Rutgers University
Institutional Biosafety Committee (IBC) – Central Campus
Meeting for NIH Guidelines Materials
Minutes of August 6, 2025

1. ATTENDEES

☐ Carol Bagnell	☐ Bhupinder Singh	☒ Blas Peixoto - REHS
☐ Nada Boustany	☐ Milind Shah	☒ Robert Adcock - REHS
☐ Jeffrey Boyd	☐ Matthew Ferguson – Local Non-Affiliated	☒ Jacquelyn Vidal - REHS
☐ Qian Cai	☐ Ellen Welch – Local Non-Affiliated	☒ Sophia Cheng - REHS
☒ Julie Caruth	☒ Thomas Boyle – Local Non-Affiliated	☒ Nancy Connell
☒ Richard Ebright	☐ James Clancy – Local Non-Affiliated	☒ Preeti Bharaj
☒ Zhaohui Feng	☐ Jeetendra Eswaraka – Ex Officio	☐
☒ John Hershey	☐ Alejandro Ruiz – Ex Officio	☐
☒ Peng Jiang	☐ Bryan Bocco – Ex Officio	☐
☒ Eric Klein	☒ Ron Hart – Co- Chair	☐
☒ John McLaughlin	☒ Ryan McAllister - REHS	☐
☒ Donald Schaffner	☒ Brian Eggert - REHS	☐

2. MEETING LOGISTICS

CURRENT MEETING		
Called to Order: 12:09 pm	**Adjourned:** 12:52 pm	**Location:** WebEx
PREVIOUS MEETING		
Minutes from June 4, 2025		**Approved (11:0:0)[1,2]**
NEXT MEETING		
Date: October 1, 2025	**Time:** 12:00 pm	**Location:** WebEx

CONFLICT OF INTEREST STATEMENT
Committee members with a conflict of interest related to the review of a specific registration may not be involved in the review or approval of a project in which he or she has been or expects to be engaged or has a direct financial interest.

3. PRE-AGENDA

TOPIC	SUMMARY

IBC Member Education:
 	
NIH Transparency
	NIH Implementation Update: Promoting Maximal Transparency Under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
	• Communication received March 28, 2025
	• To maximally meet the transparency aims of the NIH Guidelines on June 1st 2025:
	  ○ The NIH OSP will publicly post the rosters of all active IBCs registered with OSP via the IBC-Registration Management System
	  ▪ These rosters will include all members identified by name and role on the committee.
	  ▪ In addition, NIH will be posting the contact information for:
	  • IBC Chair
	  • Biological Safety officer
	  • IBC Contact
	  ○ NIH expects that approved meeting minutes from all IBC meetings occurring on, or after this date will be posted publicly on an institutional website.
	  ▪ NIH's expectation that minutes will be posted immediately after approval and once all appropriate and allowable redactions have been made
	  ▪ Please note that the provisions of this memo only apply to meetings taking place on, or after June 1, 2025. Minutes from meetings before that date do not need to be posted but still must be provided to members of the public upon request
	  ▪ Minutes must remain publicly available for 5-years
	• The June IBC minutes will be the first document posted to the Rutgers IBC website after approval by the IBC.

	• REHS will be asking PI to provide a submission summary that meets the NIH expectations and to assist reviewers in providing their review summary

IBC Member Education:
 	
Presidential Executive Order: Improving the Safety and Security of Biological Research
	Expect New/Revised Executive Orders
	• Framework for Nucleic Acid Synthesis Screening
	  ○ Within 90 days of the date of this order (8/3/25), the Director of OSTP, in coordination with the APNSA and the heads of relevant agencies, shall revise or replace the previous EO
	• Dual Use Research of Concern Policy
	  ○ #4a) Within 120 days of the date of this order (9/2/25), the Director of OSTP, pursuant to 42 U.S.C. 6627 and in coordination with the APNSA and the heads of relevant agencies, shall revise or replace the previous EO
	• Within 180 days of the date of this order (11/1/25), the Director of OSTP, in coordination with the Director of the Office of Management and Budget, the APNSA, the Assistant to the President for Domestic Policy, and the heads of the other relevant agencies, shall develop and implement a strategy to govern, limit, and track dangerous gain-of-function research across the United States that occurs without Federal funding and other life-science research that could cause significant societal consequences
	Framework for Nucleic Acid Synthesis Screening
	• No updates provided as of 8/6/2025 at 8:30 AM
	• Updates will be provided at a future IBC meeting once the new Executive Order becomes available

New Business:
 	
July 2025 Newsletter
	July 2025 Biosafety Newsletter
	• Anticipated release dates (quarterly)
	  ○ January
	  ○ April
	  ○ July
	  ○ October
	• Sent out via RAIN List and BPMS-registered PIs and Administrators
	October 2025 Newsletter Will Include
	• NIH Implementation Update: Promoting Maximal Transparency Under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
	• Submission Summaries from PIs related to their IBC submissions

New Business: **IBC Handbook Updates**	• REHS is in the process of removing IRE (aka DURCom) responsibilities from IBC Handbook • DURC reviews will happen by the PI & Biosafety Office through the Grant Submission and IBC Pre-Review • IBC will only review DURC identified projects once Risk-Benefit Analysis and Risk Mitigation Plan are reviewed and approved by the funding agency • IBC can require enhancements to the Risk Mitigation Plan • IBC will not be involved in the Risk Mitigation Plan, research findings communications aspects • Details related to Roles and Responsibilities are being developed to be approved by Rutgers Office for Research • IBC Charge (aka Charter) is being developed based on IBC Handbook language

PROTOCOL REVIEWS

The following protocols were reviewed according to the risk assessment guidelines published in the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* and the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*. The risk assessment is documented in the REHS Biosafety Protocol Management System and includes a review of the engineering controls, work practices, safety training, and medical surveillance of project personnel. Individual protocols are evaluated on the following matters as appropriate: the proposed biosafety level and safety practices, agent characteristics, source and nature of agents or recombinant/synthetic nucleic acid sequences and resulting effects of expressed proteins, host animals/ cells, and cloning vectors to be used, and the type of manipulations planned.

Note: Protocols were not necessarily reviewed in the order they appear below.

1. ADMINISTRATIVE APPROVALS

PROTOCOL	PI	MATERIAL(S) OF INTEREST	BSL
23-006	Martinez Zamudio, Ricardo	Amendment to update personnel and complete new Addendum K	2
13-566	Bello, Nicholas	Renewal without changes	2
16-004	Bartlett, Paula	Renewal with minor changes	2
13-273	Chang, Li Yun	Renewal with minor changes	2e
12-209	Pare, Denis	Amendment to update locations	2
20-042	Aston-Jones, Gary	Amendment to change the PI from Morgan James to Gary Aston-Jones	2
21-038	Kinz-Thompson, Colin	Amendment to update grant number and complete new Addendum K	1
22-032	Madireddy, Advaitha	Renewal without changes	2

13-097	Feng, Zhaohui	Amendment to add human organoids for use with a lenti-CRISPR library already in the protocol	2
22-037	Klein, Eric	Renewal without changes	2
19-058	Solesio Torregrosa, Maria	Renewal without minor changes (housekeeping edits)	2
22-003	Wang, Jun	Renewal without changes	2

2. ADMINISTRATIVE TERMINATIONS			
PROTOCOL	**PI**	**TITLE OF PROTOCOL**	**EXPIRY DATE**
None			

3. BIOSAFETY OFFICER REPORT (BSO) Approved (12:0:0) [1]			
PROTOCOL	**PI**	**TITLE & MATERIAL(S) OF INTEREST**	**BSL / GUIDELINES**
16-004	Bartlett, Paula	**Title:** Cell culture and transfection with cDNA and Adenovirus **Materials:** Mice, AAV **Submission Summary:** The goal of this project is to test the hypothesis that attenuated Ca2+ signaling induced by high fat diet results from elevated novel Protein Kinase C (PKC) activity. The project is previously approved for viral vector work in vitro and in vivo, specifically AAV Cre for in vivo knockdown and Lentivirus siRNA delivery to murine liver to silence PKC. This amendment adds a new flox mouse line for AAV Cre knockout of PKC to replace the previous Lentivirus mechanism and a calcium reporter delivered via AAV. The purpose of these new materials is to assess PKC's effect on calcium levels in the liver and how this influences hormone-generated calcium oscillations. Endpoints remain unchanged from previous approvals murine liver will be harvested for biochemical studies and hepatic mouse embryonic fibroblasts will be isolated for signal cell imaging studies for calcium levels. **Occupational Health:** In Place **Training:** In Place **BioAudit:** Facilities are Acceptable	1 / III-D-4

15-103	Scotto, Kathleen	**Title:** Cell culture and transfection with cDNA and Adenovirus **Materials:** Human cells, CRISPR/Cas9 **Submission Summary:** The goal of this project is to understand the mechanism for ABCG2 regulation of cell survival in the face of stress inducers ((i.e. nutrient deprivation, ionizing radiation, rapamycin) and identify possible therapeutic targets through the investigation. The project is previously approved for in vitro CRISPR/Cas9 genetic editing of human cell lines. This amendment adds human placental trophoblast cell lines and genetic editing of the cells via CRISPR/Cas9 for ABCG2 knock out. ABCG2 is a transporter involved in moving drugs and urate across cell membranes, out of cells. Once established, these cells, both WT and KO clones, will be subjected to various previously approved in vitro cell-based assays, including protein/RNA analyses, Forskolin-induced differentiation assay, in vitro migration assay, RNA-immunoprecipitation, western blotting, and qRT-PCR. **Occupational Health:** In Place **Training:** In Place **BioAudit:** Facilities are Acceptable	2 / III-D-2

4. AD HOC MEETING APPROVALS			
PROTOCOL	**PI**	**TITLE & MATERIAL(S) OF INTEREST**	**BSL**
None			

5. NEW PROTOCOLS			
PROTOCOL	**PI**	**TITLE & MATERIAL(S) OF INTEREST**	**BSL / GUIDELINES**
25-020	Deaconescu, Alexandra	**Title:** Structural Studies of Stress Responses **Materials:** Escherichia coli **Submission Summary:** Our main objective is to dissect mechanisms of DNA repair and response to stress signals using the tool of structural biology and	1 / III-E

		biochemistry. To this end, we are producing, purifying, characterizing and crystallizing different proteins and protein complexes involved in these processes. Our starting material is generally genomic DNA (cDNA) that is amplified via PCR and cloned into bacterial (E. coli) expression vectors. More specifically, we are working with non-infectious E.coli K12 Derivative Strains (DH5-alpha, Top10) for cloning and BL21, BL21 (DE3) and BL21(DE3) Rosetta2 strains for protein production. Additionally, we obtain our DNA via gene synthesis from outside vendors (we do not carry out DNA synthesis in the lab). Protein purification is achieved using various chromatography techniques. We then use functional characterization such as using fluorescence anisotropy, UV-VIS spectroscopy, gel shifts, SDS-PAGE, and also conduct protein crystallization and structure determination using X-ray crystallography and cryo-EM. We use strain the standard laboratory strain MG1655 (a K-12 derivative) to record growth curves under distinct environmental conditions (obtained by media supplementation with antibiotics, phosphate, metal ions or by using different sources of carbon). **Occupational Health:** Not Applicable **Training:** In Place **BioAudit:** Facilities are Acceptable **IBC Vote: Approved (13:0:0)[1]**	
25-023	Maddamsetti, Rohan	**Title:** Maddamsetti Lab IBC Protocol **Materials:** rDNA, Escherichia coli **Submission Summary:** In this initial project, the laboratory will investigate the evolution of antibiotic resistance mediated by plasmids and transposons in laboratory strains of E. coli. They study how transposon dynamics and changes in plasmid copy number affect antibiotic resistance phenotypes in E. coli. They will clone laboratory strains of E. coli with engineered transposons and plasmids, and then conduct evolution experiments of these strains under different antibiotic selection pressures. They will then conduct genome sequencing of evolved strains, and use bioinformatics to measure genetic targets of selection, and measure changes in antibiotic resistance gene copy number, and examine diversification of antibiotic resistance genes during the laboratory experiments. Additional studies focus	1 / III-E

		on phenotypic characterization of evolved strains and plasmids, using traditional microbiology (cell plating assays and growth curves) as well as fluorescent microscopy, live-dead cell staining, and flow cytometry. The antibiotic resistance genes that they are studying in this initial project have limited clinical relevance (this work uses standard antibiotic resistance markers, and not clinical antibiotic resistance genes, to maximize biosafety). All constructs will be sent for sequencing for verification. **Occupational Health:** Not Applicable **Training:** In Place **BioAudit:** Required before commencing work (Provision of approval) **IBC Vote: Approved (13:0:0)**[1]	
25-024	Zhang, Xiyuan	**Title:** Investigate the initiation, progression, and treatment of pediatric sarcomas **Materials:** rDNA, Lentiviral vector, Human cell culture, Mice **Submission Summary:** The main goal of this project is to develop the platform of small interfering RNA (siRNA), small hairpin RNA (shRNA), and CRISPR/Cas-based gene editing for human Schwann cells and malignant peripheral nerve sheath tumor (MPNST) cells to engineer malignant transformation and survival essentiality using neurofibromatosis type 1 as a disease model. This is a new submission. Specifically, they are requesting approval to transcriptionally activate transcription factors and epigenetic modulators in human neoplastic Schwann cells using the doxycycline-inducible pLvx-TetOne-Puro over-expression system. The over-expression of master transcription factors and key epigenetic modulators alone or in combination may cause the malignant transformation of neoplastic Schwann cells to form colonies in anchor-based soft agar assays or in vivo when injected near sciatic nerve or paraspinal area in NSG mice. Results of this study will help identify the key transcriptional regulators that govern the malignant transformation of human Schwann cells, which are the cell-of-origin of benign plexiform neurofibromas and MPNST. Furthermore, they also request	2 / III-D-1, III-D-2, III-D-4

		approval to the knock-out or transcriptional inhibition of the master transcription factors and epigenetic modulators in human MPNST cell lines using CRISPR/Cas9. The overexpression and gene-specific (super) enhancer of these master transcription factors and epigenetic modulators were previously identified through bioinformatic analysis done in human MPNST cell liens. These vectors will be purchased from Addgene, packaged in the lab into Lentivirus using the 2nd-generation lentivirus packaging plasmids and tested on the human neoplastic Schwann cells and MPNST cells in the lab. Testing in animals will be done by direct injection of transfected human cell lines in the sciatic nerve or paraspinal area of immune-compromised NSG mice. The overexpression of these transcription factors and epigenetic modulators in neoplastic Schwann cells may cause malignant transformation to form colonies in anchor-based soft agar assays or form tumor when injected in NSG mice. The knockout or transcriptional inhibition of these genes are expected to cause severe growth inhibition of human MPNST cells. The end point for the proposed in vitro overexpression experiment is 6 weeks after doxycycline-induction of the gene transcription, we will harvest the colonies and subject to downstream analysis by sequencing. The end point for the proposed in vivo overexpression experiment is 6 months after doxycycline-induction of the gene transcription, we will monitor the animals for tumor growth and harvest the tumors for downstream analysis by immunohistochemistry, immunofluorescent staining, and sequencing. The end point for the proposed knock-out or transcriptional inhibition of these genes is 3 weeks after doxycycline-induction of the Cas9 expression in the transfected cells, we will monitor the cell growth in Incucyte (live cell imaging analysis system) and harvest the cells for downstream analysis by sequencing. The rest of the cells will be cryopreserved and stored in liquid nitrogen or disinfected with 10% bleach for at least 30 minutes before disposal. **Occupational Health:** In Place **Training:** Required before commencing work (Provision of approval)	

		BioAudit: Required before commencing work (Provision of approval) **IBC Vote: Approved (14:0:0)**[1]	

1. AMENDMENTS			
PROTOCOL	**PI**	**TITLE & MATERIAL(S) OF INTEREST**	**BSL / GUIDELINES**
13-341	Rodriguez, Gloria	**Title:** Iron acquisition and regulation in mycobacteria **Materials:** rDNA, *Mycobacterium mariunum* **Submission Summary:** The overall aim of the project is to characterize the molecular events required for iron metabolism and control of intracellular iron levels in mycobacterium tuberculosis. We use as surrogates for some of our experiments M. smegmatis, M. bovis BCG or the attenuated M. tuberculosis mc26020 that allow us to perform experiments that can not be performed or are more complex in BSL-3 with M. tuberculosis. Depending on the aim of the experiment we use these surrogate strain as follow: M. smegmatis: For biochemical analysis and protein-protein interactions experiments. In these type of experiments we grow cultures of up to 1 L to prepare lyzates and assay enzymatic activities protein content or protein interactions. M. tuberculosis mc26020: We will use this strain to reproduce deletion mutants affected in iron metabolism or regulation that also render M. tuberculosis attenuated in vivo. By using this strain and work in BSL-2 we can perform biochemical studies, cell fractionations, microscopy, protein localization, protein labeling and protein binding assays that can not be performed with virulent M. tuberculosis in the BSL-3. Amendment May 2025: M. marinum: We will culture M. marinum in conditions of iron limitation and iron sufficiency and look at its response in terms of growth, which will be followed by measuring optical density and plating for CFUs and release of extracellular vesicles into the culture supernatant.	2 / III-D-1, III-D-2

		We will be testing mutants affected in the response to iron limitation, which we have studied before, including genes potentially important for peptidoglycan remodeling in response to iron deficiency, and that could be linked to membrane vesicle release. These mutants will be complemented with the cognate genes to validate the gene-phenotype associations. M. marinum mutant strains will not be intentionally engineered to enhance or exacerbate the fitness, virulence, and/or tropism compared to the parent wild-type strain. In addition, Mtb BSL3/Risk Group3 sequences will not be genetically introduced to M. marinum BSL2/RG2 genetically modified strains. Any findings with changes introduced to the M. marinum that result in a one-log increase in bacterial growth will be reported to the IBC. **Occupational Health:** In Place **Training:** In Place **BioAudit:** Facilities are Acceptable **IBC Vote: Approved (14:0:0)[1]**	
13-370	Wood, Teresa	**Title:** IGF and IGF Receptor Function in Mammary Epithelial and Oligodendrocyte Growth and Development **Materials:** rDNA, AAV expressing DREADDS construct, Mice **Submission Summary:** The goal of this project is to understand the function of insulin and insulin-like growth factor receptors and their downstream signaling pathways in epithelial cells. Cells that are the focus of the studies are neural progenitor cells and mammary or breast cancer cells. Prior approval included transduction with lentiviral particles to 1) delete specific gene alleles through expressing the Cre recombinase, 2 inhibit expression of signaling molecules or downstream target proteins through shRNA expression, 3) overexpress specific genes or proteins, or 4) label cells with fluorescent proteins to track them when injected into mice in vivo. This amendment adds AAV particles that will be injected into the motor cortex of mice. Specifically, adult mice will be stereotaxically injected with pAAV-hSyn-hM3D(Gq)-mCherry (Addgene viral prep # 50474-AAV2) into the motor cortex. The pAAV-hSyn-EGFP	2 / III-D-4

		control (Addgene viral prep # 50465-AAV2) will be injected into the motor cortex of the contralateral hemisphere. Viral particles will be purchased from Addgene and not generated in the laboratory. Mice will be allowed to recover for 3 weeks and then treated 2x daily with clozapine N-oxide (CNO) or saline via IP injection for a continuous period of 7 or 14 days. CNO will activate the genes carried by the AAVs allowing for stimulation of neuronal activity from the brain to the spinal cord. Mice will be euthanized and spinal cord tissues perfusion fixed and processed for electron microscopy or brain tissues perfusion fixed for immunostaining to assess mCherry/NeuN or EGFP/NeuN and cFos expression in cortical neurons as validation for the AAV cellular uptake. AAV SOP will be followed. AAV injections will be performed in the NJMS Cancer Center vivarium procedure room. All protocols for surgeries and CNO/saline injections are included in the Myelin animal protocol for Dr. Wood (PROTO202500037). Carrying virus from the lab to the animal facility procedure room will be done using secondary containment in a rigid, sealed biohazard labeled container. All work will be completed under BSL2 working conditions inside Biosafety cabinets, including loading and unloading of gasketed safety cups for centrifugation. **Occupational Health:** Not Applicable **Training:** In Place **BioAudit:** Facilities are Acceptable **IBC Vote: Approved (14:0:0)[1]**	
15-070	Xie, Lai-Hua	**Title:** Knocking down TRPC using shRNA in cardiac myocytes **Materials:** rDNA, Lentiviral vectors, Human cell lines **Submission Summary:** Recent studies suggest that ferroptosis plays a key role in heart diseases like heart attacks and heart failure. So far, most studies on ferroptosis in the heart have focused only on heart muscle cells. It is still unclear whether other heart cells, such as cardiac fibroblasts, also experience ferroptosis. Doxorubicin is a common chemotherapy drug, but it can harm the heart, leading to a condition called doxorubicin-induced cardiotoxicity (DIC). A specific molecule in this pathway, called Wnt5a, has	2 / III-D-1

		been found in higher levels in both heart failure patients and mice with DIC. We will use both pharmacological and genetic approaches to 1) Study how Wnt5a affects ferroptosis in heart muscle cells, 2) Investigate if Wnt5a also regulates ferroptosis in cardiac fibroblasts, and 3) study how Wnt5a's role in ferroptosis contributes to DIC. This amendment adds two new aspects to the previous IBC approval. 1)To add the MDA-MD-231 human breast cancer cell line. We will add MDA-MD-231 human cancer cell lines to evaluate if attenuation of Wnt5a affect Dox-induced antitumor activity. The cell line will be purchased from ATCC or obtained from Dr. Dongfang Liu (Department of Department of Pathology, Immunology and Laboratory Medicine, NJMS). Both in vitro and in vivo experiments will be conducted. a) In vitro: we will use culture MDA-MB-231 cells to evaluate if modulating ferroptosis by the Wnt5a pathway affects Dox-induced antitumor activity by the MTT assay. b) In vivo: we will use xenograft breast cancer model. Female athymic nude-Foxn1nu mice (5–6-week-old) will be anesthetized and a total of 1 × 10^6 MDA-MB-231 breast cancer cells will be injected into the mammary fat pad. When xenografts reach an average size of 4 mm (~ 3 weeks post-implantation) mice with comparable sized tumors will be randomized into various treatment groups. Dox will be administered once per week for 4 injections (total 20 mg/kg). Two weeks after the final dose of Dox, tumor progression will be evaluated and compared amongst different groups by tumor size measurements. 2) To add lentiviral vectors. We will use the lentiviral vectors that modify the Wnt5a signaling pathway to study its involvement in ferroptosis and DIC. The following lentiviral vectors will be purchased from Origene: 1) Wnt5a Rat shRNA Lentiviral Particle (Locus ID 64566); 2) Fzd2 Rat shRNA Lentiviral Particle (Locus ID 64512). Detailed information can be found in website: https://www.origene.com/. The datasheet and SDS are uploaded to the file cabinet. The pGFP-C-shLenti vector is a third generation Lentivector. Lentiviral particles (15-30 µl (0.75-1.5x 10^5 IFU)) will be added to each 35-mm dish of cells (e.g. mouse ventricular cardiac myocyte, fibroblasts and rat neonatal ventricular myocytes for transduction. Live/Dead cell viability assay will be conducted in cultured ventricular myocytes ~24-48	

		hours after transduction. Representative ferroptosis biomarkers and membrane Lipid oxidation level will be evaluated by using Western blotting and liperfluo assay. **Occupational Health:** In Place **Training:** In Place **BioAudit:** Facilities are Acceptable **IBC Vote: Approved (14:0:0)[1]**	
14-167	Bhanot, Purnima	**Title:** Studies of hemoprotozoan parasites **Materials:** rDNA, *Plasmodium falciparum* **Submission Summary:** The long-term goal of my research is to elucidate the biology of Plasmodium. Previously approved to modify a variety of genes that include kinases, ER-shaping proteins and the proteasome. Other classes of genes and similar pathways that are not expected to result in increased virulence may be targeted for modification to identify key proteins required for parasite growth. This identification can reveal novel targets for drug discovery against malaria. This amendment includes additional gene modifications that will be conditional knockdown systems to reduce the level of proteins (kinases, ER-shaping proteins and the proteasome) in Plasmodium species approved previously on this IBC. Knockdown of Plasmodium genes will be achieved using a transgenic strain of Plasmodium falciparum 3D7 (with inducible Cre-recombinase enzyme) that will be obtained from Virginia Tech. Transfection of plasmids containing LoxP sites for genes of interest into Plasmodium falciparum 3D7 (with inducible Cre-recombinase enzyme) from Virginia Tech will drive gene deletion. We will monitor parasite biology and growth in human red blood cells as previously approved IBC. The long-term goal of this research program is to elucidate the biology of Plasmodium. Previously approved to modify a variety of genes that include kinases, ER-shaping proteins and the proteasome. Other classes of genes and similar pathways that are not expected to result in increased virulence may be targeted for modification to identify key proteins required for parasite growth. This identification can reveal novel targets for drug discovery against malaria. This amendment includes additional gene modifications that will be conditional knockdown	2 / III-D-1

		systems to reduce the level of proteins (kinases, ER-shaping proteins and the proteasome) in Plasmodium species approved previously on this IBC. Knockdown of Plasmodium genes will be achieved using a transgenic strain of Plasmodium falciparum 3D7 (with inducible Cre-recombinase enzyme) that will be obtained from Virginia Tech. Transfection of plasmids containing LoxP sites for genes of interest into Plasmodium falciparum 3D7 (with inducible Cre-recombinase enzyme) from Virginia Tech will drive gene deletion. We will monitor parasite biology and growth in human red blood cells as previously approved IBC. **Occupational Health:** In Place **Training:** In Place **BioAudit:** Facilities are Acceptable **IBC Vote: Approved (14:0:0)[1]**	
23-038	Miskolci, Veronika	**Title:** Metabolic regulation of innate immunity using zebrafish **Materials:** *Staphylococcus aureus*, rDNA, zebrafish **Submission Summary:** Recent advances in immunology revealed that intracellular metabolism goes beyond simply providing energy for a cell; it plays an important role in immune cell function. Current evidence suggests that intracellular metabolism is not just a side product of immune cell activation, but rather it determines the activation state of an immune cell, thereby dictating its function. This project will study the metabolic regulation of innate immunity during inflammation and tissue repair using zebrafish as an in vivo model, with a focus on neutrophils and macrophages, and their crosstalk with stromal cells. Insights into the regulation of immune cell metabolism may provide therapeutic opportunities in disease management. The project was previously approved for larval zebrafish tail fin inflammation and tissue repair following sterile injury or infection. The tail wound infection model involves Risk Group 2 bacteria to model infected wounds with the most common bacterial species that occur in clinical settings (PMID: 34680743). The project also involves the creation of transgenic larval zebrafish via the Tol2 plasmid	2 / III-D-4

		system approach to allow study in a cell-specific manner. The amendment adds 1) Include Staphylococcus aureus in the tail wound infection protocol 2) Updates on work with Klebsiella pneumoniae strains from TBD to MKP103 (classical pathotype) and KPPR1 in zebrafish tail wound infection protocol 3) Generation of transgenic zebrafish to express KillerRed or Opto-ASC using Tol2 system in macrophages, to allow cell killing with spatial control. Opto-ASC is a light-responsive (optogenetic) form of the inflammasome adaptor protein ASC (Apoptosis-Associated Speck-Like Protein Containing a CARD), which allows tight control of inflammasome formation in vivo, to induce cell death in target cells. Expression will be driven under the macrophage promoter mfap4 to drive macrophage-specific expression. KillerRed is an optogenetic tool to kill expressing cells by reactive oxygen species production upon green or white light illumination. Expression will be driven under the macrophage promoter mfap4 to drive macrophage-specific expression. These optogenic tools will be tested first for effectiveness. These tools will allow the study of cell functions in specific locations at the wound. The group has identified a macrophage interaction with a particular subset of epithelial cells at the wound edge during wound healing. The function of these specific macrophages will be elucidated by killing the interacting macrophages at the wound edge. The group will extend Opto-ASC and/or KillerRed to neutrophils and epithelial cells, under cell-specific promoters. Innate immune response and wound healing over time will be analyzed predominantly by quantitative imaging of live or fixed samples. Imaging will be done by confocal spinning disk, macro zoom fluorescence, auto-fluorescence lifetime imaging, and second harmonic generation microscopy. The group also employs traditional molecular, cellular, and biochemical approaches, such as western blotting, flow cytometry, mass spectrometry (metabolomics), and RNA sequencing (last three will be performed at core facilities). **Occupational Health:** In Place **Training:** In Place **BioAudit:** Facilities are Acceptable	

		IBC Vote: Approved (14:0:0)[1]	
13-574	Yurchenco, Peter	**Title:** Basement membrane self-assembly, structure & function **Materials:** rDNA, AAV vectors **Submission Summary:** The long-term goal is to elucidate mechanisms of basement membrane assembly and architecture-dependent functions. These extracellular scaffolds are required for tissue growth, differentiation and adult functions. Elucidation of the mechanisms is critical to understanding the pathogenesis of basement membrane-dependent diseases of kidney, nerve, muscle, vasculature and other organs, to develop structure-correcting therapies, and (in the future) to engineer biomaterials with the desired basement membrane properties for tissue regeneration and repair. The current and renewed protocol has two parts. One part is the production and analysis of recombinant basement membrane proteins for in vitro analysis. The second part is the generation and analysis of adeno-associated virus (AAV) for somatic gene therapy of mouse models of laminin-deficient muscular dystrophy and peripheral neuropathy, a basement membrane disease complex. These two parts were previously approved. The first part is the genetic editing of the basement membrane proteins laminins, nidogen, and perlecan, as well their interacting integrin, and dystroglcyan receptors within cDNA plasmids (previously cloned in E.coli) and stably expressed in our laboratory as recombinant proteins. These recombinant proteins were expressed either by human embryonic kidney cell lines (HEK293) or, in the case of dystroglycan, by a Chinese Hamster Cell line. The above proteins are purified from the conditioned medium of stable clones and characterized by biochemical assays (e.g. ELISA, SDS-PAGE), ultrastructural analysis (electron microscopy), and cell-surface assembly on cultured wild-type rat Schwann cells and mouse C2C12 myotubes. In addition, the protocol described the organ culture of mouse laminin gamma1-floxed embryonic dorsal root ganglia rendered null for laminin protein expression with adeno-virus expressing Cre-recombinase. These modified organ	2 / III-D-4

		cultures were treated with some of the above listed recombinant proteins to study their role in Schwann cell myelination. Isolated proteins are used to determine binding affinities to each other, to measure cell adhesive properties, and to measure polymerization. Two additional culture systems that have been studied for basement membrane functions are mouse ureteric bud organ cultures (obtained from mouse embryos) and kidney inter-medullary collecting duct cells generated from the laminin gamma1-floxed mice. The second part, previously approved, is the AAV somatic gene transfer of laminin-binding linker proteins to mouse models of the LAMA2-related dystrophy (a congenital muscular dystrophy and peripheral neuropathy seen in humans) in an effort to develop a treatment. The choice of linker proteins (that bind to the altered or compensating laminins in the dystrophic mice) was constructed based on the in vitro studies with the recombinant proteins. The activities of these linker proteins that bind to laminins affect the laminin activities of polymerization and receptor binding. The amendment adds the following: (a) The further modification and fine-tuning of linker proteins to optimize functional activities in vitro and in mice. (b) The optimization of AAV dose and time of administration and the minimization of adverse effects of treatment in mice. (c) The evaluation of new constructs that combine polymerization and cell adhesion activities within a single protein. Subparts a – c utilize the universal (CBh) promoter in AAV serotype 9 virus. (d) The evaluation of a new myotrophic AAV (SLB101 from Solid Biosciences, containing mini-agrin) that will be co-infected with low-dose AAV9-CBh-aLNNdDelG2 (aLNNdDelG2 and miniagrin are laminin binding protein s) (previously approved constructs). Mini-agrin is a protein that can bind to alpha-dystroglycan, a protein crucial for connecting the muscle cell's cytoskeleton to the extracellular matrix. This myotrophic virus is currently being evaluated in human trials with supporting studies indicating reduced human toxicity. This modified protocol has the purpose of increasing muscle expression while retaining the benefit for neuropathy that requires nerve expression of aLNNdDelG2' with a universal promoter. Like previously approved AAV9, this modified virus is	

		replication-deficient, produced by triple transfection of cells, and rated BSL-1. The AAV has higher skeletal and cardiac muscle delivery and, importantly, decreased liver transduction compared to AAV9. Note that low liver transduction is desired in order to reduce hepatotoxicity, a concern in a variety of human studies using AAV. **Occupational Health:** In Place **Training:** In Place **BioAudit:** Facilities are Acceptable **IBC Vote: Approved (14:0:0)[1]**	

[1] Voting Decision (Yay: Nay: Abstain)
[2] Member(s) joined the meeting
[3] Member(s) left the meeting