add the source for each AAV vector.

3) Recombinant or Synthetic Nucleic Acid Usage: *NIH Section III-D-1* should be selected for use of AAV as a host-vector system.

4) Animal Gene Transfer, Question 4: AAV is requested for use as BSL-2. Thus, use of them *in vivo* should also be used at ABSL-2.

5) Risk Group and Containment Practices, Question 2: ABSL-2 should be selected for *in vivo* use of AAV.

6) Exposure Assessment and Protective Equipment, Question 4: Safety-engineered sharps are required for use with BSL-2 agents; revise. Describe if a biosafety cabinet will be used. Any work outside of a biosafety cabinet must include use of full-face protection (e.g. goggles and surgical mask).

7) Exposure Assessment and Protective Equipment, Question 5: Include all PPE required for entrance to the ABSL-2 animal facility (double gloves, face shield, surgical mask).

8) Waste Management, Question 1c: Pathological waste is defined as waste material consisting of only human remains, anatomical parts, and/or tissue. Animal carcasses should be sealed in double bags, labeled, and disposed according to animal facility guidelines. Revise.

9) Waste Management, Question 3: Use of AAV in the laboratory may result in a spill. Provide detail describing the process for clean-up and decontamination of a biological spill (e.g. clean up materials, contact time for disinfectant, disposal of potentially contaminated clean up materials).

Comments:

This is a modification to add the use of AAV as a delivery system to label neurons to an approved transgenic mouse only IBC. They propose to use AAV injected IP into mice to label dopaminergic cells *in vivo*. There are many form issues, including missing information about the AAV (in the virus section) that need to be addressed. Since "viruses" was not selected, no information about the AAV is provided. Most sections have not been updated to reflect the use of AAV, what its source is, if they will be cloning or generating, etc. In addition, the application is missing waste management information. Since there is so much information missing, it is suggested that the application be revised before review by the committee. Until the information requested on the

AAV is provided, a meaningful evaluation cannot be performed. This should come back for review after revision.

Review comments were provided to the investigator for response. The revised application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	MOD201900312
Title:	Amendment for **IBC201800131**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Clarify how the Adenovirus is not replication-defective. If this is incorrect, correct the response.

2) Recombinant or Synthetic Nucleic Acid Usage: *NIH Section III-D-3* should be selected for use of a virus in cell culture.

3) Exposure Assessment and Protective Equipment, Question 1: Adenovirus is infectious and must be described here. In addition, a statement should be included that cell lines may harbor unknown infectious agents.

4) Exposure Assessment and Protective Equipment, Question 2: Describe any procedures that may increase risk for accidental exposure to personnel via percutaneous or mucous membrane exposure routes or environmental release with BSL-2 reagents.

5) Waste Management, Question 1a: Describe the methods used for proper decontamination (e.g. specific disinfectant or physical decontamination method used) and disposal of solid biological waste.

6) Waste Management, Question 1b: Describe the final concentration of bleach and the time for decontamination of liquid wastes. Describe the method of disposal.

7) Waste Management, Question 3: Include how potentially contaminated clean up materials will be disposed.

Comments:

This modification will add Adenovirus expressing GFP and two genes that influence NADPH oxidase expression (PGC1-a and PPAR-g) and analyze its impact on primary mouse cells and used at BSL-2. The overall project involves use of genetically engineered mice for the study of NADPH oxidase. Tissues of rats will also be used. Plasmids and siRNA will also be used for manipulation of NADPH oxidase modification in a variety of cell lines at BSL-2. *Escherichia coli* will be used at BSL-1 for plasmid preparation. No major issues were noted. This amendment seeks to add Adenoviral vectors to the approved protocol. Adenoviral vectors (Adv-5) will be used to overexpress PGC-1alpha and PPARgamma protein in murine primary cells (driven by the CMV promoter), as part of the work described in the protocol to study the effects of these proteins on NADPH oxidase expression and activity. Adenoviral vectors will also deliver GFP, as a marker. The vectors will be obtained from *Vector Biolabs* and the work will be done at BSL-2. Other work covered in the protocol covers breeding of engineered mice to study NADPH oxidase and use of

various tissues and human, primate, murine and hamster cells for biochemical work. However, Adenoviral vectors will only be used with murine primary cells. Recommendation for approval.

Initial comment:
1) Primary Cells or Cell Lines: Add the use of Adenoviral vectors to the murine cell entry.

Review comments were provided to the investigator for response. The revised application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocol:	IBC202000011
Title:	Prevail Therapeutics, Inc./PRV-GD2-101 Gaucher Disease
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 05b_ICF_PRV-GD2-101_USA_6-9 years_Assent form_V.2.1.0_20191216_FINAL • PR001A Pediatric Pharmacy Manual v1.0 10Jan2020 signed 21Jan2020 • PRV-GD2-101_Protocol_V2_15Nov2019 • HSIC0609 • GD2_Protocol_Synopsis • PPD Prevail - PRV-GD2-101 - MA-PP Site Manual - v1 - 2019-12-30 • PRVL0003_Laboratory Manual_V1.0_27DEC2019 • HSFM0208PRO • 04 Prevail_PR001A_Investigator Brochure Version 3.0_20Nov2019_FINAL • 47.2 Prep of BSL2 Viruses (10-19) • PRV-GD2-101_Protocol Amendment 1_Summary of Changes • 05c_ICF_PRV-GD2-101_USA_Parent Travel_Accommodation Information Acknowledgment Form _v2.1.0_16Dec2019_US_FINAL • 11_AAV Safety Data Sheet 2018 Final • 05a_ICF_Prevail_PRV-GD2-101_USA_Main_ICF_vers.2.1.0_20191216_FINAL • PTCMDIV820d • PRVL0003_Laboratory Manual_V1.0_27DEC2019

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Include Adeno-Associated Virus (AAV) in the response.

2) Exposure Assessment and Protective Equipment: Question 5: Uncheck face shields, double gloves, surgical masks; Select the following: gloves, safety glasses, gown.

3) Exposure Assessment and Protective Equipment: Question 4: Study product is PR001A, not BMN 307. Correct the response.

4) Exposure Assessment and Protective Equipment: Question 2: The study product will not be administered intravenously. It will be given by injection into the cisterna magna. Correct.

5) Human Gene Transfer/Human Clinical Trial: Materials: Question 10: Describe the intended *in vivo* target cells (tissues, or organs) and the transduction efficiency. Transduction is the process by which genetic material is inserted into a cell by a virus. Transduction efficiency is a measure that indicates how well a gene is incorporated into cells by the vector.

6) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: If the agent is determined to be from a virus (a viral vector), the assay(s) used to check whether there is any infectious virus remaining in the agent preparation should be identified.

7) Human Gene Transfer/Human Clinical Trial: Materials: Question 7a: Include information regarding administration of steroids to mitigate immune responses.

8) Human Gene Transfer/Human Clinical Trial: Materials: Question 7: Check YES, as this is a first-in-humans study for this product.

9) Human Gene Transfer/Human Clinical Trial: Question 3a: Describe how the product will be transported from the pharmacy to the CRTC.

10) Viruses, Prions, or Vectors: The source of AAV is not from study participants. Correct the response.

11) Viruses, Prions, or Vectors: Describe how PR001A will be used in this study under "Description of Usage."

12) Viruses, Prions, or Vectors: Non-viral gene encoded by AAV should be entered under "Inserted Nucleic Acids Information."

13) Tissues, Blood, or Body Fluids: Due to the fact that tissues/fluids from study participants will not be administered to humans, the appropriate response to the question should be NO.

14) Tissues, Blood, or Body Fluids: Tissues/fluids from study subjects should be designated as recombinant.

15) Basic Information: Question 3: Include a brief description (2-3 sentences) of what PR001A is and how it will be administered.

Comments:

Summary: This study evaluates the safety and tolerability of a single dose of PR001A given to infants through a suboccipital injection in the cisterna magna. This method has been used for neurological gene replacement therapy in infants to deliver product into the CSF and will be injected by a neurosurgeon or an interventional radiologist under general anesthesia. PR001A is a recombinant Adeno-Associated Virus serotype 9 (rAAV9) vector-based gene therapy being evaluated in infants with neuronopathic Gaucher Disease (nGD with at least 1 pathogenic glucocerebrosidase-1 (GBA1) mutation (PD-GBA). Gaucher Disease (GD) is an autosomal recessive lysosomal storage disorder. ß-glucocerebrosidase (GCase) catalyzes the conversion of the glycosphingolipid glucosylceramide (GluCer) into glucose and ceramide. Deficiency in the GBA1encoded enzyme GCase, a key lysosomal enzyme required for the normal metabolism of glycolipids, leads to the accumulation of the GCase glycolipid substrates GluCer and glucosylsphingosine (GluSph) and ultimately results in toxicity and inflammation in conditions such as nGD. PR001A has been tested in animal models, which showed distribution in the CNS and periphery, reduction of glycolipids, and improvement in motor behavior deficits. Safety of PR001A was evaluated as part of the mouse efficacy experiments and in healthy NHPs. PR001A treatment was well tolerated at all dose levels investigated, including levels higher than the intended clinical dose. Up to 15 infants aged 0 to 24 months old who have bi-allelic GBA1

mutations c/w a diagnosis of Gaucher's Disease type 2 will be enrolled. Each infant will be followed for approximately 5 years. During the first 12 months after dosing, participants will be evaluated for safety, tolerability, immunogenicity, biomarkers, and efficacy. Enrollment will be staggered with at least 4 weeks between each of the first 3 participants receiving PR001A at a single dose of 1.3×10^{11} vg/g brain. This dose was selected to potentially achieve a therapeutic benefit while maintaining a reasonable and feasible safety margin.

Biosafety: PR0001A is a non-replicating rAAV9 gene therapy vector containing a codon-optimized human GBA1 open reading frame, a chicken β-actin (CBA) promoter element and flanking inverted terminal repeats (ITRs). PR0001A is made at The Research Institute at Nationwide Children's Hospital in Columbus, OH, according to GMP. The PR001A vector is produced using triple-plasmid DNA transfection into HEK293 cells with the AAV vector plasmid PR001-D, AAV9 helper plasmid pNLrep2-cap9 encoding the AAV rep2 and cap9 genes, and the Adenovirus helper plasmid pHELP. Risks include the possible development of an immune response to wild type GCase produced by transduced cells. Participants will receive prophylactic steroids to mitigate immune responses. There may be systemic exposure to the AAV9 in the blood outside the CSF. There are risks associated with administration into the cisterna magna, as there are with LPs (headaches, infections, hematomas). The participant may develop antibodies to AAV9.

Initial comments:
1) Basic information: Question 3: Include a brief description of what PR001A is and how it will be administered.
2) Human Gene Transfer: Question 3a: Describe how the product will be transported from the pharmacy to the CRTC.
3) Human Gene Transfer: Question 7: Check YES, this is a first-in-humans study for this product.
4) Human Gene Transfer: Question 7a: Include information regarding administration of steroids to mitigate immune responses.
5) Exposure Assessment: Question 2: The study product will not be administered intravenously. It will be given by injection into the cisterna magna. Revise.
6) Exposure Assessment: Question 4: Study product is PR001A, not BMN 307. Correct the responses.
7) Exposure Assessment Question 5: Uncheck face shields, double gloves, surgical masks; check gloves, safety glasses, gown.

The application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	IBC202000014
Title:	20-011
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• IB • Protocol • Main Consent

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Describe whether the agent(s) used in the course of this research may be infectious to humans. The response should include a) HSV-1 and b) fluids/tissues obtained from human subjects.

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: Include methods for detection of virus shedding. If the agent is determined to be from a virus (a viral vector), the assay(s) used to check whether there is any infectious virus remaining in the agent preparation should be identified.

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: Include methods for detection of virus shedding. If the agent is determined to be from a virus (a viral vector), the assay(s) used to check whether there is any infectious virus remaining in the agent preparation should be identified.

4) Human Gene Transfer/Human Clinical Trial: Materials: Question 2a: Delete the *Cemiplimab* language. It is a monoclonal antibody. Briefly summarize experience to date from the ongoing Phase 1 RP1 study.

5) Human Gene Transfer/Human Clinical Trial: Materials: Question 7a: *Acyclovir* is expected to have activity against RP1 if needed in this setting.

6) Human Gene Transfer/Human Clinical Trial: Materials: Question 7: Check YES, since there is no experience with this product in immunosuppressed organ transplant recipients.

7) Human Gene Transfer/Human Clinical Trial: Question 4: Check YES - as tumor cells die, release of tumor-associated antigens leads to an anti-tumor immune response, enhanced by the local expression of GM-CSF by RP1.

8) Viruses, Prions, or Vectors: Question 1: Clarify where participants with tumors that require injection under US guidance will receive RP1.

9) Viruses, Prions, or Vectors: Question 1: Indicate that RP1 is selectively replication competent.

10) Viruses, Prions, or Vectors: Clarify the source- from where is the HSV obtained. Sponsor is listed as *Replimune*.

11) Biosafety Summary: Question 1: Check Tissues, Blood, or Body fluids since tumors will be directly injected. Because these specimens will be collected during the study. Follow up questions will appear. Be sure to indicate that the specimens will be recombinant materials in the pop-up boxes for the agents as they will have been exposed to the viral vectors.

Comments:

Summary: This is a phase 1B, open-label study of RP1 designed to investigate the safety and tolerability of RP1 to treat 30 liver and kidney transplant recipients with advanced cutaneous squamous cell cancer. RP1 (rHSV-1hGM-CSF/ GALV-GP-R-) is a selectively replication competent HSV-1 intended for injection into sold tumors. Oncolytic viruses like RP1 are live, replicating viruses that preferentially replicate in tumor cells resulting in immunogenic cell death and induction of immune responses. This virus contains a codon-optimized sequence for hGM-CSF and a codon optimized sequence for Gibbon Ape Leukemia Virus glycoprotein (GALV-GP R-). Expression of GALV-GP R- results in fusion activity (syncytial formation in infected tumor cells since it binds to the PiT-1 receptor for GALV). GALV-GP-R- expression is under the control of the Rous Sarcoma Virus long terminal repeat (RSV LTR). This process then results in the death of the cells and enhances the spread of the virus in the tumor microenvironment. RP1 selectively replicates in tumor cells so the expression of the GALV-GP R- is minimized in normal tissues. As the tumor cells die, it is thought that the release of tumor-associated antigens leads to an anti-tumor immune response, enhanced by the local expression of GM-CSF by the virus. Participants will receive an initial dose of 1 x 10^6 plaque-forming units (PFU) of RP1 and two weeks later will receive 1 x 10^7 PFU of RP1 and continue every two weeks until study endpoints are met. RP1 is given by intra-tumoral injection into superficial or subcutaneous tumors using direct visualization or ultrasound guidance. The volume of RP1 administered is based on the longest diameter of individual tumor lesions; up to 10 mL may be given at a visit with multiple tumors injected provided they do not require > 10 mL of RP1. Participant will be active up to approximately 3 years: a 28-day screening period, up to a 1 year treatment period, and a 2-year follow-up period.

Biosafety: RP1 was made using a new isolate of HSV-1 (strain RH018). The neurovirulence factor ICP34.5 and the ICP47 protein have been deleted from this viral strain. The ICP34.5 deletion allows the virus to replicate selectively in tumors. The role of ICP47 is to block the major histocompatibility complex (MHC) class 1 antigen presentation pathway by binding to the transporter associated with antigen processing (TAP). The ICP47 deletion also allows the increased and earlier expression of RNA binding protein encoded by HSV-1, which inhibits activation of cellular PKR (US11), a gene that promotes growth in tumor cells without decreasing tumor selectivity. There is an ongoing Phase 1/2 study of RP1 alone and in combination with nivolumab in patients with advanced cancers, with single agent dose escalation, dose expansion to include nivolumab, and the combination in multiple phase 2 cohorts in individual tumor types, including CSCC. Adverse events reported by two or more participants so far include fever, fatigue, nausea, vomiting, headache, and flu-like illness. The dosing schedule is based on data from the phase 1 study. It is expected that multiple doses will be needed to maximize the anti-tumor immune response. In addition to safety and tolerability, the incidence of organ allograft rejection will be monitored from the start of the study until 30 days after their last dose of RP1. RP1 is expected to be sensitive to acyclovir (thymidine kinase inhibitor). Because the virus is attenuated in normal cells, no adverse effects would be expected with exposure to individuals who come into contact with it; universal precautions should be followed. The likelihood for RP1 infection of personal contacts, health care workers and study staff are thought to be unlikely due to ICP34.5 deletion, which should prevent effective viral replication in non-tumor tissue. The most likely possible route of transfer of RP1 from participants to others would be by accidental needle stick. Should this occur, it is not expected that any significant clinical manifestations would be evident, due to the deletion of ICP34.5. Talimogene laherparepvec (T-VEC) is an attenuated HSV-1 that encodes GM-CSF and is FDA-approved to treat advanced melanoma. There are case reports of organ

transplant recipients who received T-VEC to treat melanoma and other non-melanoma skin cancers that showed response and had a safety profile similar to that seen in non-transplant patients. T-VEC treatment did not result in allograft rejection.

Initial comments:
1) Biosafety summary: Question 1: Check tissues, blood, or body fluids since tumors will be directly injected.
2) Viruses, Prions, Vectors, Question 1: Clarify where participants with tumors that require injection under Ultrasonic guidance will receive RP1.
3) Viruses, Prions, Vectors, Question 1: Indicate RP1 is selectively replication competent.
4) Human Gene Transfer, Question 2a: Delete cemiplimab language. It is a monoclonal antibody. Briefly summarize experience to date from the ongoing Phase 1 RP1 study.
5) Human Gene Transfer, Question 4, 4a: Check YES - as tumor cells die, release of tumor-associated antigens leads to an anti-tumor immune response, enhanced by the local expression of GM-CSF by rp1.
6) Human Gene Transfer, Question 7: Check YES, since there is no experience with this product in immunosuppressed organ transplant recipients.
7) Human Gene Transfer, Question 7a: Acyclovir is expected to have activity against RP1 if needed in this setting.

The application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	IBC202000024
Title:	Rubius RTX-134-01
Investigator:	REDACTED
Highest BSL:	BSL-1 BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• HSIC0604 • RTX-134-01 Pharmacy Manual_V2.0 • Safety Data Sheet for RTX134 Drug Product_vF • Table 1.docx • RTX-134-01 Clinical Protocol_V 3.0_FINAL • 1.14.4.1 Investigator's Brochure_V3.0_FINAL • HSFM0208PRO • RTX-134-01 ICF template_V3.0_FINAL

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 5: Select "Gloves (latex or nitrile)" and "Laboratory coat."

2) Exposure Assessment and Protective Equipment: Question 5: Unselect "Bite/scratch resistant gloves/sleeve".

3) Risk Group and Containment Practices: Question 2: Unselect BSL-1.

4) Human Gene Transfer/Human Clinical Trial: Materials: Question 8: Include a statement that indicates that as a result of the enucleation process of red blood cells, the genetic material, including the Lentiviral vector DNA, are removed.

5) Human Gene Transfer/Human Clinical Trial: Materials: Question 6: State how study product will be administered to humans.

6) Human Gene Transfer/Human Clinical Trial: Materials: Question 3a: Provide a response.

7) Human Gene Transfer/Human Clinical Trial: Materials: Question 3: The clinical protocol states, "This is a Phase 1b first-in-human study in adult subjects with PKU." Correct the answer.

8) Lentivirus and Lentiviral Vectors: Question 1. Confirm whether or not the Lentivirus vector is produced at the University of Pittsburgh or is generated/made/created by the Sponsor.

9) Primary Cells or Cell Lines: Under the Description of Usage for the cells listed, expand the response to state that CD34+ hematopoietic progenitor cells will be obtained from blood type O, Rh-negative, Kell-negative donors. The CD34+ cells are expanded, transduced with a Lentiviral vector encoding AvPAL (LV1-PAL), and differentiated into enucleated red cells expressing AvPAL in the red cell cytoplasm.

Comments:

Summary: This is a Phase 1b first-in-human study in adults with phenylketonuria (PKU). RTX-134 consists of allogeneic human red cells derived from CD34+ hematopoietic progenitor cells from blood type O, Rh-negative, Kell-negative donors. The CD34+ cells are transduced with a third-generation 4 plasmid Lentiviral vector containing the *Anabaena variabilis* phenylalanine ammonia lyase (AvPAL) gene and differentiated into enucleated RBCs expressing AvPAL. AvPAL is a phenylalanine ammonia lyase (PAL) derived from the cyanobacterium *Anabaena*

variabilis that deammoniates Phe to produce trans-cinnamic acid (tCA) and ammonia. AvPAL is expected to be expressed in RTX-134 cells at concentrations of approximately 2 to 20 × $^{10\text{-}11}$ units (µmol/min/cell). In participants who receive RTX-134, Phe in the blood will enter the RTX-134 cells and metabolized by the AvPAL within the cytoplasm, leading to lower blood Phe levels. The primary study objectives are to evaluate safety and tolerability; correlate dose with percent reduction in serum Phe levels relative to baseline; determine a preliminary dose to achieve serum Phe levels < 600 µmol/L (European target) and < 360 µmol/L (US target); and evaluate the pharmacokinetics. Secondary objectives include pharmacodynamics and immunogenicity. Four different ascending dose levels will be studied in adults 18 years of age and older. Participants will receive a single IV dose of RTX-134 at 30 mL/h (0.5 mL/min). The proposed clinical doses for the first-in-human study (3×10^{10} cells to 1×10^{11} cells) of RTX-134 would be less than 1% of the total circulating erythrocyte population and would contain < 5% of the number of cells in a typical donor blood unit. Detection of RTX-134 will be evaluated using multiple pharmacokinetic (PK) and PD assessments. Participants will be monitored for 28 days after last detection of RTX-134. RTX-134 is not expected to be detected longer than 120 days after administration. The starting dose will be approximately 3 units (U) of RTX-134. Dosing will be staggered by dose level. Participants within a dose level will be dosed a minimum of 24 hours apart if no dose limiting toxicities or safety concerns are observed. Dosing in the next dose level will occur after review of available PK/PD data. The study will be overseen by a Safety Monitoring Committee (SMC).

Biosafety: There is no clinical experience with RTX-134. RTX-134 is a sterile cell suspension and variation in AvPAL activity level and cell concentration is expected. This is a pooled blood product so there is a potential risk of transmission of infectious diseases not identified (or known) at time of donor screening/collection and material handling. Donor eligibility is determined in accordance with existing regulations. The donor's health history is screened for risk factors and clinical evidence of HIV, Hepatitis B, Hepatitis C, Human Transmissible Spongiform Encephalopathies, Syphilis, HTLV, ZKV. RTX-134 is released when it meets the release criteria (eg, cell purity, viability, sterility, negative for replication competent Lentiviral vector, negative for mycoplasma), listed on the RTX--134 certificate of analysis. RTX-134 will be administered based on a rapid microbial detection test (Gram stain) and preliminary results in a 14-day US Pharmacopeia sterility test. In the event of a sterility failure based on final 14-day test results, the site will be notified. Multiple lots may be pooled for individual participant dosing. If lots are pooled, the cells will be from the same donor. Sites will be notified of the final concentration and activity of each lot before dosing. Participant dosing will be based on the resulting volume; variance in cell concentration and activity will be accounted for when analyzing PK and PD effects. Before RTX-134 dosing, crossmatch testing will be performed using RTX-134 material tested against a blood sample from the participant. The participant will be monitored for infusion reactions for 4 to 6 hours after starting the infusion. Risks and adverse reactions are expected to be similar to those seen with blood cell transfusions, although a smaller volume of RTX-134 will be given than a standard red cell transfusion - fever, chills, tachycardia, hypotension, rash, hemolysis. The infusion will be stopped if it has not been completed. Participants will be followed for 15 years for delayed AEs as per FDA gene therapy guidelines. Participants will be monitored for new malignancies; new incidence or exacerbations of pre-existing neurologic, rheumatologic, autoimmune or hematologic disorders; or delayed adverse events attributed to RTX-134, including unexpected illness or hospitalization.

Initial comments:
1) The Rubius Safety Data Sheet indicated they product "could be handled with BSL-1 containment requirements", instead of BSL-2 (Universal Precautions), which is more appropriate.
2) Lentivirus Vectors: Question 1: Confirm whether or not the Lentivirus vector is produced at the University of Pittsburgh or by the Sponsor.
3) Risk Group: Question 2: Uncheck BSL-1; BSL-2 should be checked.
4) Exposure and PPE: Question 5: Gloves (latex), gown, goggles should be checked.

The application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	IBC202000019
Title:	Zika Virus Boosted Humanoid Brain Cancer Model
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Supporting Documents: If BSL-2+ will be required, then the laboratory's Biosafety Operations Manual must be signed and uploaded in the application.

2) Waste Management: Question 1b:: If *Wescodyne* will be used to decontaminate liquid waste the final concentration of *Wescodyne* should be equivalent to 3 ounces of disinfectant per 5 gallons of liquid waste. Concentrated *Wescodyne* must be added directly to liquid waste to be decontaminated. Addition of pre-diluted *Wescodyne* to liquid waste will further dilute the active disinfectant and will result in insufficient decontamination of the waste.

3) Waste Management: Question 1b: The correct final concentration of bleach should be 1:10 (v/v) bleach to biological liquid waste. Correct the response.

4) Waste Management: Question 1a: Describe the methods used for proper decontamination of solid biological waste. In other words, how will solids be decontaminated?

5) Exposure Assessment and Protective Equipment: Question 4: Safety engineered sharps devices are required for use with biological materials at BSL-2/ABSL-2 and above. Revise to indicate use of safety engineered sharps devices.

6) Exposure Assessment and Protective Equipment: Question 2: Injection of viral vectors and/or Zika Virus into animals poses a risk of needle stick for personnel. Include this risk, and in Question 4 indicate that only safety-engineered sharps devices will be used to administer these materials to animals.

7) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells or cell lines may harbor unknown infectious agents.

8) Risk Group and Containment Practices: Question 2: Select the appropriate ABSL to be used.

9) Animals: Question 1: If using mice, then identify the associated ARO/IACUC protocols.

10) Lentivirus and Lentiviral Vectors: Question 2a: This question refers to Lentiviruses only. Remove the description of AAV. Deletion of the tat gene is not sufficient for use of Lentiviruses at BSL-2. Clarify/correct the response.

11) Recombinant or Synthetic Nucleic Acid Work Description: Question 3 a-d: Provide additional information regarding the use of shRNA and CRISPR/Cas9 to modify expression of TP53, PTEN, and NF-1. Will shRNA or CRISPR/Cas9 be targeted to reduce expression of and/or knock-out these genes? If so, will Lentiviral vectors be used to deliver shRNA or CRISPR/Cas9? TP53, PTEN, and NF-1 (if NF-1 refers to Neurofibromatosis type 1) are all tumor suppressor genes. If

shRNA targeting these genes will be delivered via Lentiviral vector this work must be performed at BSL-2+.

12) Recombinant or Synthetic Nucleic Acid Work Description: Question 3d/3e: clarify how gRNA will be introduced into cells.

13) Viruses, Prions, or Vectors: If gRNA will be encoded by a viral vector(s), these should be described under an appropriate entry.

14) Viruses, Prions, or Vectors: Lentivirus (HIV 4-plasmid system) - pLP (Vector) and Lenti-EF1a-dCas9-VPR-Puro: Cas9 should be listed under "Inserted Nucleic Acids Information."

15) Tissues, Blood, or Body Fluids: The investigator has indicated that murine tissues will be administered to animals. Confirm and describe under "Description of Usage." If this is a mistake, correct the response to the question of whether recombinant tissues will be administered to animals.

Comments:
In this protocol, the investigator plans to use a complex organoid model to study the potential of Zika Virus-derived vectors for treating tumors such as glioblastoma. iPS cells from *ATCC* will be combined with stem cells from either patients or *ATCC* and then differentiated *in vitro* to form organoids. Some of these cells will be infected with Lentivirus to drive doxycycline-inducible shRNA expression. Human HEK293T cells will be used to produce Lentivirus, and Zika Virus will be propagated in mosquito C6/36 and primate Vero cell lines. In a separate project, human brain slices in *ex vivo* culture will be used to study Zika Virus propagation in intact tissues. This description came from the virus section. Zika Virus strains to be used include FS13025, IBH30656, ZIKV-LAV, H/FP/2013, PRVABC59, and PE 242. In addition, Lentivirus will be used to infect iPS cells, and Adenovirus (AAV-1) will be used to infect differentiated organoids. All Zika strains can infect human cells and are not replication-defective; Lentivirus and AAV can infect human tissues and are replication-defective. The investigator is proposing BSL-2 containment for experiments; but will require BSL-2+ containment, especially if viral vectors encode Cas and gRNA (refer to investigator's comment). This information should be included in the application. The 'Live Animals' and 'Animal Gene Transfer' sections describe intracranial injections of virus into live nude mice and Bl6 immunocompromised mice, and both the 'Viruses and Prions' section and 'the Recombinant Nucleic Acid Work Description' mention *ex vivo* brain slices. The investigator needs to clarify whether this is a cancer protocol (Zika to target cancer organoids…will they modify Zika Virus for these studies? And if so, how?) or whether intact brain slices from live mice is also part of this protocol. Important information is missing, clarifications and potentially higher level of BSL containment are necessary. This is a protocol from a new investigator at Pitt to study Zika Virus. The investigator will perform cell culture with human, non-human primate, and insect cells and will obtain cells from humans. Replication-defective AAV and Lentiviruses encoding reporter genes (e.g. fluorescent proteins and luciferase) are described for use in cell culture at BSL-2. Both non-recombinant and recombinant strains of ZIKV are described for use in cell culture and in mice at BSL-2/ABSL-2. *E. coli* is described for propagation of plasmids at BSL-2. However, poorly described use of CRISPR/Cas9 editing is mentioned in a comment by the investigator, using a Lentiviral vector that encodes both Cas9 and gRNA, which should be used at BSL-2+. Significant missing information and discrepancies should be addressed before coming to the committee.

The application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

Protocol:	MOD202000073
Title:	Amendment for **IBC201700077**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	None
Additional Documents:	• REDACTED BSL-2+ Manual 2017_ April 6 updated • PI signed and personal training updated 20200218

Determination: Modifications Required

Required modifications:

1) Amendment Request: The protocol application only contains details for CRISPR/Cas9 in Lentiviruses *not* in AAV. If the AAV work is no longer intended to be performed - then remove it from the described changes in number 2. Otherwise provide a description of proposed research in AAV within the protocol.

2) Basic Information, Question 3: Add the use of viral delivery in the description as it directly affects the consideration of BSL level of CRISPR work. When the application states co-infection, does this mean the viral particles will be administered in one infection scenario, or that the cells will be infected with one, then later infected with the other (presumably CRISPR first, then sgRNA). Describe this important information in more detail. Clearly state the separate timing of administration of the CRISPR and then at minimum, 72 hours later the RNA. If performing the two vector administrations at the same time, then BSL-2+ is required for this procedure. Clarify.

3) Tissues, Blood, or Body Fluid: Tissues obtained from animals in which recombinant nucleic acids are administered are recombinant. Correct the answers where the questions are asking whether tissues are recombinant.

4) Tissues, Blood, or Body Fluid: Mouse (murine): The investigator has indicated that animal tissues will be administered to animals. Clarify this under "Description of Usage." If this is in error, correct the response to the answer of whether this tissue/blood/fluid will be administered to animals.

5) Tissues, Blood, or Body Fluid: Rat: The investigator has indicated that animal tissues will be administered to animals. Clarify this under "Description of Usage." If this is in error, correct the response to the answer of whether this tissue/blood/fluid will be administered to animals.

6) Primary Cells or Cell Lines: Other Human cells or cell lines: The investigator has indicated that these cells will be administered to animals. Clarify this under "Description of Usage." If this is in error, correct the response to the answer of whether these cells will be administered to animals.

7) Primary Cells or Cell Lines: Human stem cells or iPS cells (embryonic or adult): it is indicated that nucleic acids will be introduced into cells. These should be designated as recombinant.

8) Primary Cells or Cell Lines: Mouse (murine): It is indicated that recombinant nucleic acids will be introduced into cells. These should be designated as recombinant.

9) Primary Cells or Cell Lines: Other Human cells or cell lines: It is indicated that recombinant nucleic acids will be introduced into cells. These should be designated as recombinant.

10) Primary Cells or Cell Lines: Mouse (murine): It is indicated that recombinant nucleic acids will be introduced into cells. These should be designated as recombinant.

11) Viruses, Prions, or Vectors: Adeno-Associated Virus (AAV-6): For use at BSL-2, transduction of cells with a replication-defective virus (e.g. AAV)) encoding CRISPR/Cas and with a separate

replication-defective vector encoding sgRNA should be separated by more than 72 hours. Otherwise this must be performed at BSL-2+.

12) Viruses, Prions, or Vectors: Lentivirus HIV-1 4-plasmid systems (3rd generation): The application indicates that these viral vectors will be used to deliver genes or siRNA, and therefore these materials are recombinant.

13) Viruses, Prions, or Vectors: Human Immunodeficiency Virus (HIV, Types 1 and2) - HIV: It is stated in the "Description of Usage" that this virus will be introduced into mice. Correct the response that asks whether the virus will be administered to animals.

14) Viruses, Prions, or Vectors: Human Immunodeficiency Virus (HIV, Types 1 and2) - HIV: The "Description of Usage" states that the virus is replication-competent. Yet, it is designated as replication-defective. Clarify this discrepancy.

15) Viruses, Prions, or Vectors: In the amendment page, it is stated: CRISPR/Cas with sgRNAs will be used by transduction with replication-defective virus vectors, both Lentiviruses and Adeno-Associated Viruses. Add the CRISPR work to the AAV. Since it is stated that the CRISPR will not be done in mice, and the current AAV lists only *in vivo* work, make a separate entry for the AAV CRISPR work, clearly stating if this will be on separate vectors for the CRISPR/Cas and sgRNA or on the same vector.

16) Recombinant or Synthetic Nucleic Acid Work Description: Questions 3a, 3e: In the amendment summary, it is mentioned that there will be the use of AAV in CRISPR delivery-include this information in the response.

17) Animal Gene Transfer, Question 3: AAV uses a helper virus, so the answer should be YES.

18) Animal Gene Transfer, Question 1: Earlier in the application it is stated that mice will be infected with replication-competent HIV-1. Correct the response.

19) Exposure Assessment and Protective Equipment, Question 1: Include a statement that human cells or cell lines may harbor unknown infectious agents.

20) Exposure Assessment and Protective Equipment, Question 4: Use of safety-engineered sharps devices is required at BSL-2+/ABSL-2. Indicate that safety-engineered sharps will be used.

21) Supporting Documents: The signature page on the Biosafety Operations Manual should be signed by the investigator, and a staff member of EH&S.

Comments:

This is a protocol modification in response to a previously disapproved modification application. The investigator proposes to use CRISPR/Cas in a viral delivery system, in both Lentiviral and AAV (per the amendment application). However, the use of AAV is not mentioned in the protocol itself. The investigator should clarify if AAV delivering CRISPR/Cas is to be used. The protocol application now states that the CRISPR/Cas and sgRNA will be delivered on separate vectors, but also states co-transduction. If this means that the viral particles are applied to the cells at the same time, then it does not meet the IBC requirement for downgrade to BSL-2. This will not be administered to animals and only done *in vitro*. Because of the history of this protocol, as well as the lack of description of AAV work, and detail in the transduction scenario, full discussion is warranted regardless of overall risk level. This modification to Dr. REDACTED's protocol still contains inconsistencies in the wording of the proposed CRISPR/Cas studies as detailed. The applicant states that the guide RNA and CRISPR will be delivered separately (at different times) to cells, but then uses wording like co-infection that is confusing. Further, there is mention of CRISPR

delivery by AAV in the introduction, but the protocol application indicates the use of Lentivirus only. The proposed work using Lentivirus and separate timed delivery of CRISPR and sgRNA into cells is would be appropriate at BSL-2+ as proposed. The laboratory biosafety manual and detailed practices are appropriate. For context this is the re-working of the modification that was discussed at the February meeting and the committee determined that the investigator should complete a new modification submission. It appears that the new modification was very clear on the uses of CRISPR in Lentiviruses, the investigators are NOT seeking a downgrade to BSL-2, and have included the required laboratory biosafety manual. However, it is noted that the manual is not signed by a member of EH&S. There is mention of AAV in the amendment description of changes but the application does not describe any work with CRISPR in AAV. Clarification in the amendment is required, as it seems the investigator does not plan to do any AAV CRISPR work based on the descriptions in the protocol. Recommendation for approval pending minor modifications.

The application was placed on the March agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	16
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:44 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

March 23, 2020 3:00 PM

Meeting; ZOOM teleconference meeting
SARS-CoV-2 protocols only

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			yes
REDACTED, Vice Chair			yes
REDACTED			yes
REDACTED			yes
REDACTED	Absent		
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED	Absent		
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED	Absent		
REDACTED			yes
REDACTED	Absent		
REDACTED			yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			yes

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	EH&S representative

REDACTED	Co-Director, ORP (Research Protections Office)

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	20
Meeting start time:	3:02 PM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions. This meeting was conducted via teleconference due to the Governmental "Stay at Home" orders to prevent spread of the Corona Virus.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

Since this was an "emergency" meeting and not regularly scheduled, the March meeting minutes were not reviewed. They will be reviewed at the regularly scheduled April meeting.

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

No report, as this was an "emergency" meeting outside of the regularly scheduled meetings to discuss specific protocols involving research with the Corona Virus.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocol follows on the next page

Protocol:	MOD202000082
Title:	Amendment for **IBC201700256**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-2
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Waste Management: Remove ethanol from the responses of question 1 and 3.

2) Waste Management: 70% ethanol is not considered appropriate for inactivation; remove this wording and retain the 10% bleach (adding in v/v final). Surfaces can we wiped with 70% ethanol after bleach to remove residue.

Comments:

The investigator has submitted this modification for either a bacterial expression plasmid expressing COVID-19 Spike protein or the COVID-19 Spike protein itself for structural biology experiments. Bacteria will be used at BSL-2. This is technically BSL-1 work and low-risk. The amendment describes addition of expression of Corona Virus Spike protein in *E. coli* for purification and subsequent structural studies using NMR. The work will be performed at BSL-2, is low risk, and seems to be appropriate.

The protocol application was placed onto the March 23rd Emergency meeting agenda for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocol:	MOD202000130
Title:	Amendment for **IBC201700029** (construct pseudovirus)
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 3/24/2021

Required modifications:

1) Basic Information, Question 3: *Haemophilus influenzae* is mentioned here but not described elsewhere in the protocol. Clarify this discrepancy.

2) Tissues, Blood, or Body Fluids: Confirm both laboratory areas will be used for research or revise the location(s).

3) Primary Cells or Cell Lines: Confirm both laboratory areas will be used for research or revise the location(s).

4) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: Confirm the BST location or revise the location to BSP.

5) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: *Haemophilus influenzae* is described in the protocol summary. If that organism is used, it should be included here.

6) Viruses, Prions, or Vectors: Confirm both that both laboratory areas will be used for research or revise the location(s).

7) Animal Gene Transfer, Question 6: Note that Influenza Virus is mentioned; is or is not the use of Influenza in this research? Clarify.

8) Waste Management, Question 2: Confirm both BST locations will be used or revise.

Comments:

The investigator has submitted a modification to include Lentivirus pseudotyped with nCoV Spike protein for use in cell culture and in mice. A number of clarifications should be addressed throughout the protocol, many of which are likely were made in the original protocol. These include lack of acknowledgement of an oncoprotein (Akt1) to be expressed in cells, lack of clarity regarding CRISPR/Cas9 expression, designation of non-recombinant Influenza as replication-defective, etc. If Lentiviruses are used to express oncogenes or Cas9 with gRNA, they would need to be used at BSL-2+. The modification describes the addition of work using Lentiviral particles pseudotyped with the spike envelope protein from SARS-CoV-2 in cell culture and mice. There

are multiple clarifications needed including potential expression of an oncogene in a Lentiviral vector as well as use of Influenza Virus. Current language in sections of the protocol describes use of Influenza as a viral vector to deliver transgenes. This should be clarified. The work is proposed at BSL-2/ABSL-2, which may not be appropriate for overexpression of an oncogene and/or CRISPR/Cas9 and gRNA from a single Lentiviral vector. Clarification is required.

Review comments were provided to the investigator for response. The revised application was placed on the March 23rd Emergency meeting agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

The investigator provided a revised application to address the comments from the March 23rd meeting. An approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 3:15 PM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

April 2, 2020 3:00 PM

Meeting; ZOOM teleconference meeting
SARS-CoV-2 protocols only

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			yes
REDACTED, Vice Chair			yes
REDACTED			yes
REDACTED			yes
REDACTED	Absent		
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED	Absent		
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED			yes
REDACTED	Absent		
REDACTED			yes
REDACTED	Absent		
REDACTED			yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			yes

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	EH&S representative

REDACTED	Co-Director, ORP (Research Protections Office)

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	20
Meeting start time:	3:05 PM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions. This meeting was conducted via teleconference due to the Governmental "Stay at Home" orders to prevent spread of the Corona Virus.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

Since this was the second "emergency" meeting and not regularly scheduled, the regularly scheduled March 9th meeting minutes were not reviewed. They will be reviewed at the April meeting along with the first emergency meeting minutes of March 23rd.

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

No report, as this was an "emergency" meeting outside of the regularly scheduled meetings to discuss specific protocols involving research with the Corona Virus.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocols on the following pages.

Protocol:	MOD202000090
Title:	Amendment for **IBC201600015**
Investigator:	REDACTED
Highest BSL:	RBL ABSL-3
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Appendix 2 2020 • REDACTED EEE VEE WEE Chimeras RA 12-2014 Final • Appendix1B • Appendix1A • Appendix 4 • Appendix 5 2020 • Appendix 1C (3) • Appendix3A3B 2020 • REDACTED RBL manual rev5 August 2017 Final

Determination: Approved

Last day of continuing review period: 4/2/2021

Required modifications: None

Comments:
This modification adds work with transgenic hamsters expressing the human ACE2 receptor gene, work with SARS-CoV-2 in NHPs, ferrets, and hamsters, as well as work with SARS-CoV-2 viruses tagged with reporter genes (e.g. GFP). The work is proposed at the appropriate biosafety levels with all work with infectious SARS-CoV-2 viruses to be done in the RBL. The investigator's RBL biosafety manual is up-to-date and EH&S is aware of this new work. Minor clarifications are requested. This is a modification for an existing protocol to include use of transgenic hamsters, work with SARS-Cov-2 in non-human primates, ferrets and hamsters, and SARS-CoV-2 reporter expressing viruses (GFP and luciferase).

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 2nd emergency meeting for review.

Approval: No additional comments were provided; an approval letter was generated and sent to the investigator.

Supporting documents: None

Votes:

For:	17
Against:	0
Abstained:	0

Protocol:	MOD202000151
Title:	Amendment for **IBC201800039**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Include information about the newly added VSV.

2) Viruses, Prions, or Vectors: Vesicular Stomatitis Virus (VSV; non-exotic strains: Indiana 1 serotypes) - Indiana 1: This virus infects human cells. Edit and modify the answer.

3) Viruses, Prions, or Vectors: SARS-associated Coronavirus - 2019 SARS-CoV-2/USA-WA1/2020: The investigator indicates that only isolated viral RNA will be obtained. As this agent is not replication-competent, the answer to the question of whether it is replication-defective should be answered YES. Edit and modify the response to the question.

4) Recombinant or Synthetic Nucleic Acids Usage: In the Primary Cells and Cell Lines section the entry for human cells indicates that these materials are recombinant and will be administered to animals (NOD/SCID mice). If this information is accurate, this work falls under *NIH Section-III-D-4* and this box should be checked.

5) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Describe how SARS-CoV-2 RNA and VSV will be used in the study in the response.

6) Live Animals, Question 6: In the Primary Cells and Cell Lines section the entry for human cells indicates that these materials are recombinant and will be administered to animals (NOD/SCID mice). If this information is accurate the answer to question 6 should be YES.

7) Animal Gene Transfer, Question 1: As replication-competent VSV will be administered to mice, this question should be answered YES.

8) Animal Gene Transfer, Questions 5-7: In the Primary Cells and Cell Lines section the entry for human cells indicates that these materials are recombinant and will be administered to animals (NOD/SCID mice). If this information is accurate answers should be provided for questions 5, 6, and 7.

9) Risk Group and Containment Practices, Question 3: Edit (add/delete) the appropriate personnel in this section of the protocol.

10) Exposure Assessment and Protective Equipment, Question 1: Include VSV in the response.

11) Supporting Documents: Upload A signed copy of the laboratory Biosafety Manual in the Supporting Documents section of the application.

Comments:

Despite the fact that the protocol amendment states that only personnel changes were made, SARS-CoV-2 RNA (non-replication competent) and Vesicular Stomatitis Virus (non-recombinant) were added to the protocol. SARS-CoV-2 genes will be amplified and cloned into mammalian

expression vectors for use in eukaryotic cells at BSL-2+. VSV will be administered to mice at ABSL-2. Several forms issues should be corrected prior to approval.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 2nd emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	17
Against:	0
Abstained:	0

Protocol:	MOD202000159
Title:	Amendment for **IBC201700077**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED BSL-2+ Manual 2017_ April 6 updated • Investigator signed and personal training updated 20200218

Determination: Modifications Required

Required modifications:

1) Supporting Documents: The laboratory's Biosafety Manual must be updated to reflect new research. Upload the most recent version signed by EH&S and the investigator.

2) Waste Management: Question 1b: Clarify that liquid wastes will be decontaminated by addition of concentrated bleach to achieve a final concentration of 1:10, v:v, bleach:liquid waste. Adding pre-diluted bleach (1:10 dilution in water then added to liquid waste) results in incomplete decontamination as the concentration of sodium hypochlorite is too low to be effective.

3) Viruses, Prions, or Vectors: Include a separate entry for the Lentiviruses pseudotyped with SARS-CoV-2 Spike protein that will be used *in vitro*.

4) Primary Cells or Cell Lines: Include the CoV-2 work here. What cell lines will be exposed?

5) Tissues, Blood, or Body Fluids: Clarify entry for rat tissues: If tissues are harvested from rats that have been exposed to recombinant materials (administration of AAV is described in Animal Gene Transfer section) then the rat tissues entry should be marked as recombinant.

Comments:

This is an amendment to a protocol previously approved to study pulmonary hypertension in the context of HIV. The investigator requests to add SARS-CoV-2 Spike proteins to the study to characterize cell entry mechanisms, using a tagged GFP construct, in cell culture on relevant pulmonary primary cell lines. No animal work is being proposed. The amendment application is well-explained, but the information is not translated into the main protocol. Hence, most of the information for the proposed work is not listed in the protocol itself. Specifically, the cell line usage and the viral information. It should be clear why “pseudovirus” is being used to describe the replication incompetent Lentivirus. The current description seems to disregard the potential biohazard of the Lentivirus and perhaps should be removed throughout. Background: SARS-CoV-2 is a novel Coronavirus strain that is highly contagious in the human population, complicated by severe respiratory manifestations and death in a varying percentage of infected individuals. Among those infected, there is a higher prevalence and severity of SARS-CoV-2 disease in persons with co-morbid cardiovascular disease, such as hypertension and diabetes. Furthermore, >7% of patients suffer myocardial and cardiovascular injury from the infection (22% of the critically ill), particularly as disease severity intensifies. Recently, it has been reported that the SARS-CoV-2 Spike protein can bind and utilize the angiotensin-converting enzyme 2 (ACE2) receptor for gaining entry to human cells. In addition to pulmonary epithelial, brain, mucosal, and renal cells, cardiomyocytes and vascular endothelial cells express ACE2. However, data regarding direct

SARS-CoV-2 infection of any cardiovascular cell type are lacking, and it is unclear whether factors that control viral entry into the cardiovascular system could underlie SARS-CoV-2 disease characteristics. This amendment is to study SARS-CoV-2 entry into endothelial cells and cardiomyocytes, as cardiovascular injury is a known side effect of respiratory SARS-CoV-2 infection. The investigator will create pseudotyped replication incompetent viruses, carrying the SARS-CoV-2 Spike protein on its envelope membrane surface but with a Lentivirus backbone carrying a GFP reporter gene. The laboratory is approved to use Lentiviral vectors at BSL-2+. The laboratory will obtain cloned expression vectors for both the Spike protein from the Wuhan epidemic (*Sino Biologic*) and from the German epidemic (Materials Transfer Agreement pending this amendment), which are similar in sequence but not entirely identical. They will use the following constructs for this approach: pCG1_SARS-CoV-2, pCG1_SARS-CoV-2-HA (expression vectors for Spike protein); pCG1_hACE2 (expression vector for ACE2 protein, the receptor for the Spike protein), pCAGGS_VSV-G (expression vector for envelope VSV-g protein as control), and pCAGGS_TMPRSS2 (expression vector for protease that is important for viral entry). The investigator needs to clarify the target cells that will be used for infection from the list included in the approved protocol. It is not apparent that this "pseudovirus" should be listed as a separate virus or if this is just an extension of the Lentivirus system already approved. Expression of the Spike protein in replication deficient Lentivirus should not create any additional biosafety concerns beyond what the investigator is already approved.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 2nd emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	17
Against:	0
Abstained:	0

Protocol:	MOD202000160
Title:	Amendment for **IBC201700106**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Include information on SARS-CoV-2 in the response.

2) Tissues, Blood, or Body Fluids: Mouse (murine): List the source of materials under "Source."

3) Tissues, Blood, or Body Fluids: Humans: Include information regarding the administration of human tissues to animals under "Description of Usage."

4) Virus, Prions, or Vectors: West Nile Virus - Kunjin: Indicate the source of the material. Response cannot be blank.

5) Virus, Prions, or Vectors: KSHV is described later in the protocol, it should also be described in the response.

6) Virus, Prions, or Vectors: The Lentiviral vector section describes expression of Kaposi's Sarcoma-Associated Herpes Virus genes – identify which ones and elaborate.

7) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Describe the procedures and techniques to be used with the nucleic acid molecules in the project, including all recombinant viruses listed in the protocol.

8) Recombinant or Synthetic Nucleic Acid Work Description, Question 3c. This segment refers to Kaposi's Sarcoma-Associated Herpes Virus genes – identify which ones and elaborate.

9) Recombinant or Synthetic Nucleic Acid Work Description, Question 3d: Include vectors used for CRISPR/Cas9 in the response.

10) Animal Gene Transfer, Question 5: Earlier, the investigator indicates that MMLV will be administered to animals. This should be described in the Animal Gene Transfer section of the application.

11) Exposure Assessment and Protective Equipment, Question 1: Include Lentiviruses, West Nile Virus, Vesicular Stomatitis Virus, and Kaposi's Sarcoma-Associated Herpes Virus in the response.

12) Exposure Assessment and Protective Equipment, Question 4: Safety engineered sharps devices are required for work with BSL-2/ABSL-2 or higher; revise to include for use of needles, scalpels, etc.

13) Waste Management, Question 1c: Animal carcasses should be sealed in double bags, labeled, and disposed according to animal facility guidelines.

14) Supporting Documents: Upload a signed copy of the laboratory’s Biosafety Operations Manual.

Comments:
This amendment to an existing protocol updates personnel and adds human tissues, Vesicular Stomatitis Virus (VSV), West Nile Virus (WNV), and SARS-CoV-2 RNA. The protocol also describes Kaposi's Sarcoma-Associated Herpes Virus (KSHV), but this virus is not listed in the protocol. WNV and SARS-CoV-2 RNA will be used for *in vitro* only at BSL-2+. VSV will be used at BSL-2+ and administered to animals at ABSL-2. There are no biosafety concerns, but there are several discrepancies and missing information that need to be clarified. This modification request seeks to make two changes. While the first is to document various changes in personnel, the second is to make significant changes to the list of viruses and viral tools used in the protocol, with West Nile Virus (strain Kunjin), VSV (strain Indiana 1) and SARS CoV2 (strain WA01-2020) being added. The underlying protocol is focused on Polyomaviruses and specifically on the expression of components of Polyoma viral genome in various cells (mammalian and also *E. coli* and P*ichia pastoris* for significant overexpression) and then assessing the interactions of Polyoma viral proteins with mammalian cells. Occasionally intact Polyomaviruses are also used. The protocol also uses Retroviral vectors (Lentiviral, MSCV, MMLV) and transgenic mice expressing Polyomaviral proteins, as well as the propagation of cell lines transformed with Polyomaviruses. In some cases, cells transduced with Lentiviral vectors expressing various viral genes and human oncogenes will be xenotransplanted into mice to determine carcinogenic potential. Overall, the aim of the modification request is to use insight from Polyomavirus persistence to study persistence of various RNA viruses. The summary notes that BSL-2+ conditions will be used to look at persistence with attenuated forms of West Nile Virus (Kunjin strain – naturally attenuated) and VSV (strain Indiana 1). Further, non-infectious, purified RNA and corresponding cDNA fragments from SARS CoV2 will be used for persistence studies. Several human tissues are used in the underlying protocol (all at BSL-2+, source, Pitt). These are obtained directly from human participants. Polyoma Viruses are propagated in human cells. Murine cells will also be used (BSL-2+), for similar purposes. Several human cell lines are also be used (all at BSL-2+, source – *ATCC*). These are human Merkel Cell Carcinoma cell lines MKL-1, MKL-2, MS-1, MCC-1 and MCC-26; human normal and cancer cell lines, including HeLa, CaSki, HepG2, SLK, BJAB, MCF7, U2OS, SAOS2, 293, 293T, 293FT, IMR-90, WI-38, BJ-tert and HCT116. These will in general be transfected or transformed with plasmids directing expression of bits of Polyomalviral DNA. Some cells (293T or 293FT) will be used to generate Lentiviral vectors. A few other rat, mouse and monkey cell lines (all from *ATCC* and all used at BSL-2+) will be used for similar purposes. In addition to several Polyomaviruses and Papiloma Viruses, the following viruses or vectors will be used:

- Lentiviral vectors (HIV 3-plasmid system, BSL-2+ *Addgene*, *Invitrogen*, *Cell Biolab*) will be used, to express human oncogenes and viral oncoproteins, as well as shRNA and

sgRNA. Human oncogenes for expression include H-ras, c-Myc and several others will be targeted by shRNA.

- Retroviral vectors (MSCV and MMLV, BSL-2+, *Addgene*, *Sigma Aldrich*) will be used to express HPV E7 and human oncogenes, respectively.
- Rabies virus (strain SAD-B19, BSL-2+, from a collaborator at Thomas Jefferson University) molecular clones or fragments will be transfected into human cell lines.
- West Nile Virus (Kenjin, BSL-2+, source currently null) will be used for *in vitro* infection assays to study innate immune responses.
- VSV (Indiana 1, BSL-2+, from a collaborator) will be used to infect human and murine cells to assess the innate immune response.
- Finally, non-infectious, purified RNA and corresponding cDNA fragments from SARS CoV2 (WA01-2020, BSL-2+, from *BEI Resources NIAID*, a collaborator, and patient tissues) will be used to study persistence.

The protocol notes the possibility for increased virulence as a result of using cells infected with MCV and KSHV, as well as inherent risks of using gene editing with the reagents here described. It also notes that MCV and KSHV don't causes disease in healthy individuals. On verification that the laboratory's Biosafety Operations Manual is current, approval is recommended once the issues are addressed and amended.

Initial comments:
1) Project Summary: Add details of SARS CoV2 to the Project Summary.
2) Viruses, Prions, or Vectors: Provide the source of WNV
3) The Lentiviral vector section describes the expression of Kaposi's Sarcoma-Associated Herpes Virus genes – identify which ones and elaborate on the use.
4) Recombinant or Synthetic Nucleic Acid Work Description: Question 3c. This segment refers to Kaposi's Sarcoma-Associated Herpes Virus genes – identify which ones and elaborate.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 2nd emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	17
Against:	0
Abstained:	0

Protocol:	MOD202000165
Title:	Amendment for **IBC201800164**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• High-level expression of viral glycoproteins in the milk of goats by using replication-defective Adenoviral vector • 714650 MERS grant activation sheet • EBIOM_102743_edit_report • MERS ms REDACTED 2 • MERS ms REDACTED 1 • SARS ms REDACTED 1

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Add a short description of work with Spike protein of SARS-CoV-2 virus in both mice and goats to the Project Summary.

2) Tissues, Blood, or Body Fluids: Add the milk (body fluid) of the goats here as it will be collected and purified.

3) Recombinant or Synthetic Nucleic Acids Usage: Check the box: *NIH Appendix Q* - Animal research involving recombinant or synthetic nucleic acids not in standard laboratory containment housing; as goats would meet this criteria.

4) Exposure, Assessment, and Protective Equipment, Questions 4 and 5: Indicate that appropriate PPE for ABSL-2 large animal studies (e.g. liquid-barrier coverall suit, dedicated boots or shoe covers, double gloves, hair bonnet, face shield, and surgical mask or safety glasses/goggles with surgical mask) will be used.

5) Waste Management, Question 1: Note that packaging of carcasses may require training from DLAR veterinarians. Carcasses must be packaged in a leak proof manner and the weight limit of no more than 30 lbs. for each biowaste box is strictly enforced. If animals exceed 30 pounds, training from DLAR veterinarians is available to learn to safely break down and package the carcasses.

6) Waste Management, Question 1c: Describe proper carcass disposal for large animals. This includes: maintaining a permanent record for each animal noting a) method of permanently identifying individual animals, b) recombinant materials administered, c) samples/tissues collected, d) method of carcass disposal to ensure that animals treated with recombinant materials do not enter the food chain (at Pitt this method includes maintaining the date of euthanasia, e) date of carcass packaging as DLAR carcass waste, and f) disposal via incineration). These records must be retained permanently.

Comments:
Amendment to include work with the novel SARS Co-V Spike protein administered to goat mammary glands to test large scale production of possible vaccine subunit using an AAV mediated infection. The resulting glycoproteins will be purified from goat milk and tested for immunogenicity, etc. There are a couple form issues pertaining to the addition of the Spike protein to the protocol (including in the virus and body fluids sections). Once these are remedied, recommendation for approval. This is a modification to add antibody production in goat milk via administration of AdV expressing the Spike protein of SARS-CoV-2. AdV will be infused into the teat and milk will be harvested for purification of antibodies. There are multiple clarifications required, and due to administration of recombinant materials to large animals, *Appendix Q* applies to the research. An amendment to develop an Adenoviral-based 2019-nCOV vaccines to ultimately target human, camels or other reservoir animals, the laboratory will generate recombinant Adenovirus encoding codon-optimized synthetic 2019-nCoV-S (Ad.2019nCoV-S), 2019-nCoV-S1 (Ad.2019nCoV-S1 antigens and define the preliminary immunogenicity screening studies using a mouse model. Approval may be obtained upon the questions and changes being completed.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 2nd emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	17
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 3:18 PM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

April 13, 2020 10:00 AM

Meeting; ZOOM teleconference meeting

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			Yes
REDACTED, Vice Chair	absent		
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED	absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Left 10:58AM		Yes
REDACTED	absent		
REDACTED			Yes
REDACTED	absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	absent		
REDACTED			Yes
REDACTED	absent		
REDACTED			Yes
REDACTED			Yes

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	EH&S Office
REDACTED	IACUC Office
REDACTED	ORP Co-Director

GUEST NAMES

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions via the tele-meeting portal.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The March 9th, (March 23rd, and April 2nd non-standard scheduled) 2020 meeting minutes were reviewed and approved by the committee.

Votes:

For:	20
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

The IBC reviewed a proposal for reviewing research protocols that include the use of SARS-CoV-2 which have been designated as high priority research per University Leadership. The IBC will continue to hold "Emergency" meetings for high priority research (use of SARS-CoV-2) in between the regularly scheduled IBC meetings. There were no comments provided by the committee and the motion passed.

Votes:

For:	20
Against:	0
Abstained:	0

The information will be posted on the IBC website home page, to inform the research community.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocol:	IBC201900176
Title:	IGFBP7 and TIMP2 in AKI
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-4
	• NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Live Animals, Question 3: Earlier in the protocol it is stated that "Blood, urine, kidney, liver, and lung tissue will be collected from mice upon sacrifice." Thus, the answer here should be YES.

2) Exposure Assessment and Protective Equipment, Question 2: The investigator has stated safety engineered scalpels will be used as well as a knife. If a safety engineered option is not available, EH&S may offer suggestions for safe work practices and issue a safety engineered sharps exemption.

3) Exposure Assessment and Protective Equipment, Questions 4 and 5: Add in the PPE required for DLAR animal facility access.

4) Waste Management, Question 1a: Infectious solid waste should be chemically disinfected by spraying with or soaking in an EPA-registered disinfectant prior to disposal of the solid waste in approved biohazardous bags. Revise accordingly.

5) Waste Management, Question 1b: Clarify that liquid waste will be decontaminated by adding concentrated bleach to a final 1:10 dilution (v:v) bleach in liquid waste. Addition of pre-diluted 10% bleach to liquid wastes further dilutes the disinfectant and results in insufficient concentration of active ingredient for proper decontamination.

6) Waste Management, Question 3: Ensure that any materials used for cleaning (e.g. paper towels) are disposed in a biohazard bag.

Comments:

siRNA will be injected into mice to knockdown receptors to aid in the characterization of the roles IGFBP7 and TIMP2 in AKI (acute kidney injury). Human kidneys will be dissociated into primary cell culture. Mouse Blood, urine, kidney, liver, and lung tissue will be collected from mice and used for quantification of protein and RNA expression, histologic evaluation and for immunofluorescence staining. Cell lines will be used to better define the etiology of AKI and a

viable target to investigate HB-mediated AKI therapeutics. Additionally, knockout mice will be bred.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	IBC202000028
Title:	Cutaneous Physiology
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Describe how the other rDNA materials will be used (cell lines, Lentivirus, siRNA).

2) Tissues, Blood, or Body Fluids: Under the "Description of Usage" describe how these cells will be transduced and what transgenes will be studied.

3) Primary Cells or Cell Lines: Include production of Lentiviruses in the "Description of Usage" for the appropriate entry.

4) Primary Cells or Cell Lines: Edit the entry and describe the administration of mouse cells to animals in the appropriate place.

5) Viruses, Prions, or Vectors: Inserted Nucleic Acids Information: List non-viral genes and/or shRNA that will be expressed from the Lentiviral vectors, not a description of the Lentiviral vector itself.

6) Viruses, Prions, or Vectors: In the "Description of Usage" describe in what cells/cell lines the Lentivirus will be used.

7) Recombinant or Synthetic Nucleic Acid Usage: If Lentiviral vectors will be amplified in *E. coli*, the *NIH Guidelines Section III-D-2* should be selected.

8) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include a description of recombinant/synthetic nucleic acid work in mice and if cells are administered to animals, then describe.

9) Live Animals, Question 1: Insert the linked IACUC protocol number.

10) Animal Gene Transfer, Question 3: Clarify what helper viruses will be delivered to mice.

11) Animal Gene Transfer, Question 4: If animals are not receiving any rDNA materials, it is unclear why ABSL-2 is needed.

12) Exposure Assessment and Protective Equipment, Question 5: Select additional PPE: hair bonnet, coverall suit, and shoe covers.

Comments:

This protocol is for a project creating transgenic mice to investigate the role of neurons, neuroglia, and necroptotic death pathways in skin pathophysiology. The project will include mice, human and mouse cell lines, *E. coli* for plasmid production, and 4-plasmid Lentiviral transduction system for gene expression or knockdown. The Recombinant or Synthetic Nucleic Acid Work Description indicates that four genes are going to be knocked down while the Viruses, Prions, or Vectors section indicates two plus an unspecified set of additional genes will be knocked down. This

discrepancy needs to be clarified. The work is proposed at BSL-2 and ABSL-2, which is appears to be appropriate but needs clarification. Pending minor modifications, this protocol could be approved. The goal of the project is to create transgenic mice for the studies pertaining to the role of the neurons and neuroglia in the skin pathophysiology processes (including inflammatory disorders and cancer), and the role of the necroptotic (i.e. ferroptotic) death in the skin pathophysiology. ABSL-2 is an appropriate level for the work. Approval is recommended pending minor modifications. This protocol is for a project that will use tissue from mice as well as mouse and human cells lines to investigate the role of neuronal tissue and necroptotic cell death in skin pathophysiology. While the project summary states the goal of creating transgenic mice, no *in vivo* work is described in the proposal. In addition to mouse tissue and cell lines, the project will use *E. coli* for plasmid production, and a Lentivirus 4-plasmid system for gene expression and knockdown. BSL-2 and ABSL-2 appears appropriate. The Viruses, Prions, or Vectors section as well as the Recombinant or Synthetic Nucleic Acid Work Description section (3b) needs to be clarified to include all genes that will be knocked down, and the protocol could be pending minor modifications.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	IBC202000033
Title:	DNA Sensing in FRC
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Risk Group and Containment Practices, Question 1: Based on the *NIH Guidelines* "RG2 : *Escherichia coli*—all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli O157:H7*". Risk Group 2 (RG2) should be selected.

Comments:

The investigator has submitted a protocol for the study of sepsis. Transgenic mice will be bred, and sepsis will be induced by a variety of methods (including pathogenic *E. coli*). The group will test the efficacy of modified adipose fibroblastic reticular cells via adoptive transfer. Several form issues should be addressed prior to approval including the animal biosafety level designation. The protocol is appropriate for BSL-2/ABSL-2 containment.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	IBC202000038
Title:	NanI Sialidase Effects on CPE Activity
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 4/13/2021

Required modifications: None

Comments:

This protocol contains research of human gastrointestinal diseases caused by *Clostridium perfringens* type F strains. The group will examine the impact of rNanI and *Clostridium perfringens* enterotoxin on enterocyte-like cell lines that do or do not produce mucus. CPE and recombinant CPE will be purified in the laboratory. Minor modifications are required and BSL-2 is appropriate for this work. The investigator will receive animal tissue exposed to *C. perfringens* from UC Davis; USDA permit or clarification may be required. This project describes work by an experienced investigator to characterize the role of the NanI Sialidase in *Clostridium perfringens* pathogenesis, especially with respect to NanI enzymatic degradation of mucous and activation of *C. perfringens* enterotoxin (CPE) by intestinal proteases. Work at Pitt will be *in vitro* and use enterocyte-like cell lines, recombinant wild-type and mutant NanI protein and test the impact of these proteins on bacterial binding, cytotoxicity, and paracellular transit. Mouse and rabbit tissue from experiments performed by a collaborator at UC Davis will be sent to Pitt for analysis and used at BSL-2. Several human cell types (Caco-2, HT29, etc.) will be used for *in vitro* pathogenesis experiments with recombinant proteins and live Clostridium bacteria at BSL-2. *Escherichia coli* will be used at BSL-2 for generation of recombinant NanI and CPE variants at BSL-2. *C. perfringens* will be used as a source for CPE toxin that will be used *in vitro* at BSL-2. Small clarifications requested – no major concerns. The project investigates the role of the NanI Sialidase in the activation of *Clostridium perfringens* pathogenesis by intestinal proteases. Work at Pitt will be *in vitro* and will generate recombinant CPE and/or attenuated forms of it for *in vitro* experiments as well as the use of enterocyte-like cell lines. The effect of NanI on bacterial binding and cytotoxicity will be measured. Animal work will be performed off-site by a collaborator. Bacterial cultures will be used at BSL-2.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Approval Recommended: No additional comments were provided by the committee. Recommendation for approval with no comments.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000084
Title:	Amendment for **IBC201800147**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description, Question 2: Identify the expression system(s) used for expression of c-Myc.

2) Recombinant or Synthetic Nucleic Acid Work Description: The protocol is still missing the information for Lentiviral vector production to comply with BSL-2. Titers smaller than 10^9 50% tissue culture infectious dose (TCID50). Culture volume less than 100 ml would be needed. Check also University of Pittsburgh Institutional Biosafety Committee Guidance on Biosafety Level Assignment for Primate Lentiviral Vectors.

3) Waste Management, Question 3: Provide additional detail describing the process for clean-up and decontamination of a biological spill (e.g. disinfectant, contact time for disinfectant, disposal of potentially contaminated clean up materials).

Comments:

The modification request seeks to add approval for use of Lentiviral vectors to allow stable silencing of Sirt2 by delivery of shRNA in two *in vitro* models of liver cancer. Effects of knockdown on Myc will be assessed. The work will use human cell lines (HepG2, Huh7, HEK293T, obtained from *ATCC*). The HEK293T will be used to generate the viral vector, which will, in turn, be used to infect HepG2 and Huh7 cells. The vector will be assembled using the 3-plasmid system. The transfer vector (encoding the shRNA or a scrambled control) will be obtained from *ABM* and the packaging and envelope (pseudotyped with VSV-G) are from *Addgene*. Viral vector work will be done at BSL-2. Recommend approval pending clarifications. This amendment is for a previously approved protocol investigating the role of sirtuins in post translational modification of mitochondrial proteins. The reason for this amendment is to include Lentiviral vectors to overexpress a gene construct in cultured mammalian cells. The work includes tissues and cells harvested from mice, human cell lines, and *E. coli* for protein expression. A 3-plasmid Lentiviral transduction system from *Addgene* and *ABM* will be used to express shRNA to silence sirt2 expression in human cell lines. In the Recombinant or Synthetic Nucleic Acid Work Description, HEK293 T-cells are included but need to be added to the Primary Cells or Cell Lines section. The work is proposed at BSL-2, which appears to be appropriate. Once the cell line issue, and other concerns are addressed, approval is recommended. For BSL-2 approval, HEK293T cell supernatant for Lentiviral vector production needs to be below 100ml. Titers need to be below 10^9 50% tissue culture infectious dose (TCID50). Sirt1 is targeted by shRNA. No oncogene or tumor suppressors are used.

Initial comments:
1) Recombinant or Synthetic Nucleic Acid Work Description: The protocol states that plasmids of the 3-plasmid system will be propagated in NEB Stable cells. This will require addition of the bacteria under the section of Bacteria, Yeasts, Fungi, or Parasites/Invertebrates in the application.
2) Recombinant or Synthetic Nucleic Acids Usage: Select *NIH Section III-D-2*
3) For work with Lentivirus, safety glasses or goggles are recommended.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0
Abstained:	1; recused for involvement

Protocol:	MOD202000102
Title:	Amendment for **IBC201700226**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: For the Source of the mouse specimen(s), provide the specific commercial vendor.

2) Tissues, Blood, or Body Fluids: For the Source of the NHP specimen(s), provide the specific vendor from where the collaborator's laboratory obtained them.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include a description of *in vivo* work with recombinant nucleic acids.

4) Genetically Engineered Animals, Question 3a: The response should be removed if cells will not be transplanted.

Comments:

This protocol seeks to add an Adenoviral system as a backup to the AAV system for the viral delivery of pdx1, MafA and MafB in pancreatic cells lines. The investigator states that their final experiment will involve infusing the pancreas of non-human primates with viruses shown to be effective in cell culture. Samples collected will be expanded to include stool and blood. Experiments involving primary cells lines will now include acinar cells (266-6) and additional clarity was added into the use of PPE and recombinant DNA. This is a low-risk review at ASBL-2. The researchers want to add Adenovirus to their protocol. Minor comment, the transduced cells will be administered to animals and this should be explained more clearly. The investigator has submitted an amendment to add Adenovirus for increased transduction efficiency of pancreatic islet cells. Minor modifications are required prior to approval at BSL-2/ABSL-2.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000116
Title:	Amendment for **IBC201800040**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Lentivirus: CRISPR/Cas9 and gRNA has not been listed under "Inserted Nucleic Acids Information." Also, include a list of the "other genes".

2) Viruses, Prions, or Vectors: This information was entered in the comments: "We will strictly follow the requirements for gene editing research. When using CRISPR/Cas9 technology for gene editing, we will separate gene deliveries into two steps. We will first deliver the Cas9 expressing plasmid (such as lentiCas9-Blast (*Addgene* #52962) or lentiCas9-EGFP (*Addgene* #63592)) into the studied cells by transfection. At least waiting for 72 hours (usually about a week), we will transfer the studied sgRNAs- encoding replication-defective viruses into the studied cells." Place this information in the body of the protocol application, as comments are removed from the final revision of the protocol.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: This information was entered in the comments: "For the CRISPR/Cas9 knockout experiments, pSpCas9(BB)-2A vectors and lentiCRISPR v2 will be used to express sgRNAs for targeting genes of interest. Most of times, we will directly transfect the sgRNA constructs into cultured cells using transfection reagents. Mammalian expression plasmids using commonly used transfection reagents, such as Lipofectamine 200 or 3000 for cell line transfection. Occasionally, for the cells hard to transfect, we will use the third generation of packing system to make lentiviral vectors for transduction. We will use two steps: first deliver Cas9-expressing plasmids; more than 72 hours later, replication-defective viruses will be used to deliver sgRNAs." Place this response with the detailed information in Question 1 in the application itself and not just in the comment section.

4) Exposure Assessment and Protective Equipment, Question 2: Will any needles, scalpels or other sharps be used? If so, then enter this information.

5) Exposure Assessment and Protective Equipment, Question 4: If any sharps will be used (e.g. needles, scalpels), safety-engineered sharps devices are required for work at BSL-2/ABSL-2. Revise.

Comments:

For use at BSL-2, transduction of cells with a replication-defective virus encoding CRISPR/Cas and with a separate replication-defective vector encoding sgRNA should be separated by at least 72 hours. Otherwise the work must be performed at BSL-2+. This has been placed as a comment in the protocol. In addition, Cas9 and gRNA should be listed under inserted nucleic acids.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000120
Title:	Amendment for **IBC201700051**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: If rat cells will be exposed to Lentivirus, they should be designated as recombinant.

2) Viruses, Prions, or Vectors: The investigator has stated all viruses are replication defective; however, the entries for Adenovirus, Lentivirus, and AAV-8 all note "Agent is *not* replication-defective". Correct all three entries, as this is incorrect information.

3) Recombinant or Synthetic Nucleic Acid Usage: If plasmids expressing viral vectors will be amplified in bacteria, then select the *NIH Guidelines Section III-D-2*.

4) Animal Gene Transfer, Question 3: As AAV requires a helper virus, select YES.

Comments:

In this amendment the investigator wants to add the use of AAV vectors; AAV-2 for cell culture experiments and AAV-8 for *in vivo* work. Regarding the protocol, the investigator investigates glucose and energy homeostasis using a mix of cell culture and *in vivo* model systems. In cultured cells, Adenovirus and Lentivirus are used to drive expression of proteins of interest (POI's), while Adeno-Associated Virus (AAV-2) will be used to drive either expression or knockdown of POI's. In mice and rats, Lentivirus is injected ICV to the hypothalamus and IV to target the liver for protein overexpression, and AAV-8 will be used to drive overexpression or knockdown of POI's. The work in the protocol is being conducted in BSL-2 and ABSL-2 conditions. Information related to the use of AAV has been inserted in the protocol. Since work does not involve NHP or NHP tissue/cell, remove mention of NHP use in question 1 of the 'Exposure Assessment and Protective Equipment'. This amended IBC protocol will investigate the regulation of glucose and energy homeostasis using *in vivo* and *in vitro* models by adding the use of AAV *in vivo* in mice and *in vitro*. AAV-8 mediated overexpression and knockdown of gene, including IRF3, IRF7, STAT1 and IFI202b, will be used in in AIM2-/- mice to evaluate whether changes in these proteins will affect adipogeneis and lipogenesis, whereas AAV-2 mediated overexpress or knockdown of same genes will be applied to murine, human and rat cell lines. All work was claimed to be performed at level of BSL-2 which is suitable. Overall, the protocol can be approved pending modifications.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000181
Title:	Amendment for **IBC201600075**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-2 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED Lab SOP

Determination: Approved

Last day of continuing review period: 4/13/2021

Required modifications: None

Comments:
This is a protocol modification to add a tetracycline-inducible mammalian expression plasmid and plasmids encoding the SARS-CoV-2 Spike protein, receptor binding domain (RBD), and N protein. All work will use plasmid DNA in various cell lines at BSL-2, which is appropriate. No biosafety concerns. This is a modification to add plasmid expression system and plasmids encoding various domain proteins of the SARS-CoV-2 virus. This work is strictly cell culture work and plasmid transfection in various cell lines, including human.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0
Abstained:	1; recused for involvement

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocols on the following pages

Protocol:	IBC202000021
Title:	HCC 20-040
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• Safety Data Sheet for RTX240 Drug Product_vF • 2020-01-31 Main ICF_v2.0_20-040 • 2019-09-30 HSIC0604 Bloodborne Pathogen Standard Exposure Control Plan_20-040 • 2020-01-27 Investigator's Brochure_v2.0_20-040 • Rubius_RTX-240-01 Drug Product Manual V4 12Mar20 • 2019-04-26 HSIC0616 Guidelines for Handling Sharps_20-040 • 2018-06-29 HSFM0208PRO Waste Management Procedure_20-040 • 2020-03-12 Protocol_v3_20-040 • LV1 240 illustration for IBC

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Indicate risk of needle stick exposure (slow IV push administration).

2) Human Gene Transfer/Human Clinical Trial: Materials: Question 5: Describe the genetic content of the transgene or the nucleic acid delivered (e.g. gene of interest in viral vector), including the species source of the sequence and whether any modifications have been made.

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 6: Describe the delivery method for the gene transfer agent (e.g. intravenous).

4) Human Gene Transfer/Human Clinical Trial: Question 1: Link the related PittPRO protocol in question 1.

5) Biosafety Summary: Check the box for Recombinant or Synthetic Nucleic Acids.

Comments:

Summary: This is an open-label phase 1 / 2 study of RTX-240 to evaluate its safety, tolerability, determine the MTD, PD, and optimal dosing interval. Part 1 will focus on safety, PK, and optimal dose. The study population consists of persons with refractory or locally advanced solid tumors. Part 2 will consist of expansion cohorts enrolling persons with specific solid tumors. RTX-240 consists of allogeneic cultured human enucleated red cells expressing 4-1BB ligand (4-1BBL) and interleukin-15 trans-presentation (IL-15TP), a fusion of interleukin-15 (IL-15) and IL-15 receptor alpha (IL-15Rα), on the cell surface. 4-1BBL and IL-15TP are expressed as fusions to the n-terminus of glycophorin A (GPA), a transmembrane protein expressed at high levels on red blood cells. RTX-240 is made starting with CD34+ stem cells from healthy donors (blood type O, Rh-negative, Kell-negative). The cells are transduced with two Lentiviral vectors, expanded, differentiated, and matured into enucleated red cells co-expressing 4-1BBL and IL-15TP on the cell surface. LV1-IL-15TP and LV1-4-1BBL are third-generation, replication-defective, self-inactivating, HIV-1 based Lentiviral vectors. The product is designed to stimulate antitumor

activity of NK cells and T-cells through direct cell-to-cell interaction, with the goal of improving anti-tumor activity and overcoming resistance to immunotherapy in participants with solid tumors. In *in vitro* studies, RTX-240 led to NK and T-cell activation, proliferation, and cytotoxicity in a dose dependent manner. In a mouse model, mRBC-240 treatment at a dose of 1 x 10^9 cells significantly inhibited tumor growth, decreased metastatic tumor burden and resulted in the expected PD changes in the blood and tumor. RTX-240 will be administered by slow IV push every 6 weeks, except during the safety cohort, when it will be administered every 4 weeks. During the Phase 1 dose escalation study, up to 6 dose levels will be studied, starting at 1 x 10^8 to 6 x 10^{10}. Up to 32 participants will be evaluated in phase 1. In Phase 2, up to 140 persons with NSCLC, melanoma and other specific solid tumors will receive the optimal dose(s) identified in Phase 1. There is a first-in-humans study with this product. An independent DSMC will oversee the study.

Biosafety: RTX-240 consists of cells expressing both 4-1BBL and IL-15TP (double positive cells), cells that express only 4-1BBL or IL-15 (single positive cells), and cells that express neither 4-1BBL nor IL-15TP. The specification for purity as measured by enucleated cells is ≥ 70%. All lots are tested for the expression of CD233, which indicates that the drug product contains terminally differentiated red blood cells. Positive co-expression of 4-1BBL and IL-15TP is confirmed as part of release testing, and the specification is that ≥ 40% of the cells express both 4-1BBL and IL-15TP. Dose levels are expressed in terms of the total number of RTX-240 cells/dose. Adjustments may be made to account for lot-to-lot variability in the contribution of each of these components. When dose reduction of RTX-240 is indicated, the dose will be reduced to approximately 50% of the targeted number of RTX-240 cells. Additional adjustments may be required to account for lot-to-lot variability; the resulting dose will ensure that the total exposure to both 4-1BBL and IL15TP positive cells has been reduced relative to the previously administered dose. Up to 2 dose reductions of RTX-240 will be allowed. The drug product is formulated at a concentration of approximately 1 × 10^9 cells/mL in a formulation containing human serum albumin, sodium bicarbonate, sodium phosphate dibasic, HEPES, calcium chloride dihydrate, Adenine, magnesium chloride hexahydrate, potassium hydroxide, sodium hydroxide, Adenosine, mannitol, and lactobionic acid. A type and screen will be performed during screening and prior to each RTX-240 administration and to check for the presence of circulating antibodies against red blood cells. RTX-240 will be administered based on preliminary, rapid sterility results. In the event of a subsequent sterility failure, the investigator will be notified by the Sponsor and a plan for additional safety monitoring will be developed. The biodistribution of RTX-240 is expected to be limited to the vasculature, which could minimize on-target off-tissue toxicity. Possible toxicities include blood group incompatibility (will be using Group O donors, crossmatching), CRS, hemolysis, infusion-related reactions, transmission of infection/sterility failure, GVD (very unlikely), and unknown others. Participants will be followed for up to 15 years or until death or withdrawal of consent.

Initial comments:
1) Biosafety Summary: Select the box for recombinant or synthetic nucleic acids.
2) Exposure assessment: Indicate risk of needlestick exposure – slow IV push administration.

The application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0
Abstained:	1; recused for involvement

Protocol:	IBC202000039
Title:	20-047
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-08-20 Tmunity CART-TnMUC1 Product Manual_Version 1.0_20-047 • 2019-04-09 CART-TnMUC1-01_Prescreen ICF_20-047 • 2019-11-15 CART-TnMUC1-01_Tmunity Product Manual_Version 1.1_20-047 • 2019-06-05 TnMUC1-01_HistoGeneX_Lab Manual_v1.0_20-047 • 2019-03-28 TnMUC1 IHC Assay Validation_20-047 • 2019-03-26 TnMUC1-01_Investigator Brochure v1_20-047 • 2019-04-23 CART-TnMUC1-01_Main ICF_20-047 • 2019-05-03 CART-TnMUC1-01 Protocol v2_20-047 • 2019-07-15 CART-TnMUC1-01_Eurofins Laboratory Manual_V1.0_20-047

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells may harbor unknown infectious agents.

2) Exposure Assessment and Protective Equipment: Question 2: As this material will be introduced via intravenous injection, there is a risk of accidental exposure to personnel via percutaneous or mucous membrane exposure routes or environmental release. Modify the response.

3) Human Gene Transfer/Human Clinical Trial: Materials: Question 6: Describe the delivery method for the gene transfer agent (e.g. intravenous).

4) Human Gene Transfer/Human Clinical Trial: Question 1: Provide/identify the linked PittPRO protocol that will be associated with this IBC protocol.

5) Human Gene Transfer/Human Clinical Trial: Briefly outline steps taken to ensure chain of custody once product arrives on site.

6) Lentivirus and Lentiviral Vectors: Question 2a: Provide information about the safety aspects of the vector – how it is made to be safer than another vector system, rather than simply identifying the genes in the system.

7) Viruses, Prions, or Vectors: The viral vector used for transduction of cells is a Lentivirus, not Vesicular Stomatitis Virus. Correct the virus designation.

8) Viruses, Prions, or Vectors: The viral vector should be designated as recombinant.

9) Viruses, Prions, or Vectors: List all transgenes under "Inserted Nucleic Acids Information."

Comments:

Summary: This Phase I two arm parallel dose study evaluates safety, tolerability, prelim efficacy of transduced autologous T-cells to express a chimeric antigen receptor that can recognize a tumor antigen known as TnMUC1 and activate the T-cells (CART-TnMUC1 cells). MUC1 expression

is widespread in numerous organs, and tumors may express the abnormally glycosylated TnMUC1 glycoform. The CART-TnMUC1 is prepared from the participant's PBMCs obtained via leukapheresis. T-cells are enriched from the autologous leukapheresis product, activated with anti-CD3 and anti-CD28 antibody coated magnetic beads followed by transduction with a self-inactivating Lentiviral vector containing the 5E5-CD2ζ CAR transgene. The TnMUC1 CAR consists of the murine anti-human TnMUC1 single chain variable fragment (scFv) derived from the monoclonal antibody (mAb) 5E5; the CD8α hinge and transmembrane domain; and the dual CD2 and CD3ζ intracellular signaling domains. TnMUC1 positivity is determined in the pre-screening setting using a central IHC assay performed on archival tumor tissue. There are two phases to the study; phase 1 is designed to identify the dose and regimen to be given to participants with advanced solid TnMUC1 tumors (pancreatic, breast, NSCLC) or multiple myeloma in a dose escalation manner. The second expansion Phase, 1a, a single arm study, is designed to assess efficacy in women with TnMUC1+ platinum-resistant ovarian cancer. Approximately 80 participants will be enrolled overall. The first dose cohort of CAR-TnMUC1 will be given to participants with solid tumors in Phase 1, Arm 1 in the absence of a lymphodepletion regimen to assess the preliminary safety of the CART cells alone. Lymphodepletion is associated with a strong *in vivo* proliferation signal of CAR-T. Once safety is confirmed in Dose Level 1 in participants with solid tumors, the trial will continue to enroll in Dose Level Cohorts 2 and beyond with both Arms enrolling in parallel.

Biosafety: This is a first-in-humans study. The product is manufactured and released by the Clinical Cell and Vaccine Production Facility at U Penn. RCL detection assays are used to screen the vector product as well as the vector-transduced cells. The sponsor oversees final product release. The product is frozen in cryopreservation bag(s) a target concentration of 1×10^8 total nucleated cells per mL with greater than 1×10^9 total cells per bag, or with a minimum of 10 mL cell suspension if less than 1×10^9 total cells. Other cell populations, including monocytes, natural killer (NK) cells, and B-cells, may be present. Toxicities include fever, infusion reactions, CRS, CART-related encephalopathy, TnMUC1-specific on-target, off-tumor effects such as gastritis or acute kidney injury. Participants will be assessed for presence of RCL up to 15 years. Clonal expansion of a given T-cell clone within the CART-TnMUC1 population infused is theoretically possible. This toxicity has not previously been reported; considered to be of low risk. Participants will be monitored for clonal expansion of CART-TnMUC1. Lymphodepletion (chemotherapy) is associated with hematologic toxicities and increased risk of infections.

Initial comments:
1) Human Gene Transfer: Outline steps to ensure chain of custody once the product arrives on site.

The application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0

Abstained: 1; recused for involvement

Protocol:	IBC202000041
Title:	20-048
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-12-17 CTC collection schedule_20-048 • 2019-05-30 CART-PSMA-TGF_RDN-02 Protocol v1_20-048 • 2019-12-04 CART-PSMA-TGFBRDN-02_Product Manual v1.1_20-048 • 2016 CAPLABSCMb Ventana Lab Manual_20-048 • 2020-02-10 CART-PSMA-TGFbRDN-02_Investigator Brochure v2_20-048 • 2020-02-20 PRC Checklist Program Director Letter_20-048 • 2019-09-17 CART-PSMA-TGFBRDN-02_Eurofins Laboratory Manual v1_20-048 • 2019-06-20 CART-PSMA-TGFBRDN-02_Main ICF_20-048 • 2019-08-21 Apheresis Infusion Manual _20-048

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells may harbor unknown infectious agents.

2) Exposure Assessment and Protective Equipment: Question 2: As this material will be introduced via intravenous injection, there is a risk of accidental exposure to personnel via percutaneous or mucous membrane exposure routes or environmental release. Modify the response.

3) Human Gene Transfer/Human Clinical Trial: Materials: 2a. Add preliminary findings from the first in humans study conducted by Penn.

4) Human Gene Transfer/Human Clinical Trial: Materials: Question 6: Describe the delivery method for the gene transfer agent (e.g. intravenous).

5) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: If the agent is determined to be from a virus (a viral vector), the assay(s) used to check whether there is any infectious virus remaining in the agent preparation should be identified.

6) Human Gene Transfer/Human Clinical Trial: Briefly describe chain of custody procedures for study product upon receipt from U Penn facility.

7) Lentivirus and Lentiviral Vectors: Question 2a: Provide information about the safety aspects of the vector – how it is made to be safer than another vector system, rather than simply identifying the genes in the system.

8) Viruses, Prions, or Vectors: The viral vector used for transduction of cells is a Lentivirus, not Vesicular Stomatitis Virus. Correct the virus designation.

9) Viruses, Prions, or Vectors: The source cannot be left blank. Edit the entry and provide a response to the question regarding where the materials were obtained.

10) Viruses, Prions, or Vectors: Lentiviruses *can* infect human cells. Correct the response.

Comments:
Summary: This is a multicenter phase 1 study of transduced autologous T-cells modified to express a chimeric antigen receptor (CAR) designed to recognize the tumor specific antigen known as prostate-specific membrane antigen (PSMA) and a dominant negative TGFβ receptor, thereby activating the T-cell. The study population is men with advanced prostate cancer. The PSMA-TGFβRIIDN CAR comprises a) the murine anti-human PSMA single chain variable fragment (scFv) derived from the monoclonal antibody (mAb) J591; b) the cluster of differentiation (CD)8α hinge and transmembrane domain, and c) the 4-1BB and CD3ζ intracellular signaling domains. The Lentivirus used is Vesicular Stomatitis Virus G. This is a dose escalation study to determine the MTD and/or the RP2D of CART-PSMA-TGFβRDN cells that can be safely administered to participants following lymphodepletion. The CART infusion will begin approximately 3-5 days after lymphodepletion. Some data from the first in humans study sponsored by UPenn is available. Findings from this study listed: a) in the absence of lymphodepletion, the CART-PSMATGFβRDN cells demonstrated minor degrees of expansion at the higher dose level tested; b) the cell expansion at the highest cell dose tested (1-3 × 108/m2) was much higher with lymphodepletion than without it; and, c) severe toxicity (grade 4 CRS) was observed at the highest dose level when administered following the standard lymphodepleting regimen in 1/1 participants in Cohort 3 compared with grade 3 CRS in 2/3 participants when CART-PSMA-TGFβDN was given without lymphodepletion. Based on these observations, the goal of this study is to explore lower total doses of the CART-PSMA-TGFβRDN cells, and to identify the optimal dosing schedule, either as a single infusion or as a fractionated infusion (balancing toxicity and activity), for future studies. The starting dose in this study is one half-log lower than the starting dose in the CART-PSMA-01 study. The study plans to evaluate fractionated dosing once the $1\text{-}3 \times 10^8$ transduced cells dose level is reached, or if DLT is observed in the lower dose cohorts. The first dose of the fractionated dose will contain 30% of the total dose and will be administered on Day 0. The second dose will contain the remaining 70% of the total dose and will be administered on any one day between Days 3 to Day 7 (inclusive) based on observed toxicity following the Day 0 dose. The study will be monitored by a PSMC.

Biosafety: Leukapheresis product is cryopreserved and then shipped to UPenn Clinical Cell and Vaccine Production Facility, which is accredited). Final product release is conducted by *Tmunity*. Manufacturing of the CART-PSMA-TGFβRDN product takes approximately 4-6 weeks. Dosing is formulated based on anti-PSMA CAR expression on the T-cells (number of transduced cells). The total dose of transduced cells will depend on their cohort assignment. The product may contain other cells such as monocytes, natural killer (NK) cells, and B-cells. Participants will be admitted for the CART-PSMA-TGFβRDN infusion (given IV) and monitored for at least 2 days afterwards. Possible toxicities include CRS, CRS encephalopathy, uncontrolled clonal T-cell proliferation, insertional oncogenesis (unlikely), off-tumor/on-target toxicity, immunogenicity (reject CAR T-cells), RCL (unlikely, screening for VSV-G DNA, HIV+ excluded). Participants will be screened for RCL until there are 2 sequential tests that show no persistence of the CAR. RCL testing is only required at Week 12 (±1 week), Week 24 (±2 weeks), Week 52 (±2 weeks) and annually thereafter. RCL blood sampling will be done for a minimum of 15 years or until the CAR is no longer

detectable in blood by PCR. Participants will be tested for anti-mouse antibody development (immunogenicity).

Initial comments:
1) Briefly describe chain of custody procedures for study product upon receipt from UPenn facility.
2) HGT/Materials: Question 2a: Add preliminary findings from the first-in-humans study conducted by Penn.

The application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0
Abstained:	1; recused for involvement

Protocol:	IBC202000047
Title:	SARS-CoV Biology and Antivirals
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Include the SARS-CoV-2 virus/replicon, as the application earlier indicated that the replicon can enter/infect cells.

2) Exposure Assessment and Protective Equipment: Questions 2 and 4: It is unclear why blades and needles will be used in this study. If they are, safety-engineered sharps should be used.

3) Exposure Assessment and Protective Equipment: Question 1: Will the "pCMVΔP1ΔenvpA, pSVIIIenv" packing system from the NIH AIDS Reagent Program be used in these studies to generate replication-competent Lentiviral vectors? If so, this work must be described appropriately throughout the protocol and this work may need to be performed at BSL-2+. Clarify.

4) Risk Group and Containment Practices: Question 1: Check the box for Risk Group 3 (RG-3) for use of Lentivirus vectors.

5) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Include viral vectors expressing CRISPR/Cas9, gRNA, and shRNA.

6) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Include information regarding SARS-CoV-2 recombinant/synthetic nucleic acids. Be clear on what exactly will be introduced into cells and how.

7) Viruses, Prions, or Vectors: SARS-associated Coronavirus - SARS-CoV-2: List collaborators and commercial sources under "Source."

8) Viruses, Prions, or Vectors: Lentiviruses expressing CRISPR/Cas9 and gRNA should be included, as well as identifying the source of these materials.

9) Viruses, Prions, or Vectors: SARS-associated Coronavirus - SARS-CoV-2: Clarify how the virus can enter or infect human cells.

10) Viruses, Prions, or Vectors: SARS-associated Coronavirus - SARS-CoV-2: the statement, "SARS-CoV and SARS-CoV-2 Spike protein expression plasmid, will be obtained from Loyola University and University of Illinois at Chicago, and used to make pseudotyped HIV-1 GFP or luciferase reporter virus" is unclear. How does this differ from the Lentivirus entries? It is suggested that Lentivirus entries be separate from Coronavirus reporter viruses.

11) Viruses, Prions, or Vectors: Coronavirus - SARS-CoV-2 entry: Confirm that no full-length RNA genome of either SARS-CoV-1 or SARS-CoV-2 will be obtained from collaborators. Work with material expressing a single protein (e.g. Spike 1) from SARS-CoV-1 or SARS-C0V-2 is acceptable. However, the full-length RNA genome of SARS-CoV-1 is regulated by the Federal Select Agent Program and must not be acquired without pre-approval from the University Responsible Official and Alternate Responsible Officials (Director EH&S, and BSOs EH&S).

12) Viruses, Prions, or Vectors: If Lentiviral vectors will be used to express shRNA, then list that work in the appropriate Lentivirus entry in this section of the application.

Comments:
This is a new protocol to study SARS-CoV-2 biology and to screen for therapeutics using the following: a) replication-defective Lentiviral reporter viruses pseudotyped with SARS-CoV-1 and SARS-CoV-2 Spike proteins, b) transfection of plasmids encoding individual SARS-CoV-2 genes into cells for protein expression, and c) a SARS-CoV-2 replicon system in which plasmids encoding the viral genome (with the exception of the S, M, and E genes) will be transcribed into RNA that will be transfected into cells for stable expression. Human, nonhuman primate, and hamster cells will be used for cell culture experiments and *E. coli* will be used for amplification of plasmids and protein expression. Lentiviruses expressing shRNA and CRISPR/Cas separate from gRNA will also be used. All work will be performed at BSL-2, which is likely appropriate. Several clarifications are requested, particularly in regard to the SARS-CoV-2 replicon system. Recommendation for approval pending modifications with member review. This new protocol application describes work to examine SARS-CoV-2 mechanisms of infection and use of pseudotyped Lentiviral particles or a SARS-CoV-2 replicon system for screening for potential therapeutics. Replication-defective Lentiviral vectors will be pseudotyped with S1 proteins from SARS-CoV-1 and SARS-CoV-2 Spike proteins, and plasmids encoding single SARS-CoV-2 genes will be transfected or electroporated into cells for protein expression. Generation of a SARS-CoV-2 replicon system lacking the S, M, and E structural proteins will be performed using a cDNA library as starting materials. Human, NHP, and hamster cell lines will be used in *in vitro* experiments and *E. coli* will be used for propagation of plasmids and protein expression. Lentiviral vectors will be used to express shRNA and CRISPR/Cas separate from gRNA. All work is proposed at BSL-2, which is appropriate. Many clarifications are requested, with several required for better description of the SARS-CoV-2 replicon system. Recommendation for approval pending modifications with member review.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the meeting. The protocol application was placed onto the April agenda for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0
Abstained:	1; was in ZOOM waiting room

Protocol:	MOD202000072
Title:	Amendment for **IBC201700155**
Investigator:	REDACTED
Highest BSL:	RBL ABSL-3
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Supporting Documents: Upload the laboratory's Biosafety Operations Manual that has been signed by a member of the EH&S Department and the Investigator.

2) Lentivirus and Lentiviral Vectors: It is unclear why this section is left blank. Complete the application.

3) Viruses, Prions, or Vectors: For the Lentiviral work, it is unclear what sgRNA will be used in conjunction with CRISPR/Cas. This has direct bearing on the BSL assigned to the work. Describe what genes will be edited by the CRISPR technology.

Comments:

This is an amendment to add clinical isolates of a Select Agent that is already approved for work in the RBL. Also, the investigator is adding in Lentiviral work for *in vitro* use only. The Lentivirus will express CRISPR/Cas and shRNA (though not sgRNA)? The expressed or silenced genes are not described and should be identified on the application to be sure that no oncogenes are involved. There are many sections of the protocol that need to be updated with Lentivirus information in general. This work proposed may or may not be considered to be low risk, but since the application is conducted within the RBL overall, the modification should come to convened review. This is a modification of an existing protocol for the study of Rift Valley Fever Virus (which is a Select Agent). The investigator is currently approved for work in the RBL with the ZH501 strain. The revision will include work with clinical isolates as well. The investigator is also adding Lentivirus for transduction of cells which will later be infected with RVFV. Several modifications are required prior to approval. Additional sections will need to be added for information regarding CRISPR/Cas9 and Lentivirus work.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000094
Title:	Amendment for **IBC201800060**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Supporting Documents: Upload the final version of the laboratory's Biosafety Operations Manual. The final version must be signed by a member of the Department of Environmental Health and Safety (EH&S) and the Investigator. Then it should be uploaded to the application.

2) Exposure Assessment and Protective Equipment: Question 5: Correct the PPE for a BSL-2+ laboratory.

3) Viruses, Prions, or Vectors: Expression of oncogenes from Lentiviral vectors requires use at BSL-2+. The application lists SMAD1 and SMAD5 as oncogenes. Correct to indicate the biosafety level at BSL-2+.

4) Protocol Team Members: Question 1: Personnel listed are not current for training and certain individuals must complete the Bloodborne Pathogens training. Other personnel must complete Bloodborne Pathogens and Chemical Hygiene trainings.

Comments:

This a request for a modification to an existing protocol to add new experiments requiring breast cancer cell lines to be injected into zebrafish embryos and the use of a Lentiviral system not previously present in the protocol. There are questions regarding biosafety levels that must be addressed at the next convened meeting prior to approval, specifically, work with Lentivirus involving oncogenes which would move this protocol to BSL-2+ containment requirements. This amendment is being submitted to add new personnel, addition of breast cancer cell lines, zebrafish xenograft model to assess tumor angiogenesis and metastasis and addition of *Sigma Mission* Lentiviral system. The original protocol was designed to use rDNA to manipulate gene expression zebra fish and cell culture models. In zebrafish, the investigator proposes to inject synthetic mRNA to overexpress genes of interest; DNA plasmids to generate transient or stable transgenics; morpholinos to effect transient knockdown; and TALENs or guide RNAs + Cas9 mRNA to generate mutations. In cultured cells, the investigator proposes to transfect or transduce (using Lentivirus) DNA plasmids or siRNAs. Current work is primarily focused on understanding the role of ALK1 signaling in normal vascular development and human disease processes. The investigator is submitting review to add personnel and to update their protocol based on new experiments involving xenographs of human cell lines into zebrafish embryos. The modifications

clearly describe the inclusion of a new 3-vector HIV Lentiviral system which should be Risk Group 3, but all other changes are appropriate.

Initial comments:
1) Primary cells and cell lines: Since the investigator is proposing the use of Lentivirus now, the genes to be manipulated using the Lentivirus should mentioned in the application, and if any gene is an oncogene then the work would need to be conducted under BSL-2+ containment conditions.
2) Viruses prions and vectors: Smads are oncogenes. Therefore, the Lentiviral work needs to be done at BSL-2+. The investigator should mention all the genes that are planned to be to be manipulated in human cells using the Lentivirus.
Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4281477/
3) Recombinant and synthetic nucleic acid work: The response to question 2 should be YES. See comment on previous page. Smad pathway genes are being manipulated and these genes are oncogenes.
4) Recombinant and synthetic nucleic acid work: Clarify, based on the answer to 3D it seems the investigator is planning to inject Lentivirus into zebra fish. If this is true, then *Section III-D-4* would apply.
5) Risk groups: Based on use of oncogenes with HIV, it should be BSL-2+ in question 2.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000101
Title:	Amendment for **IBC201700079**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED lab plasmid inventory • signed IBC Protocol 22Nov19

Determination: Modifications Required

Required modifications:

1) Supporting Documents: An updated laboratory Biosafety Manual is required. Once the updated manual is approved by EH&S and signed by the investigator, then the final version of the manual must be uploaded in the Supporting Documents section of the application.

2) Exposure Assessment and Protective Equipment: Question 1: Include Adenoviruses in the response.

3) Animal Gene Transfer: Question 3: Clarify what helper virus will be administered to animals. If this is not the case, correct the response.

4) Viruses, Prions, or Vectors: CRISPR/Cas9 should be listed under "Inserted Nucleic Acids Information" for viral vectors that will be used to express it.

5) Viruses, Prions, or Vectors: Adenovirus type 5 - Human Adenovirus Type 5 (dE1/E3): List non-viral genes of interest that will be expressed from this vector under "Inserted Nucleic Acids Information."

6) Viruses, Prions, or Vectors: Lentivirus (HIV 4-plasmid system) - pLV[ncRNA]-mCherry;T2A:Puro: List non-viral genes of interest that will be expressed from this vector under "Inserted Nucleic Acids Information."

7) Viruses, Prions, or Vectors: Adenovirus (Adv, human; all serotypes) - pAdenoG-HA-mTET2: List non-viral genes of interest that will be expressed from this vector under "Inserted Nucleic Acids Information."

8) Viruses, Prions, or Vectors: Adenovirus (Adv, human; all serotypes) - pAdenoG-HA-mTET2: this virus should be designated as recombinant.

9) Viruses, Prions, or Vectors: Adenovirus type 5 - Human Adenovirus Type 5 (dE1/E3): Clarify how this virus is unable to enter or infect human cells. If this is incorrect, change the response to that question.

10) Viruses, Prions, or Vectors: Adenovirus type 5 - Human Adenovirus Type 5 (dE1/E3): This AdV should be designated as recombinant.

11) Primary Cells or Cell Lines: Cell/cell line: Other human cells or cell lines (HEK293): Since HEK293 cells will be used to generate Lentivirus-like particles, including those encoding Tet oncogenes, this work should be performed at BSL-2+.

Comments:

The modification request seeks to make several changes. The underlying protocol examines the epigenetics of vascular differentiation and plasticity in development and cardiovascular diseases, with emphasis on the roles of lineage-specific signatures. The work alters these signatures using epigenome editing tools. These rely on Lentiviral vectors to deliver various proteins and fusion constructs. Some changes are administrative. The main changes are to update and modify the lists of genes targeted for Lentiviral vector-mediated overexpression or silencing for use *in vitro* and *in vivo*, to modify the list of cells used for Lentiviral vector-mediated transduction, and to add Adenoviral vectors for overexpression of target genes in various cells. Many tissues or cells will be isolated from mice infected with Lentiviral vectors. These vectors will deliver a range of overexpression constructs including Myocardin-LSD1 fusion protein, TET1, TET2, Myocardin, LSD1, microRNA miR145, long non-coding RNA CASC15. Lentiviral vectors will also be used to silence TET1, TET2, LSD1, Myocardin, SET1-2, SMYD2, MLL1-4, lnc CASC15. Mice are from *Jackson Laboratories* or from the laboratory's own colony. Rat and murine aortic smooth muscle cells (from University of Virginia) will be transduced with Adenoviral vectors, delivering TET2, Myocardin, LSD1 and a Myocardin-LSD1 fusion. Rat smooth muscle cells will also be transduced with Lentiviral vectors for stable expression of several proteins, RNAs or wild type or mutant fusion constructs. It is noted that the TET enzymes (Tet1, Tet2, Tet3) are oncogenes but vectors expressing these will not be injected into mice. Several genes will also be targeted for silencing and sgRNA will be generated targeting the CTA2 promoter, the MYH11 promoter or the miR145 promoter. Lentiviral vectors will be generated using the HIV 4-plasmid system (key vectors from *Addgene*). Where oncogenes will be expressed, the work will be done at BSL-2+, otherwise BSL-2. Vectors will be used to transduce cultured cells or will be injected into mice. For the latter, cells will be harvested after 2-4 weeks. Adenoviral vectors (from *Addgene*, *Vector Biolabs* or from a colleague at Yale University) will be used to overexpress Myocardin-LSD1 and other proteins *in vitro*. Work will be done at BSL-2+ and ABSL-2. The modification describes numerous changes including the addition of Lentiviral and Adenoviral vectors for expression of proteins in cultured cells and to target expression of different proteins *in vivo* in a project focusing on epigenetic control of vascular cell differentiation and plasticity. Lentivirus and Adenovirus will be used to express and silence gene expression in live mice and murine tissues. Expression of several genes (Myocardin-LSD1 fusion protein, TET1, TET2, Myocardin, LSD1, microRNA miR145, long non-coding RNA CASC15. Lentiviral particles will also encode for shRNA to mediate silencing of: TET1, TET2, LSD1, Myocardin, SET1-2, SMYD2, MLL1-4, lnc CASC15) including oncogenes TET1/2, will be included. A variety of cell lines including human cell lines and rodent cell lines will be used at BSL-1, BSL-2, and BSL-2+. Lentiviruses with oncogenes will not be used with mice, other Lentivirus will be administered by retro-orbital or tail intravenous injections of gels containing virus, additionally virus will be used *in vitro* and *ex vivo*. Additionally, laboratory *Escherichia coli* will be used at BSL-1. TET enzymes: Tet1, Tet2 and Tet3. TETs are

classified as oncogenes; so, their use should be listed as BSL-2+ throughout. ABSL-2/BSL-2+ are appropriate. There are some forms issues to be addressed prior to approval.

Initial comments:
1) Tissues, Blood, or Body Fluids: Work should be done at BSL-2+ due to the use of the oncogenes.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000119
Title:	Amendment for **IBC201800237**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• NIH Section III-D-1 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• citiCompletionReport3798651 • EOH-Memo REDACTI 2019

Determination: Modifications Required

Required modifications:

1) Supporting Documents: Upload the most recent and signed laboratory Biosafety Operations Manual. If work will be requiring BSL-2+ containment, then the laboratory Biosafety Operations Manual will need to be signed by the investigator and a member of the Department the Environmental Health and Safety (EH&S).

2) Exposure Assessment and Protective Equipment: Question 4: Describe procedures, work practices, and/or engineering controls that will be used to mitigate potential risks associated with centrifugation.

3) Exposure Assessment and Protective Equipment: Question 1: Include Lentivirus in the response.

4) Risk Group and Containment Practices: Question 3: Some personnel are not current for Bloodborne Pathogens training or Chemical Hygiene training.

5) Risk Group and Containment Practices: Question 1: Lentiviruses are Risk Group (RG-3).

6) Risk Group and Containment Practices: Question 2: BSL-2+ likely should be selected.

7) Animals: Question 1: The investigator name on the related ARO protocol does not match the investigator name on this IBC protocol application. Review response.

8) Lentivirus and Lentiviral Vectors: Question 2: If Lentivirus will be produced from a single plasmid, it requires use under BSL-2+ containment and safety conditions. Note that LentiCRISPRv2 requires plasmids encoding structural proteins and envelope to produce infectious virus.

9) Lentivirus and Lentiviral Vectors: Question 3: Clarify whether CRISPR/Cas9 knockout cells will not be generated stably or not.

10) Recombinant or Synthetic Nucleic Acid Work Description: If the investigator is using Lentivirus to transfer CRISPR/Cas9 and sgRNA at the same time, the protocol should be conducted under BSL-2+ containment. In addition, the Lentivirus information should be completed.

11) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Describe the use of recombinant Lentiviruses in the response.

12) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-2* should also be selected.

13) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-3* should also be selected.

14) Viruses, Prions, or Vectors: All virus vectors must be entered. Expression of CRISPR/Cas9 in the same vector with gRNA (e.g. LentiCRISPRv2) requires use at BSL-2+ per University of Pittsburgh IBC guidance for research with gene editing technology.

15) Viruses, Prions, or Vectors: If the investigator is using a Lentivirus vector, then the Viruses section of the application needs to be completed.

16) Primary Cells or Cell Lines: Cells used for production of or infection with BSL-2+ materials must be used at BSL-2+.

17) Amendment Introduction: Add a short amount of background for the addition including what Pink1 is and why it is being tested in mouse fibroblasts. More information regarding the pLenti6-DEST plasmid is required to clarify that this is not a Lentivirus, but rather a plasmid-based system. Also, the *Addgene* website says it is unavailable; it is always best for the investigator to describe the information rather than just providing a link to the committee. The investigator needs to clarify if Lentiviral vectors will be used on the existing protocol as delivery of CRISPR/Cas9 and sgRNA is also indicated on the application.

Comments:
This protocol is looking at ubiquitin-dependent protein degradation. The modification is to add staff, plasmids, and funding. In the work described however, there is mention of using Lentiviruses to deliver CRISPR/Cas and guide RNAs but none of the relevant sections regarding Lentiviruses have been filled completed. If CRISPR/Cas and guide RNAs are being given by one Lentivirus, then the work must be done at BSL-2+. Also, under the animal work section, the application mentions giving the mice small molecule compounds called "TFEB activator series" but there are no further details on what these compounds are. Under the work description, it is not entirely clear which genes are being regulated, or how, which could be important as some genes are tumor suppressors and XBP1 may be oncogenes. The purpose of this amendment is to add new personnel, new plasmids and new funding. This protocol deals with manipulation of gene expression in human cell lines in order to generate stably transduced lines. The modification is adding the use of Lentiviral delivered CRISPR/Cas and sgRNA to human cells *in vitro*. The investigator states that they will be delivered at the same time on the same vector. This will require a change to BSL-2+ and updating of safety procedures accordingly.

Initial comments:
1) Amendment page: Provide more information on this page regarding the plasmid to be added.
2) Personnel: The amendment suggests addition of new personnel but there are no new entries.
3) Primary cells and cell lines: The investigator suggested the reason for the amendment is the addition of new plasmids. Describe those plasmids in the response for this section of the application.
4) Bacteria, Yeast, Fungi: Again, describe the new plasmids that the investigator wants to add to the protocol.
5) Recombinant and synthetic nucleic acid: Question 5 should be YES. The investigator is proposing to use a Lentiviral vector.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000137
Title:	Amendment for **IBC201800125**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED Signature page 2018 • REDACTED-BSL-2+ Manual Template Revision • BSL-2+ Manual Signature Page

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluid: Clarify how murine cells/tissues will be administered to animals under "Description of Usage." If that is incorrect, change the response to the question.

2) Live Animals, Question 1: The IACUC protocol that is linked to the IBC protocol is expired. Link a current IACUC protocol.

Comments:

This amendment is to add the use of SRp40 CRISPR/Cas9 Knockout Plasmid and the correlating SRp40 HDR plasmid for co-transfection to select for positively transfected cells, and the use of NTERT cells, which are immortalized primary human keratinocytes. The work is listed at the appropriate level. The protocol's goal is to advance our understanding and treating of cutaneous inflammation. To do this, the researchers utilize both mouse and human xenotransplant models to understand and treat cutaneous inflammation. In this regard, they use transgenic mice and knockout mice to establish and interrogate inflammatory reactions. When transgenic and knockout mice are not available, they utilize vectors (gene gun and Lentiviral) to over express proteins in mouse skin, human skin explants, and in cell lines. This modification adds a new immortalized keratinocyte cell line NTERT and plasmid based CRISPR/Cas9 plasmids to delete SRp40, which is a splicing factor that modified the purine receptor P2X7R, a target in skin inflammation. The new studies at BSL-2 are only *in vitro* and do not include Lentiviral vectors. The protocol does contain Lentivectors for gene expression in mice, but this work does not involve CRISPR. Recommendation for approval.

Review comments were provided to the investigator for response. The revised application was placed on the April agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0

Abstained: 0

Protocol:	MOD202000177
Title:	Amendment for IBC201800153
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED BSM

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Regarding "Our lab plan to study inhibition of viral entry and infection of SARS-CoV-2." Some more information would be useful: a) entry into what cells or animal? B) general approach for inhibition - will it require recombinant techniques?

2) Primary Cells or Cell Lines: Cells that have been designated for administration to animals should include this information in the “Description of Usage”.

3) Bacteria, Yeast, Fungi, or Parasites/Invertebrates: Animal work involving bacteria should be described under "Description of Usage."

4) Viruses, Prions, or Vectors: Confirm that Human Immunodeficiency Virus (HIV) - Types 1 is replication-defective.

5) Viruses, Prions, or Vectors: Confirm that Lentivirus (HIV 3-plasmid system); pCMVΔP1ΔenvpA, pSVIIIenv is NOT replication-defective.

6) Viruses, Prions, or Vectors: In the Animal Gene Transfer section, it appears that KSHV is administered to mice and rats. If this is correct, this information should also be included in the virus section and designated as being administered to animals.

7) Viruses, Prions, or Vectors: Missing information on the pseudovirus. Add information on whether SARS-CoV-2 genes with the VSV virus and if so, which genes and from what plasmid.

8) Viruses, Prions, or Vectors: Later in the protocol it is stated that CRISPR/Cas9 and gRNA will be expressed using viral vectors. Include CRISPR/Cas9 and gRNA under "Inserted Nucleic Acids Information" in the appropriate entries below.

9) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Briefly describe the recombinant work with animals including the administration of cells, bacteria, virus, etc.

10) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Provide additional information regarding the use of SARS-CoV-2, pseudovirus and DNA plasmids. Describe the work that will be conducted for this modification.

11) Animal Gene Transfer, Question 6: Earlier in the protocol, it is stated that bacteria will be administered to animals. These bacteria should be described.

12) Exposure Assessment and Protective Equipment, Question 1: Human cell lines and nonhuman primate cells may harbor unknown infectious agents and should be included in the response. VSV should also be included in the response.

13) Exposure Assessment and Protective Equipment, Question 5: Include PPE required for entrance to the ABSL-2 animal facility (e.g. hair bonnet, coverall suit, shoe covers).

14) Exposure Assessment and Protective Equipment, Question 1: SARS-CoV-2 pseudovirus should be included in the response.

15) Exposure Assessment and Protective Equipment, Question 1: Clarify what pCMVΔP1ΔenvpA, pSVIIIenv is. This is not listed in the NIH AIDS Reagent Program.

16) Waste Management, Question 1c: Animal carcasses should be sealed in double bags, labeled, and disposed according to animal facility guidelines. Revise.

17) Supporting Documents: The investigator should contact the EH&S laboratory safety specialist to complete the COVID19 checklist.

Comments:
The investigator has submitted a modification to add work with a pseudovirus of SARS-CoV-2, using VSV, additional plasmids, and additional cell lines. General form issues were noted including animal work and consistency in entries. The new project was not described in great detail; additional information is requested regarding the experiments and description of use. The investigator is currently approved for BSL-2+/ABSL-2 work however, the COVID19 checklist has not been provided to EH&S. This modification adds a pseudovirus of SARS-CoV-2 and new plasmids to a study of viral gene biology. With regards to SARS-CoV-2, experiments to block viral entry and infection of SARS-CoV-2 pseudovirus will be tested with a variety of kidney and lung cell lines as listed as BSL-2+. A variety of other cell lines will be used from BSL-1 to BSL-2+, which seem appropriate. Many types of pathogenic bacteria will be used to test whether bacterial induced inflammation influence KSHV cancer cells and tumorigenesis *in vitro* at BSL-2. A variety of viruses will be used including the SARS-CoV-2 pseudovirus, which, is a modified Vesicular Stomatitis Virus that will be made with plasmids that express SARS-CoV-2 surface glycoproteins. There is missing information on the pseudovirus. The investigator needs to include information on whether SARS-CoV-2 genes with the VSV virus and if so, which genes and from which plasmid.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the meeting. The protocol application was placed onto the April agenda for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0
Abstained:	0

Protocol:	MOD202000187
Title:	Amendment for IBC201700180
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissue, Blood, or Body Fluids: Mouse (murine): If blood will be obtained from genetically engineered mice or mice with recombinant nucleic acids, this entry should be designated recombinant.

Comments:

This amendment request seeks to add replication-deficient Adenoviral vectors to facilitate knockout of MyD88. The vectors will deliver, separately, CRISPR and sgRNA. MyD88 is an adaptor downstream of toll-like receptors in pro-inflammatory signaling. The aim here is to assess whether the reduction in inflammation in mouse models of Influenza improves outcomes from viral pneumonia. As hyper-inflammation is a major complication in SARS-CoV2 infections, a reduction in inflammation may be beneficial in this disease as well. The underlying protocol is based on analysis of the Type 17 pathway which is required for immune response against several pathogens, many explicitly named in the protocol. But excessive or persistent inflammation is detrimental. So, this work is based on knockout of various of these target genes in mice to examine the effects on host defenses in response to infection with these agents and to assess the effects on disease progression. *In vivo* and *in vitro* work will be done, the latter including, amongst several other assays, challenging isolated cells with bacterial or viral named pathogens for uptake and response studies. The underlying protocol (not this amendment) also does some work with ferrets and in studying asthma by transferring antigen-specific T-cells into SCID mice. The protocol lists several bacterial pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *E. coli*) and the fungal pathogen *Aspergillus fumigatus*. All are used at BSL-2 for the same purpose: to challenge mice or murine cells. Several Influenza virus strains are also used (but all at BSL-2). The new addition is Adenoviral vectors which will separately deliver sgRNA and CRISPR as described above. These tools well be provided by a colleague at Pitt. The laboratory has used the same replication-deficient vectors to overexpress several target genes. Work will be done at BSL-2. The modification request notes that CRISPR and the sgRNA will be delivered separately at least 72 hours apart. Animal work will be done at ABSL-2. The amendment adds a new use of Adenoviral vectors to express CRISPR machinery and then sgRNA in mice via subsequent infections. The target is Myd88, a protein that mediates both immune protection from Influenza A viruses and pathogenic inflammation. There are no specific safety concerns with this amendment. In the original proposal, the investigator seeks to understand regulators of inflammation in lung infection using *in vivo* studies of mice and ferrets, along with *ex vivo* studies using cells derived from them. To drive CRISPR-Cas9 mutagenesis, mice will be injected with two strains of replication-defective Adenovirus that drive expression of

CRISPR machinery and then sgRNAs, at least 72 hours apart. Which virus strains the investigator will use to prevent superinfection inhibition is not specified, nor is the mode of injection. CRISPR-modified mice and externally generated transgenic mice will be used as a source of primary cells (bone marrow, lung monocytes, epithelia, and fibroblasts) for experiments that including challenge with viral, bacterial, and fungal pathogens (Influenza virus types B/H3N2/H1N1, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *A. fumigatus*, and *E. coli*). The same pathogens may also be used to study infection and pathogenesis in live mice or ferrets. Immune reconstitution will be done by transplanting mouse bone marrow into irradiated mice. Immune cells (T-cells or PBMCs) will be transferred from mice or ferrets into immunocompromised SCID mice to study the how clonal isolates that target individual antigens fight infection and/or inflammation. Insights developed by these studies could have an immediate application to the current crisis involving COVID-19, a relative of influenza that kills through inflammatory pneumonia. The pathogens in this study are classified as RG-2, and work will be done at BSL-2 and ABSL-2.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the meeting. The protocol application was placed onto the April agenda for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	18
Against:	0
Abstained:	1; recused for involvement

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 11:08 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

April 27, 2020 10:00 AM

Meeting; ZOOM teleconference meeting
SARS-CoV-2 protocols only

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			Yes
REDACTED, Vice Chair			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED			yes
REDACTED			yes

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	EH&S representative

REDACTED REDACTED	Co-Director, ORP (Research Protections Office) IACUC Office

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	5
Meeting start time:	10:00 PM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions. This meeting was conducted via teleconference due to the Governmental "Stay at Home" orders to prevent spread of the Corona Virus.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

Since this was the third emergency meeting and not regularly scheduled, the regularly scheduled April 13th meeting minutes were not reviewed. They will be reviewed at the regularly scheduled May meeting.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

No report, as this was an "emergency" meeting outside of the regularly scheduled meetings to discuss specific protocols involving research with the Corona Virus.

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocols on the following pages

Protocol:	MOD202000194
Title:	Amendment for **IBC201700104** BSL upgrade
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• List of plasmids potentially used with Lentivirus system

Determination: Modifications Required

Required modifications:

1) Supporting Documents: The Laboratory's final signed Biosafety Operations Manual must be uploaded into the application before approval can be provided.

2) Exposure Assessment and Protective Equipment: Elaborate in question 4 what additional safety precautions will be undertaken for the work with the COVID-19 tissues?

3) Exposure Assessment and Protective Equipment: Question 4: Update information to reflect BSL-2+ status/requirements.

4) Exposure Assessment and Protective Equipment: Question 5: Select all PPE required for BSL-2+ and entrance to ABSL-2 animal facility (e.g. double gloves, hair bonnet, coverall suit, shoe covers, solid-front wrap around gown).

5) Animals: Remove the "completed" ARO protocols, and if appropriate, link an active ARO protocol instead. Not linking ARO protocols to the IBC application could delay IACUC approval.

6) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: If SARS-CoV-2 RNA will be reverse transcribed and/or PCR amplified, this should be described.

7) Viruses, Prions, or Vectors: Coronavirus (CoV) species: other not listed - SARS-CoV-2: Clarify how SARS-CoV-2 that is not replication-defective cannot enter or infect human cells.

8) Tissues, Blood, or Body Fluids: Tissue/Blood/Fluids - Humans: clarify how exosomes and extracellular vesicles isolated from human blood and tissues will be free of infectious SARS-CoV-2 from COVID-19 patients. If procedures will differ between SARS-CoV-2-infected and uninfected individuals, it is recommended that separate entries be listed in the application.

9) Tissues, Blood, or Body Fluids: Provide an entry for obtaining blood/tissue/fluid from patients with COVID-19. Describe if the laboratory is obtaining patient samples and not the actual virus.

10) Protocol Team Members: Personnel identified are not current for Bloodborne Pathogens training. Other personnel are due for Chemical Hygiene training.

Comments:

This modification of an existing protocol seeks to add capacity to analyze RNA expression levels in the placenta of Covid-19 positive mothers. Samples would be obtained at Columbia University Medical center. Future samples may also come from Magee's. The investigator wishes to reinstate BSL-2+ standards in a large equipment room where samples will be stored prior to RNA extraction. The sample preparation room already at BSL-2+. The extraction of RNA from these samples is straight forward. Personnel are being updated. The investigator is approved at BSL-2

currently so this would be a step up in safety level. Changes to the protocol also describe transplanting extracellular vesicles from cultured placental cells into mice, but it is not clear if this is to be done with the tissues from Columbia. If the storage room still has not been altered since it was last a BSL-2+ rated then this should be straightforward transition, however this should be assessed. The modification seeks to add placental tissues from women who were pregnant and infected with SARS-CoV-2. These tissues will be obtained from Columbia University Medical Center or Magee Womens Hospital and will be stored at in a freezer until used at BSL-2+. These tissues will be immersed in *Trizol* for RNA extraction. It is unclear whether or not SARS-CoV-2 RNA will be reverse transcribed and PCR amplified. This and several other clarifications are requested prior to approval.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 27th emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD202000197
Title:	Amendment for **IBC201600115**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Section III-D-7 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• SOP • CDC Import Approval Letter • CDC Inspection and Adequate Response Letter • CDC Import Permit Form Approval

Determination: Modifications Required

Required modifications:

1) Viruses, Prions, or Vectors: Vesicular Stomatitus Virus (VSV): GFP should be listed under "Inserted Nucleic Acids Information" for this virus.

2) Viruses, Prions, or Vectors: Vesicular Stomatitus Virus (VSV): Clarify how VSV will be pseudotyped. Will the glycoprotein (VSV-G) will be replaced by the SARS-CoV-2 Spike protein in the viral vector? Or will it be supplied in trans? What will be the source of the genetic material encoding the Spike protein?

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Clarify the materials used to express or produce the SARS-CoV-2 Spike protein.

4) Animal Gene Transfer, Question 1: The answer should be YES for administration of replication-competent Influenza Virus.

5) Animal Gene Transfer, Question 2: The answer should be YES for administration of replication-competent Influenza Virus.

Comments:

This amendment request seeks to make two changes, in addition to personnel updates:
a) To add two strains of H1N1 (strains A/PR/8/34 and A/CA/07/2009; both BSL-2) for *in vitro* studies and to use in a mouse model of lung injury.
b) To add recombinant and replication-deficient delta G VSV (VSV with its fusion protein removed) which will be pseudotyped with the SARS CoV2 S protein.
The amendment request builds on the investigator's work showing that thrombospondin 1 (TSP1) is protective in a mouse model of lung injury induced by *P. auruginosa*. TSP1 is a serine protease inhibitor that is used by several bacterial and viral pathogens to enter the cell. Hence the proposal to study the effects on TSP1 and related serine protease inhibitory activity on TMPRSS2 and human airway trypsin-like protease. TMPRSS2 is used for cleavage and activation of the Influenza Virus fusion protein. SARS CoV2 also needs TMPRSS2 to enter the cell. This work will therefore aim to test whether TSP1 is protective against Influenza and Coronavirus entry. The work will use a pseudotyped replication deficient VSV displaying the SARS CoV2 S protein. The underlying protocol focuses on study of mouse models of lung inflammation and injury so a range of C57BL/6

and derivative mouse strains, cell ablation and adoptive transfer protocols are conducted. The underlying protocol also approves Lentiviral vectors. *P. auruginosa* transposon-generated mutants and in-frame deletions (of the common PA14 strain) are also used. The protocol also uses *Streptococcus pneumoniae* (including clinical isolates), *Pseudomonas aeruginosa* (several clinical isolates and mutant constructs), *Staphylococcus aureus* (including clinical strains) and *Klebsiella pneumoniae* (including clinical isolates). Approved viruses and viral vectors prior to the amendment include Lentiviral vectors (3-plasmid) and replication-defective Adenoviral vectors. Vaccinia Virus (strain vTF7-3 [Wr], BSL-2, Source *ATCC*) will be used to express bacteriophage T7 RNA polymerase and to express and recover the pseudotyped VSV delta G. H1N1 strains and pseudotyped VSV delta G. Work will be done at BSL-2. CRISPR/Cas9 will be used to knockdown of TSP1 and other closely related genes. Two Lentiviral vectors will be used. In this protocol modification, the investigator proposes to add two viral vectors and personnel to an existing protocol. Influenza H1N1 clinical strains will be obtained from collaborators for infection of cells and mice at BSL-2/ABSL-2, which is appropriate. In addition, Vesicular Stomatitis Virus (VSV) encoding GFP and pseudotyped with the SARS-CoV-2 Spike protein is proposed. Typically, this is performed by producing VSV lacking its envelope glycoprotein from a plasmid with a separate plasmid encoding another viral entry protein. The investigator seems to state that the VSV glycoprotein will be replaced with the SARS-CoV-2 Spike protein in the viral vector to infect cells at BSL-2. It is not clear if this new system will produce replication-defective virus or not. Clarifications are requested prior to approval.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 27th emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD202000168
Title:	Amendment for **IBC201700156**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Amendment Request, Question 2: Remove the room number as the described work will not be conducted in the identified location.

2) Protocol Personnel, Question 1: There are personnel listed under Personnel or Handlers that will need to update or complete their training. Some must complete Chemical Hygiene and Bloodborne Pathogens trainings. Others must update/renew their Bloodborne Pathogens training. Additional personnel will soon be due for BBP and Chemical Hygiene training.

3) Tissues, Blood, or Body Fluids: Remove the room number as the described work will not be conducted in the identified location.

4) Exposure Assessment and Protective Equipment, Question 4: Include a statement that all flow cytometry samples will be stained within the Biosafety Cabinet (BSC) and fixed prior to removal from the BSC and used in the flow cytometer equipment.

5) Exposure Assessment and Protective Equipment, Questions 4 and 5: A room-dedicated, solid-front wrap around gown is required for BSL-2+ work; select and revise.

6) Waste Management, Question 1a: BSL-2+ solid waste should be decontaminated with a 1:10 dilution of bleach and placed in a biohazard bag within the Biosafety Cabinet. The bag should be closed and sprayed with bleach prior to removal from the Biosafety Cabinet and then it should be placed in a second biohazard bag and box. Revise.

Comments:

In this protocol modification, the investigator will obtain blood from COVID-19 patients for isolation of plasma and PBMC for RNA sequencing and measurement of immune markers. The samples will be handled at BSL-2+, which is appropriate. There are no biosafety concerns. Recommendation for approval pending approval of the laboratory biosafety operations manual. This amendment is to add work with isolates from COVID-19 positive patients, including blood and tracheal aspirates, to be used for *in vitro* experiments at BSL-2+ in the School of Public Health. There is also the addition of new personnel. The amendment seeks to add a systems immunological approach to finding biomarkers of COVID19. The investigator will evaluate IL6, IL1, and CRP levels. The amendment will add personnel. They are requesting an increase in biosafety; change from BSL-2 to BSL-2+. They will be using blood and aspirates from the trachea of COVID19 patients as samples for RNAseq, flow cytometry, and MesoScale protein analysis. These isolates

will be infectious to humans and thus the change from BSL-2 to BSL-2+. Increased PPE is described in the application, as is the disposals of wastes.

Initial comments:
1) Research Personnel: Ensure training of all new personnel is completed.
2) Note: it is mentioned that risk is low due to low "Traffic" when using BSL-2+. Will this still be the case when work restrictions are removed?

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 27th emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	1; recused for involvement

Protocol:	MOD202000217
Title:	Amendment for **IBC201600203**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Genes of interest_042020

Determination: Modifications Required

Required modifications:

1) Primary Cells or Cell Lines: Because of the increased surveillance of work involving SARS CoV-2 proteins, it may be prudent to add NSP2 to the cell lines it will be expressed in in the "Description of Usage" within the application.

2) Primary Cells or Cell Lines: Cells in which recombinant materials will be administered should be designated as recombinant.

3) Waste Management, Question 1b: Clarify that liquid culture and supernatants will also be inactivated with the 10% v/v final of bleach (or if *Virkon S*, provide the concentration used) and add 20 minutes to the contact time with the wastes.

4) Waste Management, Question 1a: Describe the EPA-registered disinfectant (e.g. 1:10 dilution of bleach) and contact time that will be used to decontaminate surfaces, plasticware, etc.

5) Waste Management, Question 3: Ethanol solutions are not an EPA-registered disinfectant and should not be used as primary chemical disinfection for work at BSL-2. Revise.

Comments:

This is a protocol modification to express the SARS-CoV-2 protein NSP2 in yeast (presumably from a bacterial plasmid or YAC) and in *E. coli* and mammalian cells. As this is a single gene from the virus, this is low risk and use at BSL-1 (yeast, *E. coli*) or BSL-2 (mammalian cells) is appropriate. The amendment is to add NSP2 (non-structural protein 2) of the SARS CoV to an existing protocol which studies protein trafficking. Work will be done in bacteria, yeast, and mammalian cell lines to study endosomal trafficking and functions. The protein will be expressed using plasmids only; no viral work is proposed. The amendment is clearly written and there are no comments other than perhaps listing the NSP2 in the "Description of Usage" under which mammalian cell lines it will be used, and for clarity, a very minor wording change in the liquid waste disposal.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 27th emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	1; recused for involvement

Protocol:	IBC202000049
Title:	Cloning with SARS-CoV-2 Genome Fragments
Investigator:	REDACTED
Highest BSL:	BSL-1
NIH Guidelines:	• NIH Section III-D-2 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description, Question 3a: It is mentioned that "siRNA and shRNA screen of human cellular proteins required for SARS-CoV-2 infection". However, there is no reference to siRNA/shRNA this in the rest of the document. Clarify.

2) Recombinant or Synthetic Nucleic Acid Work Description, Question 3d: It is noted that siRNA via transfection or shRNA via Lentivirus transduction, but it is stated there will not be work with cells or virus. Clarify.

3) Risk Group and Containment Practices, Question 2: As per University Guidance in the SARS-CoV-2 Laboratory and Research Guidelines, this work may need to be performed at BSL-2: https://www.ehs.pitt.edu/sites/default/files/docs/ResearchBiosafetyGuidelinesCOVID-19.pdf

4) Exposure Assessment and Protective Equipment: Indicate if the work will be conducted in a biosafety cabinet or if face protection will be used.

Comments:

This is a new protocol to clone cDNA encoding the SARS-CoV-2 genome in several bacterial expression plasmids or BACs for cloning of reporter genes. There is no tissue culture, virus or animal work proposed- only bacterial work. New protocol to clone cDNAs of SARS-CoV-2 genome in bacterial expression plasmids or BACs as reporter genes. No tissue culture, virus or animal work involved. This is a protocol for cloning cDNAs for fragments of the SARS-CoV-2 in *E coli*. There is no work with the virus or eukaryotic cells proposed and no concerns are noted.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the April 27th emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	10
Against:	0
Abstained:	2; recused for involvement

NEW BUSINESS

A question was asked about the update to the MyIBC software that was scheduled to be completed at the beginning of May. The testing phase was completed, however was discovered with some errors that require the Software Developer to fix. This is expected to be completed and the updated software is expected to be installed the weekend of May 16th. The software will be upgraded during off-hours and over the weekend, to help limit downtime on the users. A message of the scheduled downtime and upgrade will be posted on the Login page. The message will indicate the dates and expected time that the system will be unavailable. Once the IT team has completed the upgrade and the system is again live, the message will be removed so that users will be able to sign in and begin using the upgraded software as soon as possible.

With no further questions or comments, the meeting was adjourned by the IBC Vice-Chair.

Meeting end time: 10:35 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

May 11th, 2020 10:00 AM

Meeting; ZOOM teleconference meeting

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			Yes
REDACTED, Vice Chair			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	EH&S Office
REDACTED	IACUC Office
REDACTED	ORP Co-Director

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions via the tele-meeting portal.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The April 13th (regularly scheduled) and April 27th, (non-standard scheduled) 2020 meeting minutes were reviewed and approved by the committee.

Votes:

For:	20
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

MyIBC update: The IBC online review system has been scheduled for a software update which will be initiated on Friday, May 22nd over the Memorial Day weekend. A message will be posted on the MyIBC website during the expected downtime and will be removed once the system is available. The changes should not affect how the IBC Office and Committee perform the review functions.

NON-COMPLIANCE, EVENT, INJURY REPORT

Discussion around the possible use of Lentiviral vectors encoding TERT oncogenes was discussed. The investigator will be contacted to clarify and if necessary, an incident reporting form will be completed and sent to the investigator to be reviewed at the next meeting.

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocols on the following pages

Protocol:	IBC202000029
Title:	SV2A and Traumatic Brain Injury
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-2 • NIH Section III-D-4
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Usage: *Section III-D-1* should be selected.

2) Recombinant or Synthetic Nucleic Acid Usage: The investigator has not indicated use of prokaryotes or eukaryotes in the protocol. Clarify why *Section III-D-2* was selected.

3) Animal Gene Transfer, Question 3: As AAV production uses a helper virus, the answer should be YES.

4) Exposure Assessment and Protective Equipment, Question 5: Add PPE required for ABSL-2 work (hair bonnet, double gloves, shoe covers, face shield).

5) Waste Management, Question 1c: Animal carcasses should be sealed in double bags, labeled, and disposed according to animal facility guidelines. Revise.

Comments:

In this project investigators will utilize a traumatic brain injury (TBI) to study the role of clathrin in the cortex and hippocampus. AdenoAssociated Virus will be microinjected into the dentate gyrus of rats to increase the expression of the protein clathrin. Brain tissues and liquids will be collected such as blood and CSF. The work is appropriately listed at ABSL-2. This is a project with aim of characterizing cortical and hippocampal expression of clathrin in a controlled cortical impact (CCI) model of traumatic brain injury (TBI). The group will determine if genetic intervention to increase clathrin expression can attenuate cognitive deficits after TBI. AAV will be microinjected into the dentate gyrus of rats to increase the expression of the protein clathrin. No known oncogenes or toxins will be expressed. Immunoblotting and immunohistochemical techniques will be utilized on blood/CSF/other tissues from rats to assess protein expression and localization after injury. All work is proposed at BSL-2/ABSL-2 which is appropriate. Approval is recommended once revisions are made in the application.

No primary review comments were provided. The application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	IBC202000035
Title:	Neuronal Transporters
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-A-1-a or Section III-B-2 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Columbia_MTA • OHSU_MTA

Determination: Modifications Required

Required modifications:

1) Toxins: Clarify that the laboratory will not be receiving or purifying Botulinum Toxin. Even work with exempt quantities of a select agent must be registered with the University.

2) Toxins: The investigator may work with this binding domain sequence using chemical precautions at BSL-1. Handling of powders or manipulations of solutions that may create aerosols must be conducted within a chemical fume hood or biosafety cabinet. As the investigator is working with human cells, that work should remain at BSL-2. Only the work *in E. coli* may be conducted at BSL-1. Any work involving the human cells or Baculovirus must be conducted under BSL-2.

3) Recombinant or Synthetic Nucleic Acid Usage: Confirm that the project involves the deliberate transfer of drug resistance into organisms that do not acquire them naturally and provide additional information.

4) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Briefly describe the work involving recombinant materials.

5) Waste Management, Question 1a: Infectious solid waste should be chemically disinfected by spraying with or soaking in an EPA-registered disinfectant prior to disposal of the solid waste in approved biohazardous bags. Revise the application questions accordingly.

6) Waste Management, Question 1b: Clarify that liquid waste will be decontaminated by adding concentrated bleach to a final 1:10 dilution (v:v) bleach in liquid waste. Addition of pre-diluted 10% bleach to liquid wastes further dilutes the disinfectant and results in insufficient concentration of active ingredient for proper decontamination.

7) Waste Management, Question 3: Provide additional detail describing the process for clean-up and decontamination of a biological spill (e.g. clean up materials, contact time for disinfectant, disposal of potentially contaminated clean up materials).

Comments:

The aim of the proposed research is to elucidate the molecular function, architecture, and high-affinity drug binding sites of synaptic vesicle transporters by determining single particle cryo-EM structures. The overall goal of this research is the design of better antiepileptic drugs with higher specificity and fewer side-effects and will also advance efforts toward understanding the function of these transporters. The investigator has developed methods for large-scale expression, stabilization by drugs, and for the production of toxins which recognize the extracellular domain of these transporters, which will be used in these studies to generate the quantities of transporter proteins necessary for performing cryo-EM reconstructions. Variants of the HEK293 cell line

(HEK 293T/17 and HEK293S GnTI$^-$) as well as the Sf9 insect cell line will be used for recombinant protein expression, and the Sf9 line also used for Baculovirus production. Three *E. coli* lines will be used for DNA cloning and plasmid propagation (DH5a), recombinant protein expression (BL21 DE3), and production of bacmid (DH10Bac). The AcMNPV Baculovirus will be used to generate BacMan and Bac-Bac for transduction of mammalian and insect cell lines, respectively, using a commercially available kit (*ThermoFisher*). While the protocols to be used have been optimized and published, there is a heavy reliance on citations in lieu of full descriptions, and additional information is required. All vectors to be used need to be listed along with the inserted nucleic acids (Viruses, Prions, and Vectors), the Toxin description needs to be clarified (unclear where synthesis and subcloning takes place), and the citation used to justify BSL-1 for the toxin doesn't actually address BSL level for the toxin fragment to be used (Toxin, Recombinant or Synthetic Nucleic Acid Work). Confirmation of BSL-1 for use of the binding domain of BoTN is required. Overall, approve, but the issues noted above need to be addressed before approval. This project will study the structure and function of synaptic vesicle transporter and their high affinity drug binding sites. Work will include use of human and insect cell lines. Baculovirus and plasmid vectors will be used in creation of recombinant proteins. BSL-2 is proposed and appropriate for the work described. This new protocol by a new investigator describes a study that will analyze synaptic vesicle transporters and their role on seizures. Human and insect cell lines will be used at BSL-2 and BSL-1 respectively. Baculovirus will be used for protein production, using an insect cell line and "mammalian cell lines", but it is not clear what mammalian cell line will be used. Some missing information on how the recombinant proteins will be made in the cell lines. Laboratory *Escherichia coli* strains will be used at BSL-1. A non-toxic Botulinum Toxin amino acids 872-1296 will be synthesized and cloned in an *E. coli* expression vector. The toxin domain will be used in experiments with neuronal transporters. RG-2 should be checked for the use of human cells. There are a lot of minor revisions required.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	IBC202000040
Title:	MFN1 Knockout Mice Breeding Protocol
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 5/11/2021

Required modifications:

1) Tissues, Blood, or Body Fluids: If materials will be harvested from animals that have been administered Adenovirus, the materials must be used at BSL-2. Revise the biosafety level.

2) Primary Cells or Cell Lines: If cells will be harvested from animals that have been administered Adenovirus, the materials must be used at BSL-2. Revise the biosafety level.

3) Viruses, Prions, or Vectors: Adenovirus should be designated as recombinant; revise.

4) Viruses, Prions, or Vectors: Adenovirus can infect human cells; revise.

5) Viruses, Prions, or Vectors: Genes of interest to be expressed from Adenovirus (e.g. MFN1) should be listed under "Inserted Nucleic Acids Information."

6) Live Animals, Question 1: List the IACUC protocol to be associated with this IBC protocol.

7) Animal Gene Transfer, Question 2: The answer should be YES. Revise the response.

8) Exposure Assessment and Protective Equipment, Question 1: Adenovirus can infect human cells; revise.

9) Exposure Assessment and Protective Equipment, Question 4: Indicate in the response if a biosafety cabinet (BSC) will be used. Full face protection is required if working with BSL-2 agents outside of a biosafety cabinet. Safety-engineered sharps devices for needles and scalpels must be used at BSL-2/ABSL-2. Revise accordingly.

10) Waste Management, Question 1b: Clarify that liquid waste will be decontaminated by adding concentrated bleach to a final 1:10 dilution (v:v) bleach in liquid waste. Addition of pre-diluted 10% bleach to liquid wastes further dilutes the disinfectant and results in insufficient concentration of active ingredient for proper decontamination.

11) Waste Management, Question 1b: Solidifying liquid waste is not appropriate. If the amount generated is too large to chemically disinfect, the waste may be autoclaved.

12) Waste Management, Question 3: It is not recommended to solidify waste. Refer to Environmental Health and Safety Guideline #05-006 and revise the response accordingly.

Comments:

In this proposal, the investigator seeks to understand the role of mitochondrial fusion in thrombosis and aging using transgenic mouse models. The investigator will inject a Type 5 Adenovirus vector (*Vector Biolabs*) intraperitoneally to drive expression of MFN1 protein in a MFN1 knockout mouse line. Experiments will be conducted in BSL-1 and ABSL-1 conditions. There were no biosafety concerns. The goal of this proposal is to study the role of the MFN1 protein in fusion of

mitochondria in platelets. The laboratory will generate mice with a platelet-specific knockout in the MFN1 gene, which will then be crossed with MitoTimer mice in order to measure mitochondrial turnover. In aged MFN1-knockout mice, Adenovirus type 5 will be used to reintroduce the MFN1 gene via an IP injection. The experiments will be performed at BSL-1/ABSL-1. The proposed studies will create a platelet-specific knockout mouse for the gene MFN1. The knockout strain will be further crossed onto a transgenic overexpressing a gene called MitoTimer. Some groups of all genotypes will then be treated with as single i.p. injection of an Adenovirus bearing the MFN1 gene in order to rescue the phenotype.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	IBC202000052
Title:	Legionella Pathogenesis in Amoebae
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-2 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment, Question 4: Work on the bench top with BSL-2 agents requires full face protection (safety goggles and surgical mask or full-face shield). If any needles or sharps will be used, safety-engineered sharps are required at BSL-2. Revise.

2) Exposure Assessment and Protective Equipment, Question 5: Select the full-face protection that will be worn when working with BSL-2 agents outside of a Biosafety Cabinet (BSC).

3) Waste Management, Question 3: Ethanol solutions are not an EPA-registered disinfectant and should not be used as primary chemical disinfection in the BSC. Revise.

4) Waste Management, Question 3: PPE should be removed before leaving the lab space; revise the language in all entries.

5) Waste Management, Question 3: Materials used to clean a spill should not be disposed in the garbage; revise to state materials will be discarded in the biological hazard waste. Revise the language in all entries.

Comments:

In this protocol, the investigator seeks to study proteins necessary for the pathogenic bacterium *Legionella sp.* to infect and grow in their amoeba hosts. Both *Legionella sp.* bacteria and amoebas (*Acanthamoeba castellani* or *Dyctyostelium discodeum*) will be transfected with plasmids by electroporation. Plasmids will use Crispr/Cas9 to delete or mutate endogenous genes or express transgenes with fluorescent proteins or luciferase for imaging and quantitation. Proteins to be introduced by Crispr/Cas9 include endogenous genes from the species in question (*Dictyostelium* or *Legionella*) conjugated with affinity tags (FLAG, Myc, HA, or Strep), fluorescent proteins, or luciferase. *Legionella* and *Acanthamoeba* can both infect humans; hence work will be done under BSL-2 conditions. This new protocol studies *Legionella*, its replication in the environment and infection of human hosts. The work will use several strains of *Legionella* as well as amoeba hosts of the bacteria, and *E. coli* for plasmid propagation. These plasmids will be used to alter the expression of genes of interest in both the *Legionella* and the amoebae and to express tag proteins for tracking/counting purposes. CRISPR/Cas will be employed, but via plasmid electroportation, not using any viral vector as delivery. No animal work is proposed. Work will be done at BSL-2 to be cautious about human infection of *L. pneumophila* and *Acanthamoeba* and within a Biosafety Cabinet (BSC). The investigator gives detail about mitigation strategies to reduce exposures.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	IBC202000059
Title:	Corneal Stem Cells
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Approval letter-18022511-till 02282021 • 18022511 - Molecular mechanism and therapy for corneal scarring • Approval letter-19054694-till 05292021 • 19054694 - Stem Cells for Corneal Engineering

Determination: Approved

Last day of continuing review period: 5/11/2021

Required modifications:

1) Tissues, Blood, or Body Fluids: The question regarding administration to animals should be changed to YES for rabbit tissue/blood/fluid as it is noted in question 2 "cells from human and rabbit sources to rabbit eyes."

2) Primary Cells or Cell Lines: Other Human cells or cell lines: The source should identify where the cells were/will be obtained, not a description of usage.

3) Primary Cells or Cell Lines: Human stem cells or iPS cells (embryonic or adult): Clarify what recombinant materials (e.g. AAV) will be administered to the cells under "Description of Usage."

4) Animal Gene Transfer, Question 3: AAV is produced in the presence of a helper virus. Thus, the answer should be YES.

5) Exposure Assessment and Protective Equipment, Question 1: Remove "Most protocols allow BSL-1 use of these vectors." from the response as AAV is designated at BSL-2 because it is generally produced using human cells.

6) Exposure Assessment and Protective Equipment, Question 5: Select all PPE for entrance to the ABSL-2 animal facility (e.g. coverall suit).

Comments:

This newly submitted protocol application is for work that was previously approved by the IBC under another investigator. There were only minor pre-screening revisions requested and the revised application is appropriate for review by the committee as a low risk study.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0
Abstained:	1; recused for involvement

Protocol:	MOD202000148
Title:	Amendment for **IBC201900059**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Approved

Last day of continuing review period: 5/11/2021

Required modifications: None

Comments:

This amendment requests an update to laboratory personnel, the Lentivirus kit to be used, and expands on descriptions of nucleic acid work. The overall protocol is designed to identify mechanisms that maintain and reinforce ectopic non-centromeric CENP-A during tumorigenesis, as well as mechanisms that maintain positional stability of CENP-A within centromeric DNA throughout cellular proliferation. Specific changes include a transition from a Lentiviral Tet-One inducible expression system (*Clontech*) to the TransIT® Lentivirus System (*Mirus Bio*), use of a pSMPUW-IRES-NEO lentiviral vector (*Cell Biolabs*) and inclusion of the 293/17 cells (*ATCC*) packaging cell line (specific for Mirus kit). The genes to be used in the amended protocol are unchanged (CENP-A, HJURP, MCM2, CENP-C, CENP-T, CENP-X, CENP-S), and questions 4 and 6 of the Recombinant or Synthetic Nucleic Acid Work Description section are answered in detail. The modification adds new laboratory members and updates Lentivirus information for a study of chromosome biology. Particularly, the role of CENP-A in chromosome maintenance during tumorigenesis will be characterized. A variety of human cell lines will be used at BSL-2. *Escherichia coli* will be used at BSL-1 for plasmid propagation. Lentiviral and Retrovirus will be used for expression for a variety of fluorescent proteins and kinetochore/centromere related genes. Lentivirus will also be used for shRNA and expression of CRISPR/Cas9 and sgRNAs to target CENP-X, and other proteins – Cas and sgRNA are delivered separately. No major problems. This amendment is for a previously approved protocol and is being submitted to add personnel, change the Lentiviral kit being used, and update details associated with nucleic acid usage. The original project investigates CENP-A, a histone H3 variant that plays a role in chromosome segregation during cell division. Their project will include gene silencing and overexpression of cell division-related genes in human cell lines. The protocol also includes *E coli*, Molony Murine Leukemia Virus, and a 4-plasmid Lentiviral transduction system. Cells will be transduced with several genes encoding several reporter genes, CENP variants, MCM2, and HJURP, none of which appear to be oncogenes, will be added or modified by approaches including CRISPR/Cas9, shRNA, or viral transduction. MCM2 is a tumor suppressor that will be tagged but not knocked down or overexpressed. No major issues are noted, although the application indicates in question 4 of the Recombinant or Nucleic Acid Work Description that there is potential for increased virulence associated with the modifications, but this choice seems to be inappropriate for the context. The work is proposed at BSL-2, which appears appropriate. Approval is recommended.

No comments were provided. The application was placed on the May agenda.

Approval: No additional comments were provided by the committee. Pending committee review.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000158
Title:	Amendment for **IBC201900043**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Amendment Introduction: Question 2: FOXO1 and SIRT1 are tumor suppressors. Knock-out has potential oncogenic potential and will affect BSL.

2) Primary Cells or Cell Lines: Describe whether CRISPR/Cas-9 will be incorporated using Lentivirus and if any of the genes modulated by CRISPR/Cas-9 are oncogenes. If YES, then describe whether CRISPR/Cas-9 and gRNA will be on the same or different vectors. If all answers are YES, then BSL-2+ should be indicated.

3) Viruses, Prions, or Vectors: FOXO1 has been shown to have oncogenic and tumor suppressor properties. Clarify if FOXO1 will be overexpressed or knocked down via Lentivirus.

4) Viruses, Prions, or Vectors: CRISPR/Cas9 and gRNA should be listed under "Inserted Nucleic Acids Information." Clarify whether or not CRISPR/Cas9 and/or gRNA are expressed in the same viral vectors.

5) Recombinant or Synthetic Nucleic Acid Work Description, Question 3c: If tumor suppressors will be edited, then identify the genes in the response.

6) Exposure Assessment and Protective Equipment, Question 5: Surgical mask should be worn for Lentiviral work.

Comments:

The investigator is submitting this amendment to incorporate CRISPR/Cas 9 methodology to modify selected gene sequences (i.e. SIRT1, SLC16A11, BSG1, PNPLA3, FOXO1, Col1A1) in iPSC derived and primary human hepatocytes and possibly human stellate, Kupffer and liver sinusoidal endothelial cells. The researchers are studying in a liver based metastatic model and propose to use CRISPR/Cas9 editing of several genes: SIRT1, SLC16A11, BSG1, PNPLA3, FOXO1, Col1A1 in primary human liver cells and other human liver cell lines. They will use a two guide Lentiviral system. This is low risk although it should be clearly stated that the cells will or will not be used for *in vivo*/animal work. This project is designed to monitor disease processes associated with Non-Alcoholic Liver Disease, Type 2 Diabetes, a liver-based metastatic melanoma model and drug treatment in a human liver microphysiology, microfluidic model. The Lentiviral systems were in the original application and this amendment will add the CRISPER/Cas9 system for editing SIRT1, SLC16A11, BSG1, PNPLA3, FOXO1, Col1A1 in primary human liver cells and other human liver cell lines. CRISPR/Cas9 and guide RNA are expressed in separate vectors, and the laboratory is no longer using recombinant DNA technology to modify tumor suppressor genes. *NIH-III-D-4* remains checked and should presumably be un-checked as they state that they are not using animal models. The laboratory is also adding personnel.

Initial comments:
1) Amendment page: Describe whether CRISPR Cas9 will be incorporated using Lentivirus and if any of the genes are oncogenes. If the response is YES, then identify whether CRISPR Cas9 and gRNA will be in the same or different vectors. If all answers are YES then BSL-2+ should be used for the research.
2) Cell line: Describe whether CRISPR Cas9 will be incorporated using Lentivirus and if any of the genes modulated by CRISPR Cas9 are oncogenes. If YES, then identify whether CRISPR Cas9 and gRNA will be in the same or different vectors. If all answers are YES then BSL-2+ should be used for the research.
3) Risk group: Question 1: Lentivirus is a Risk Group 3 (RG3) agent.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000216
Title:	Amendment for **IBC201600041**
Investigator:	REDACTED
Highest BSL:	BSL-1 ABSL-2
NIH Guidelines:	• NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment, Question 5: Include PPE required for entrance to the animal facility.

2) Waste Management, Question 1b: Clarify that liquid waste will be decontaminated by adding concentrated bleach to a final 1:10 dilution (v:v) bleach in liquid waste. Addition of pre-diluted 10% bleach to liquid wastes further dilutes the disinfectant and results in insufficient concentration of active ingredient for proper decontamination.

Comments:

Protocol involves changes that indicate that the investigator wished to remove the AAV from the research. New work described is adoptive transfer involving genetically engineered animals.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocols on the following pages

Protocol:	IBC202000051
Title:	20-076
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 2019-08-15 rAd-IFN_Investigator's Brochure v3 0_20-076 • 2018-06028 Template PreScreening ICF_US_V1.0_20-076 • 2019-08-14 Protocol_v4.0_20-076 • 2019-11-04 Template ConsentForm_20-076

Determination: Modifications Required

Required modifications:

1) Human Gene Transfer/Human Clinical Trial: Question 1: Update the response to include the related IRB protocol.

2) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells and fluids may harbor unknown infectious agents.

3) Human Gene Transfer/Human Clinical Trial: Materials: Human Gene Therapy/clinical trial: Question 2a: Briefly describe Phase 1 and 2 study findings with this product in participants with MPM and non-muscle invasive bladder cancer.

4) Human Gene Transfer/Human Clinical Trial: Materials: Human Gene Therapy/clinical trial: Question 2: The response should be marked NO; safety data is available from earlier clinical trials of this product.

5) Human Gene Transfer/Human Clinical Trial: Materials: Question 9: The protocol indicates that viral shedding will be monitored in a subset of study participants. Modify the response to reflect this information.

6) Biosafety Summary: The protocol indicates that blood, saliva, urine, and pleural fluid will be obtained for measuring viral shedding, therefore "Tissues, Blood, or Body Fluids" should be selected. Complete any follow-on questions.

Comments:

Summary: In this Phase 3 study, participants with advanced mesothelioma are randomized to receive a recombinant Adenovirus vector containing the human interferon (IFN) alpha-2b gene (rAd-IFN), a replication deficient Adenovirus-based IFN alpha-2b (IFN-α2b) gene transfer vector, with chemotherapy, or chemotherapy alone. It is given through an intrapleural catheter into the pleural space. The vector transfects normal mesothelial and malignant mesothelioma cells, resulting in the production of IFN-α2b protein within the pleural space and tumor. Transduction of the mesothelioma cell with rAd-IFN results in tumor cell death and stimulates the immune system through a variety of mechanisms. This approach is being tried to ensure local continuous delivery of production of IFNs to compensate for short half-life of IFNs and increase duration of tumor exposure to IFN- alpha 2b. Participants randomized to the treatment group will receive rAd-IFN (3×10^{11} viral particles) once on Study Day 1, diluted to a total volume of 25 mL using sterile normal saline and administered into the pleural space. Study participants in both arms (treatment and control) will receive: celecoxib 400 mg twice daily orally on Study Days 1 to 14; and gemcitabine starting on Study Day 14, 1250 mg/m2 administered IV on Days 1 and 8 of a 21-day

gemcitabine cycle and continued every 3 weeks until disease progression /ET. The primary endpoint is overall survival (time to death). Two clinical studies assessing the safety and clinical activity of rAd-IFN in participants with MPM have been completed. The overall response rate was 25% and the disease control rate was 88%. Median overall survival for all participants with epithelial histology was 19 versus 6.5 months for participants with non-epithelial histology. Median overall survival in the first-line cohort was 12.5 months, whereas MOS in the second-line cohort was 17 months. Most participants experienced only expected mild toxicities from the vector and transgene expression. The rAD-IFN vector is also being developed for treatment of non-muscle invasive bladder cancer, and three studies have been conducted. Up to 300 participants will be enrolled. The study will be monitored by an independent DSMB.

Biosafety: rAd-IFN was generated by recombination between an IFN-α2b-containing plasmid and a derivative of Adenovirus Type 5, co-transfected into human embryonic kidney (HEK) 293 cells. During viral propagation, the proteins necessary for viral replication are supplied in trans by the HEK 293 host cells. The viral vector is made replication-deficient by deleting the early transcription gene (E1–E3) region. Expression of recombinant IFN-α2b by the recombinant rAd-IFN is driven by the cytomegalovirus immediate-early enhancer/promoter, followed by the Adenovirus Type 2 tripartite leader (TPL), which serves as a translational enhancer. The IFN-α2b coding region is downstream of the TPL. Exposure is possible during handling of product or participant body fluids. Occupational exposure is possible via needle stick, ingestion and splashing of the mucous membranes of the eyes, nose, and mouth. In Phase 1 and 2 studies, the rAd-IFN vector was generally well-tolerated. Toxicities included CRS, anemia, lymphopenia, and hypoalbuminemia. Toxicities usually resolved within 24-48 hours. High levels of IFN-alpha2b led to flu-like symptoms that could last for a week or more. Side effects associated with infusion of product included pleuritic chest pain, Shortness of Breath (SOB), hypoxia with pulmonary edema, cellulitis at catheter site, supraventricular tachycardia with SOB. Other events were reported more than 28 days after infusion of study product. Viral shedding will be assessed in first 30 participants (saliva, urine, pleural access site swab, pleural fluid sample); the 28-day sampling duration for viral shedding is based on data with a similar vector containing the IFN-β gene, where vector shedding was observed on skin at 24 days and longer in the pleural fluid of some participants. Data from earlier studies indicates that no shedding in other biological materials is expected. The study containment processes are intended to prevent horizontal transmission from the site of administration. Viral shedding will be monitored by PCR in 30 participants to balance the need to obtain further data (in addition to data available for this and similar vectors) while minimizing the sample burden for participants. Persons with pre-existing Adenovirus neutralizing antibodies may enroll. There is some evidence that the presence of high titers of Adenovirus nAbs may influence gene transfer as measured by pleural IFN levels; however, the sponsor acknowledged that the measurement of pleural IFN concentrations is technically challenging, which may confound the interpretation of gene transduction data. There is no established relationship between pleural IFN levels and clinical outcomes (survival). Samples for titers of nAbs to Ad5 will be obtained at baseline and batch tested and analyzed according to a pre-defined but retrospective analysis. Participants will not be excluded from the study based on any threshold level of nAbs. Celecoxib is an FDA-approved COX-2 inhibitor (NSAID) that may provide synergy to IFN-based therapy; and gemcitabine is an FDA-approved nucleoside analogue that inhibits tumors.

Initial comments:
1) Multiple forms issues have been addressed by pre-screening prior to review.
2) Human Gene Therapy/clinical trial 2a: The response should be marked NO; safety data is available from earlier clinical trials of this product
3) Human Gene Therapy/clinical trial 2a: Briefly describe Phase 1 and 2 study findings with this product in participants with MPM and non-muscle invasive bladder cancer.

The application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000156
Title:	Amendment for **IBC201800343**
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• 01.13.16.01 IPIM - Investigational Product Supply and Requirements (IPSR) • MASTERKEY-318 - Cohort 5 Non-HCC DLRT Meeting 14Nov18 Outcome Memo_final • RAC-letter • Schedule M_Amgen Biovex_ TVEC_20140318_for IBC Response • CC instruction manual • Amgen TVEC 01.20.01 Protocol Amend 3 English_21Oct2019 • Memo RE safety data 2018 renewal 2-1-19 • 01.13.16.04 IPIM - Investigational Product Preparation and Administration (IPPA) TVEC • HCP CC authorization form • 01.13.04.02 Investigational Product Instruction Manual Addendums • Merck Pembro IB-18 • MASTERKEY-318 - Cohort 5 HCC DLRT Meeting Outcome Memo • HCP CC authorization form - Medinfo • T-VEC_318_ IPIM_v5.0 12Dec17 • 01.13.16.04 IPIM - Investigational Product Preparation and Administration (IPPA) • Talimogene laherparepvec IB 15.1 Ver 2.0 EU RSI Change Summary Form • 20140318 ICF Phase 1_Track Change • MASTERKEY-318 - Cohort 3 NonHCC DLRT Meeting Outcome Memo 03Feb20 • TVEC IB 15.1 • 20140318_ICF_V6 Track Changes Template • 20140318 ICF Phase 2_Track Change • Signed Assurance Page • MASTERKEY-318 - Cohort 5 Non-HCC DLRT Meeting 14Nov18 Outcome Memo_final • PA3 Study Protocol Summary of Changes

Determination: Modifications Required

Required modifications:

1) Human Gene Transfer/Human Clinical Trial: Materials: Question 1: Update the scientific abstract to include changes related to product administration, as outlined within the amendment.

2) Human Gene Transfer/Human Clinical Trial: The IRB protocol linked to this new IBC protocol has been "terminated" so it is NOT a valid response. Link the appropriate IRB submission to this IBC protocol.

3) Viruses, Prions, or Vectors Viruses/Prions: Clarify if the study product will be stored exclusively at the Inpatient Pharmacy when participants with other solid tumors enroll in the study.

4) Tissues, Blood, or Body Fluids: Tissues/Blood/Body Fluids: Clarify if participants with other solid tumor types will also receive study product at the location.

5) Biosafety Summary: Select the box "Recombinant or Synthetic Nucleic Acids", as this is a human gene transfer clinical trial.

Comments:
This is an amendment to a currently-approved study. Talimogene laherparepvec is an investigational, oncolytic immunotherapy based on a modified Herpes Simplex Virus type-1 (HSV-1) administered by injection. This study evaluates the safety and efficacy intrahepatic injection of this modified virus alone and in combination with systemic IV administration of pembrolizumab. Two additional disease cohorts have been added: additional Breast Cancer (BC) cohort to include separate cohorts for Triple Negative Breast Cancer (TNBC) and hormone receptor positive participants, and additional hepatocellular carcinoma (HCC) cohort to include separate cohorts for HCC participants with and without viral Hepatitis. Recent literature has supported the use of immunotherapy for these newly included indications. Accordingly, the study schema has been revised to accommodate inclusion of these tumor types (for example, allowing for cutaneous, subcutaneous and involved lymph node intratumoral injections; expansion of allowable TVEC therapy in Part 2 for up to 35 weeks). Further, the safety data gathered from Part 1 of the study has facilitated the removal of specific lab testing, post-treatment specimen collection and the reduction of the required observation period in Part 2. There are no related changes to biosafety; however, the scientific abstract should be modified to include changes related to product administration, as outlined within the amendment. Recommend approval pending these minor corrections. Summary: This is a modification to an existing approved IBC study (2018). Talimogene laherparepvec is an FDA-approved oncolytic immunotherapy based on a live attenuated Herpes HSV-1 (strain JS-1) designed to selectively replicate in tumor tissue and stimulate an antitumor immune response. In the study product, HSV-1 genes ICP34.5 (“neurovirulence factor” that promotes viral replication in normal cells with an intact anti-viral response but is not required for replication in tumor tissue in which anti-viral responses are often defective) and ICP47 (which blocks antigen presentation by major histocompatibility complex molecules of infected cells) have been deleted. Deletion of ICP47 leads to increased and earlier expression of US11, which enhances viral replication in infected tumor cells. The coding sequence for human GM-CSF is inserted in place of ICP34.5, to enhance the immune response to tumor antigens released during oncolysis. Because talimogene laherparepvec lacks ICP34.5, it cannot replicate as effectively in normal cells as wild type HSV-1. In many cancer cells, the antiviral response pathway is dysregulated and permits talimogene laherparepvec replication eventually leading to oncolysis. The study product is injected into tumors with the anticipated result of lytic cell destruction and local release of progeny virus as well as of tumor cell antigens. GM-CSF is also produced locally to recruit and stimulate antigen-presenting cells, required for the initiation of a systemic antitumor immune response. Multiple administrative/study schema/editing changes were made that do not affect biosafety. The main change is that participants with additional advanced solid tumors besides HCC may enroll. These include hormone receptor positive breast

Adenocarcinoma [BC], Triple Negative Breast Cancer [TNBC], Colorectal Adenocarcinoma [CRC], Cutaneous Squamous Cell Carcinoma (CSCC), Basal Cell Carcinoma (BCC). There is also an additional Hepatocellular Carcinoma (HCC) cohort to include separate cohorts for HCC participants with well-controlled Hepatitis and without viral Hepatitis.

Other relevant changes include, but are not limited to:

- Allow intratumoral injection of talimogene laherparepvec into cutaneous, subcutaneous, and liver lesions and involved lymph nodes in Part 2 of the study, as safety of intrahepatic injection of talimogene laherparepvec in combination with systemic pembrolizumab will be established in Part 1. Intrahepatic injection is not required in Part 2, but it is allowed if there are injectable liver lesions
- Clarify that liver injection is not a requirement or priority in Part 2
- Expand allowable injectable disease to include subcutaneous and cutaneous tumor lesions and involved lymph nodes
- Create additional BC cohort to include separate cohorts for triple negative breast cancer (TNBC) and hormone receptor positive participants
- Change the sample size in Part 1 and Part 2 to reflect the number of cohorts/arms now under study
- Update the study schema to reflect new cohort in Part 1 and new tumor types in Part 2
- Update the eligibility criteria based on new cohorts and revised tumor types
- Shorten 23-hour observation window to 6 hours in Part 2, after review of safety data from monotherapy and combination cohorts enrolled to date in Part 1
- Add collection of Patch 1 (PTCH) mutation status in BCC cohort if available
- Add collection of BRCA1 and 2 mutation status in BC cohort if available
- Add optional liver ultrasound to schedule of assessments for monotherapy and combination cohorts enrolled to date in Part 1 and combination cohorts Part 2
- Remove 24-hour and 48-hour timepoints in Week 1 and Week 4 from Part 2 schedule of assessments, after review of available data from Part 1
- Remove assessments for anti-pembrolizumab antibodies, and pembrolizumab pharmacokinetics (PK) in Part 2
- Remove blood, urine, and swab collection at 24, and 48-hours in Part 2, after review of available data from Part 1
- Allow continuation of talimogene laherparepvec after cycle 12 in Part 2 if in the opinion of the investigator, the participant is deriving clinical benefit from the study regimen, the

participant is still receiving pembrolizumab, and the investigator obtains approval from the sponsor medical monitor
- Clarify that participants can receive a maximum of 12 cycles of talimogene laherparepvec in Part 1 and 35 cycles in Part 2
- Allow resumption of talimogene laherparepvec at progression if previously discontinued
- Allow up to 8 mL of talimogene laherparepvec to be used in Part 2 if 8 mL is shown safe in either Group A or B in Part 1
- Add clinical tumor assessments in Part 2
- Create a separate schedule of assessment table for Part 2 to reflect the changes to cohort, tumor types, and time points
- Revise the list of laboratory analytes to align with new tumor types
- Add language regarding events of clinical interest specifically for Group B HCC participants
- Add disease related events (DRE) language that instructs sites to transmit serious DREs in the same manner that they transmit serious adverse events and with the same timeline expectations
- Update HCC data in the Background and Rationale section to reflect new available clinical data since the last amendment

Biosafety: The HSV -1 thymidine kinase gene is intact, so the study product containing modified HSV-1 remains sensitive to acyclovir. Most adverse events reported in individuals administered talimogene laherparepvec are non-serious, primarily include flu-like symptoms, injection site reactions.

Initial comments:
1) Tissues/Blood/Body Fluids: Clarify if participants with other solid tumor types will also receive study product at the site indicated.
2) Viruses/Prions: Clarify if the study product will be stored exclusively at the Inpatient Pharmacy when participants with other solid tumors enroll in the study.

The application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000202
Title:	Amendment for **IBC201900033**
Investigator:	REDACTED
Highest BSL:	BSL-2 (Universal Precautions)
NIH Guidelines:	• NIH Section III-C-1 • NIH Section III-D-1
Additional Documents:	• Protocol Amendment 2 • HSFM0208PRO • HMI-102-101_Clinical Study Protocol_v1.0_15Feb2019_FINAL • HMI-102-101_Main ICF_v2.0_dd 17Dec2019_FINAL • HMI-102-101_Investigator's Brochure_15Feb2019_V2.0_FINAL • HMI-102 Pharmacy Manual (Version 21-Feb-19) • PTCMDIV820d • Admin Letter - Update on Dosing • Protocol Amendment 2 Update Letter • HSIC0609 • Admin Letter - Steroid Timing • Protocol Amendment 2 Dosing Justification Letter • FINAL - Appendix M - Homology Medicines - 02152019 • 47.2 Prep of BSL2 Viruses (10-19)

Determination: Modifications Required

Required modifications:

1) Human Gene Transfer/Human Clinical Trial: Materials: Question 1: Abstract: Include information from the participants enrolled in Cohorts 1 and 2.

Comments:

Summary: This is an amendment to an open-label, dose escalation study of single ascending doses of HMI-102 in up to 18 adult participants with phenylalanine hydroxylase (PAH) deficiency. HMI-102 is a recombinant Adeno-Associated Virus expressing codon-optimized human PAH. The vector is packaged into AAVHSC15 capsid. The capsid is a natural Clade F AAV variant isolated from CD34+ human peripheral blood stem cells from healthy adults. The capsid is closely related to AAV-9, also Clade F, differing by two amino acids in the VP3 protein (T346A, G505R). The AAVHSC15 capsid was selected based on tropism for the liver in mice and non-human primates in early studies. The HMI-102 vector is composed of a liver-specific expression cassette. HMI-102 is expected to target PAH deficiency through delivery of a hPAH cDNA to the nucleus of hepatocytes, where it becomes established as episomes. Transcription and translation of the inserted hPAH cDNA results in in the production of a functional PAH enzyme. It is administered by a single IV infusion and is a first-in-humans study. This amendment changes the highest dose in the study (dose Cohort 30 to 1^{14} vg/kg. The sponsor states this change is supported by non-clinical safety data and a review of the emerging safety and efficacy data from the ongoing clinical study. To date, two participants in Cohort 1 have received 2^{13} vg/kg of HMI-102 and two in Cohort 2 have received 6^{13} vg/kg of HMI-102. One non-treatment-related Serious Adverse Event (SAE) was reported (shingles). This event is currently status grade 1/resolved. All other AEs are

considered mild (Grade 1) and not treatment-related. All participants' transminases levels have remained within the normal range. A review of the Cohort 1 data conducted by the DMC allowed dose-escalation from the 2^{13} vg/kg to the 6^{13} vg/kg dose in Cohort 2. This change is thought to be warranted because the phenylalanine levels of Cohort 1 and 2 participants have not decreased from the pre-treatment baselines. The sponsor now believes the previously planned top dose of 8^{13} vg/kg may not achieve the predicted maximal response in humans. Based on the safety data and the plasma Phe concentration observed in Cohorts 1 and 2, the Sponsor intends to amend the planned dose for Cohort 3, from 8^{13} vg/kg to 1^{14} vg/kg. The sponsor is also adding a scale to assess a participant's perception of change during the study (Patient's Global Impression of Change), to support the use and development of the NIH Toolbox for participants with PKU.

Biosafety: The no observed adverse effect level (NOAEL) in the GLP mouse toxicology study was 2^{14} vg/kg, the highest dose tested and the maximum feasible dose, which provides a two-fold safety margin over the proposed high clinical dose of 1^{14} vg/kg. The sponsor finds the safety margin from the toxicology study together with the observed safety profile of HMI-102 in humans supports the proposed clinical dose of 1^{14} vg//kg. This is an amendment to a currently-approved study of a gene therapy for phenylalanine hydroxylase (PAH) deficiency. The goal of the treatment is to provide a long-term reduction of phenylalanine levels following a single dose of HMI-102, a recombinant Adeno-Associated Virus expressing codon-optimized human PAH. The reason for the amendment is to increase the third dose level of HMI-102 from 8^{13} vg/kg to 1^{14} vg/kg. This change is supported by non-clinical safety data and a review of the emerging safety and efficacy data from the ongoing clinical study. Due to the modest response noted to date in Cohort 2, the Sponsor believes that the top dose of 8^{13} vg/kg may not achieve the predicted maximal response in humans. Accordingly, based on the safety data and the plasma Phe concentration observed in Cohorts 1 and 2, the Sponsor intends to amend the planned dose for Cohort 3, from 8^{13} vg/kg to 1^{14} vg/kg. To-date no Treatment Emergent Adverse Events (TEAEs) or Serious TEAEs have been identified. One non-treatment-related Serious Adverse Event (SAE) was reported (shingles). This event is currently status grade 1 and has resolved. All other Adverse Events (AEs) are considered mild (Grade 1) and not treatment-related. The justification for the dose increase is well-documented and approval of the proposed amendment is recommended.

Initial comments:
Human Gene Transfer Abstract: Include information from the participants enrolled in Cohorts 1 and 2.

The application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000126
Title:	Amendment for **IBC201700334**
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• BSL2+_Manual

Determination: Modifications Required

Required modifications:
1) Viruses, Prions, or Vectors: Include FAP-mCer-TRF1, FAP-mCer-CENPA, H2B-mCer-FAP and the FAP-mCer-LacR under "Inserted Nucleic Acids Information."

Comments:
The investigator is submitting this amendment to add new constructs and personnel. LentiCas9-Blast expresses human codon-optimized *S. pyogenes* Cas9 protein and blasticidin resistance from EFS promoter in a 3rd generation Lentiviral backbone. pLentiGuide-Puro expresses *S. pyogenes* CRISPR chimeric RNA element with customizable sgRNA from U6 promoter and puromycin resistance from EF-1a promoter in a 3rd generation Lentiviral backbone. These two vectors will be used separately. First, pLentiCas9-Blast will be used to stably express Cas9 in cells and selected by Blasticidin. Next, a guide RNA against a DNA repair protein (for example, DDB2) will be cloned into pLentiGuide-Puro and expressed in cells expressing Cas9 to disrupt the specific gene of interest. Cells will be expanded by single-cell cloning. Proteins thought to play a role oxidative DNA damage (8-oxoguanine) repair will be targeted using this system. pLentiGuide-Puro and pLentiCas9-Blast will be used separately one after the other to knockout expression of the genes of DNA repair proteins (for example, DDB2 or OGG1). Investigators amended this protocol because they will use Lentiviral vectors to express CRISPR- Cas9 and guide RNAs separately in mouse fibroblast cells. The protocol has been amended to be carried out at BSL-2+ level according to recent University of Pittsburgh IBC guidance recommendations.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000152
Title:	Amendment for **IBC201600182**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Stat3 is a potential oncogene. Clarify the use of this gene in the Project summary.

2) Basic Information, Question 8: Question 8 should be completed if the investigator wishes to have an additional person be notified of correspondence.

3) Tissues, Blood, or Body Fluids: For the human cells, clarify in the "Description of Usage" how cells are recombinant.

4) Tissues, Blood, or Body Fluids, Question 2: Spleen and adipose tissue transplantation is listed in the project summary. If this work will be conducted, then describe it in the "Description of Usage"

5) Primary Cells or Cell Lines: Cells infected with viruses expressing both CRISPR/Cas9 and gRNA from a viral vector should be used at BSL-2+. Correct the BSL designations.

6) Viruses, Prions, or Vectors: The response for AAV currently indicates that it will be administered to humans; revise the response to be NO, as the agent will not be administered to humans.

7) Viruses, Prions, or Vectors: AAV: Identify non-viral genes (not sequences) to be expressed under the "Inserted Nucleic Acid Information."

8) Viruses, Prions, or Vectors: CRISPR/Ca9 and gRNA should be listed under "Inserted Nucleic Acids Information." Note that the use of viral vectors expressing both Cas9 and gRNA must be used at BSL-2+ per University of Pittsburgh IBC guidance recommendations.

9) Recombinant or Synthetic Nucleic Acid Work Description: The investigator has stated that CRISPR Cas9 will be used for gene editing with viral delivery on the same vector. Additional information is required and should be placed in the response for Question 1 and in the Virus section of the application.

10) Live Animals, Question 1: Remove the 2 listed IACUC protocols as they have expired and provide a current IACUC/ARO protocol number.

11) Risk Group and Containment Practices, Question 2: BSL-2+ should be selected for use of viruses expressing both CRISPR/Cas9 and gRNA.

12) Exposure Assessment and Protective Equipment, Question 1: Clarify how Retroviruses can infect human cells if only eco-tropic viruses will be used.

13) Exposure Assessment and Protective Equipment, Question 4: Safety-engineered sharps devices are required for BSL-2/ABSL-2; revise.

14) Supporting Documents: Upload the laboratory's Biosafety Operations Manual (BSM) that is signed by both the investigator and one of the staff from the Department of Environmental Health and Safety (EH&S) Office.

Comments:
The modification requests amending an approved protocol to use AAV1-based vectors to overexpress STAT3 in murine cardiomyocytes after apical dissection in neonatal mice. These mice express GFP under ubiquitin promoter or Serpin B2-/-. Spleen transplantation will be performed in congenitally different mice. In addition, CX3CR1 will be over-expressed in endothelial cells using a Lentiviral vector to investigate the effect of CX3CR1 expression in endothelial cell apoptosis. Human autopsy samples and mouse tissue will be used at BSL-2. *E. coli* will be used at BSL-2 to amplify the pMIG plasmid. The modification adds AAV-1 in order to overexpress Stat3 in the heart of neonatal mice in a study for hematopoietic stem cells in cardiovascular and pulmonary diseases in order to promote healing of hearts post heart attacks. Transgenic mice with serpin mutation or expressing GFP under control of the ubiquitin promoter will be used. Transplantation of spleens will be performed. Lentivirus will be used to express CX3CR1 in endothelial cells using Lentivirus. Human autopsy samples (CORID number provided) and mouse tissue will be used at BSL-2. Recombinant human and mouse cell lines will be used to express CX3CR1 from Retroviral and Lentiviral vectors. *Escherichia coli* will be used at BSL-2 to amplify the pMIG plasmid. Mice will be injected with Retrovirus and possibly other viruses (this is not 100% clear). There are many issues and missing information. The amendment request seeks to add approval to use AAV-1 vectors to an existing approval. The overall aim of the protocol is to understand the causes of heart attack and the processes of healing after heart attack. This amendment will use AAV-1 based vectors to overexpress STAT3 in murine cardiomyocytes after apical dissection in neonatal mice. The protocol will also use a range of human autopsy samples (BSL-2) and murine tissue (BSL-2, ultimately from *Jackson Labs* mice) for analysis. Adipose tissue from mice of various background will be transplanted. The protocol also uses 293T cells (BSL-2, *Clontech*), to make Retroviral and Lentiviral vectors, which will be used to transduce human pulmonary aortic endothelial cells, to express CXCR1. Murine immune cells, hematopoietic stem and progenitor cells (BSL-2, *Sigma-Aldrich*) are used for analysis and for transduction using Retro- and Lentiviral vectors, again expressing CX3CR1. The protocol already uses MESV (BSL-2, *Addgene*) and Lentiviral vectors (HIV 3-plasmid system; BSL-2, *Addgene*) to transduce murine and human aortic endothelial cells, respectively. The AAV-1 vector (BSL-2, *Vector Biolabs*) will be used to overexpress STAT3 in cardiomyocytes of neonate mice. The underlying protocol is confusing and contains many contradictions and issues. These all need to be addressed before approval.

Initial comments:
1) Viruses, Prions, or Vectors: The application is confusing; the MESV entry describes the inserted nucleic acid as encoding serpin B2 but the sequence provided and the descriptions elsewhere are for CXCR1. Review and amend appropriately.

2) Recombinant or Synthetic Nucleic Acid Work Description: Again the infection of mice by IP with serpin B2 is mentioned. Clarify the application and amend throughout if needed. Is serpin B2 being transduced or just CX3CR1?
3) Recombinant or Synthetic Nucleic Acid Work Description: CRISPR/Cas9 is mentioned as being delivered by Lentivirus. This should be described in the Viruses, Prions, or Vectors section.
4) Animal Gene Transfer: Question 3: The response should be YES, with the addition of AAV-1 vector AAV-9? Elsewhere the vector was described as AAV-1. Clarify.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000178
Title:	Amendment for **IBC201700281**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description: Oncogene/toxin was checked not applicable (N/A). But ATF3 and PTEN are tumor suppressors and at least for PTEN shRNA use is indicated with Lentiviral vectors. This would require BSL-2+.

2) Recombinant or Synthetic Nucleic Acid Work Description: pLKO-PTEN-shRNA-1320 // n/a // pLKO.1-puro // shRNA; It is not clear how this Lentiviral vector with a knock-down of a tumor suppressor gene is used. If as a Lentiviral vector, then it does require BSL-2+ containment and practices.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 3c: PTEN is a tumor suppressor and it is indicated in Question 1 that it will be knocked down using pLKO-PTEN-shRNA-1320 // n/a // pLKO.1-puro // shRNA. Note that expression of shRNA targeting tumor suppressors via Lentiviral vectors requires BSL-2+ per University of Pittsburgh IBC recommendations.

4) Risk Group and Containment Practices, Question 2: Note that expression of shRNA targeting tumor suppressors via Lentiviral vectors requires BSL-2+ per University of Pittsburgh IBC recommendations.

Comments:

The amendment is for an approved protocol to add CRISPR/Cas9 with sgRNA on the same Lentiviral vector. Thus, requiring a change to BSL-2+ from BSL-2. The investigator has submitted a modification to update CRISPR work per IBC recommendations. The work may require upgrading the laboratory to BSL-2+. The existing protocol includes *in vitro* and *in vivo* work with MHV68, Adenovirus, and Lentivirus. The laboratory is part of the Aging Institute and does not currently have BSL-2+ approval. This is an amended IBC protocol with a change from BSL-2 to BSL2+ because CRISPR/Cas and the sgRNA(s) are expressed using the same viral vector.

The application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000234
Title:	Amendment for **IBC201700203**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Deferred/Reconsidered

Required modifications:

1) Supporting Documents: If BSL-2+ work practices are required for this research, then the laboratory must have a biosafety manual reviewed and approved by both the investigator and a member of the staff of EH&S. The signed copy must be uploaded to the application.

2) Exposure Assessment and Protective Equipment: Question 1: In the Recombinant and Synthetic Nucleic Acids Work Description the investigator indicates that TERT, an oncogene, will be overexpressed using Lentiviral vectors. The investigator also indicates that Cas9/gRNA delivered by Lentiviral vector may be used to disrupt POT1, a tumor suppressor gene. Clarify the statement that "we do not anticipate cloning any oncogenes into these viruses" as it appears that this may be inaccurate. If the work description in the previous section of the protocol is accurate, then BSL-2+ work practices are required.

3) Exposure Assessment and Protective Equipment: Question 1: Include SARS-CoV-2 Spike protein as an additional method of Lentivirus pseudotyping.

4) Risk Group and Containment Practices: Questions 3 and 4: All personnel listed in the response need to be moved to the "Research Personnel" if the study will be conducted under BSL-2+.

5) Risk Group and Containment Practices: If BSL-2+ will be used for the work described, then it would need to be selected under the first column in Question 2.

6) Recombinant or Synthetic Nucleic Acid Work Description: It appears that in addition to potential overexpression of TERT (oncogene) in Lentiviral vectors, the described work may also include use of Cas9/gRNA system to disrupt POT1, a tumor suppressor gene also delivered via Lentiviral vectors. This should be clarified as IBC guidance recommends BSL-2+ practices for this work.

7) Recombinant or Synthetic Nucleic Acid Work Description: Question 2: If TERT will be expressed in cells, it should be described and the expression system used to express it should be identified.

8) Viruses, Prions, or Vectors: Indicate under "Description of Usage" (not Inserted Nucleic Acids Information) that Lentiviruses will be pseudotyped with SARS-CoV-2 Spike protein.

9) Protocol Team Members: If this work will require BSL-2+ safety practices, then all of the laboratory personnel will be required to be listed on the application.

10) Basic Information: Question 8: The investigator will always be notified of all communications from the online IBC system. Question 8 should be completed if the investigator wanted to have an additional person be notified of correspondence.

Comments:

The investigator is requesting to add two transgenic proteins HACE2m SARS-CoV2 Spike to his IBC protocol and to modify his Lentiviral packaging protocols. He would like to generate a Lentivirus that is psuedotyped with Spike protein from SARS-CoV-2 to test strategies for preventing infection with SARS-CoV-2. The laboratory has been encouraged to help with the effort to understand the pathogenicity of SARS-CoV-2 to identify potential therapies. Based on experience with Lentiviruses, the development of a Lentivirus that has been pseudotyped with the envelope protein might be a simple and safe method for studying viral entry and eventually neutralization. This virus would likely be "safer" than Lentiviruses currently pseudotyped with VSV-G due to the limited tropism mediated by the Spike protein from SARS-CoV-2. This pseudotyped virus will be used as a proof-of-concept that will be tested in several cell lines for its ability to infect cells. The investigator is also requesting to add hACE2 protein. This protein will be stably expressed in HEK293 cells to make them susceptible to viruses that have pseudotyped with SARS-CoV-2 Spike protein. These experiments (with hACE2 and Lentiviruses pseudotyped with SARS-CoV-2 Spike protein) will only be conducted *in vitro* (tissue culture) and no animal experiments are planned. The investigator has relevant expertise in studying lung disease and working with Lentiviral vectors. The new additions are acceptable as detailed. There is a question regarding BSL status regarding Lentiviral vectors that can infect human cells and expression of TERT, a known oncogene, in the previously approved protocol. It is likely that the investigator should be working at BSL-2+ already. This amendment is to add Lentiviral expression of SARS-CoV-2 Spike and hAce2 to the application. Minor questions about the expression vector for Ace2 receptor need clarification. The investigator is transfusing cells with viral vectors expressing proto-oncogenes (ie TERT) at BSL-2. Constitutive expression of human TERT is the classical way of transforming primary cells into cell lines. Based on IBC recommendations - this is should be at BSL-2+. They are also using CRISPR/Cas9 in a 2-plasmid system to silence these proteins. There is a commentary suggesting that these proteins are not oncogenes https://www.nature.com/articles/1205076 ; perhaps this was the basis for the decision to not treat this protocol as a Lentiviral expression of an oncogene.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Reconsideration: Additional comments were provided by the committee, which will need to be revised and returned to the IBC for assignment to the next available agenda.

Supporting documents: None

Votes:

For:	20
Against:	0
Abstained:	0

Protocol:	MOD202000210
Title:	Amendment for **IBC201900133**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Approved BSL-2+ Manual

Determination: Modifications Required

Required modifications:

1) Virus, Prions, or Vectors: Lentivirus (HIV 3-plasmid system) - pCCLc/pFUGW: all proteins to be expressed from Lentiviruses, including SARS-CoV-2 proteins, should be listed under "Inserted Nucleic Acids Information."

2) Recombinant or Synthetic Nucleic Acids Usage: Select the box for *Section III-D-6* as there is more than 100 ml of virus produced or handled by the laboratory at any one time.

3) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Clarify *in vivo* work that involves animals.

4) Lentivirus and Lentiviral Vectors, Question 2a: Correct the last sentence to state, "All work with Lentiviruses expressing SARS-CoV-2 genes will be done at BSL-2. . . ."

5) Animal Gene Transfer, Question 2: Administration of viruses to animals is not described anywhere else in the protocol. Clarify this discrepancy.

6) Animal Gene Transfer, Question 5: Clarify the response the application indicates YES to Question 2 in this section.

7) Supporting Documents: The IBC understands that the investigator must submit a the signed COVID-19 checklist to EH&S for final review.

Comments:

The modification details a study of protein-protein interactions of SARS CoV-2 by adding Lentivirus encoding SARS CoV-2 proteins to be cultivated in HEK293 cells and expressed in K562 cells at BSL-2+. The study uses a variety of cell types and CRISPR-CAS including primary human blood cells and human or mouse cell lines at BSL-2 or 2+. *Escherichia coli* will be used at BSL-1 for plasmid work. Murine stem cell virus and Lentivirus will be used at BSL-2+ when oncogenes and CRISPR-Cas are delivered from the same viral vector. A variety of genes will be expressed or inhibited including T-cell receptors, leukocyte antigens, and potentially oncogenic miRNAs. Some clarifications are needed, but overall the proposal is well-written. The work will be performed at BSL-2, which is appropriate. No work will be performed with SARS-CoV-2 virus. Several minor forms issues should be corrected or clarified, including whether or not viruses will be administered to animals. Recommendation for approval pending clarifications.

The application was placed on the May agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	19
Against:	0
Abstained:	1; recused for involvement

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 11:25 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

May 26th, 2020 10:00 AM

Meeting; Zoom teleconference meeting
SARS-CoV-2 protocols only

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			Yes
REDACTED, Vice Chair			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	EH&S Office

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions via the tele-meeting portal.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

Since this was the fourth "emergency" meeting and not regularly scheduled, the May 11th regularly scheduled meeting minutes were not reviewed. They will be reviewed at the regularly scheduled June meeting.

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

None reported

NON-COMPLIANCE, EVENT, INJURY REPORT

Discussion around the possible use of Lentiviral vectors encoding TERT oncogenes was discussed. Upon IBC inquiry, it was found that the investigator was not using any of the TERT oncogenes with the Lentiviral vector mentioned in the application. Initial approval of the application was appropriately determined to be BSL-2. One reviewer indicated that the BSL assigned to the parental protocol in 2017 was incorrect.

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

This meeting was scheduled to accommodate only protocol applications involving SARS-CoV-2. All other protocol applications will be assigned on the next regularly scheduled agenda.

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocols on the following pages

Protocol:	IBC202000069
Title:	Study of Coronaviruses Using Pseudovirus Particles and CoV RNA Replicon
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED 2019 lab manual

Determination: Deferred/Reconsidered

Required modifications:

1) Primary Cells or Cell Lines: For the Source under "Cell/CellLine - Other Human cells or cell lines"; clarify if the cells were obtained from a vendor or a collaborator.

2) Exposure Assessment and Protective Equipment: Question 1: Revise the wording to indicate the CoV systems used are replication-defective (i.e. still able to infect/enter cells).

3) Exposure Assessment and Protective Equipment: Question 1: Describe all agents that can infect human cells. In addition, human and non-human primate cell lines may harbor endogenous viruses that are not identified and these may have some risk, albeit low, to humans. Even well-established human cell lines may harbor unknown human pathogens. Revise the response.

4) Exposure Assessment and Protective Equipment: Question 5: BSL-2+ laboratory garb requirements include use of a solid-front wrap-around gown.

5) Recombinant or Synthetic Nucleic Acid Work Description: Clarify how full length CoV cDNA cloned in Bacterial Artificial Chromosome (BAC) will be used. This has the potential to produce replication-competent virus, which would require use at BSL-3.

6) Recombinant or Synthetic Nucleic Acid Work Description: Provide a description of the use of the SARS-CoV1 HSR and Urbani strain, MERS-CoV EMC2012 strain, and SARS-CoV2 WA1 and Wuhan strains that will be obtained from *BEI Resources*. The experiments described may need to be conducted in a BSL-3 facility for safety testing of the non-functional replicons.

7) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Identify the collaborators that will provide the cDNA.

8) Recombinant or Synthetic Nucleic Acid Work Description: What steps will the laboratory take to insure there is not an accidental transfection of cells with full length CoV DNA? Will there be spatial and temporal separation between working with cDNA of SARS-CoV, SARS-CoV-2 and MERS-CoV to prevent any possibility of recombination?

9) Recombinant or Synthetic Nucleic Acids Usage: Clarify why *Section III-D-1* (Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems) is checked.

10) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-3* should be checked.

11) Viruses or Prions: Clarification regarding the specific materials to be acquired from *BEI Resources* is needed. All three viruses listed, SARS-CoV-1, SARS-CoV-2, and MERS-CoV, are risk group 3 agents and the investigator does not have an approved BSL-3 laboratory for work with these viruses. Of critical importance, SARS-CoV-1 and any isolated nucleic acids/full genomes, are regulated by the Federal Select Agent Program and the University is not approved

for possession of these materials. Indicate the specific materials and provide the *BEI Resources* catalog number for the reagents to be acquired.

12) Viruses or Prions: Clarify how the pseudoviruses cannot enter cells, yet the "Description of Usage" states, "To study CoV infection entry. . . ." Note that viral infectivity/entry is different than viral replication.

13) Viruses or Prions: SARS-associated Coronavirus - SARS-CoV1 HSR and Urbani strain, MERS-CoV EMC2012 strain, and SARS-CoV2 WA1 and Wuhan strains: These viruses should be designated as recombinant if they are not the wild type strain.

14) Viruses or Prions: Provide the *BEI Resources* catalog number for each strain being obtained from the company. Confirm that the investigator or laboratory will not receiving virus as this work would need to be conducted in a BSL-3 facility.

- Note that SARS-CoV-1 AND full-length isolated RNA are both regulated as Select Agents; the University is not registered for possession of these agents.

15) Viruses or Prions: Since it appears that the investigator will be working with full length CoV cDNA, how will they insure that they do not transfect cells with full length cDNA? Also the application states that the agent cannot enter or infect human cells, but replicons are capable of infecting cells and undergoing limited replication, yes? Clarify.

16) Risk Group and Containment Practices: Question 1: Risk Group 3 (RG-3) should be selected.

17) Basic Information: Question 8: The investigator will always be notified of all communications from the online IBC system. The investigator will receive all correspondence and cannot opt out of the correspondence.

Comments:
The investigators plan to produce pseudotype viruses and replicons of Corona Viruses (CoV). These will be used to study virus attachment and entry, as well as immunology including the potential use as a vaccine. The protocol does not indicate how the vaccine potential will be evaluated and there are no indications that any work will be done in animals or humans, this is strictly *in vitro* work. They indicate they will be working with full length cDNA from SARS-CoV, SARS-CoV2, and MERS-CoV to generate the pseudotype viruses and replicons. Replicons will lack the S, M, and E genes. They do not indicate how they will insure that they do not accidentally transfect cells with full length cDNA. There is no plan for prevention of cross-contamination or recombination between the viruses. There is also a question of how non-infectious pseudotype viruses will be used to study virus attachment and entry; key steps in the infection process. These points need to be clarified and explained. The investigator has submitted a protocol to produce pseudovirus particles and replicons of SARS-CoV-1, SARS-CoV-2, and MERS-CoV. Work will be conducted *in vitro* and plasmids will be propagated in *E. coli*. Form issues have been identified, particularly sources of agents, and will need to be revised. Safety issues/questions must be clarified and addressed.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the May 26th emergency meeting for review.

Reconsideration: The committee recommended for Reconsideration for the research after the investigator provides responses and clarifies information about the agents that are planned to be

used. Once the investigator has provided a revised application to address the comments provided, then the protocol application will be placed onto the next available agenda for review.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD202000234
Title:	Amendment for **IBC201700203**
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• Pitt_BSL-2+ REDACTED Lab_May2020_signed

Determination: Approved

Last day of continuing review period: 5/27/2021

Required modifications:

The committee requested modifications within the submission in order to obtain approval status:
1) Viruses or Prions: Confirm or correct the building location. In addition, identify a location for use of research materials that is approved for BSL-2+ work.
2) Bacteria, Yeasts, Fungi, or Parasites: Confirm or correct the building location for the agents listed.
3) Primary Cells or Cell Lines: Confirm or correct the building location. In addition, identify the location for use of materials that is approved for BSL-2+ work.
4) Primary Cells or Cell Lines: Use of cells with BSL-2+ agents should be designated as BSL-2+ in the protocol as the cells are exposed and should then be handled under the appropriate biosafety level. Revise the applicable responses for cells.
5) Tissues, Blood, or Body Fluids: Confirm that these experiments will be conducted in Montefiore University Hospital; the investigator was last inspected in BST West.

Comments:

Determination: Reconsidered from the May 11, 2020 meeting
Required modifications from the May 11th meeting:
1) Supporting Documents: If BSL-2+ work practices are required for this research, then the laboratory must have a biosafety manual reviewed and approved by both the investigator and a member of the staff of EH&S. The signed copy of the Biosafety Operations Manual must be uploaded to the application.
2) Exposure Assessment and Protective Equipment: Question 1: In the Recombinant and Synthetic Nucleic Acids Work Description the investigator indicates that TERT, an oncogene, will be overexpressed using Lentiviral vectors. The investigator also indicates that Cas9/gRNA delivered by Lentiviral vector may be used to disrupt POT1, a tumor suppressor gene. Clarify the statement that "we do not anticipate cloning any oncogenes into these viruses" as it appears that this may be inaccurate. If the work description in the previous section of the protocol is accurate, then BSL-2+ work practices are required.
3) Exposure Assessment and Protective Equipment: Question 1: Include SARS-CoV-2 Spike protein as an additional method of Lentivirus pseudotyping.

4) Risk Group and Containment Practices: Questions 3 and 4: All personnel listed in the response need to be moved to the "Research Personnel" section of the application if the study will be conducted under BSL-2+ containment and safety practices.

5) Risk Group and Containment Practices: If BSL-2+ will be used for the work described, then it would need to be selected under the first column in Question 2.

6) Recombinant or Synthetic Nucleic Acid Work Description: It appears that in addition to potential overexpression of TERT (oncogene) in Lentiviral vectors, the described work may also include use of Cas9/gRNA system to disrupt POT1, a tumor suppressor gene also delivered via Lentiviral vectors. This should be clarified as the IBC guidance document for work with gene editing requires BSL-2+ practices for this work.

7) Recombinant or Synthetic Nucleic Acid Work Description: Question 2: If TERT will be expressed in cells, it should be listed and the expression system used to express it should be identified.

8) Viruses, Prions, or Vectors: Indicate under “Description of Usage” (not Inserted Nucleic Acids Information) that Lentiviruses will be pseudotyped with SARS-CoV-2 Spike protein.

9) Protocol Team Members: If this work will require BSL-2+ safety practices, then all of the laboratory personnel will be required to be listed on this page of the application.

10) Basic Information: Question 8: The investigator will receive all correspondence and cannot opt out of the correspondence. Question 8 should be completed if the investigator wishes to have an additional person be notified of correspondence from the system.

Comments:
The investigator is requesting to add two transgenic proteins HACE2m SARS-CoV2 Spike to his IBC protocol and to modify his Lentiviral packaging protocols. He would like to generate a Lentivirus that is psuedotyped with Spike protein from SARS-CoV-2 to test strategies for preventing infection with SARS-CoV-2. The laboratory has been encouraged to help with the effort to understand the pathogenicity of SARS-CoV-2 to identify potential therapies. Based on experience with Lentiviruses, the development of a Lentivirus that has been pseudotyped with the envelope protein might be a simple and safe method for studying viral entry and eventually neutralization. This virus would likely be "safer" than Lentiviruses currently pseudotyped with VSV-G due to the limited tropism mediated by the Spike protein from SARS-CoV-2. This pseudotyped virus will be used as a proof-of-concept that will be tested in several cell lines for its ability to infect cells. The investigator is also requesting to add hACE2 protein. This protein will be stably expressed in HEK293 cells to make them susceptible to viruses that have pseudotyped with SARS-CoV-2 Spike protein. These experiments (with hACE2 and Lentiviruses pseudotyped with SARS-CoV-2 Spike protein) will only be conducted *in vitro* (tissue culture) and no animal experiments are planned. The investigator has relevant expertise in studying lung disease and working with Lentiviral vectors. The new additions are acceptable as detailed. There is a question regarding BSL status regarding Lentiviral vectors that can infect human cells and expression of TERT, a known oncogene, in the previously approved protocol. It is likely that he should be working at BSL-2+ containment and practices already. This amendment is to add Lentiviral expression of SARS-CoV-2 Spike and hAce2 to the application. Minor questions about the expression vector for Ace2 receptor need clarification. The application indicates that the investigator is transfusing cells with viral vectors expressing proto-oncogenes (ie TERT) at BSL-2. Constitutive expression of human TERT is the classical way of transforming primary cells into

cell lines. Based on IBC recommendations - this is should be at BSL-2+. They are also using CRISPR/Cas9 in a 2-plasmid system to silence these proteins. There is a commentary suggesting that these proteins are not oncogenes https://www.nature.com/articles/1205076 ; perhaps this was the basis for the decision to not treat this protocol as a Lentiviral expression of an oncogene.

Review comments were provided to the investigator for response. The revised application was placed on the May agenda.

Reconsideration: Additional comments were provided by the committee, which will need to be revised and returned to the IBC for assignment to the next available agenda.

The investigator provided a revised protocol application which was placed onto the May 26th emergency meeting for review.

Modifications Required: Reconsideration from the May 11th meeting. Additional comments were provided by the committee, which were sent to the investigator.

One reviewer indicated that the BSL assigned to the parental protocol in 2017 was incorrect. Meeting discussion centered on use of a Lentiviral vector encoding the gene for human TERT that was approved at BSL-2 in the original 2017 protocol. The Chair and Biosafety Officer recall meeting with the investigator on September 22, 2017 regarding the original protocol. In that meeting, the investigator stated that Lentiviral vectors expressing human TERT would only be transfected into cells, which is appropriate at BSL-2 under current IBC recommendations. This was approved by the IBC in 2017 at BSL-2. The Chair told the Committee that after the May 11, 2020 IBC meeting, she emailed the investigator and asked how the Lentiviral vectors expressing human TERT had been used in his laboratory. He replied, "We have not produced any Lentiviruses that express TERT in our laboratory since I arrived here. We are planning to do so, but we have not yet produced them. . . . When I arrived here, you and I discussed the idea of TERT being an oncogene. It does not fit the definition of a classic oncogene and I wonder how the IBC determines which genes are oncogenes (and tumor suppressors…we were also informed that POT1 in a tumor suppressor!)." After a vote to approve the current protocol amendment with modifications at BSL-2+, one Committee member stated that the original protocol did not clearly state that only transfection would be used and that the IBC made an error in approving the protocol at BSL-2, which should be reported to NIH. The Chair indicated that a subcommittee will be formed at a later date to review how the IBC will define oncogenes and tumor suppressors.

The investigator provided responses to comments from the meeting and provided a laboratory biosafety operations manual. The investigator provided the revision of the application to address remaining minor issues in the protocol, and an approval letter was generated.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

Protocol:	MOD202000244
Title:	Amendment for **IBC201700029**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Section III-D-3 • NIH Section III-D-4 • NIH Exempt: Sections III-E or III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 4: Work on the bench and outside of a biosafety cabinet with BSL-2 agents must include full face protection (goggles and surgical mask or face shield). Revise.

2) Exposure Assessment and Protective Equipment: Question 1: 70% ethanol is not an EPA-registered disinfectant and should not be used as a primary means of disinfection; revise.

3) Exposure Assessment and Protective Equipment: Question 5: Include coverall suit for entrance to ABSL-2 animal facility.

4) Recombinant or Synthetic Nucleic Acids Usage: If the Baculovirus will be administered to animals while they are alive, then the *NIH Guidelines Section III-D-4* will apply to this research and should also be selected.

5) Animals: Question 1: The IACUC protocol linked is no longer a valid/active ARO protocol; provide a new IACUC/ARO protocol.

6) Viruses or Prions: Recombinant or Synthetic Nucleic Acid Work Description section lists administration of Baculovirus to animals. Review the questions for the agent and change the current response to YES and then be sure to describe how the Baculovirus is administered to the animals in the response for the "Description of Usage" for the Baculovirus.

7) Viruses or Prions: In the Exposure Assessment and Protective Equipment section (Question 1) it is stated "MemBac Baculovirus expression vectors may infect human cells". Provide clarification and correct. If the Baculovirus can infect human cells, then BSL-2 should be selected.

8) Primary Cells or Cell Lines: MLE-12 cells are designated BSL-2 by *ATCC*; revise the biosafety level of this entry to BSL-2.

9) Tissues, Blood, or Body Fluids: The Project Summary notes expressing gene products in rat tissue. If rat tissue will be used, then provide an entry for those specific rat tissues in the application.

Comments:

The investigator has submitted an additional modification for SARS-CoV-2 related research. DNA sequences corresponding to the protein-coding sequences of the SARS-CoV-2 viral proteome have been added for the use of cloning into expression vectors for studies of protein stability and interaction *in vitro*. Cells will be transfected with SARS-CoV-2 protein-coding sequences in expression vectors alone or in combination with human ubiquitin E3 ligases, then

transfected with RNAi. Clarifications and modifications are required. This investigator is adding an amendment for SARS CoV work where Beas 2B cells to study the interaction of SARS CoV2 proteins with ubiquitin ligases. Cells will be transfected with SARS-CoV-2 protein-coding sequences along or with E3 ligases.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the May 26th emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:37 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

June 8, 2020 10:00 AM

Meeting; Zoom teleconference meeting

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			Yes
REDACTED, Vice Chair	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Late 10:13		Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED	Late 10:02		Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED	Late 10:05		
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	EH&S Office
REDACTED	IACUC Office
REDACTED	ORP Co-Director

GUEST NAMES

REDACTED, Office of Trade Compliance

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present. Guest Ms. REDACTED was attending by invitation from the Biosafety Officer.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions via the tele-meeting portal.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

The May 11th (regularly scheduled) and May 26th, (non-standard scheduled) 2020 meeting minutes were reviewed and approved by the committee.

Votes:

For:	12
Against:	0
Abstained:	0

IBC members were reminded of the Conflict of Interest (COI) policy.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

None reported

NON-COMPLIANCE, EVENT, INJURY REPORT

None reported

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

Protocols on following pages.

Protocol:	IBC202000046
Title:	GCN5L1 in Heart
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-1
NIH Guidelines:	• Section III-F • Section III-D-1 • Section III-D-2 • Section III-D • Section III-D-3
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: The application indicates these materials for administration to animals Describe the administration under the "Description of Usage" question.

2) Viruses, Prions, or Vectors: In other sections of the protocol the investigator lists that the Lentivirus will be used for gene expression and knockdown, but only list shRNA in the inserted nucleic acids. Is there another protein that will be overexpressed with the Lentiviral system as well?

3) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system) - pLenti2A/B: questions regarding infectivity and replication should be answered.

4) Exposure Assessment and Protective Equipment, Question 5: Include the hair bonnet, coverall suit, and shoe covers to the list of PPE for work within the animal facility.

Comments:

This protocol is for a project investigating GCN5L1-regulated energy homeostasis and mitochondrial bioenergetics. The work will use tissues from wild type and transgenic mice, human, mouse, and rat cell lines, and *E. coli* for plasmid cloning. Cells will be transfected with GCN5L1 with Adv-5 and shRNA against GCN5L1 with a 3-plasmid Lentiviral vector. The Recombinant or synthetic nucleic acid work description section also indicates TFAM will be expressed (silenced by Lentiviral transduction?) but this is not noted in the viruses, prions, or vectors section. The work is proposed at BSL-2 and ABSL-1, which appears to be appropriate. Approval is recommended pending requested clarifications that need to be addressed. This new IBC protocol will be examining the role of GCN5L1 on cardiac energetics. GCN5l1 is a major acetyltransferase in mitochondria, regulating energy homeostasis and mitochondrial bioenergetics. *In vitro*, the Adenovirus will be used to transfect neonatal mouse or rat cardiac myocytes for gene overexpression of GCN5L1, whereas Lentivirus containing shRNA against GCN5L1 is to be transfected into mouse/rat neonatal , H9C2 and AC 16 cells to knockdown GCN5L1 protein expression. In animal studies, Cardiac specific GCN5L1 KO mice will be used to determine the importance of GCN5L1 signaling in cardiac pathophysiological stress. No other virus or oncogenes or toxins will be used in this protocol. All work was claimed to be performed at level of BSL-2/ABSL-1 that are suitable. A few minor corrections are needed to some of the questions in the application. This protocol involves the role of GCN5L1 in physiological or exercise induced cardiomyopathy. Protocol will use tissue culture and mouse models. Mice will be purchased or bred on-site, no transplantations, work is proposed at ABSL-1. BSL-2 seems fine for cell culture. GCN5L1 is an acetyl-transferase which could alter gene expression levels but unaware of other

more pathogenic effects. Overall, this protocol is not clear. The application should clearly state which types of experiments they are proposing, tissue culture or studies in mice, or both.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	IBC202000054
Title:	Opto-Steel-Trodes
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• Section III-D-4 • Section III-F • Section III-D-1 • Section III-D
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: The purpose of this question is to explain to the Institutional Biosafety Committee members about the research that involves recombinant or synthetic nucleic acid molecules. Include a brief description of the research.

2) Tissues, Blood, or Body Fluids: Work with human serum is mentioned in the Exposure Assessment and Protective Equipment section. If human serum is used, then provide an entry.

3) Viruses, Prions, or Vectors: Adeno-Associated Viral Vector (AAV) - AAV-hSyn-Jaws-KGC-GFP-ER2: It is unclear why questions regarding infectivity and replication are not answered.

4) Live Animals, Question 3: Earlier in the application, it is stated that nervous tissues will be obtained from nonhuman primates. Thus, the answer to the question should be YES.

5) Risk Group and Containment Practices, Question 1: The use of nonhuman primate tissues and cells is considered RG-2.

6) Exposure Assessment and Protective Equipment, Question 4: Describe if a biosafety cabinet will be used or if safety-engineered sharps devices are used.

7) Waste Management, Question 3: Absorbent material should be placed on the spill to contain the liquid prior to pouring disinfectant. Revise.

Comments:

This protocol proposes to use a high-density multimodal probe (optoelectrode) on stainless steel to record and manipulate neural activity deep into NHP brains with high spatiotemporal resolution. This interface enables the design of sophisticated stimulation and recording paradigms, and advances the development of new prosthetics. The ability of AAV and AdV to deliver therapeutic and experimental transgenes will be tested. The viral vectors (AAV and AdV) will deliver transgenes to the Central Nervous System (CNS) of Rhesus Macaque monkeys. Optogenetic experiments will be carried out in primary auditory cortex to test and validate the features of the proposed opto-steel-trodes. There were forms issues. Approval is recommended pending minor changes. This protocol is for a project where AAV and Adenoviral vectors will be injected into *Rhesus macaques* to deliver genes for fluorophores, opsins, and ion channels for the purpose of testing an electro-optical brain interface. The AAV will come from a collaborator's laboratory at the University of Pittsburgh and will be injected intracerebrally and CNS tissue will be harvested for *ex vivo* assays. No major concerns are noted although several forms-related issues have been identified. The work is proposed as a BSL-2 project, which appears to be appropriate safety containment. Approval is recommended pending indicated clarifications. Understanding the brain

function and dysfunction in non- human primates (NHP) is crucial to design appropriate interventions for humans. Despite the recent progress in designing neural probes for rodents, designing high-density implantable neural interfaces for NHPs remains elusive, mainly because of the required long form-factor and the expected level of reliability. Here, they propose a high-density multimodal probe (optoelectrode) on stainless steel, for the first time, to record and manipulate neural activity deep into NHP brains with high spatiotemporal resolution. In addition, the integrated high density photonic waveguides with embedded micromirrors offer the significant advantage of artifact-free closed-loop optogenetic stimulation and electrical recording. This novel interface enables the design of sophisticated stimulation and recording paradigms, and advances the development of new prosthetics. They will use established replication deficient AAV - from viral vector cores and from collaborators - to deliver transgenes to the CNS of Rhesus Macaque monkeys that drive expression of opsins, fluorophores, and ion channels, to study the functionality of a new electro- optical brain interface. All work done in the laboratory is in non-human primate *in vivo*. The protocol contains NHP tissues, viruses (two AAV vector rep-def vectors), live NHPs, recombinant DNA at BSL-2 (BSL-2 is for the NHP tissues, AAV vector work is listed at BSL-1). Needs to add the associated IACUC protocol link and one other box regarding helper virus should be unchecked. Recommendation for approval with revisions.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	IBC202000064
Title:	Influenza-Associated Cardiovascular Diseases
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• Section III-D-7 • Section III-D-4 • Section III-F • Section III-D-1 • Section III-D-2 • Section III-D • Section III-D-3
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 8: The investigator will always be notified of all communications from the IBC. Question 8 should be completed if the investigator wishes to have an additional person be notified of correspondence.

2) Primary Cells or Cell Lines: The murine cell line entry (not marked as recombinant) states cells may be from transgenic or knockout mice. Designate these cells as recombinant.

3) Primary Cells or Cell Lines: Other Human cells or cell lines: If recombinant materials will be administered to human cells, the cells should then also be designated as recombinant.

4) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: If using a strain of methicillin-resistant *Staphylococcus aureus* (such as USA300), then designate it as MRSA.

5) Viruses, Prions, or Vectors: Confirm Influenza Virus- C/Taylor/1233/47 cannot infect human cells. Also, confirm is this is wild type (WT) or recombinant.

6) Recombinant or Synthetic Nucleic Acid Usage Section: (*NIH Guidelines*) *Section III-D-4* (Experiments Involving Whole Animals) and *Section III-D-4* (Experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic animals)) should be selected.

7) Recombinant or Synthetic Nucleic Acid Usage Section: (*NIH Guidelines*) *Section III-D-7* should be *un*selected, as this section of the *NIH Guidelines* pertains ONLY to specific strains of Influenza, namely the non-contemporaneous and High Path Avian Influenza viruses, which are classified as Risk Group 3 agents and should be handled with more strict containment practices. *un*select *III-D-7*. It does not appear that this research uses these strains. Revise.

8) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Work with recombinant Influenza Viruses should be described.

9) Exposure Assessment and Protective Equipment, Question 4: Provide details of when working with any BSL-2 agents outside of a Biosafety Cabinet (BSC) that full face protection (goggles and surgical mask) will be worn. In addition, the Animal Gene Transfer section notes the use of needles. Safety-engineered sharps devices are required at BSL-2/ABSL-2. Revise.

10) Exposure Assessment and Protective Equipment, Question 5: Select surgical mask for required PPE for ABSL-2 facilities.

11) Waste Management, Question 1a: Infectious solid waste should be chemically disinfected by spraying with or soaking in an EPA-registered disinfectant prior to disposal of the solid waste in approved biohazardous bags. Revise the responses accordingly.

Comments:
This study will examine the role of viral and bacterial co-infection on cardiac disease in mice. The investigator will generate Adenovirus vectors that are replication defective (E1/E3 deleted) to overexposes cytokines within mice. Then infect with human seasonal and laboratory-adapted Influenza type A, B or C viruses and then with bacteria (*Streptococus*, *Staphlocus* or *E.coli*). The Influenza viruses and bacteria are not described as recombinant and so it is unclear why they are listed in this IBC protocol; this needs clarification from the investigator. There are few details such as the source and antibiotic resistance of the bacterial strains that should also be detailed. However, overall the studies are appropriate at BSL-2 and may be approved. The application examines the connection between Influenza and cardiovascular disease. Mouse and cell culture models will be used. Replication-deficient Adenoviral vectors are used. Multiple strains of infectious Influenza Virus are also used. The investigator will transplant bone marrow from transgenic mice into irradiated mice. The effect on cardiovascular disease after expression is being explored but it is not clear how outcomes will be measured. Additionally, the summary states that Adenoviral systems will be used, but these are not clarified in question 3d. This is a study of impact of Influenza infection on cardiovascular disease and lung pathogenesis in mice. Use of genetic mouse models that will be infected with Influenza strains and recombinant Adenoviral vectors to express cytokines. A variety of cell types will be isolated and used in culture. Additional human and mouse lung cell lines will be used. Influenza Virus are replication competent and can infect humans. The used strains are classified as RG-2 based on the *NIH Guidelines Appendix B-II-D* and *B-III-D*. Thus, the use is recommended under ABSL-2 and BSL-2. The investigator needs to include a statement that the used strains are indeed Risk Group 2 (RG-2) and not RG-3, as some Influenza Viruses are, which would require BSL-3 containment.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	IBC202000065
Title:	Modulating Gene Expression with Virus and ASO
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• Section III-D-4 • Section III-F • Section III-D-1 • Section III-D • Section III-D-3
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Biosafety Summary: Use of tissue is noted in the Project Summary. If tissue will be harvested from mice for analysis, then select the box for Tissues, Blood, or Body Fluids and provide the appropriate entry information.

2) Viruses, Prions, or Vectors: Provide the location for storage and use.

3) Viruses, Prions, or Vectors: Questions regarding infectivity and replication are not answered for Lentivirus (HIV 3-plasmid system), provide a response.

4) Animal Gene Transfer, Question 2: YES should be selected for administration of AAV.

5) Animal Gene Transfer, Question 3: YES should be selected for administration of AAV.

6) Exposure Assessment and Protective Equipment, Question 4: Safety-engineered sharps devices are required for work at BSL-2/ABSL-2; revise.

7) Exposure Assessment and Protective Equipment, Question 5: Select all of the PPE required for entry to the ABSL-2 animal facility (e.g. double gloves, hair bonnet, coverall suit, shoe covers).

8) Waste Management, Question 1a: Infectious solid waste should be chemically disinfected by spraying with or soaking in an EPA-registered disinfectant prior to disposal of the solid waste in approved biohazardous bags. Revise this section accordingly.

9) Waste Management, Question 1c: The sealed bag containing the carcass must be placed in a sealed secondary container labeled with a biohazard symbol.

10) Waste Management, Question 3: The spill should first be contained with absorbent materials, such as paper towels, then the bleach solution may be poured. The contact time should be at least 15 minutes. Revise.

Comments:

This protocol will aim to understand the role of intestinal lipoproteins and apolipoproteins in metabolic disease. Particularly, the studies will aim to understand the role of chylomicrons in IBD, CF and cardiovascular disease. The work will use cell lines and mouse models, as well as intestinal organoids. Gene expression will be manipulated in cells and mouse models using antisense oligonucleotides, AAV- and Lentiviral vectors. Lentiviral vectors will also be used to drive gene expression in isolated murine intestinal enteroids. Murine enterocytes (BSL-2, from the laboratory colony) will be isolated and transduced with Lentiviral vectors. Lentiviral vectors (3-plasmid system, BSL-2, *Addgene*) will be used to deliver shRNA and express various proteins, including PCSK-9, apoC3, LDLr, or CFTR gene. Mouse overexpressing and knockdown organoids will be

generated this way. AAV vectors (currently BSL-2 – no downgrade requested, *Addgene* and Harvard Viral Core) will deliver PCSK9 gene for APOC3 LDLr CFTR. These vectors will be used for an atherosclerosis mouse model. After 1 week, livers will be examined for changes in gene expression. The proposed projects are designed to determine the role of intestinal lipoproteins, and their associated apolipoproteins, in metabolic diseases. The studies proposed here will investigate the mechanisms by which chylomicrons drive inflammatory bowel disease (IBD), intestinal disfunction in cystic fibrosis (CF), and inflammation in cardiovascular disease (CVD). We will use cells and mice as model systems. The use of cells as model systems is necessary to determine the feasibility and relevance of specific hypotheses prior to progressing to mouse model systems. To modify gene expression in cells and in mouse tissues, we will use antisense oligonucleotides (ASOs), Adeno-Associated Virus (AAV), and Lentivirus will be used to transduce gene expression in isolated murine intestinal enteroids. The ASOs are made by *IONIS Pharmaceuticals*. The Adeno-Associated Virus will be obtained commercially as replication-defective host-vector systems. The protocol plans to use primary intestinal enterocytes and organoids. AAV to deliver APO or LDL to mice to induce atherosclerosis, and use of KnockOut (KO) mice. This protocol is focused on gene transfer in cells and mice. The proposed studies are appropriate at BSL-2/ABSL-2. There is a statement about adoptive transfer of T-cells, Tregs, and bone marrow transplant in mice that is covered by another IBC protocol. This should be removed or these cells need to be added to this IBC. There are some minor safety precaution issues including the need for double gloves at ABSL-2. Recommend for approval pending modifications.

Initial comments:
1) Genetically Engineered Animals: Source: Question 3a. Work describes adoptive transfer of T-cells, Tregs and bone marrow. This has not been mentioned elsewhere. Amend or remove.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	IBC202000066
Title:	Perceptual Learning and Plasticity in Auditory Neural Circuits
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• Section III-D-4 • Section III-F • Section III-D-1 • Section III-D
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Tissues, Blood, or Body Fluids: The application indicates there will be administration of tissue, blood, or fluids to animals. If the materials will not be administered to animals, then revise the response.

2) Tissues, Blood, or Body Fluids: The application indicates that rat and mouse tissues will be administered to animals. This information should be included under Description of Usage. If this is incorrect, the answers to those questions should be corrected.

3) Recombinant or Synthetic Nucleic Acid Usage Section: (*NIH Guidelines*) *NIH Section- III-D-4* should be checked for administration of recombinant materials to animals.

4) Live Animals (Related IACUC Protocols), Question 1: Include the linked ARO protocol application.

5) Live Animals, Question 2: Earlier in the protocol it is stated that rat and mouse tissues will be used *in vitro*. Correct the answer here to YES.

6) Genetically Engineered Animals - Source: Answer questions 3 and 4.

7) Exposure Assessment and Protective Equipment, Question 4: Describe if a biosafety cabinet (BSC) will be used when working with BSL-2 materials. Full face protection is required when manipulating these agents outside of a Biosafety Cabinet (BSC). In addition, if sharps will be used for tissue harvest, safety-engineered sharps devices are required at BSL-2/ABSL-2.

8) Exposure Assessment and Protective Equipment, Question 4: Glass pipettes should be placed in a puncture resistant sharps container; revise.

9) Exposure Assessment and Protective Equipment, Question 5: Include all PPE required for entrance to the ABSL-2 animal facility (e.g. face shield, hair bonnet, coverall suit).

10) Waste Management, Question 1b: While the liquid waste is assumed to be small amounts of unused virus, liquids should not be disposed of in biohazard boxes. Liquids can be disinfected with 10% bleach final v/v for 20 minutes and disposed of in the drain.

11) Waste Management, Question 3: Prior to pouring disinfectant on the spill, it should be contained with absorbent materials. Revise.

Comments:

This new IBC protocol will examine perceptual learning and plasticity in auditory neural circuits. In animal studies, transgenic mice or rats expressing specific Cre recombinase will be used. Also AAV-1 vectors will be used to introduce fluorescent reporters and light-gated ion channels into

the rodent brains via intracranial injections. No other virus or oncogenes or toxins will be used in this protocol. All work was claimed to be performed at level of BSL-2/ABSL-1 that are suitable. The investigators need to add detailed information on related IACUC protocols. In this proposal, the investigator seeks to understand neuronal correlates of auditory learning by expressing optogenetic constructs or fluorescent reporters in transgenic rats or mice using intracranial injection of Adeno-Associated Virus vectors from Penn Vector Core, UNC Vector Core and *Addgene*. The viruses (strains AAV-1 and AAV-5) will drive expression using Cre- or Flp-recombinase. Wild-type and transgenic rodents [mice expressing specific Cre- or Flp-recombinase driver lines; rats with BAC vectors to selectively express Cre recombinase in parvalbumin-expressing neurons] will be outsourced; in particular from *Jackson, Charles River, Taconic* and University of Missouri RRRC. Experiments will be conducted in BSL-2 and ABSL-2. The investigator indicated that they submitted the IACUC protocol.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	MOD202000136
Title:	Amendment for **IBC201800204**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• Section III-F • Section III-D-1 • Section III-D-2 • Section III-D • Section III-D-3
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Funding Sources, Question 1a: Provide the Grant Identifier or Award number within the application, not just as a comment.

2) Primary Cells or Cell Lines: An entry for the Sf9 cells should be included within the protocol.

3) Viruses, Prions, or Vectors: Include "in Sf9 cells" to the “Description of Usage” for clarity.

4) Recombinant or Synthetic Nucleic Acid Work Description: Enter the information provided regarding Baculovirus into the actual protocol application.

5) Exposure Assessment and Protective Equipment, Question 4: If the human cells will be manipulated outside of a biosafety cabinet (BSC), full-face protection (goggles and surgical mask or full-face shield) is required; revise.

Comments:

This amendment to a protocol studying parathyroid hormone receptor signaling adds a Baculovirus expression system for recombinant protein expression. The modification request seeks to add the Baculovirus expression system for overexpression of protein. The project studies parathyroid hormone type I receptor signaling via G proteins, which is important for calcium homeostasis and phosphate excretion. The receptor has two activating conformations with differing signaling outcomes. This work aims to get structural, pharmacological and biochemical information about these conformations. The amendment request will add Baculoviral vectors to express the parathyroid hormone receptor in insect cells. The vectors will be generated using the Bac-to-Bac system (*Invitrogen*, BSL-2). Low risk but several issues need to be addressed before approval.

Initial comments:

1) Funding Sources: Complete grant information.

2) Primary Cells or Cell Lines: Most baculoviral vector expression systems use Sf9 or HiFive cells. These should be added if this is the cell type to be used for the expression.

3) Recombinant or Synthetic Nucleic Acids Usage: *Sections III-D-1* and *III-D-2* become necessary as a result of this amendment.

4) Recombinant or Synthetic Nucleic Acid Work Description: Include Baculoviral vector work.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	14
Against:	0
Abstained:	0

Protocol:	MOD202000256
Title:	Amendment for **IBC201600046**
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• Section III-D-4 • Section III-F • Section III-D-1 • Section III-D-2 • Section III-D • Section III-D-3
Additional Documents:	• Vaccinia Signage • Sharps exemption • Safety Manual • 05-010SelectAgent

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 4: Safety-engineered sharps devices are required for work at BSL-2/ABSL-2; revise.

2) Exposure Assessment and Protective Equipment: Question 4: Confirm the location of work with Vaccinia Virus; BST 1003A was not listed in the virus section of the protocol. Provide the appropriate location for work with this agent.

3) Exposure Assessment and Protective Equipment: Question 1: Include Botulium neurotoxin in the response.

4) Exposure Assessment and Protective Equipment: Question 1: Include *P. aeruginosa* in the response.

5) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells may harbor unknown infectious agents.

6) Exposure Assessment and Protective Equipment: Question 5: Include all PPE required for entrance to the ABSL-2 animal facility (e.g. double gloves).

7) Recombinant or Synthetic Nucleic Acid Work Description: Question 2: All toxins should be listed in the response.

8) Recombinant or Synthetic Nucleic Acid Work Description: hSynapsin or hSynaptin?

9) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: For completeness, include a description of work with *P. aeruginosa* in the response.

10) Toxins: Botulium neurotoxin is a Select Agent; even exempt quantities must be registered and the Biosafety Officer must be notified of each purchase/acquisition. Provided the appropriate information to EH&S (https://www.ehs.pitt.edu/sites/default/files/docs/05-010SelectAgent.pdf).

11) Toxins: Indicate how the Botox will be administered.

12) Viruses or Prions: Ecotropic MSCV is appropriate at BSL-1/ABSL-1+. The BSL should be changed in the protocol if a downgrade is being requested.

13) Bacteria, Yeasts, Fungi, or Parasites: *S. epidermis* is listed later in the protocol. It should be entered in the response.

14) Amendment Introduction: The changes requested as written here would suggest both AAV2 and Botox are to be used *in vitro* but the protocol says AAV2 is to be injected into mice. This needs to be re-written so it correctly reflects what will be done.

15) Amendment Introduction: Is it "hSynaptin" not hSynapsin? Is it module not modulate?

Comments:
This is a modification of an existing protocol, requesting addition of AAV vectors that will be administered to mice to express diptheria toxin receptor driven by synaptin promoter to target neurons for subsequent DT mediated depletion. Second change is adding Botox administration intradermally. In this amendment, the investigator wants to add the use of AAV-2 in some mice to ablate SST+ neurons as an alternative to cell line deletion by diphteria toxin; and the use of botulinum toxin by intradermal injection to block neuronal vesicle fusion. This will test whether vesicle fusion in neurons influences the suppression of mast cells. Experiments in the original protocol (see below) are already conducted at biosafety level 2. There are a few comments and clarifications that need to be addressed before approval. In particular, some sections of the protocol have not been updated with the use of the AAV2 and Botox. Importantly, the investigator has requested a biosafety level downgrade for the use of MSCV in the original protocol. If this is still current, the downgrade will need to be addressed and approved by the committee. Regarding the original protocol- The investigator seeks to understand the role of dendritic cells in regulating infections of the skin. *In vivo* experiments use transgenic mice with specific immune cell lines ablated by diphtheria toxin, and fluorescent reporters introduced by BAC cDNA injection. T-cells are transplanted from syngenetic T-cell receptor-transgenic mice, after infecting the T-cells *in vitro* with replication-incompetent murine stem cell virus (MSCV) to express transgenes of interest. Finally, these mice are challenged with pathogenic infections such as *S. aureus* (USA300 or RN4220-2W), *S. pyrogenes*, *P. aeruginosa*, *C. albicans*, *Trichophyton*, *C. albicans*, *A. fumigatus*, and/or Vaccinia Virus (modified to express an ovalbumin-conjugated fluorescent reporter) to measure the influence of ablated cell lines and T-cell-expressed transgenes on the progress of infection. In some cases Staphylococcal toxins are administered to the skin. The experiments are performed at BSL-2 and ABSL-2. The amendment is proposing the addition of AAV2 and Botox to be used *in vitro*. SST-DTR mice will be generated via AAV mediated expression of either loxP-STOP-loxP DTR/GFP or loxP-STOP-loxP GFP under control of hSynaptin. Botox will be used intradermally in mice to module vesicle formation.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	13
Against:	0
Abstained:	1; recused for involvement

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocol:	IBC202000034
Title:	Study of Coronaviruses Using Virus-like Particles and CoV RNA Replicon
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• Section III-F • Section III-D-1 • Section III-D-2 • Section III-D
Additional Documents:	• SHUDA Lab BSL-2+ Manual 2020

Determination: Deferred/Reconsidered

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description: In the investigator's notes in responses to changes requested by the IBC pre-reviewers, the investigator indicates that RNA from SARS-CoV-1 and SARS-CoV-2 infected cells will be obtained from *BEI resources*. Genomic RNA isolated from cells infected with SARS-CoV-1 is regulated as a Select Agent by the Federal Select Agent Program and neither the University nor this investigator are registered for possession and use of these materials (SARS-CoV-1). The investigator must NOT obtain SARS-CoV-1 genomic RNA without prior consultation with the University's RO/AROs for Select Agents. Contact the Department of Environmental Health and Safety (J. Frerotte or M. Sitt-Fischer) for information regarding the Select Agent program.

2) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: A statement should be included in the response that S, E, and M proteins will not be introduced into replicon cells in trans.

3) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-3* should be selected for any cell culture work with viruses.

4) Viruses or Prions: A detailed description of how SARS-CoV-1 and SARS-CoV-2 are replication defective and why they should be downgraded from BSL-3 should be provided under "Description of Usage."

5) Viruses or Prions: Clarify how replication-defective SARS-CoV-1 and SARS-CoV-2 viruses cannot enter human cells.

6) Viruses or Prions: SARS-CoV-1 and SARS-CoV-2 are different viruses and should be listed in separate entries.

7) Protocol Team Members: All personnel working in this research should complete the IBC-specific training to be listed on the personnel page of the application.

8) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells and nonhuman primate cells may harbor unknown infectious agents.

9) Risk Group and Containment Practices: Question 1: Per *NIH Guidelines* regarding SARS-CoV (https://osp.od.nih.gov/biotechnology/interim-lab-biosafety-guidance-for-research-with-sars-cov-2/), RG-3 should be selected.

Comments:
To study the early infection mechanism and identify a potential inhibitor for Coronavirus (CoV) including SARS-CoV2, a causal agent of Covid-19, the investigator will aim to produce pseudovirus particles by expressing structural (S, M, N, and E) proteins from Coronavirus using SARS-CoV2 and SARS-CoV, MERS-CoV cDNA. A) full length CoV cDNA cloned in Bacterial artificial chromosome (BAC) and B) segmented CoV cNDAs cloned in multiple plasmids and BAC. Using the reagents above, they will perform (a) pseudovirus production and establish (b) CoV subgenomic RNA replicon-harbouring cells by using following methods:
(1) There is a report demonstrating that SARS-CoV pseudovirus particles can be produced by expressing structural Spike (S), Membrane(M), Nucleocapsid(N), and Envelope(E) proteins. To produce non-infectious pseudoviral particules, only structural proteins cDNAs will be cloned into expression plasmids and expressed in cell lines that support virus replication and virus production. Psudoparticles will be harvested from supernatant used for experiments to study early steps of viral infection.
(2) Subgenomic viral RNA replicon is non-infectious viral RNA that autonomously replicates in cytoplasm of the viral RNA-transfected cells. CoV subgenomic replicon is successfully established in human CoV229E, SARS-CoV, SARS-CoV2, Mouse Hepatitis Virus 9(MHV).
It is unclear what the investigator is proposing. Are they working with live SARS-CoV2 or are these *in vitro* generated pseudoviruses? The investigator should add additional details in order for a clearer understanding of what is being done and for a proper assessment of biosafety concerns. In the following sections it sounds like the investigator is not working with SARS-CoV2, but rather cDNA for structural and non-structural proteins. Is this the correct selection? It appears that the laboratory is making two things – a pseudovirus with SARS structural proteins that can not replicate so they can study binding, and then they are transfecting replication machinery into cells without the structural proteins to study replication. It is also unclear what makes this research BSL-2+? The investigator already has approval for BSL-2+ with other viruses so that may explain why it is listed in this manner. The investigator is proposed to generate pseudoviruses and replicons of Coronaviruses (CoV) to study early infection mechanisms and potentially identify inhibitors for CoV infection. A number of details are missing and need to be addressed for this protocol to be approved. The investigator does not provide details on how the pseudoviruses are to be made. It is not clear if the investigator is proposing work with SARS-CoV-2 as well as other CoV including SARS-CoV-1 and MERS-CoV. If multiple CoV, what assurances are there that steps will be taken to prevent recombination? Spatial and temporal separation would be considered wise. Some assurances need to be provided that cells will not be transfected with full-length CoV cDNA, and how the investigator will insure cells are not accidentally transfected with full-length cDNA. There is also concern about the investigator's expressed desire to have undergraduate volunteers performing this work. It is unclear how a pseudovirus generated to study early infection mechanisms is considered non-infectious, but this is not the first investigator to make this statement. Some clarity on what they mean by non-infectious is necessary.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Deferred/Reconsideration: Significant concerns and comments were provided by the committee, which will need to be addressed by the investigator prior to placement onto the next IBC meeting agenda for Reconsideration Review.

Supporting documents: None

Votes:

For:	15
Against:	0
Abstained:	0

Protocol:	IBC202000053
Title:	Bar-coded SIVmac239
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• Section III-D-4 • Section III-F • Section III-D-1 • Section III-D
Additional Documents:	• approval letter from EH&S • BSL-2+ protocol and signed cover sheet

Determination: Modifications Required

Required modifications:

1) Exposure Assessment and Protective Equipment: Question 1: Include a statement that nonhuman primate tissues and cells may harbor unknown pathogens.

2) Recombinant or Synthetic Nucleic Acid Work Description: Question 5: SIV is a Lentivirus. The answer to this question should be YES.

3) Viruses or Prions: The source of where the agents were obtains cannot be null. Provide a response to indicate from where the agents were obtained.

4) Viruses or Prions: SIV can infect human cells. Correct the response.

Comments:

The aim of this project is to model human HIV-TB co-infection and use this as a model for HIV-TB-related immune reconstitution inflammatory syndromes (TB-IRIS). Digitally barcoded SIVmac239 will be used to infect *Rhesus macaques* to model human SIV infection. After infection for 6 months, macaques will be infected with barcoded *Mycobacterium tuberculosis*. Work is proposed at BSL-2+ which is appropriate. The work is well-described, and approval is recommended. In this protocol the authors describe a barcoded SIV that will be put into NHP animal models for a tractable disease model. The barcoded virus has been published by authors at the NIH and authors include the link the published paper in the application. The approved IACUC number is provided as is the IBC for the barcoded TB referenced sporadically in the application. All of the BSL-2+ safety requirements seem to be in order. There are minor comments about the PPE for laboratory work and one potential forms issue. Since it is BSL-2+ it is recommended to be discussed, but it is appropriate for approval.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	15
Against:	0
Abstained:	0

Protocol:	IBC202000056
Title:	HIV-1 Pathogenesis
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• Section III-D • Section III-D-3
Additional Documents:	• BSL-2+ Manual (REDACTED lab)-signed

Determination: Modifications Required

Required modifications:

1) Waste Management: Question 1b: The decontamination time should be 20 minutes; correct the response.

2) Exposure Assessment and Protective Equipment: Question 4: Double gloves are required for work at BSL-2+. Select double gloves in the PPE checklist in the question.

3) Exposure Assessment and Protective Equipment: Question 1: Include a statement that human cells may harbor unknown pathogens.

4) Protocol Team Members: Since this research is conducted under BSL-2+, then all of the laboratory personnel must be listed in the personnel section of the application.

5) Recombinant or Synthetic Nucleic Acid Work Description: Question 3: The application describes the use of Lentiviral vectors to express miRNA. Thus, the answer to this question should be YES.

6) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Describe work with all recombinant nucleic acids, including HIV-1 vectors and viruses.

7) Recombinant or Synthetic Nucleic Acid Work Description: Question 2: Clarify that HIV-1 proteins Vpr and Nef are oncogenes. If that is incorrect, remove the agents from the response.

8) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-1* should also be selected for use of a Lentivirus to express a non-viral protein in cells.

9) Viruses or Prions: It appears that the protocol will use both replication-competent HIV-1 (listed below) and replication-defective HIV-1 ("The Lentivirus uses multiple components such as gag-Pol, VSV-G-Env, Rev and miRNA expression vector"). Include a separate entry for the latter and describe all inserted nucleic acids under "Inserted Nucleic Acids Information."

Comments:

HIV-1 viral accessory genes will be cloned into the mammalian expression vector system (pcDNA 3.1) and transfected into eukaryotic cells to study the effect of these gene products individually. This is not for use in animals. This is requested at BSL-2+, which requires convened review. This protocol is to examine the role HIV viral replication in a wide variety of cells lines, no animals. Viral proteins will be expressed using expression vectors (pCDNA), and GFP HIV viruses will be constructed and worked at BSL-2+. Additional information on the virus is needed, as well as a signed Biosafety Operations Manual.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	15
Against:	0
Abstained:	0

Protocol:	MOD202000189
Title:	Amendment for **IBC201800077**
Investigator:	REDACTED
Highest BSL:	BSL-2
NIH Guidelines:	• Section III-D-4 • Section III-F • Section III-D-1 • Section III-D-2 • Section III-D • Section III-D-3
Additional Documents:	• REDACTED IACUC Protocol 2018 Updated 11.2.2018

Determination: Modifications Required

Required modifications:

1) Supporting Documents: If viruses expressing both the Cas9 and gRNA are used in the study, a signed laboratory operations manual should be uploaded.

2) Waste Management: Question 1a: 70% ethanol is not an EPA-registered disinfectant and must not be used as a primary means of decontamination of solid materials. Revise to remove reference to 70% ethanol as a primary disinfectant.

3) Exposure Assessment and Protective Equipment: Question 1: It is unclear how it is known that human iPS cells may not contain unknown pathogens. In addition, the application states that infectious viruses will be administered to iPS cells. Thus, it is suggested that the last sentence of the response be modified.

4) Risk Group and Containment Practices: Question 2: If viruses expressing Cas9 and gRNA are used in the study, then BSL-2+ should be selected.

5) Recombinant or Synthetic Nucleic Acid Work Description: Question 3e: This question asks if viral vectors are used to express CRISPR/Cas9 and guide RNA, are they on the same vector or separated. Per University of Pittsburgh IBC guidelines, expression of CRISPR/Cas9 and gRNAs from the same viral vector requires use at BSL-2+. This includes Adenovirus and AAV, which the investigator has indicated express Cas9. Clarify the response. If the viral vectors are used with CRISPR/Cas9 and the guide RNA, then make appropriate revisions to the application throughout.

6) Viruses or Prions: If Cas9 and gRNA are expressed from the same viral vector, then they should be used at BSL-2+. Correct the BSL designation(s) where appropriate.

7) Funding Sources: In addition to the grant number, the funding source should be identified.

Comments:

Modifications required with Member Review required post-revisions submitted. The investigators have made a modification where they will use of CRISPR/Cas9 for heart muscle regeneration at BSL-2. Adenovirus will be used for *in vitro* and *in vivo* work at ABSL-2. This modification is linked to their animal protocol and Lentivirus will be used for stable cell line generation. There are some minor forms issues but in general, the research for CAS9n should be conducted at BSL-2+. Additionally, Lentiviral stable cell lines will only be used after passage 2 which may be of concern. The modification adds new vectors for imaging analysis and adds the use of CRISPR/Cas9 to edit gene expression in a study on mechanisms of heart muscle cell regeneration. Human heart muscle samples and murine and rat heart samples will be used at BSL-2. A variety

of mammalian stem cells including human stem cells will be used with plasmids or Adenovirus at BSL-2, and none will be transferred to animals. *Escherichia coli* will be used at BSL-1 for plasmids. Adenoviral and AAV vectors will be used for expression of a variety of proteins for imaging and genetic manipulation such as Cre and Cas9 at BSL-2 – this should probably be BSL-2+. Lentivirus will be used to make stable cell lines, but only 2 passages will be performed prior to experimental use. A variety of genetically modified mice will be used and will be injected with Adenovirus and AAV at ABSL-2.

Review comments were provided to the investigator for response. The revised application was placed on the June agenda.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	15
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:23 AM

University of Pittsburgh
Institutional Biosafety Committee (IBC) Meeting Minutes

June 22, 2020 10:00 AM

Meeting; ZOOM teleconference meeting
SARS-CoV-2 protocols only

VOTING MEMBER ATTENDANCE			
Name of Regular/Alternate Member	**Status**	**Substituting For**	**Present by Teleconference?**
REDACTED, Chair			Yes
REDACTED, Vice Chair	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED	Late: 10:20		Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED			Yes
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		
REDACTED	Absent		
REDACTED			Yes
REDACTED	Absent		

NON-VOTING MEMBER ATTENDANCE	
Name of Regular/Alternate Member	**Role of Non-Voting member**
REDACTED	IBC Office
REDACTED	IBC Office
REDACTED	EH&S Office
REDACTED	IACUC Office

REDACTED	ORP Co-Director

GUEST NAMES

None

QUORUM INFORMATION

Committee members on the roster:	26
Number required for quorum:	5
Meeting start time:	10:00 AM

The Chair called the meeting to order. A quorum of members was present.

All members received all pertinent material before the meeting and were able to actively and equally participate in all discussions via the tele-meeting portal.

REVIEW OF MEETING MINUTES from Previous IBC Meeting

Since this was the last (fifth) "emergency" meeting set up to discuss SARS-CoV-2-specific protocols and not regularly scheduled, the standard June meeting minutes were not reviewed. They will be reviewed at the July meeting.

ENVIRONMENTAL HEALTH AND SAFETY REPORT

None reported

IBC OFFICE REPORT

No report, as this was an "emergency" meeting outside of the regularly scheduled meetings to discuss specific protocols involving research with the Corona Virus.

NON-COMPLIANCE, EVENT, INJURY REPORT

The committee reviewed and discussed an incident report that described a proposed "fee for service" viral core facility that had not yet obtained IBC approval for the production and distribution of the viral materials as a "core" facility. The investigator clarified that the core work had not been started and that they were in the processes of creating and submitting an application for the approval for the core facility. The committee determined that as the work had not yet been started, and that there was timely intervention that no non-compliance had occurred. Since there had been no non-compliance, the committee determined that the incident was not reportable to the NIH.

Votes:

For:	11
Against:	0
Abstained:	0

The incident also invoked a discussion of "unconscious bias". There has been a request to have committee membership undergo the University training program for all committee members. The IBC Office will be removing investigator names from all future Incident Reports so as to "blind" the committee for any perceived biases to ensure that there is no biases during review. Training information will be provided to the committee as it will be scheduled.

REVIEW OF SUBMISSIONS – LOW-RISK REVIEW SUBMISSIONS

This meeting was scheduled to accommodate only protocol applications involving SARS-CoV-2. All other protocol applications will be assigned to the next regularly scheduled agenda.

REVIEW OF SUBMISSIONS - CONVENED DISCUSSION SUBMISSIONS

Protocol:	IBC202000069
Title:	Study of Coronaviruses Using Pseudovirus Particles and CoV RNA replicon
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• NIH Section III-D-1 • NIH Section III-D-2 • NIH Exempt: Sections III-E or III-F
Additional Documents:	• REDACTED 2019 lab manual

Determination: Modifications Required

Required modifications:

1) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include the information provided from the investigator's response that indicates that "viral RNA will never be transduce into cells to avoid a risk of infectious virus production" to the protocol information within the application itself, so that the application itself is updated.

Required modifications from the May 26th meeting (Deferred/Reconsidered):

1) Primary Cells or Cell Lines: For the Source under "Cell/CellLine - Other Human cells or cell lines"; clarify if the cells were obtained from a vendor or a collaborator.

2) Exposure Assessment and Protective Equipment: Question 1: Revise the wording to indicate the CoV systems used are replication-defective (i.e. still able to infect/enter cells).

3) Exposure Assessment and Protective Equipment: Question 1: Describe all agents that can infect human cells. In addition, human and non-human primate cell lines may harbor endogenous viruses that are not identified and these may have some risk, albeit low, to humans. Even well-established human cell lines may harbor unknown human pathogens. Revise the response.

4) Exposure Assessment and Protective Equipment: Question 5: BSL-2+ laboratory garb requirements include use of a solid-front wrap-around gown.

5) Recombinant or Synthetic Nucleic Acid Work Description: Clarify how full length CoV cDNA cloned in Bacterial Artificial Chromosome (BAC) will be used. This has the potential to produce replication-competent virus, which would require use at BSL-3.

6) Recombinant or Synthetic Nucleic Acid Work Description: Provide a description of the use of the SARS-CoV1 HSR and Urbani strain, MERS-CoV EMC2012 strain, and SARS-CoV2 WA1 and Wuhan strains that will be obtained from *BEI Resources*. The experiments described may need to be conducted in a BSL-3 facility for safety testing of the non-functional replicons.

7) Recombinant or Synthetic Nucleic Acid Work Description: Question 1: Identify the collaborators that will provide the cDNA.

8) Recombinant or Synthetic Nucleic Acid Work Description: What steps will the laboratory take to insure there is not an accidental transfection of cells with full length CoV DNA? Will there be spatial and temporal separation between working with cDNA of SARS-CoV, SARS-CoV-2 and MERS-CoV to prevent any possibility of recombination?

9) Recombinant or Synthetic Nucleic Acids Usage: Clarify why *Section III-D-1* (Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems) is checked.

10) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-3* should be checked.

11) Viruses or Prions: Clarification regarding the specific materials to be acquired from *BEI Resources* is needed. All three viruses listed, SARS-CoV-1, SARS-CoV-2, and MERS-CoV, are risk group 3 agents and the investigator does not have an approved BSL-3 laboratory for work with these viruses. Of critical importance, SARS-CoV-1 and any isolated nucleic acids/full genomes, are regulated by the Federal Select Agent Program and the University is not approved for possession of these materials. Indicate the specific materials and provide the *BEI Resources* catalog number for the reagents to be acquired.

12) Viruses or Prions: Clarify how the pseudoviruses cannot enter cells, yet the "Description of Usage" states, "To study CoV infection entry. . . ." Note that viral infectivity/entry is different than viral replication.

13) Viruses or Prions: SARS-associated Coronavirus - SARS-CoV1 HSR and Urbani strain, MERS-CoV EMC2012 strain, and SARS-CoV2 WA1 and Wuhan strains: These viruses should be designated as recombinant if they are not the wild type strain.

14) Viruses or Prions: Provide the *BEI Resources* catalog number for each strain being obtained from the company. Confirm that the investigator or laboratory will not receiving virus as this work would need to be conducted in a BSL-3 facility.

- Note that SARS-CoV-1 AND full-length isolated RNA are both regulated as Select Agents; the University is not registered for possession of these agents.

15) Viruses or Prions: Since it appears that the investigator will be working with full length CoV cDNA, how will they insure that they do not transfect cells with full length cDNA? Also the application states that the agent cannot enter or infect human cells, but replicons are capable of infecting cells and undergoing limited replication, yes? Clarify.

16) Risk Group and Containment Practices: Question 1: Risk Group 3 (RG-3) should be selected.

17) Basic Information: Question 8: The investigator will always be notified of all communications from the online IBC system. The investigator will receive all correspondence and cannot opt out of the correspondence.

Comments:
The investigators plan to produce pseudotyped viruses and replicons of coronaviruses (CoV). These will be used to study virus attachment and entry, as well as immunology including the

potential use as a vaccine. The protocol does not indicate how the vaccine potential will be evaluated and there are no indications that any work will be done in animals or humans, this is strictly *in vitro* work. They indicate they will be working with full length cDNA from SARS-CoV, SARS-CoV2, and MERS-CoV to generate the pseudotype viruses and replicons. Replicons will lack the S, M, and E genes. They do not indicate how they will insure they do not accidentally transfect cells with full length cDNA. There is no plan for prevention of cross-contamination or recombination between the viruses. There is also a question of how non-infectious pseudotype viruses will be used to study virus attachment and entry; key steps in the infection process. These points need to be clarified and explained before the protocol can be approved. The investigator has submitted a protocol to produce pseudovirus particles and replicons of SARS-CoV-1, SARS-CoV-2, and MERS-CoV. Work will be conducted *in vitro* and plasmids will be propagated in *E. coli*. Forms issues have been identified, particularly sources of agents, and will need to be revised. Safety issues/questions must be clarified and addressed prior to approval.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the May 26th emergency meeting for review.

May meeting: Deferred/Reconsideration: The committee recommended for Reconsideration for the research after the investigator provides responses and clarifies information about the agents that are planned to be used. The investigator provided a revised application to address the comments provided, and the protocol was placed onto the June 22nd agenda for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	0

Protocol:	IBC202000084
Title:	SARS-CoV-2 Vaccine
Investigator:	REDACTED
Highest BSL:	BSL-2+
NIH Guidelines:	• Section III-D-4 • Section III-D-4 • Section III-D-1 • Section III-D
Additional Documents:	• PIIS2352396420301183

Determination: Modifications Required

Required modifications:

1) Waste Management, Question 1b: Liquid waste should be decontaminated using a 1:10 v:v bleach:final volume liquid waste. Revise.

2) Supporting Documents: The investigator should upload a signed copy of the most recent version of the laboratory Biosafety Operations Manual.

Comments:

In this proposal, the investigator seeks to test the safety and effectiveness of a nasally-delivered Retroviral vaccine for SARS-CoV2 in a non-human primate (NHP) model. The candidate vaccine is a strain of Adv-5 Adenovirus that expresses the SARS-CoV2 Spike protein, and is replication-defective (Ad5.SARS-CoV-2-S1 from a collaborator's laboratory). Two populations of *Rhesus macaques* will be given the virus intranasally: control (healthy) individuals and NHPs infected with Simian Immunodeficiency Virus (SIV) and treated with antiretroviral (ARV) drugs. A third group of SIV-infected NHPs treated with the same ARV drug regimen will be administered with a 'sham' vaccine. This latter requires clarification. Blood and other tissues will be collected to measure the generation of antibodies against SARS-CoV-2-S1. In this protocol, the investigator will study a replication-defective Adenovirus encoding the SARS-CoV-2 Spike protein as a vaccine in SIV-infected rhesus macaques. Tissues, blood, and fluids will be obtained from the animals for *in vitro* assays. As the SIV is replication-competent, all work will be performed at BSL-2+/ABSL-2+, which is appropriate. No Coronavirus will be used in the protocol. A "sham" vaccine will also be administered to some animals and it is not clear what this means. Multiple form issues and clarifications should be made prior to approval at BSL-2+.

Review comments were provided to the investigator for response. The revised application was placed on the June 22nd emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	11
Against:	0
Abstained:	0

Protocol:	MOD202000287
Title:	SARS-CoV-2 Spike Protein (**IBC201700141**)
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• Section III-D-1 • Section III-D-3 • Section III-D-4 • Section III-F
Additional Documents:	None

Determination: Modifications Required

Required modifications:

1) Waste Management: Question 1b: Revise the response statement to indicate that liquid wastes will be adjusted to 1:10 v:v bleach:infectious waste. Addition of 1:10 v:v bleach:water to liquid waste results in further dilution of the active disinfectant ingredient leading to insufficient inactivation of potentially infectious materials.

2) Exposure Assessment and Protective Equipment: Question 1: Correct the statement regarding Lentiviruses. Although the Lentiviruses described in the protocol are replication-defective, they still can infect human cells.

3) Recombinant or Synthetic Nucleic Acids Usage: *Section III-D-2* should be selected for expression of SARS-CoV-2 Spike protein in bacteria.

4) Viruses or Prions: Lentivirus: Infectivity and replication information is missing. Provide the information under the question "Additional Virus Information".

5) Viruses or Prions: AAV: Infectivity and replication information is missing. Provide the information under the question "Additional Virus Information".

6) Tissues, Blood, or Body Fluids: For Tissues: If the tissues are from genetically modified animals and/or animals that have received recombinant materials, then they should be identified as recombinant.

Comments:

This amendment is being submitted to add expression of the SARS-CoV-2 Spike protein to an existing protocol investigating therapeutic strategies for cerebral ischemia, novel analgesic pathways, and mechanisms underlying analgesic function. Their work will use frogs and frog eggs in electrophysiology experiments, rats and mice for *in vitro* and *ex vivo* experiments, human stem cells and cell lines, and rat and mouse cell lines. *E. coli* will be used for protein expression and DNA manipulation, and AAV, 4-plasmid Lentiviral vectors, and HSV will be used for viral transduction into animals and cells. None of the expressed proteins appear to be oncogenes. siRNA/shRNA are indicated as being used with the viral vectors but this is not included and the targeted genes are not indicated in the biohazard summary. The work is proposed at BSL-2/ABSL-2, which appears to be appropriate. Approval is recommended pending recommended changes and clarifications. The investigator proposes to express the SARS-CoV-2 Spike protein using mammalian vectors in cells at BSL-2. These vectors and modified cells will not be administered to mice. The biosafety risk is minimal. Recommendation for approval pending a few form corrections.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the June 22nd emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD202000305
Title:	Covid-19 Proteins on Necroptosis (**IBC201800224**)
Investigator:	REDACTED
Highest BSL:	BSL-2+ ABSL-2
NIH Guidelines:	• Section III-D-1 • Section III-D-2 • Section III-D-3 • Section III-D-4 • Section III-F
Additional Documents:	• BSL-2+ manual 2019

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Work with SARS-CoV-2 proteins should be briefly described.

2) Primary Cells or Cell Lines: Infection of cells with BSL-2+ viruses should be performed at BSL-2+. Correct the responses in the application.

3) Primary Cells or Cell Lines: Include the COVID transfection/transductions to the description of usage for the cells entries and specify it won't be used for *in vivo* research experiments.

4) Viruses, Prions, or Vectors: Lentivirus (HIV 3-plasmid system): Questions regarding infectivity and replication competence should be answered.

5) Viruses, Prions, or Vectors: Retrovirus (MESV): It is stated earlier in the protocol that Retroviruses will be used to deliver shRNA to cells. Include shRNA in "Inserted Nucleic Acids Information."

6) Recombinant or Synthetic Nucleic Acids Usage: *NIH Section III-D-4* should be marked for use of animals and use of transgenic animals.

7) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Describe the new work that is being added to the protocol.

Comments:

This is a modification to include Lentiviral vectors expressing individual SARS-CoV-2 proteins in human or mouse cells for *in vitro* experiments. These experiments will be performed at BSL-2, which is appropriate. Forms issues noted should be corrected prior to approval. This is an amendment to determine if SARS CoV2 proteins affect necroptosis in mammalian cells. They propose to use transient and Lentiviral transfection- genes of interest will be introduced into standard human and mouse cell lines then stimulated with agents to initate necroptosis. All work is *in vitro*, no animal work is proposed. Single genes will be expressed at a time, and no complete virus is used. The work can be done at BSL-2, the laboratory area is approved for BSL-2+. Other than some form issues the amendment can be approved.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, the protocol application was placed onto the June 22nd emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

Protocol:	MOD202000338
Title:	SARS-CoV-2 Recombinant S1 Protein and Vaccine (**IBC201800310**)
Investigator:	REDACTED
Highest BSL:	BSL-2 ABSL-2
NIH Guidelines:	• Section III-D-1 • Section III-D-2 • Section III-D-3 • Section III-D-4 • Section III-F
Additional Documents:	• Transfer permit • BH460 Host strain for protein purification • HBL purification

Determination: Modifications Required

Required modifications:

1) Basic Information, Question 3: Information about SARS-CoV-2 should be included in the Project Summary.

2) Tissues, Blood, or Body Fluids: Include information regarding SARS-CoV-2 reagents used in mice.

3) Primary Cells or Cell Lines: Include information regarding cell work with VSV, Adenoviruses, and Lentiviruses in the application. This includes production of Lentiviruses.

4) Bacteria, Yeasts, Fungi, or Parasites/Invertebrates: *Bacillus anthracis* - BH500: clarify what this statement means: "The strain can used as a host strain to make SARS-CoV-2 recombinant S protein vaccine." Does this mean that this bacterium will encode SARS-CoV-2 proteins? Clarify.

5) Viruses, Prions, or Vectors: Lentivirus (HIV 4-plasmid system) - HIV-based Vector (3rd generation): It is recommended that the investigator clarify that Cas9 and gRNA will be encoded in separate viruses under "Description of Usage."

6) Viruses, Prions, or Vectors: Vesicular Stomatitus Virus (VSV) - Pseudotyped ΔG-GFP (G*ΔG-GFP) rVSV: clarify how the virus is unable to enter or infect human cells. If this is incorrect, modify the response.

7) Viruses, Prions, or Vectors: Lentivirus (HIV 4-plasmid system) - HIV-based Vector (3rd generation): Questions regarding infectivity and replication should be answered.

8) Viruses, Prions, or Vectors: Lentivirus (HIV 4-plasmid system) - HIV-based Vector (3rd generation): Cas9 and gRNA should be included under "Inserted Nucleic Acids Information."

9) Toxins: Define "HBL toxin" in the application.

10) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Describe all recombinant and synthetic nucleic acid work involving animals in the response.

11) Recombinant or Synthetic Nucleic Acid Work Description, Question 1: Include VSV in the response.

12) Recombinant or Synthetic Nucleic Acid Work Description, Question 2: Include *Clostridium perfringens* enterotoxin, Diphtheria toxin, Anthrax toxin, Anthrax lethal toxin, HBL toxin, and *Bacillus cereus* non-hemolytic enterotoxin in the response.

13) Animal Gene Transfer, Questions 6 and 7: The following agents are described earlier in the protocol as being administered to animals and should be included in the response: Anthrax toxin, Anthrax lethal toxin, HBL toxin, and *Bacillus cereus* non-hemolytic enterotoxin.

14) Exposure Assessment and Protective Equipment, Question 1: Include VSV in the response.

Comments:
The amendment is to develop rVSV viruses expressing SARS-CoV2 S1 protein. As mentioned, the SARS-CoV2 work should be added to the summary and the rVSV recombinant Pseudovirus was incorrectly described as not being able to infect human cells but the cell line being used to propagate it is not described. A more complete description in the use of recombinant DNA would be helpful. This is an amendment to add *Bacillus anthracis* strains expressing SARS-CoV-2 Spike protein, which will be administered to mice, and Vesicular Stomatitis Virus Pseudotyped with the SARS-CoV-2 Spike protein, which will be used *in vitro* only. Both will be used at BSL-2/ABSL-2, which is appropriate. Several additional clarifications and forms issues should be corrected regarding the addition of these reagents to the protocol. In addition, the protocol describes the use of Lentiviral vectors for expression of CRISPR/Cas9 in cells. The investigator incorrectly states that the vector used (pSpCas9(BB)-2A) does not express both Cas9 and gRNA. These viruses require BSL-2+, not BSL-2.

Due to the mandate for fast-track processing of protocols for work with the CoViD-19 agents, there was no time to provide the reviewer comments to the investigator prior to the emergency meeting. The protocol application was placed onto the June 22nd emergency meeting for review.

Modifications Required: Additional comments were provided by the committee, which will need to be addressed by the investigator prior to final IBC approval.

Supporting documents: None

Votes:

For:	12
Against:	0
Abstained:	0

NEW BUSINESS

With no other business, the meeting was adjourned.

Meeting end time: 10:37 AM