**NHGRI ACUC MINUTES**
**29 January 2020**
**1:00 pm**
Redacted by agreement **Conference Room**

**Members present**: Drs. Bodine, Clark, Redacted by agreement Redacted by agreement

**Members absent**: Our Nonscientific member position was vacant for this meeting as Redacted by agreement resigned. Redacted by agreement Redacted by agreement has been appointed as his replacement but is in the process of completing training.

**OACU Observer**: None

**Visitors:** Redacted by agreement

Note: The Facility Veterinarian and Facility Manager of the facilities where protocols are performed are given the opportunity to review all new Animal Study Proposals, resubmissions and significant amendments prior to the meeting. Their comments are provided in the text below, when applicable.

## OLD BUSINESS

1. The minutes of the 6 December 2019 meeting were approved with one modification. The reference to Redacted by agreement Redacted by agreement comment on the surgical details was modified. Completed 1/29/20.

2. OACU Refresher Course update – Redacted by agreement was due to complete the refresher course in November but she is currently on maternity leave. She will be reminded to take the refresher course when she returns. Redacted by agreement were due to complete the refresher course in December. Redacted by agreement completed the course on 12/7/19, Redacted by agreement completed the course on 12/9/19, Redacted by agreement completed the course on 12/23/19 and Redacted by agreement completed the course on 12/31/19. Redacted by agreement and Redacted by agreement were due to take the refresher course in January. Redacted by agreement and Redacted by agreement completed the course on 1/13/19. Redacted by agreement Redacted by agreement are due to complete the refresher course in February.

3. Update on Spring and Fall Semiannual Report Deficiencies

   Redacted by agreement gave a summary of the pending deficiencies from the Fall Semiannual Program Review. She noted that the two hoods in Redacted by agreement were certified this month. The water leak in Redacted by agreement has been resolved but the strong rat odor remains in Redacted by agreement The rat odor is NEI's responsibility. Dr. Clark will follow up with them.

## NEW BUSINESS

1. No ***new*** **Animal Study Proposals were** reviewed at this meeting.

2. Five **three-year renewals were** reviewed at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor):

   Redacted by agreement "Mouse Models for Human Red Cell Production Disorders." P.I.; Redacted by agreement

   Defective production of red blood cells results in anemia. The process by which immature bone marrow cells decide to become red blood cells is not entirely understood. The PI is interested in mouse models of disorders where red cell production is inhibited.

   Current models of heme production state that iron is imported into cells as transferrin, which is converted to heme by enzymes in the mitochondria. The problem is that at the moment when heme synthesis needs to be at its peak to match globin synthesis, the mitochondria (where heme is synthesized) are all extruded

along with the nucleus. The PI is trying to determine how heme gets into these developing red cells. In collaboration with a group at the University of Maryland, they are studying a heme transport protein, HRG1, which is encoded by the *Slc48a* gene. Using CRISPR/Cas9 they have generated knockout and knockin mice for *Slc48a*. They found that HRG1 deficient mice have an iron deficient anemia and massive iron accumulation in the macrophages of the spleen and marrow, consistent with a defect in heme transport. These animals will prove to be useful models for iron deficient anemia, and they have preliminary data that certain types of iron refractory anemia are associated with mutations in the human counter parts of these genes.

They have also generated mice with reporter genes knocked into the *SpiC*, *MafB* and *Slc48a* loci. SPIC and MAFB are expressed in specific subsets of macrophages. By either crossing their macrophage reporter mice to the Slc48a reporter mice or transplanting the marrow of the reporter mice into each other, they hope to demonstrate the interaction of HRG1 expressed in erythroblaats interacting with macrophages. These studies will be important to demonstrate what happens to the heme when it is transported.

Finally, they are evaluating the role of HRG1 in the severity of Sickle Cell Disease. Patients with Sickle Cell Disease often have massive iron overload due to the breakage of the sickled red cells and the release of heme into the circulation. They have crossed the *Slc48a* mutant allele onto the Sickle Cell mouse background. They hypothesize that the inability to transport heme will lead to less iron storage in the tissues and a less severe disease. If these experiments are successful, they hope that an HRG1 inhibitor can be developed to use to help iron overload patients, including Sickle Cell patients.

Redacted by agreement presented his resubmission. Redacted by agreement explained that this protocol focuses on the process by which red blood cells (rbc) are made and has evolved into studies of the iron transporter protein HRG1. A paper describing studies with his knockout mouse was just published. Macrophages identify aged rbc, engulf them and reprocess the iron to make new red cells. If they remove HRG, heme cannot get out of the macrophage, where it accumulates into a non-toxic crystal called hemozoin. . The mice look reasonably healthy but their bone marrow and spleen are jet black. HRG1 is also expressed on developing erythrocytes. The rbc that express HRG1 is taking up the heme and the PI is trying to determine how the heme gets into the rbc. They are breeding mice to use as models to see if they can identify the heme transporter. Redacted by agreement noted that there are no surgeries performed under this ASP. Dr. Clark asked about injections and Redacted by agreement responded that he will have to submit an amendment if he does this. Dr. Clark noted that the $Npc1^{m1N/m1N}$ mice were not on the renewal. Redacted by agreement responded that he thought they would not be using them but a question has come up concerning transport and they do need to be added to the ASP. They will be added administratively to this renewal. Redacted by agreement asked if Redacted by agreement should be added and Redacted by agreement responded that although he is a special volunteer, he will not be handling mice so does not need to be on the ASP. There were no further comments or questions.

**Decision**: Approved by unanimous vote (8-0, with Redacted by agreement recusing himself as the PI) with the addition of the $Npc1^{m1N/m1N}$ strain to section B. Completed 1/31/20.

Redacted by agreement "Metabolism and Immunity in Mouse Models of Inborn Errors of Mitochondrial Metabolism." P.I.; Redacted by agreement Redacted by .

Inborn errors of mitochondrial metabolism (IEMM) encompass a large group of rare genetic diseases with a variety of presentations. Enzyme deficiencies in protein, fat, carbohydrate, and energy metabolism lead to biochemical imbalances, which can present a myriad of metabolic, nutritional, immune, neurologic and developmental problems. The multitude of clinical features associated with IEMM leads to challenges for clinical care. Patients with IEMM oftentimes suffer from bouts of metabolic instability (e.g. hypoglycemia, acidosis), termed metabolic decompensation. Using mouse models of IEMM, they will reproduce the metabolic instability seen with infection in patients as a platform for 1) defining the mechanisms involved in precipitating metabolic instability, 2) evaluate potential treatments.

For the past three years, the Redacted by agreement lab has focused on developing model systems of infection in IEMM. Using an animal model of mitochondrial hepatopathy, they have shown that infection with influenza exacerbates the disease due to immune activation. Depletion of the innate immune system abrogates these effects. They have recently expanded their studies to include CNS disease in IEMM, a major cause of neurologic decline in IEMM. Since infection can be devastating in IEMM, they have created mouse models that are deficint in specific immune cells. They previously published a paper on complex IV deficiency (oxidative phosphorylation) in T-cells. They are currently trying to correct the immune deficits in this model by bypassing complex IV deficiency with an alternative oxidase. Additional models of mitochondrial disease in cells of the innate and adaptive immune systems are underway.

Redacted by agreement presented his protocol. He explained that he has patients in the Clinical Center with mitochondrial disease with a multisystem disorder that affects many organ systems. Sepsis is the leading cause of death for these patients. This protocol uses mouse models to answer questions about immune function. Immune function is impaired in the mice with mitochondrial disease. Redacted by agreement explained that in his mouse model of influenza, the mice are euthanized at day 5 so they are USDA pain/distress category "C". If maintained beyond day 5, they would become severely ill and would die by day 10. Dr. Bodine added that the infected animals feel much like we do when we get a flu shot but their distress would increase if they were not euthanized at day 5. Redacted by agreement asked for an explanation of the USDA pain/distress category "E" mice. Redacted by agreement explained that patients become very ill due to virus or bacterial infection. Giving the mice Poly I:C simulates viral infection and LPS simulates bacterial infection without the use of toxic live organisms. The immune system of the mice thinks there is an infection present and responds, which causes pain/distress to the mice. Dr. Bodine added that analgesia cannot be used as it would mask the response to infection that the PI is studying. Redacted by agreement is using the minimum number of animals needed to generate significant results for these studies. Redacted by agreement asked if the sheep rbc had been tested for contamination. Dr. Clark responded that as a non-murine product it does not need to be certified, however she will work with Redacted by agreement to see what information the company provides. Redacted by agreement Redacted by agreement provided rewording for section K. Redacted by agreement noted that the Redacted by agreement Facility Manager had provided corrections to the room numbers in section B, she had asked about current ABSL2 training status and asked whether the Redacted by agreement procedure room would be needed for this study. It was determined that Redacted by agreement and Dr. Clark would provide the ABSL2 training and the Redacted by agreement procedure room would not be used. Redacted by agreement noted that the Mouse Imaging Facility (MIF) should be added to section B.

After the meeting, it was determined that the Mouse Imaging (MIF) would not be needed so all references to the MIF and Redacted by agreement were removed from sections B, C, F and O. With the removal of Redacted by agreement no ABSL-2 training is required.

**Decision**: Approved by unanimous vote (9-0) with the removal of the MIF, Redacted by agreement and with the changes to section K provided by DOHS. Completed 1/31/20.

Redacted by agreement **"Development** of a Mouse Model for Proteus Syndrome (PS)." P.I.; Redacted by agreement

The PI is studying a rare disease called Proteus syndrome (PS). This disease causes a progressive overgrowth of tissue and can affect any tissue in the body. Common manifestations include skin and bony overgrowth, vascular malformations, benign fatty tumors, loss of fat, and other specific types of skin malformations. The disease is mosaic; that is in any given individual, overgrowth occurs in only some of their tissues. This is because the pathway alteration that causes PS occurs in a single cell during embryogenesis and only the altered cell's descendants carry the mutation. They have shown that PS is caused by a mutation in the *AKT1* gene. AKT1 is part of a pathway that transmits signals from the environment to the machinery responsible for many cellular functions including growth and proliferation. The mutation causes the AKT1 protein to remain active at times when it would normally be inactive in unaffected individuals. This project was initiated in order to develop an animal model to better understand how PS lesions develop and progress and to investigate therapeutic options for treating patients with this

disease. They found that mice with mosaic expression of mutant *Akt1* develop several of the same types of overgrowth as seen in patients. Furthermore, they saw that if all cells in a mouse embryo express the mutation, their blood vessels failed to develop properly and they did not survive. Their studies are now investigating how the overgrowths and lesions are forming. They are also beginning studies using the embryonic mouse model to test different drugs to see if they can correct some of the vascular defects and improve survival.

Redacted by agreement presented her resubmission. She explained that she is using a mouse model to study the AKT1 gene mutation. It is a systemic disorder so it affects many tissues. They will use the mouse model to address questions they can't in people. The animal model has mosaic expression of the mutation that does mimic the human model. They are trying to understand how the overgrowth occurs. If they express the mutation in all cells, it is not compatible with life because extensive vascular changes occur. They will use the mouse model to test therapeutics.

Redacted by agreement asked which treatments would be tested. Redacted by agreement responded that she would start with mirasentib. It is a chemotherapeutic drug with limited studies and not much efficacy data in humans. Redacted by agreement Redacted by agreement noted that "ABSL1" should be added to the registration. Dr. Bodine asked if Redacted by agreement Redacted by agreement could be removed from the ASP. Redacted by agreement noted that we were previously told that since he was the PI on the RDNA registration that had to be on the ASP. Redacted by agreement responded that if Redacted by agreement was listed on the registration, it should be OK to remove Redacted by agreement Dr. Bodine suggested that the text in asterisk in section B be moved to section K. Redacted by agreement asked if there was any evidence in the literature that running on a wheel would contribute to the development of the overgrowth (Proteus Syndrome). Redacted by agreement responded that there was no evidence but if the Proteus does develop on the front feet it could be initiated by pressure generated by the wheel. Redacted by agreement added that eventually the mice would develop cerebriform connective tissue nevus or CCTN but they do not live long enough. She is attempting to speed up the process by using the exercise wheels. Dr. Clark commented that the mice like running on the wheels. There were no further questions or comments.

**Decision**: Approved by unanimous vote (9-0) with the removal of Redacted by agreement from section A, with the addition of a note to section K that the registration is in Redacted by agreement name but Redacted by agreement is on the registration and with the addition of "ABSL1" to section K. Completed 1/31/20.

Redacted by agreement **"Model** of SPTAN1 Disease: Understanding Disease Pathogenesis Provides Clues to Therapeutic Options." P.I.; Redacted by agreement

This protocol was inactivated at the request of the PI.

Redacted by agreement **"Functional** Analysis of Candidate Genes Associated with Human Disease." P.I.; Redacted by agreement

This protocol was inactivated at the request of the PI.

3. One **Animal Study Proposal was subjected to annual review** at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor):

Redacted by agreement "Bacterial Colonization and Infection." P.I.; Redacted by agreement

The goal of this protocol is to establish a robust and clinically relevant experimental model to explore *Klebsiella pneumoniae* colonization and infection *in vivo*. This project aims to 1) establish an experimental model of *K. pneumoniae* gastrointestinal colonization; and 2) assess the capacity of the microbiota to control KPC-*K. pneumoniae* colonization; 3) provide a platform to screen novel antimicrobial therapies, including pre- and probiotic strategies.

During the last year they have demonstrated that wild-type immune competent mice (C57BL/6) will become asymptomatically colonized with KPC-*K. pneumoniae* for up to two weeks. They discovered that only certain classes of antibiotics result in the outgrowth of KPC-*K. pneumoniae*, demonstrating that the indigenous community provides colonization resistance to this pathogen. Their goal for this year is to try to identify which members of the gut microbial community confer this colonization resistance and to see if this is modifiable through diet or other pre-biotic, probiotic strategies. Dr. Bodine was the primary reviewer for this annual review.

Dr. Bodine presented this annual review. He explained that Redacted by agreement studies the microbiome. *Klebsiella pneumonia* can live happily on the skin without any issues but can suddenly flare up and cause significant health problems. Redacted by agreement is looking at what can promote and what can prevent flare ups. Dr. Bodine commented that the literature search was well done. There were no questions or additional comments. Dr. Bodine recommended that this protocol be continued.

**Decision**: Approved by unanimous vote (9-0) without modification.

4. Seven **protocol amendments** were submitted at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement "Tyrosine Kinases in Hematopoietic and Bone Development." P.I.; Redacted by agreement

To evaluate factors affecting susceptibility to autoimmunity in their mouse models of immunodeficiency, the PI proposes to induce systemic lupus erythematosus (SLE) like symptoms including auto-antibodies, proteinuria, and nephritis using a method that is triggered by immunization with chromatin as a self-antigen, with Complete or Incomplete Freund's Adjuvant (CFA or IFA).

Chromatin will be prepared from activated splenocytes of donor C57BL/6J mice precipitated with ethanol and resuspended in sterile pharmaceutical-grade injectable saline. Chromatin (100 ug) will be emulsified with 100 ul of CFA or IFA. A total of <200 ul of emulsion will be injected per mouse, subcutaneously at the tail base (100ul/side). Mice will be immunized three times, at 0, 2 and 4 weeks. The initial immunization will use CFA and subsequent two boosters will use IFA. Mice will be bled retro-orbitally, no more than 70 ul at one time, at 2, 4 and 6 weeks and thereafter once/month for measurement of autoantibodies. Mice will be euthanized at no later than 6 months post-immunization. Any mouse that becomes lethargic, dehydrated or has open unhealed ulcers will be euthanized.

As a potential alternative refinement, mice will be immunized with activated splenocytes of C57BL/6J mice subcutaneously. Cells will be harvested from C57Bl/6 mice and cultured with sterile Concanalvin A for three days, extensively washed, resuspended at $10^7$ cells/ml in sterile saline. 100ul of cells will be injected subcutaneously on day 0, 7 and then 2 weeks later. Mice will be bled as above starting at one month, every 2 weeks for evaluation of autoantibodies and euthanized no later than 6 months.

The PI will test these protocols on 2 cohorts of 5-10 WT mice (total 10-20 mice for the 2 methods) to determine the efficacy of generating autoantibodies. Depending on results at 2 months, they will immunize approximately 5-10 WT and 5-10 mutant mice with either chromatin/CFA adjuvant or activated cells alone to assess pathology at different time points. In the event that the cells (without adjuvant) present a clearly detectable response, they will not continue with the use of CFA. They will perform the experiments a total of 3 times (a maximum of 80 mice in total including the 20 from the initial trial).

The mice injected with CFA are accounted for in USDA Pain/ Distress Category "E". Mice injected with activated cells and the donor mice are USDA Pain/Distress Category "C".

Redacted by agreement presented her amendment. She explained that she had submitted a paper and the reviewers have requested an amplified immune response. She is confident that she can get this response using the chromatin/CFA/IFA protocol but she would also like to try a less inflammatory method using activated splenocytes. Redacted by agreement explained that CFA has mycobacterial cell walls like LPS. Redacted by agreement asked if the question posed by the reviewers was important. Redacted by agreement responded that the question is interesting but if it turns out to be too difficult, they just will not do it. Redacted by agreement asked if this amendment would increase the number of USDA pain/distress category "E" mice from 20 mice to 80 mice? Redacted by agreement responded that there are currently no category "E" mice on her protocol so this will increase the number from "0" to "80". Dr. Clark noted that there is an ARAC Guideline on the use of CFA that categorizes it as USDA pain/distress category "C". ARAC has recently updated the guideline on endpoints and referenced increased inflammation with the use of CFA so she would like to go the conservative route and call it a category "E". There were no further questions or comments.

**Decision**: Approved by unanimous vote (9-0) without modification.

Redacted by agreement 'Molecular Genetic Analysis of Zebrafish Development." P.I.: Redacted by agreement

The PI would like to add adult zebrafish (juveniles/adults) imaging to his protocol. They currently have fry and larvae imaging approved.

Zebrafish adults will be photographed using microscope equipment either in the aquatics facility in Redacted by agreement Images will be taken, transferred and saved only on government devices. For imaging, the animals will be anesthetized as described in section I. Some adults may also be imaged in Redacted by agreement (already listed in the animal protocol). After imaging, the adults are placed in clean system water and monitored for recovery from anesthesia. They are then returned to the system in Redacted by agreement Redacted by or euthanized. Euthanasia of the animals will be performed in Redacted by agreement or in Redacted by agreement as described in the ASP. This procedure is anticipated to be used on 300 animals.

Dr. Clark presented this amendment. She explained that the PI is adding imaging of adult zebrafish to his ASP. Dr. Bodine asked how long fish could survive out of water. Redacted by agreement responded that the imaging is usually done in wet agar. He added that larvae do not need to breathe via gills and don't need blood until two weeks of age. There were no further questions or comments.

**Decision**: Approved by unanimous vote (9-0) without modification.

Redacted by agreement 'Molecular Genetic Analysis of Zebrafish Development." P.I.: Redacted by agreement

The PI would like to add zebrafish origin hematopoietic cell injection into embryos as a procedure to his ASP.

The procedure is similar to the microinjection procedure already described in the ASP. To collect the zebrafish cells for injection, they will euthanize existing zebrafish strains housed in Redacted by agreement and collect hematopoietic cells for transplant. Cells are purified and resuspended in sterile Phosphate Buffered Saline (PBS). PBS is not available in pharmaceutical-grade however, they are using it to generate data that is comparable to previous work.

Injections of 2-5 dpf embryos will occur in procedure room Redacted by agreement Embryos will be anesthetized with tricaine. A glass needle is used to inject cells into circulation. There are no anticipated phenotypes due to this injection. The embryos will be housed in an incubator in Redacted by agreement and placed on system by 6 dpf. They have been doing this procedure in irradiated adult fish. However, by using embryos they take advantage that at this age they lack an acquired immune system and therefore do not need to be irradiated.

This is a refinement to the adult procedure. Up to 1,000 animals are anticipated to undergo this procedure and will be accounted for under USDA Pain/Distress Category "C."

Dr. Clark presented this amendment. She explained that they initially used irradiated adult fish but these fish developed secondary complications so they want to try it now in younger animals. Redacted by agreement questioned the source for the cells. Dr. Clark responded that the cells are collected from adult fish in the facility. Redacted by agreement questioned the term "fry". Redacted by agreement responded that it is an indistinct term but generally refers to the life stage that need to be fed and are housed on the system. There were no further questions or comments.

**Decision**: Approved by unanimous vote (9-0) without modification.

Redacted by agreement "Studies of the Hutchinson-Gilford Progeria Syndrome Mutation in Transgenic Mice." P.I.; Redacted by agreement

The PI would like to include a detailed description for the known phenotypes of their Hutchinson-Gilford Progeria mouse models. These animals are involved in aging studies and while they describe some predicted phenotypes in the ASP, they now have very solid data about the effects of the different mutations. Due to experimental needs, no treatment should be initiated before speaking with investigators or should be in accordance with the Emergency Animal Treatment and Care form. Humane endpoints have also been updated. They will be utilizing a Grading scale to better assess both experimental and humane endpoints.

HGPS G608G Scale of severity:

| Rating | Description | Criteria |
|---|---|---|
| 1 | Normal | no observable pain or distress with health observation, subjective to condition, health observations should be set at intervals to monitor. |
| 2 | Mild | progression of any expected phenotype or health issue previous or new, may have associated transient to mild pain or distress, treatment and/or health obs / supportive care are necessary and set at intervals to monitor as per vet direction. |
| 3 | Mild to Moderate | mild-moderate pain or distress and/or hinderance to health and/or normal activity, treatment and health obs / supportive care are necessary and set at intervals to monitor as per vet direction. |
| 4 | Moderate to Severe | moderate to severe pain or distress and/or hinderance to health and/or normal activity, treatment and health obs / supportive care are necessary every day. If condition worsens within 1-2 days or a sharp health decline is observable then required investigator notification and euthanasia by close of business. |
| 5 | Severe | severe pain and/or hinderance to health and/or normal activity, considered humane endpoint criteria with investigator notification and animal euthanasia performed within 2 hours of notification. |

Any mice that reach a scale of 5 or any mice that generate concern by the facility staff, the investigator will be notified immediately to determine if the facility can treat or euthanize or if the investigator will need to euthanize for tissue and preclinical trial data collection. If the investigator requires tissue from an animal at a Scale of 5, they will collect within 2 hours of notification.

Redacted by agreement presented his amendment. He explained that they are studying aging animals and how they compare with wild-type mice of the same age. The Redacted by agreement lab had determined that the Redacted by agreement personnel were making decisions on the endpoints for these mice without their input. The mice were being

euthanized before they had a chance to collect the vessels. It was determined that the endpoints were unclear so this amendment was submitted. This amendment is an attempt to document the endpoints and how they vary by strain. Redacted by agreement asked if the kyphosis was really a skeletal deformity or if the mice were just hunched. Redacted by agreement responded that it was indeed skeletal. He noted that bone issues are more pronounced than in a routine old mouse. Dr. Clark added that the bone deformities can be seen using imaging. Redacted by agreement questioned how they ensure that the grade 5 are euthanized and the mice don't suffer. Dr. Clark responded that the PI is required to take action and euthanize the mouse within two hours of notification and she will help the facility in determination of the endpoints. Redacted by agreement asked if this grading scale is applicable to sick animals on other protocols and Dr. Clark responded that it was. Redacted by agreement Redacted by agreement noted that the Facility Vet questioned whether dystocia animals would be treated or euthanized. A tech request was submitted that indicated that the animals would be euthanized. Redacted by agreement asked if a C-section would be attempted and Dr. Clark responded that it would not. There were no further questions or comments.

**Decision**: Approved by unanimous vote (8-0, with Redacted by agreement recusing himself from the vote as the PI) without modification.

Redacted by agreement "Functional Analysis of Folic Acid and Vitamin B12 Metabolic Pathways in Early Zebrafish". P.I.; Redacted by agreement

The PI would like to add the tcnba-FLAG tagged zebrafish transgenic/mutant line to his ASP. Eight fish will be transferred from the NHGRI Fish Core. These fish have been engineered to remove two additional vitamin B12 transport proteins. They will be incorporated into the existing work being carried out under this protocol. This includes observation of fish for phenotypes associated with specific genotypes and manipulating the levels of vitamin B12 and folic acid to which the fish are exposed during development. These experiments have already been carried out using fish deleted for the Tcn2 gene and the new members of this gene family they have recently characterized. This new line will allow them to follow the expression of the Tcnba protein. There are number of different experiments described in the protocol and all will be performed as described in the ASP. They will also use a large number of fish for biochemical studies. All of these studies utilize fish after they have been euthanized.

They may observe developmental abnormalities in the "second generation" of fish that are homozygous for alleles that interfere with the function of the Tcnba protein. If any adverse phenotype becomes evident, an additional amendment will be submitted to describe them and endpoints will be updated if needed.

Estimated number of fish generated by breeding of these fish: 8,000
This increase in numbers is required as several hundred fish will be used to maintain the lines. The balance of the increase reflects the number of fish that will be used for the developmental studies described in the protocol. In brief, several hundred fertilized embryos are required for each data point of experiments designed to understand the developmental and consequences of disrupting vitamin B12 metabolism. These fish are followed from fertilization to day six or seven of development.

Dr. Bodine presented this amendment. He explained that this is a significant amendment due to the increase in animal numbers. Back when the USDA report was submitted in October, it was determined that the PI had exceeded his animal numbers. Dr. Clark met with him and found that he was counting embryos which inflated the numbers. The protocol was resubmitted at the end of October so instead of trying to adjust the numbers via an amendment, the increase in numbers was addressed in the resubmission and now in this amendment. Dr. Clark reported that the lab is now keeping a more accurate count of their fish by using a thermometer gauge. There were no further questions or comments.

Decision: Approved by unanimous vote (9-0) without modification.

Redacted by agreement "Genetic and Functional Studies of Familial Mediterranean Fever and Autoinflammatory Diseases". P.I.; Redacted by agreement

The PI would like to adjust the USDA Column E numbers of mice on this protocol.

Originally, they asked for 96 mice/year based on projected experiments. However, due to increased breeding for FMF-KI mice (untreated homozygotes of V726A-KI and M680I-KI mice) to revise a manuscript that was submitted to *Nature Immunology* and for follow up studies of other inflammatory diseases (PAPA and TRAPS), in which the FMF protein, pyrin, mediates inflammation, they would like to increase this number by 100 for a total of 196 mice/year.

(196 mice per year) x 3 = 588 animals.

Dr. Bodine presented this amendment. He explained that the PI exceeded the number of column "E" animals at the end of last year. Dr. Clark added that this amendment is two-fold, it addresses the number of column "E" animals from 2019 as well as the column "E" animals he will use over the next three years. There were no further questions or comments.

Decision: Approved by unanimous vote (9-0) without modification.

Redacted by agreement "Mouse Models of Glycosphingolipid Storage Disorders: Understanding Disease Pathogenesis Provides Clues to Therapeutic Options". P.I.; Redacted by agreement

The PI would like to update section M of her ASP and follow the NHGRI ACUC Guideline on avoiding Overcrowding in Animal Housing to include trio breeding (2 females and 1 male). Many of the mice are small and have smaller sized litters. Having two litters and two mothers helps to ensure care of the litters and increases the rate of survival of the pups. Breeding units are set up with 2 females at the same time to minimize differences in ages of the litters. Prompt weaning or separation of litters is done when there are more than 14 animals older than 14 days/cage.

Dr. Bodine presented this amendment. He explained that harem breeding performance standards date back to the 1920's. He added that Redacted by agreement provided appropriate justification for using the practice. The facilities are concerned about overcrowding of the cages but the number of animals will be monitored closely. There were no further questions or comments.

Decision: Approved by unanimous vote (9-0) without modification.

5. The following minor amendments and significant amendments approved administratively were announced for concurrence of the committee for the **addition or removal of personnel, location and/or normal strains**. All the new personnel have completed the NIH OACU Animal User training course, registered with AEP and have been specifically trained by OLAM personnel. The additional strains will not increase animal numbers by more than 10% and require no special care beyond that described in the original Animal Study Proposal (Change in the Principal Investigator or change of species requires resubmission of the protocol.) In the case of adding an animal holding location, the signature of the veterinarian and facility manager of the affected facility is required.

Redacted by agreement Redacted by Add two strains
Redacted by agreement two personnel
Redacted by agreement Add two personnel
Redacted by agreement Add personnel
Redacted by agreement Add personnel
Remove personnel

Redacted by agreement Redacted by agreement Add personnel
Redacted by agreement Add personnel

Significant Amendments for Administrative Approval
None

6. **NHGRI Guidelines**

02.2 – Animal Study Proposal Amendment Guideline

Dr. Clark presented this guideline. She explained that it was an administrative burden to have two people sign a minor amendment to add a new co-investigator to a protocol so she proposed that only one person sign these amendments. She added item D "Unconditional Administrative Process" to the guideline to cover the addition of personnel, grammatical errors, check box problems, contact information updates, addition of procedures to the training and experience form and updates to the Emergency Animal Treatment and Care Form. Redacted by agreement asked who could sign these amendments and Dr. Clark responded that the ACUC coordinator, the ACUC Chairperson or the Animal Program Director could sign. Redacted by agreement Redacted by agreement commented that this should be applied to anything that does not explicitly require two signatures by the NIH. Redacted by agreement asked if the VVC was recently approved and if there had been any activity? Dr. Clark responded that it was recently approved and so far, there had been no activity. It was decided that any future VVC amendments would be added to the minor amendment sheet that is distributed in every ACUC meeting packet and this should be clarified in section B of this guideline. There were no further questions or comments.

Decision: Approved by unanimous vote (9-0) with the change listed above. Completed 1/31/20.

7. **NHGRI SOPS**

Standard Operating Procedure for Topical Association of Mice with Staphylococcus, Streptococcus and Corynebacteria sp.

Dr. Clark presented this SOP. She explained that this is a study specific SOP and she merged two SOPs into one. Dr. Bodine asked if this SOP should have the OLAM title page and Dr. Clark responded that it should not since it is technically not an OLAM SOP.

Decision: Approved by unanimous vote (9-0) without modification.

Using the Heat Tape and Vivarium Electronics VE-200 to Provide Heat to Cages on the Lab Products Rack

Dr. Clark presented this SOP. She explained that it describes the use of the strips with copper that are used to provide warmth to cages of fragile, weaker phenotype mice on the animal housing racks. It explains how to set them up and how to monitor their use. Dr. Bodine asked who used them and Dr. Clark responded that they are used by Redacted by agreement

Decision: Approved by unanimous vote (9-0) without modification.

Zebrafish Care Off System

Dr. Clark presented this SOP. She explained some of the fish housed in the Redacted by agreement Fish Facility need to be housed off the system for drug studies and other purposes. This SOP helps the facilities manage who

is doing what in caring for the fish. It ensures that the housing and care is adequate. She added that only adult fish are housed off the system. [Redacted by agreement] asked if system water was used for the off system tanks and [Redacted by agreement] responded that it was. [Redacted by agreement] noted that they could be left off the system for weeks or months if we purchased some small recirculating pumps. Dr. Clark responded that Zeolite is being tested and may be a good alternative to the pumps.

Decision: Approved by unanimous vote (9-0) without modification.

Rules for NHGRI [Redacted by agreement] Shared Procedure Room [Redacted by agreement]

Dr. Clark presented this SOP. Dr. Clark responded that people were not following the required procedures in this room, including proper storage of reagents and the proper disposal of euthanized animals, so we needed to update the rules. Dr. Bodine asked if this SOP would be printed and used for formal training of co-investigators and Dr. Clark responded that it would.

Decision: Approved by unanimous vote (9-0) without modification.

Care of Zebrafish in Satellite Incubators

Dr. Clark presented this SOP and explained that this is a new SOP that will used to maintain documentation for satellite rooms. Incubators in these satellite rooms offer a stable environment so the parameters only need to be documented every three days. Documentation will only be made when animals are present in the incubators.

Decision: Approved by unanimous vote (9-0) without modification.

8. Other new business:

- Hot Topic – Assessing Pain and Distress in Animal Research Models

  Dr. Clark explained that some facilities consider breeding and holding protocol animals as USDA pain/distress category "B". Dr. Bodine responded that we use the official NIH pain/distress categories that consider these animals category "C". [Redacted by agreement] added that most breeding animals get genotyped or receive hormone injections anyway. Dr. Clark explained that the ACUC pays special attention to pain/distress category "D" and "E" animals. We look for ways to reduce pain and distress. Dr. Bodine added that we could use today's discussion of the column "E" animals on the complete freund's adjuvant amendment as an example. Dr. Bodine noted that each member has a specific area of review they are responsible for: [Redacted by agreement] represents the general community interests in the proper care and use of animals; [Redacted by agreement] reviews nomenclature; [Redacted by agreement] addresses safety concerns; [Redacted by agreement] ensures that animal numbers are appropriate; [Redacted by agreement] looks at the animal model in relationship to human disease; [Redacted by agreement] assures the appropriateness of the animal model especially as it relates to developmental models; Dr. Clark participates in the pre-review process and addresses veterinary concerns; [Redacted by agreement] represents the non-scientist view and assures that section D is understandable to the layperson; Dr. Bodine assures that the literature search is appropriate and [Redacted by agreement] participates in the pre-review process and assures that animals are correctly classified and justified in the appropriate pain or distress category. [Redacted by agreement] added that [Redacted by agreement] always has good comments on the pain/distress categories and [Redacted by agreement] responded that he thinks that the public would have concerns about this.

- New Non-Scientific Member

  Dr. Bodine announced that [Redacted by agreement] will replace [Redacted by agreement] as the non-scientist member.

- Animal Number Overage on Animal Study Proposals

  This topic was discussed in relationship to the G-08-1 and G-10-5 amendments above.

- OLAM Announcements

  **AAALAC 2020**

  Dr. Clark explained that AAALAC will be visiting the NIH campus in June. A training for all investigators will be held in May in Redacted by agreement

  Dr. Clark explained that the AVMA has updated their Guidelines for Euthanasia and there will be changes for mice and fish.

  ***Post Approval Monitoring Report***

  Post Approval Monitoring was performed on the following protocols:

  Redacted by agreement

  Dr. Clark and Redacted by agreement observed Redacted by agreement perform embryo transfer. Sterile gloves were not used but Redacted by agreement did identify appropriate sterile gloves and is now using them.

  Redacted by agreement

  Redacted by agreement observed the balance beam behavioral test that is performed by Redacted by agreement on mice less than 10 days old. Redacted by agreement asked if the mice fall and Redacted by agreement explained that there is bubble wrap underneath the beam and after more than two falls, the test is halted. No deficiencies were observed. Redacted by agreement questioned the procedures for toe clipping and Redacted by agreement stated that it is done on mice less than 10 days old. The protocol states that toe clipping is done on mice less than 8 days old so Dr. Clark will follow up. Redacted by agreement asked if toe clipping interferes with the balance beam and Dr. Clark responded that the toe clipping is done on different mice.

  Redacted by agreement

  Redacted by agreement observed Redacted by agreement perform tail snips on 22 day old mice using ethyl chloride. At this age, topical and systemic analgesia is required. Dr. Bodine suggested that OLAM offer a tail snip training session.

  Redacted by agreement

  Redacted by agreement observed the olfactory test where Redacted by agreement fasts the mice and hides Fruit Loops for them to find. No deficiencies were observed. Redacted by agreement did note that there was no sign-up sheet for the behavior room and no cleaning schedule. She talked to Redacted by agreement about it and she will look into it. Redacted by agreement will make some signs to note that the room is in use.

The next meeting will be held on February 26, 2020 at 1:00 pm in the Redacted by agreement conference room.

Redacted by agreement

_____

David Bodine, Ph.D.
Chairperson NHGRI ACUC

**NHGRI ACUC MINUTES**
**26 February 2020**
**1:00 pm**
Redacted by agreement **Conference Room**

**Members present**: Drs. Bodine, Clark, Redacted by agreement
Redacted by agreement
**Members absent**: Redacted by agreement
**OACU Observer**: None
**Visitors:** Redacted by agreement
Note: The Facility Veterinarian and Facility Manager of the facilities where protocols are performed are given the opportunity to review all new Animal Study Proposals, resubmissions and significant amendments prior to the meeting. Their comments are provided in the text below, when applicable.

## OLD BUSINESS

1. The minutes of the 29 January 2020 meeting were approved without modification. Redacted by agreement asked what the USDA pain categories meant and Dr. Bodine provided a detailed description of each of the pain categories.

2. OACU Refresher Course update – Redacted by agreement was due to complete the refresher course in November but she was on maternity leave. She returned and completed the course on 2/20/20. Redacted by agreement Redacted by agreement were due to complete the refresher course in February. Redacted by agreement completed the course on 1/2/10. Redacted by agreement completed the course on 2/26/20. Redacted by agreement Redacted by agreement are due to complete the refresher course in March.

3. Update on Spring and Fall Semiannual Report Deficiencies

   Redacted by agreement noted that the strong rat odor remains in Redacted by agreement The rat odor is NEI's responsibility. Dr. Clark reported that we are waiting on Siemens to complete the HVAC repairs.

## NEW BUSINESS

1. No ***new*** **Animal Study Proposals were** reviewed at this meeting.

2. No **three-year renewals were** reviewed at this meeting.

3. Two **Animal Study Proposal were subjected to annual review** at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor):

   Dr. Bodine explained the procedures for the annual review of Animal Study Proposals for Redacted by agreement benefit.

Redacted by agreement "Maintenance of Mouse Breeding Colonies for General Use in the NHGRI." P.I.; Redacted by agreement

The Transgenic Core presently has 13 breeding units. Of these 13, 11 lines have been bred to homozygosity onto the C57BL/6J background. All lines have been cryopreserved and are available upon request. There were ~ 60 mice transferred to investigators this past year. Once the remaining 2 lines have been bred to homozygosity, this protocol will be merged with Redacted by agreement. Dr. Bodine was the primary reviewer for this annual review.

Dr. Bodine presented this annual review. He explained that the NHGRI Transgenic Mouse Core generates mouse models for the investigators. The investigators do not meed to maintain their own mouse strains. The Core breeds and cryopreserves the mouse lines. This reduces the number of animals on the self overall because it precludes multiple investigators breeding the same mouse strains. Redacted by agreement questioned the meaning of the text "Any GEM mice not used can be taken off the shelf and can be resurrected later." Dr. Bodine responded that the Core can freeze the sperm and bring the mouse strains back when needed. This is both a refinement and a reduction. There were no questions or additional comments. Dr. Bodine recommended that this protocol be continued.

**Decision**: Approved by unanimous vote (8-0) without modification.

Redacted by agreement "Systematic Mutagenesis and Genetic Analysis in Zebrafish." P.I.; Redacted by agreement

The Redacted by agreement lab mutated another 50 gene candidates for regeneration studies and have generated 3 manuscripts for publication, including one on utilizing the DTR transgenic fish line. In the next year they will continue mutagenizing candidate regeneration genes and will perform hair cell ablations to study chromosomal structural dynamics during regeneration. In addition, this past year they have published two manuscripts related to this protocol. Dr. Clark was the primary reviewer for this annual review.

Redacted by agreement presented his annual review. He explained that he studies the genetics of the regeneration of hearing (hair cells) and other tissues and he models human diseases in fish. He noted that the studies are going well. Dr. Bodine asked if Redacted by agreement had found anyone else that knocked out these same genes in fish and Redacted by agreement responded that he had not found any reports about knocking out these genes. There were no further questions or comments. Dr. Clark recommended that this protocol be continued.

**Decision**: Approved by unanimous vote (7-0, with Redacted by agreement recusing himself from the vote as the PI) without modification.

4. Four **protocol amendments** were submitted at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement "Generation of Genetically Engineered Mice Using Targeted and Conventional Transgenics and Embryo Rederivation in the NHGRI Embryonic Stem Cell and Transgenic Mouse Core." P.I.; Redacted by agreement

The PI would like to remove N-ethyl-N-nitrosourea (ENU) from the Animal Study Proposal listed above. It will not be used for generating mutations at this time. This amendment does not change the number of animals, the endpoints or the USDA Pain/Distress Category.

Dr. Bodine presented this amendment. He explained that Redacted by agreement is generating mutations in mice at targeted locations and is no longer using ENU to generate random mutations. There were no questions or comments.

**Decision**: Approved by unanimous vote (8-0) without modification.

Redacted by agreement "Genetic and Embryological Studies of Murine Mouse Models for Neural Crest Disorders." P.I.; Redacted by agreement Redacted by agreement

The PI requested the following changes:

*Modify euthanasia procedure*. The NPC1 murine studies sometimes require mice to be euthanized on weekends or after working hours. The PI would like to include two additional methods of euthanasia:

1. An overdose of Avertin as the primary method of euthanasia, followed by transcardiac perfusion as the secondary method. Avertin will be delivered via intraperitoneal injection at an overdose of 0.04 mL/gram.
2. Anesthesia via isoflurane as per ASP, followed by a secondary method of decapitation with sharp scissors.

*Blood draw:* After injection with either Avertin or ketamine/xylazine or gas anesthesia, investigators will perform large volume blood draws (~250 uL) for plasma collection. A retro-orbital blood draw with a capillary tube will be used for blood draws and this procedure will be immediately followed by a transcardiac perfusion (secondary euthanasia method).

Total number of animals euthanized via deep anthesia followed by transcardiac perfusion with a terminal blood draw is estimated to be 250 per year.

*Metabolics studies:* For a subset of studies, they need to collect tissues for metabolomics assays. Given the nutrient sensitive nature of the mebalomics measurements, they need to fast the mice for at least 12 hours, though 24 hours is preferred, before blood and tissue collection from fasted and non-fasted *Npc1*$^{-/-}$ and $^{+/+}$ mice.

They propose a small pilot study to validate the metabolomics assays. Mice, at 9 weeks of age, will be fasted one time for 16 hours. Mice will still have access to water *ad libitum* but food will be removed by the investigator 16 hours prior to procedures. If mice appear lethargic or otherwise unwell, they will be immediately euthanized. These procedures will be considered USDA Pain/Distress Category "C". Total number of animals used for the metabolomics pilot study is **16** mice (8 mice fasted and 8 mice non-fasted).

Dr. Bodine presented this amendment. He explained that the NPC1 mice have a cholesterol transport defect that causes profound neurological syndrome. The PI wants to add additional euthanasia methods that can be used on weekends or holidays when controlled substances (ketamine) are not easily available. In these cases, the ketamine would be replaced with the use of Avertin. The PI would also like to add fasting to his ASP. They will fast the mice for 16 hours in order to validate the metabolomics assays. The PI would also like to add terminal blood collection to this ASP. They will anesthetize the mice and collect large volumes of blood prior to perfusion. Redacted by agreement asked if the mouse would be anesthetized for the perfusion procedure. Dr. Bodine responded that the mouse would be under the same deep anesthesthetic plane that was initiated for the terminal blood collection. There were no further questions or comments.

**Decision**: Approved by unanimous vote (8-0) without modification.

Redacted by agreement "Mouse Models of Methylmalonic Acidemia and Propionate Metabolic Disorders." PI.; Redacted by agreement

Recently the PI evaluated the extent of liver regeneration in mice (n=7) that had undergone the partial hepatectomy surgery approved in this protocol (1 lobe). There was minimal regeneration of liver tissue. Therefore they would like to increase the amount of liver resected to 2/3 partial hepatectomy, which is the standard in the field. The ability of liver cells (hepatocytes) to proliferate is in direct proportion to the amount of resected liver tissue. The approach to the surgery or post-surgical management does not change but they will also collect the median lobe as described by Mitchell C, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice [published correction appears in Nat Protoc 2014 Jun;9(6). doi: 10.1038/nprot.2014.122]. *Nat Protoc*. 2008;3(7):1167–1170. doi:10.1038/nprot.2008.80 All animals that have had the surgery have recovered very well with full activity within 30-60 minutes post-surgery. They do not foresee any adverse effect from the increased tissue resection.

In addition, they will add a low dose ketamine dose 20mg/kg IP as part of the pre-medication procedure. This is to help reduce the level of isoflurane used during the surgery while still maintaining an anesthetic

plane as well as assists pain management. This is to help reduce the perioperative liver injury that may occur with isoflurane.

Dr. Clark presented this amendment. She explained that several partial hepatectomies (one liver lobe removed) had been performed under the previous amendment. The mice were euthanized and no regrowth of liver was observed. The PI proposes to take more liver (2/3) in order to encourage the liver to regenerate. Dr. Clark noted that the PI is also adding a low dose of ketamine in order to reduce the dose of isoflurane to minimize liver damage related to this anesthetic. Redacted by agreement asked if the mice were going to survive. Dr. Bodine responded that healthy mice would survive the procedure but they were unsure that these sick mice would. Redacted by agreement asked if there would be a large blood volume loss with the procedure. Dr. Clark responded that each lobe would be tied off. Redacted by agreement questioned the pain category and Dr. Clark responded that it would be "D". Redacted by agreement asked if removing more of the liver would cause more pain. Dr. Bodine responded that it should not be more painful to remove more of the liver. Redacted by agreement Redacted by agreement added that the pain is mainly associated with the incision. Dr. Bodine noted that regeneration of the liver is a well known procedure. Redacted by agreement asked if removing 2/3 of the liver was common. Dr. Clark responded that there is a paper that describes taking 2/3 of the liver and the gall bladder. There were no further questions or comments.

**Decision**: Approved by unanimous vote (8-0) without modification.

Redacted by agreement "Animal Handling and Biomethodology Training for NHGRI Investigators." P.I.; Redacted by agreement

The PI needs to add the Mouse Imaging Facility (MIF) as a procedure room for an ongoing collaboration with the MIF to establish MRI capabilities for zebrafish from embryo to adult.

As part of this collaboration, they need to anesthetize adult zebrafish in a specialized plastic holder compatible with MRI and designed by MIF physicists and OLAM. Adults will be anesthetized with MS-222 bath, as previously described in the ASP. Once in a state of unconsciousness, the fish will be placed within the holder with or without a 22 gauge 'feeding tube' modified for intubation. The holder is connected to a syringe pump and MS-222 will be slowly pumped across the gills to maintain the anesthetic plane. Anesthesia is required for restraint and the procedures are not considered painful or distressful.

They will begin with a pilot study of 10-20 adults and establish the flow rate and dose of MS-222 across the gills necessary to maintain an anesthetic plan. This will be done on a bench within the MIF, which will allow the animal to be monitored at all times and make appropriate adjustments to the flow rate. Anesthesia time will be prolonged to mimic MRI scanning time and may range from 30 minutes to 3 hours. At the end of the anesthetic period, animals will be recovered in fresh system water and monitored for up to 1 hour to determine if there are any adverse effects, such as respiratory distress. After they recover, all animals will be euthanatized per methods outlined in the approved ASP.

Once the standard operating procedure and best practice for this method of anesthesia is established, they will conduct another pilot study (20-30 animals) to undergo the imaging procedures as per the MIF SOP. Redacted by agreement or Redacted by agreement will be handling the animals and supervising the anesthesia, however the MRI procedure will be completed by the MIF Staff. After the imaging, animals will be recovered and monitored for 1 hour to determine if there are any adverse effects such as respiratory distress. Animals will be euthanatized per methods outlined in approved ASP. Total number of animals is 50 additional adult zebrafish.

Redacted by agreement presented her amendment. She explained that this protocol is used for training investigators and she needs to add the Mouse Imaging Facility (MIF) as a procedure room for a pilot study. They are working with the MIF physicist to develop a mechanism for doing MRIs on fish. They have developed a device that can be used to maintain fish on anesthesia. They can intubate the fish and wash the gills with

MS-222. [Redacted by agreement] asked if the procedure was terminal and [Redacted by agreement] responded that ultimately the goal is to be a survival procedure. [Redacted by agreement] questioned the procedure for intubating a fish. [Redacted by agreement] [Redacted by agreement] explained that they use a flexible, plastic rodent gavage needle. [Redacted by agreement] asked why it was necessary to intubate the fish. [Redacted by agreement] responded that they want to direct the water so it goes just through the gills. Dr. Bodine added that the end result is to be able to do MRIs of the fish. [Redacted by agreement] asked what would happen to the fish in this pilot study after the procedure and [Redacted by agreement] responded that they would be euthanized. [Redacted by agreement] questioned the purpose and asked why they did not just do the longitudinal studies. [Redacted by agreement] responded that it would be an important technique to use in the study of metastasis. Dr. Bodine added that they may need to test interventions so they would need long term survival. The device was passed around the table to all committee members. There were no further questions or comments.

**Decision**: Approved by unanimous vote (7-0, with [Redacted by agreement] recusing herself from the vote as the PI) without modification.

5. The following minor amendments and significant amendments approved administratively were announced for concurrence of the committee for the **addition or removal of personnel, location and/or normal strains**. All the new personnel have completed the NIH OACU Animal User training course, registered with AEP and have been specifically trained by OLAM personnel. The additional strains will not increase animal numbers by more than 10% and require no special care beyond that described in the original Animal Study Proposal (Change in the Principal Investigator or change of species requires resubmission of the protocol.) In the case of adding an animal holding location, the signature of the veterinarian and facility manager of the affected facility is required.

[Redacted by agreement] Remove personnel.
[Redacted by agreement] Add personnel
Add three strains
Add strain
[Redacted by agreement] Add personnel
[Redacted by agreement] Add personnel

<u>Significant Amendments for Administrative Approval</u>
None

<u>Amendments Approved Via VVC</u>

[Redacted by agreement] Add new dose and route for Baytril treatment.

Dr. Bodine explained the approval process for minor amendments, significant amendments for administrative approval and the VVC for [Redacted by agreement] benefit. [Redacted by agreement] asked if this was the first meeting that included a VVC approval. Dr. Clark responded that it was the first VVC approval. The ASP included Baytril but there was no dose. The VVC included approval of a dose and added the subcutaneous route of injecting the drug. Dr. Clark added that Baytril is a common antibiotic.

6. **NHGRI Guidelines**

01.1 Guidelines for Euthanasia of Mice

Dr. Clark presented this guideline. She explained that the change in flow rate had already been discussed as part of the Hot Topic (see below). There were no further questions or comments.

Decision: Approved by unanimous vote (8-0) with no further changes.

01.2 Guidelines for Euthanasia of Zebrafish

Dr. Clark presented this guideline. She explained that in addition to the 30 minutes in anesthetic, it was added that embryos up to 7 days could be frozen in a -20 ºC or -80 ºC freezer. Redacted by agreement argued that the embryos can not feel pain at this age and a -80 ºC freezer was expensive and not always available. Dr. Clark agreed to add back the choice of a -20 ºC freezer. It was decided that the text would read "-20 ºC freezer or colder." Dr. Clark noted that formaldehyde derivatives were removed from the guideline. Redacted by . Redacted by agreement responded that zebrafish are sometimes fixed directly with formalin. It was decided that the formalin would be added back to the guideline. Dr. Bodine suggested that this be added to the ASPs. Redacted by Redacted by agreement added that a justification for using it should be included as well. There were no further questions or comments.

Decision: Approved by unanimous vote (8-0) with the changes listed above. Completed 2/27/20.

7. **NHGRI SOPS**

Standard Operating Procedure for Topical Association of Mice with Staphylococcus, Streptococcus and Corynebacteria sp.

Dr. Clark presented this SOP. She explained that this SOP is research specific and we need to review it every three years. Redacted by agreement asked what was used to spray the cages and Dr. Clark responded that they are sprayed with disinfectant. Dr. Bodine added that it protects the people from microbes and the mice from people. There were no further questions or comments.

Decision: Approved by unanimous vote (8-0) without further modification.

8. Other new business:

- Hot Topic – 2020 AVMA Guidelines on Euthanasia

  Dr. Clark explained the Hot Topic is a training opportunity for the ACUC members at every meeting. She noted that the AVMA Guidelines had been updated in 2020. The changes included two areas that affect NHGRI directly. They changed the $CO_2$ displacement volume for rodent euthanasia from 10-30% to 30-70%. Redacted by agreement asked if the investigators would be retrained in this technique. Dr. Clark responded that she will walk around and check the directions posted by every euthanasia machine. For euthanizing fish, the fish need to stay in the euthanasia solution for 30 minutes or more or they can do two method euthanasia, for example they can anesthetize the fish and then decapitate it. Redacted by agreement asked why the flow rate for the mice was so low to begin with. Dr. Clark explained that lesions in the nasal cavity of mice had been observed which were attributed to the higher flow rates. This was considered painful. The 2020 AVMA Guidelines now start the flow rate at 30%. Dr. Bodine noted that this is much better for the animals and he made a motion that we implement the new flow rates immediately, even though the changes have been put out by OLAW for a comment period prior to final approval. In regard to the changes for zebrafish, Redacted by agreement commented that 30 minutes seemed excessive. Dr. Clark responded that the time was based on a Goldfish study. Everyone agreed that the changes should be implemented right away and this was documented when a vote was taken to approve the changes to the rodent and zebrafish euthanasia guidelines.

- Exception to the Guide – Floor Feeding

  Dr. Clark explained that the Guide specifically states that animals should not be fed on the cage floor. This is OK for most rodents but in practice when we have fragile animals that have trouble reaching the feeder or when animals are newly weaned, it helps to offer fruit/mush on the cage floor. A copy of this memo will be placed in every rodent animal study proposal so we are covered to have food on the floor of the cages. Dr. Clark noted that this memo has been resurrected from a previous exception memo. She also noted that as part of standard veterinary care, she can approve to have animals fed on the cage floor without an exception to the Guide. Dr. Bodine added that when a protocol comes up for resubmission, these feeding requirements should be listed in section M.

  Decision: Approved by unanimous vote (8-0) without modification.

- New NonScientific Member

  Dr. Clark introduced Redacted by agreement as the new Nonscientific member, replacing Redacted by agreement (appointment letter dated 1/16/20).

- PBS Update

  Dr. Clark noted that at an FDA AAALAC site visit, the use of PBS was a concern as it is not available as pharmaceutical-grade. Dr. Bodine commented that the buffer is sometimes required and it depends on what is being dissolved. A discussion ensued on how to keep the PBS sterile. Redacted by agreement suggested that it always be sterile filtered. Dr. Clark suggested that we need a guideline on making sure that its use is justified and that it remains sterile after opening.

- OLAM Announcements

  **OLAW Audit**

  Dr. Clark explained that OLAW will do a routine audit of the whole NIH animal program March 2-6. We need to provide them with our guidelines, our SOPs, our animal study proposals, our minutes, and our semiannual reports. There will be an inbriefing Monday morning at 7:30 am. Dr. Bodine, Dr. Clark, Redacted by agreement and Redacted by agreement will participate in the policy review on Tuesday morning and Dr. Clark and Redacted by agreement will participate in the records review Tuesday afternoon. They will visit the Transgenic Core, the aerosolization chamber lab and other procedure areas.

  ***Post Approval Monitoring Report***

  Post Approval Monitoring was performed on the following protocols:

  Redacted by agreement

  Redacted by agreement observed Redacted by agreement and Redacted by agreement perform intracranial AAV injections of mice under anesthesia. The animals recovered well. They were observed placing the mice in the stereotaxic frame prior to shaving and prepping the injection site. Dr. Clark advised them to shave and prepare the site prior to placing the animal in the frame. They were using the wrong ophthalmic solution, which was present in the room. Redacted by agreement identified that the wrong ophthalmic solution was being used and Redacted by agreement Redacted by removed it and replaced it with appropriate lubricant . Redacted by agreement noted that the surgery cards remain on the cages until the wound clips are removed, as appropiate. In addition to post-operative

observations, the eyes of the mice with the wrong ophthalmic solution used were checked for several days and no adverse effect was noted.

Redacted by agreement

Redacted by agreement observed Redacted by agreement perform retro-orbital blood collection and euthanasia. She was given re-training on the blood collection, specifically the retraint and was asked to practice on training animals prior to collecting blood from study animals.

Redacted by agreement

Redacted by agreement observed Redacted by agreement perform tail amputation on adult fish. No discrepancies were noted. Redacted by agreement will need to remove Redacted by agreement from the ASP.

Redacted by agreement

Mice were written up on the health report as having gone past their protocol humane endpoints. The mice were euthanized and Dr. Clark trained the Co-PI Redacted by agreement on how to better assess the endpoints in the mice.

Redacted by agreement

Redacted by agreement observed Redacted by agreement perform the marble burying behavior testing. An ear punch was used that was not sterile. The Co-PI was reminded that the ear punch must initially be sterile.

Redacted by agreement

Redacted by agreement observed post procedural mice inoculated intranasally with the flu vaccine by Redacted by agreement Redacted by agreement Redacted by agreement and Dr. Clark observed the animals and subsequent days and there was no evidence that the animals show signs of pain or distress during the study period and prior to euthanasia. No discrepancies were noted.

The next meeting will be held on March 25, 2020 at 1:00 pm in the Redacted by agreement conference room.

Redacted by agreement

David Bodine, Ph.D.
Chairperson NHGRI ACUC

**NHGRI ACUC MINUTES**
**25 March 2020**
**1:00 pm**
**WebEx Meeting due to Covid-19**

**Members present**: Drs. Bodine, Clark, Redacted by agreement
Redacted by agreement
**Members absent**: None
**OACU Observer**: Redacted by agreement
**Visitors:** Redacted by agreement
Note: The Facility Veterinarian and Facility Manager of the facilities where protocols are performed are given the opportunity to review all new Animal Study Proposals, resubmissions and significant amendments prior to the meeting. Their comments are provided in the text below, when applicable.

## OLD BUSINESS

1. The minutes of the 26 February 2020 meeting were approved with the change to the Post Approval Monitoring summary for protocol Redacted by agreement noted that he observed fin clips on adult fish, not larvae. Completed 3/26/20.

2. OACU Refresher Course update – Redacted by agreement were due to complete the refresher course in March. Redacted by agreement completed the course on 3/7/20. Redacted by agreement Redacted by agreement completed the course on 3/16/20. No one is due to complete the refresher course in April.

3. Update on Spring and Fall Semiannual Report Deficiencies

   Redacted by agreement noted that the strong rat odor remains in Redacted by agreement The rat odor is NEI's responsibility. Dr. Bodine reported that he talked to the lead institute (NEI) about moving the rat room to a new location and they seemed amenable to that possibility.

## NEW BUSINESS

1. No ***new*** **Animal Study Proposals were** reviewed at this meeting.

2. One **three-year renewals was** reviewed at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement "Study of Fanconi Anemia Pathway in Zebrafish." PI.; Redacted by agreement

The aim of this study is to understand the disease process associated with Fanconi anemia (FA), a rare genetic disorder. The majority of FA patients are born with abnormalities of multiple organ systems, most develop childhood bone marrow failure (BMF) resulting in depletion of all types of blood forming cells. They also experience infertility and an increased risk for cancer, particularly acute myeloid leukemia (AML) and squamous cell carcinomas of the head and neck. Mutations in one of 22 genes are known to cause FA; these genes encode proteins which orchestrate repair of DNA damage caused by interstrand crosslinks. Understanding the FA disease process may provide an opportunity to prevent/manage this devastating disease.

The PI has generated mutations in zebrafish homologs of 17 FA and 2 FA-associated protein (FAAP100 and FAAP24) genes. General characterization of these mutants—such as embryonic sensitivity to DNA interstrand crosslinking agents, growth, viability to adult stage, gender bias, and fertility—was completed and published in *PLoS Genetics* (PMID: 30540754). (b)(4)

(b)(4)

They will continue characterization of all their mutant zebrafish lines for a better understanding of blood disease and cancer associated with FA. In addition, they will evaluate the effect of concurrent mutations in 1) the *tp53* gene on tumor development and 2) in a modifier gene, *adh5*, on FA specific characteristics. Utilization of transgenic lines expressing hematopoietic stem/progenitor cell (HSPC) markers—such as CD41 and GATA1—will hopefully detect any FA gene mutant associated differences in HSPCs, which may enable them to explore drugs that could help in repopulation of hematopoietic stem cells.

Dr. Bodine explained that every three years an Animal Study Proposal has to be resubmitted, reviewed and approved in order for the study to continue. Redacted by agreement presented his resubmission. He explained that three years ago he generated Franconi Anemia (FA) mutations in zebrafish. FA is a rare disease that causes many congenital problems and carrries an increased risk of cancer. Redacted by agreement Redacted by agreement has generated mutations in 24 Franconi related disease causing genes. One gene is a modifier of FA and absence of this gene in people causes an FA-like syndrome. Over the next three years he will look at adult null fish to see if there are defects in blood cell development. No current animal model has been developed to study cancer development in FA. He has generated FA fish on the p53 null background to see if this increases the frequency of tumors. He will grow the zebrafish to different ages and look at pathology (cancer development). Dr. Bodine asked if breeding and observations were the the main procedures performed on this ASP and Redacted by agreement responded this was correct. No surgical procedures are performed. Redacted by agreement asked if any "squeezing" procedures had been performed over the past three years and Redacted by agreement responded that none were done. Redacted by agreement noted that Dr. Clark explained squeezing to her in the ACUC member training the day before the meeting. Dr. Bodine added that "squeezing" is in the ASP even though it is not done very often. Dr. Bodine asked if the homozygote fish were fertile and Redacted by agreement responded that in most cases they were and that he did not get many female homozygotes. Redacted by agreement noted that the animal numbers were reasonable. Redacted by agreement Redacted by agreement asked if he had any idea why a mouse model did not show any of the defects documented in people? Redacted by agreement responded that the mouse model does not develop the hematopoietic defect. It is not clear how cancer development occurs so no real effort has been made in mice to study it. Dr. Clark noted that tumor endpoints are not included in the ASP. Redacted by agreement responded that they will observe the zebrafish over time and if they show signs of tumors, they will euthanize them. Dr. Clark noted that if they see tumors, they will need an amendment to add the endpoints. Dr. Bodine added that the literature search was excellent. Redacted by agreement noted that only paraformaldehyde, o-Dianisidine, Diepoxybutadiene, Mitomycin C and Cisplatin should be listed in section K. She also provided safety language to add to section K. There were no further questions or comments.

**Decision**: Approved by unanimous vote (10-0) with the changes to section K. noted above. Completed 3/26/20.

3. Five **Animal Study Proposals were subjected to annual review** at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor):

Redacted by agreement "Transgenic Mice to study Functions of CBFB in Development and Disease." P.I.: Redacted by agreement

The objective of this study is to use transgenic mice as models to understand the function of the gene *CBFB* (Core binding factor b) in diseases and normal development. *CBFB* encodes the protein CBFbeta, which binds three related proteins (RUNX1, RUNX2 and RUNX3) and together they function as transcriptional regulators. *CBFB* and *RUNX1* are frequently mutated in human leukemias through chromosome translocations, resulting in the generation of fusion genes such as *CBFB-MYH11* (MYosin Heavy chain 11). They have established a mouse model of human leukemia by introducing the fusion gene *CBFB-*

*MYH11*, which is seen in patients with a subtype of acute myeloid leukemia, into mice. They have demonstrated that this *CBFB-MYH11* fusion gene represses normal functions of CBFbeta and RUNX1 and blocks normal blood cell formation and drives leukemia development. Additional genetic changes, such as activation of other cellular oncogenes, are needed for full leukemic transformation. One of these genes is *Gata2*, and they recently showed that *Gata2* deficiency delayed leukemogenesis while contributing to aggressive leukemia phenotype in *Cbfb-MYH11* knockin mice (Saida et al., Leukemia 2019). They are continuing their studies on the interplay between *Cbfb*/*Runx1* and *Gata2* during leukemia development. They are also finishing their studies on the role of *Runx1* in leukemogenesis driven by *CBFB-MYH11*, and they expect to submit a paper on this study soon. Finally, they have used CRISPR genome targeting to tag RUNX1 in the mouse models so they can better characterize the molecular mechanism of blood cell formation and leukemia development involving this important protein. Dr. Bodine was the primary reviewer for this annual review.

Dr. Bodine presented this annual review. He explained that Redacted by agreement studies acute myeloid leukemia which is the most common leukemia in people. CBFB binds to DNA and other proteins that can turn leukemia genes "on and "off". Redacted by agreement has different experiments where he puts in mutant CBFB or he crosses to mice defient in the partner genes. This helps him determine how quickly and severely mice get leukemia by observing the mice and analyzing the blood. He is not trying to treat the mice at this point. Dr. Bodine commented that the literature search was good. There were no further questions or additional comments. Dr. Bodine recommended that this protocol be continued.

**Decision**: Approved by unanimous vote (10-0) without modification.

Redacted by agreement "Cryopreservation of Mouse Embryos and Spermatozoa." P.I.; Redacted by agreement

*In Vitro Fertilization* During this past year, the Core has cryopreserved ~94 genetically engineered mouse lines. This is reflective of the number of CRISPR mutations/gene edits that have been created. Therefore, with the efficiency of CRISPR editing, they expect to have at least 100-150 lines to cryopreserve each year. The Core has streamlined the cryopreservation program by reducing the number of IVFs required for quality control and have updated and simplified protocols. Both of these measures have resulted in less use of animals (Reduction and Refinement) and more efficient use of personnel. Dr. Bodine was the primary reviewer for this annual review.

Redacted by agreement presented her annual review. She explained that this is the Transgenic Mouse Core cryopreservation ASP. They have 99-100 lines per year being frozen down, mainly due to the efficiency of CRISPR/Cas gene editing. Redacted by agreement said that she had streamlined this ASP. They do quality control by freezing the sperm and doing *in vitro* fertilization (IVF) to make sure they can bring them back. They are now testing cryoprotective media by IVF each time they make it. This cryopreservation program is saving large numbers of mice (**reduction).** Dr. Bodine asked Redacted by agreement to explain IVF. Redacted by agreement responded that they harvest oocytes from the female mice, they give hormones, they take frozen or fresh sperm and add together with the oocytes in a pertri dish to produce fertilized eggs. The eggs are cultured over night and then the embryo transfer is performed. There were no further questions or comments. Redacted by agreement commented that this was fascinating and would like to observe the procedure at some point. Dr. Bodine recommended that this protocol be continued.

**Decision**: Approved by unanimous vote (9-0, with Redacted by agreement recusing herself from the vote as the PI) without modification.

Redacted by agreement "Regulation of Epithelial Differentiation." P.I.; Redacted by agreement

Studies performed on this Animal Study Proposal investigate the complex relationships between the skin's epithelial barrier, the microbes (bacteria, fungi) that inhabit the skin and the immune cells that perform

surveillance of the skin. Many of their experiments seek to understand the complexity of skin/microbe/immune cell interactions under normal development, and then explore changes that may contribute to disease. They also seek to catalog and understand the bacteria that colonize the skin of normal mice. In the past year, they have both cultured and sequenced the microbiome of mice to develop isolates and genomic datasets. One of their major goals for this year is to explore the murine microbiome at a more functional level, exploring the genes expressed by the bacteria as they colonize mouse skin, using RNA-Seq. They are interested to learn about the transcriptional profile that bacteria express when colonizing an epithelial surface which are distinct from the bacteria's growth in media. This will help them to understand genes involved in attachment and nutrient utilization. This next year they would like to continue to explore how the communities of bacteria are formed and the molecular cues that they use to promote or inhibit each other's growth. Through these studies, they aim to improve skin care treatment of humans and other vertebrate species. Dr. Bodine was the primary reviewer for this annual review.

Dr. Bodine presented this annual review. He explained that Redacted by agreement is studying epithelial differentiation. She studies how skin keeps water in and the microbiome. She colonizes mouse skin with bacteria from patients with different skin diseases like eczema. She euthanizes the mice and analyzes the cells underneath the skin layers to see the effects of genes on mouse skin. Redacted by agreement asked if there was any pain or distress and Dr. Bodine explained that there was none beyond minimal and transient pain associated with restraint. If there were a change, for example a skin rash, an amendment would be needed. Dr. Bodine explained the annual review process. He noted that he was pleased with the literature search.

There were no further questions or comments. Dr. Bodine recommended that this protocol be continued.

**Decision**: Approved by unanimous vote (10-0) without modification.

Redacted by agreement "Studies of the Hutchinson-Gilford Progeria Syndrome (HGPS) Mutation in Transgenic Mice." P.I.; Redacted by agreement Redacted by agreement

Hutchinson-Gilford Progeria Syndrome (HGPS) is an extremely rare, premature aging syndrome. The typical patient has severe growth retardation, low weight and short stature, hair loss, wrinkled skin, cardiovascular disease, incomplete sexual maturation, delayed dentition and stiffness of joints. Mutations in the LMNA gene encoding the inner nuclear lamina proteins lamin A and C, have been associated with HGPS. HGPS is a dominant disorder. This makes it critical to observe what expression of the mutant lamin A protein (called progerin) does in the presence of normal lamin A. They are studying the function of the HGPS mutation in transgenic mice that have the human LMNA gene with the HGPS mutation [codon G608G (ggc>ggt)] and the normal mouse LMNA genes. Mice carrying the mutant human gene exhibit accelerated aging, including cardiovascular disease, hair loss, skin rigidity, inflammation and dryness of the eye, curving of the spine, joint contractures and loss of fat stores. While these symptoms are common in mice greater than 2 years of age, mice homozygous for the mutant Lamin A transgene develop these symptoms in as little as 2-3 months.

Using their mouse models they are currently incorporating two new therapeutic approaches to treat HGPS. The first is a morpholino (PPMO706) that blocks the aberrant splicing event that leads to the production of progerin, the pathogenic mutant form of Lamin A. The second is a gene editing approach. Using an Adeno Associated Virus vector, they introduce a guide RNA directed at the HGPS mutation and a modified Cas 9 nuclease designed to correct the mutation.

Because the mouse life span is much shorter than even a human HGPS patient's, the goal of these mouse studies is to determine whether drugs have efficacy while human trials of the drugs are in progress. Currently they have no active studies of this type, but previously they have evaluated drugs that interfere with the processing of mutant Lamin A or, like rapamycin, have been shown to extend a normal mouse's life span. They conducted these studies with combinations of drugs and they hope that these studies will lay

the groundwork for clinical trials of these drugs in patients with cardiovascular disease as well as HGPS patients. They are also testing new analogues of these drugs to see if they have an increased potency. Redacted by agreement Redacted by agreement was the primary reviewer for this annual review.

Redacted by agreement presented his annual review. He explained that this ASP the lab is studying a mouse model for Progeria. Progeria is a famous disease but very, very, rare. It only takes one mutant gene in people to cause this premature aging. They know the mutation and they have a mouse model that expresses it. The mice are not balding but do have cardiovascular disease in the great vessels of the heart. These mice live two years or less. Redacted by agreement asked if this was the same life span as the wild-type mice and Redacted by agreement responded that it was about 1+ years shorter than normal littermates. Redacted by agreement asked if they used caloric restrictions, which has been shown to increase the life span in many mammals. Redacted by agreement responded that the Redacted by agreement lab is using drugs to treat the disease. Redacted by agreement noted that the literature search makes reference to various mouse models. Redacted by agreement responded that the Redacted by agreement lab is using all three. Two out of the three mouse models were generated by Redacted by agreement and one was imported. There were no further questions or comments. Redacted by agreement recommended that the protocol be continued.

**Decision**: Approved by unanimous vote (9-0, with Redacted by agreement recusing himself from the vote as the PI) without modification.

Redacted by agreement "Mouse Models for the Study of Saul-Wilson Syndrome and Other Skeletal Dysplasias." P.I.; Redacted by agreement

The PI recently obtained the desired knock-in heterozygous *Cog4* mutant mice, reproducing the genotype of Saul-Wilson syndrome. At present, they are expanding their colony (F2s born within the last two weeks, with normal litter sizes), and during the course of the upcoming year they expect to start characterizing the phenotype of the mutant mice, to see if it differs from wild-type, and if recapitulates human disease. Dr. Clark was the primary reviewer for this annual review.

Dr. Bodine presented this annual review. He explained that the PI studies Saul-Wilson Syndrome and other skeletal dysplaisas. This new PI is just getting started. He has characterized the phenotype to see how it recapitulates the human disease. Redacted by agreement added that the disease manifests as a bone deformity. Dr. Clark noted that right now the protocol is breeding and if they see a phenotype they will do an amendment. There were no further questions or comments. Dr. Clark recommended that the protocol be continued.

**Decision**: Approved by unanimous vote (10-0) without modification.

4. Four **protocol amendments** were submitted at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement "Transgenic Mice to study Functions of CBFB in Development and Disease." P.I.; Redacted by agreement

The PI would like to clarify that bone marrow will not be transduced with recombinant retroviral vectors. He would like to make the following modification to the text in section F of the Animal Study Proposal:

Please replace "The B6129F1/J mice will be used for injection of leukemia cells developed in the Cbfb-MYH11 transgenic mice, or bone marrow cells from normal mice (B6 or B6.129 mixed background) transduced with retroviral vectors expressing transgenes, in order to study leukemia biology" with "The B6129F1/J mice will be used for injection of leukemia cells developed in the Cbfb-MYH11 transgenic mice in order to study leukemia biology."

Dr. Bodine presented this amendment. He explained that Redacted by agreement is removing the transduction of retroviral vectors from his Animal Study Proposal. Redacted by agreement noted that the pain category was not included in the amendment. Dr. Bodine responded that the USDA Pain/Distress Category is "C" and it will be added. Redacted by agreement asked for an explanation of transduction of retroviral vectors and Dr. Bodine responded that it is a biological method to deliver genes to mouse cells. Retroviruses have the ability to transform their single-stranded RNA genome into a double-stranded DNA molecule that integrates into the genome of dividing target cells. In the past Redacted by agreement used retroviral transduction in his leukemia research. There were no further questions or comments.

**Decision**: Approved by unanimous vote (10-0) with the addition of the USDA Pain/Distress Category "C". Completed 3/26/20.

Redacted by agreement "Cryopreservation of Mouse Embryos and Spermatozoa." P.I.: Redacted by agreement

The PI would like to clarify the humane endpoints and the procedures that will be followed in the event of a dystocia.

They will use the standard clinical endpoints. These include significantly hunched posture, significantly reduced activity or impaired mobility that interferes with normal eating and drinking, significantly rough fur, a weight loss of > 20%, a body condition score (BCS) of 1 (out of 5), respiratory distress, dystocia without clinical response, a pain score (PS) of 3 or 4 (out of 4) when therapy is ineffective or needs to be withheld for research purposes, or weak or no response to external stimuli (moribund).

For surgical recipients experiencing difficulty with birth or dystocia, either a C-section will be performed or the animal will be euthanized. If a C-section is not indicated, the recipient mother will be euthanized by $CO_2$ narcotization followed by cervical dislocation. If a C-section is indicated, they will need to perform cervical dislocation without prior narcotization, as to not harm the feti with $CO_2$ gas. This method of euthanasia has been scientifically justified in the Animal Study Proposal.

Redacted by agreement presented her amendment. She explained that her amendment was submitted to define the endpoints and clarify how to handle mice in dystocia. If they are trying to rescue the pups, they do not use $CO_2$ narcotization because it will decrease the chances of survival for the pups. Dr. Bodine explained that dystocia was when female mice have difficulty giving birth to pups. Redacted by agreement added that if the female mouse has dystocia, they can do a caesarian section to rescue the pups. Once the pups are removed, they are placed on a warmer and then fostered to a FVB/N female. Redacted by agreement noted that the pain category was not included in the amendment. Dr. Clark responded that since the surgery was terminal, the USDA Pain/Distress Category is "C" and it will be added. Redacted by agreement asked what causes dystocia and Redacted by agreement Redacted by agreement responded that genes play a role but she has to rescue the pups so to decrease the number of the mice made. There were no further questions or comments.

**Decision**: Approved by unanimous vote (9-0, with Redacted by agreement recusing herself from the vote as the PI) without modification.

Redacted by agreement "Systematic Mutagenesis and Genetic Analysis in Zebrafish." PI.: Redacted by agreement

The PI would like to add larval caudal fin amputation. The zebrafish larvae are highly regenerative at this stage with rapid regeneration within 2-3 days and the assay is to study the process of tissue regeneration. The 3dpf larvae are exposed to 0.02% w/v Tricane (MS-222) until they stop moving and then a single incision is made with a scalpel amputating the tissue at the "pigment gap" in the tailfin. The fish are then allowed to regenerate their tail over the course of 72 hours with all animals being euthanized at specific time intervals.

After amputation the fish are exposed to one of three different chemicals, 2-Deoxy-D-glucose at 100mM, an inhibitor of hexokinase, SB431542 at 50$\mu$M, an inhibitor of TGFb receptors, or 2-NBDG at 500$\mu$M, a fluorescent analog of glucose. These chemicals will remain in the embryo water with larvae grouped in 6-well plates for up to the duration of the fin regeneration time of 3 days. They estimate 1000 larvae will be subjected to this procedure in the next year but will not significantly change total animal numbers. Because the fish cannot experience pain at this age, they are considered column "C".

For the hDtR procedure already approved in the ASP, the PI would like to increase the animal numbers: He would like to add an additional 500 fish to the hDTR procedure (hair cell ablation via diphtheria toxin injection) using the same toxin dose and the same recovery timeframe as has been previously done. This will provide 25 fish for control and experimental conditions for 3 timepoints done in triplicate. This number was sufficient for similar experiments performed previously. The fish will be used for a new experiment where they will be assaying chromatin remodeling during regeneration via single cell Hi-C sequencing. The assay is performed after the fish are euthanized at each timepoint.

Redacted by agreement presented his amendment. He explained that he needs to increase his zebrafish numbers in order to add a new three dimensional chromatin confirmation experiment. He is also adding a new technique to test tail regeneration in larval fish. They will remove the tip of the fin deep enough to penetrate the notochord and it will grow back in 2-3 days. After the amputation they will expose the fish to one of three different drugs. Dr. Bodine asked if this procedure was done on older fish and Redacted by agreement responded that genotyping older fish is on his ASP but, in contrast to this procedure, removes relatively less fin with no surrounding tissue. Redacted by agreement asked if the larvae experienced pain? Redacted by agreement responded that this procedure is performed before the larvae have developed pain receptors. Redacted by agreement Redacted by agreement asked if adding 500 fish was a significant increase. Redacted by agreement responded that he uses hundreds of thousands of larvae/fish on his ASP per year so 500 is a small percentage of the total. There were no further questions or comments.

**Decision**: Approved by unanimous vote (10-0) without modification.

Redacted by agreement "Animal Handling and Biomethodology Training for NHGRI Investigators." P.I.; Redacted by agreement

The PI would like to add Perox-Aid to assess the clinical treatment options for zebrafish. It is a buffered 35% pharmaceutical-grade hydrogen peroxide solution produced for the aquaculture market to use as an external microbicide for the control of mortality due to *Flavobacterium species* bacteria (FDA approved).

Perox-Aid is widely used for control of pathogens (such as bacteria and mold) in aquaculture as the byproducts are primarily water and oxygen. It has no current published use as a clinical treatment for zebrafish and they would conduct a pilot study to determine the zebrafish senstitivity at different life stages with varying doses (0-100 mg/L) .).

**Pilot study for zebrafish sensitivity at different life stages to effective dose/treatment:**

Exposure to normal embryos would occur at 3 dpf with the embryos remaining in the solution until 7 dpf. They would be monitored for signs of aversion to the initial dose as well as mortality/deformity. They anticipate using less than 100 animals and account for these animals as USDA Pain or Distress Category C.

Exposure to normal juveniles and adults (30+ dpf) would be a bath treatment of the dose every other day for a total of three treatments. They will be monitored for signs of distress and change in clinical health. Throughout the treatments, fish will be euthanized and their gill structures analyzed for signs of damage/irritation. They anticipate using no more than 100 fish, (50 juveniles and 50 adults) for this pilot study with no change in humane endpoints and acccount for these animals as USDA Pain or Distress Category C.

Redacted by agreement presented her amendment. She explained that she is adding an FDA approved medication for treatment of bacterial infection and respiratory issues in fish. She is proposing a pilot study to determine the effective dose. Once the dose is determined she will move forward to determine if the medication can be used clinically. Dr. Bodine asked if this was the first pharmaceutical-grade drug used to treat fish. Redacted by agreement responded that MS-222 is also pharmaceutical-grade and is used for euthanasia. Dr. Bodine asked if Redacted by agreement would be doing the study and she responded that Redacted by agreement would be doing it. Redacted by agreement commented that it appeared that the general ASP was not changing, they were just adding something new and Redacted by agreement responded that this was correct. There were no further questions or comments.

**Decision**: Approved by unanimous vote (9-0, with Redacted by agreement recusing herself from the vote as the PI) without modification.

5. The following minor amendments, significant amendments approved administratively and VVC amendments were announced for concurrence of the committee. For amendments for the **addition or removal of personnel, location and/or normal strains**, all the new personnel have completed the NIH OACU Animal User training course, registered with AEP and have been specifically trained by OLAM personnel. The additional strains will not increase animal numbers by more than 10% and require no special care beyond that described in the original Animal Study Proposal (Change in the Principal Investigator or change of species requires resubmission of the protocol.) In the case of adding an animal holding location, the signature of the veterinarian and facility manager of the affected facility is required.

Dr. Bodine explained the process for the submission of minor amendments.

Redacted by agreement Add personnel
Redacted by agreement Add two strains
Redacted by agreement Add personnel
Redacted by agreement Remove two personnel
Redacted by agreement Remove personnel
Redacted by agreement Remove personnel
Redacted by agreement Remove personnel
Add personnel

<u>Significant Amendments for Administrative Approval</u>

None

<u>Amendments Approved Via VVC</u>

Redacted by agreement Clarify euthanasia method

Dr. Clark explained that the method of euthanasia described by Redacted by agreement was already in an ACUC Guideline as well as in the AVMA Guidelines. Dr. Bodine added that if he wanted to add cervical dislocation without prior narcotization as a primary method of euthanasia he would need a scientific justification and a major amendment would be needed. Redacted by agreement noted that the pain category was not included in the amendment. Dr. Bodine responded that the USDA Pain/Distress Category is "C" and it will be added. Redacted by agreement questioned the "other methods in the guideline" and asked if that covered all other euthanasia methods described in the guideline and Dr. Bodine responded this was correct. Redacted by agreement Redacted by agreement asked for a an explanation of terminal perfusion. Dr. Bodine explained that it is the procedure used for fixing tissues. After death, tissues degrade quickly so they need to "fix" the tissues before the degradation begins. Dr. Bodine explained that it is a terminal procedure performed under a heavy dose of anesthesia. The heart is still beating and fixative is pumped into the tissues. Redacted by agreement asked if perfusion

was USDA Pain/Distress Category "D" and Dr. Clark explained that the mice are under deep anesthesia and will not wake up so the USDA Pain/Distress category "C". Dr. Bodine added that the pain category is not determined by whether or not anesthesia is given. Pain category is determined by whether or not the procedure generates pain or distress that is more than momentary. Redacted by agreement asked if perfusion is an overdose of anesthetic. Dr. Bodine responded that technically the animals could survive the anesthetic. Redacted by agreement asked if the mouse was under deep sedation and Dr. Clark responded that some people use gas anesthetic and some use Avertin. She added that perfusion is a two step procedure, gas or chemical anesthesia followed by perfusion of fixative. Redacted by agreement asked if anesthesia was in the ASP and Redacted by agreement Redacted by agreement responded that it was.

6. **NHGRI Guidelines**

None

7. **NHGRI SOPS**

Import/Export of Mice

Dr. Clark presented this SOP. She explained that this was an old SOP that was put in the new format. There were no questions or comments.

Decision: Approved by unanimous vote (10-0) without further modification.

JAX nomenclature (fact sheet replacing the previous SOP)

Redacted by agreement presented this fact sheet as a proposed replacement for the nomenclature SOP. Redacted by agreement explained that she liks this because it is a simple, quick reference that covers most scenarios. She added that the only majopr change from our previous nomenclature is that "GE" changed to "EM" for gene editing. Dr. Bodine asked if this reference could be provided to the investigators when they are writing new ASPs or resubmitting old ones. Redacted by agreement can do this. Redacted by agreement added that the correct nomenclature also needs to be used on imports/exports. There were no further questions or comments.

Decision: Approved by unanimous vote (10-0) without further modification.

Operation of VetEquip Anesthesia Units for Mice

Dr. Clark presented this SOP. She explained that this SOP is for the anesthesia unit used by the Mouse Core in the Redacted by agreement surgery suite. The SOP was put in the new format and there were no real changes. There were no questions or comments.

Decision: Approved by unanimous vote (10-0) without further modification.

General Practices for the Weighing and Measuring of Zebrafish

Dr. Clark presented this SOP. She explained that this is a new SOP so that investigators can weigh and measure fish as part of other procedures they are doing with anesthetized fish. These procedures will be added to all of the NHGRI zebrafish ASPs. There were no further questions or comments.

Decision: Approved by unanimous vote (10-0) without further modification.

Procedures for Transporting Animals to and From NIH Facilities

She explained that OLAW questioned this procedure so we decided to put the information together in an SOP. Redacted by agreement asked if this SOP referred only to campus transfers. Dr. Bodine responded that this was correct and that off campus transfers are referred to as imports and exports. There were no further questions or comments.

Decision: Approved by unanimous vote (10-0) without further modification.

Standard Operating Procedure for Topical Association of Mice with Staphylococcus, Streptococcus and Corynebacteria sp.

Dr. Clark presented this Investigator SOP. She explained that Redacted by agreement had identified some differences between facility procedures versus this SOP and changes will be made after the meeting to make sure the SOP is in compliance with the facility. Redacted by agreement questioned the differences between the SOP and the facility requirements and Redacted by agreement responded that they use different chemical hazard cards and stickers. The SOP states the animals are housed in Redacted by agreement and this room is for biohazards. There were no further questions or comments.

Decision: Approved by unanimous vote (10-0) without further modification.

8. Other new business:

- Hot Topic – Veterinary Verification and Consultation (VVC)

  See the OLAW Site Visit Update below.

- New OACU Observer

  Redacted by agreement introduced Redacted by agreement She has been with NIH for ten years, but is new to OACU. The members introduced themselves as well.

* Continuity of operations for the ACUC/Pandemic Response

Dr. Clark explained that our Disaster Plan/Pandemic response did not clearly describe how our ACUC would function in a situation such as the pandemic we are dealing with today. Our plan will be updated and submitted for review at the April ACUC meeting. The Pandemic Plan will be merged with the Disaster Plan and details on the ACUC will be added. Dr. Clark reported that under the current pandemic, science has slowed down but the animals are being taken care of and their health and well being are our top priority. Dr. Bodine added that ARAC made a point to report that right now our animal staff is here at 100%. Staffing and supplies are sufficient to care for the animals. At some point it its conceivable that space for weaning will fill up and staff may get sick and be unable to come to work. NHGRI is making an effort to decrease animal numbers by routine colony management at this point. Redacted by agreement added that we need to be proactive in regard to rack space. She expressed concern that a limited number of people are on campus to genotype and investigators could run out of space for weaning. Dr. Bodine responded that investigators can be approved to come in and genotype/wean under maintenance of resources. Dr. Clark will confirm with Redacted by agreement that genotyping is an essential function.

* Spring Semiannual Program Review

Dr. Clark explained that we may be able to use the facility walk throughs that have been performed by the lead Institutes for the facilities where our animals are housed. The personnel participating in the walk throughs can be approved by our ACUC as ad hoc consultants. She added that laboratory visits are only required annually and we visited all our labs in October. That would leave us with our satellite facilities. Redacted by agreement noted that only one person is required to perform a walk through for species not covered by the USDA. Ms. Smith noted that there is a pending waiver that has been submitted to OLAW concerning the semiannual walk throughs. We should receive a response from OLAW either late this week or early next week. OACU will provide guidance on how to document the waivers for ad hoc inspections in the semiannual reports. The reports are still due April 30.

- OLAM Announcements

**OLAW Site Visit Update**

Dr. Clark reported that our OLAW Site Visit was successful. NHGRI was one of the ICs that was called out with positive comments on our ACUC. The site visitors complimented the Transgenic Mouse Core on the testing of the Avertin using performance standards. Avertin is a non-pharmaceutical anesthetic andso it was noteworthy that they would single Avertin use out for positive comments because of how the Core handles it. The OLAW site visitors also had positive comments concerning our post approval monitoring program and were impressed with the secondary protocol files that Redacted by agreement and Redacted by agreement maintain with the safety data sheets, DNA registrations, pathogen registrations, PAM findings and other protocol specific documents. Dr. Clark explained that OLAW had concerns related to our VVC procedure, which is new to our ACUC. The VVC amendment being reviewed at this meeting is only the second time our ACUC has used this avenue of approval. OLAW suggested that we establish guidelines, SOPs, drug formularies and other references that are approved by the ACUC and used by the veterinarian for the VVC approvals. The VVC amendment should reference the guideline or document used in the approval process. Dr. Clark will review the VVC OLAW Webinar and revise the ACUC guideline. Dr. Bodine noted that the VVC problem was not unique to NHGRI. Several other ICs were not in compliance. Redacted by agreement noted that NHGRI was following the ARAC guideline which also needs to be revised. Redacted by agreement added that all of NIH was deficient in this regard, including the ARAC guideline which is currently under revision. She is happy to work with the Institutes on the VVC and noted that OLAW did not give the best guidance initially.

Dr. Clark noted that the AAALAC Site Visit had been postponed until August.

***Post Approval Monitoring Report***

Post Approval Monitoring was performed on the following protocols:

Redacted by agreement

The researcher was sick so Redacted by agreement reviewed the ASP. The Emergency Treatment and Care form needs to be updated with Redacted by agreement as the primary contact.

Redacted by agreement

Redacted by agreement observed Redacted by agreement setting up breeders and using microisolator technique. No deficiencies were noted.

Redacted by agreement

Redacted by agreement observed Redacted by agreement setting up breeders and using microisolator technique. No deficiencies were noted.

Improper Disposal of Zebrafish Embryos

Redacted by agreement observed two incidents of improper disposal of zebrafish embryos (<72 dpf) in the lab sink. The room entrance logs were pulled and five people were retrained. Dr. Bodine asked how many embryos were observed in the sink and Redacted by agreement responded that it was approximately 5. Dr. Clark noted that the embryos are not animals yet. Redacted by agreement wanted to know how improper disposal was identified. Redacted by agreement responded that he inspects the sinks daily. He collected them and properly disposed of them. Dr. Bodine questioned the size of the screen and Redacted by Redacted by agreement responded that it is not small enough for embryos. Dr. Clark added that the second incident appeared to involve NHLBI so she communicated the problem to the PI and APD. Dr. Clark explained that we are calling it "inadvertent" but it is actually accidental.

The next meeting will be held on April 27, 2020 at 1:00 pm in the Redacted by agreement conference room.

Redacted by agreement

David Bodine, Ph.D.
Chairperson NHGRI ACUC

**NHGRI ACUC MINUTES**
**27 April 2020**
**1:00 pm**
**WebEx Meeting due to Covid-19**

**Members present**: Drs. Bodine, Clark, Redacted by agreement Redacted by agreement
**Members absent**: Redacted by agreement
**OACU Observer**: Redacted by agreement
**Visitors:** Redacted by agreement
Note: The Facility Veterinarian and Facility Manager of the facilities where protocols are performed are given the opportunity to review all new Animal Study Proposals, resubmissions and significant amendments prior to the meeting. Their comments are provided in the text below, when applicable.

## OLD BUSINESS

1. The minutes of the 25 March 2020 meeting were approved without modification.

2. OACU Refresher Course update – No one was due to complete the refresher course in April. Redacted by agreement Redacted by agreement are due to complete the refresher course in May.

3. Update on Fall and Spring Semiannual Report Deficiencies

   Redacted by agreement noted that the strong rat odor remains in Redacted by agreement The rat odor is NEI's responsibility. Dr. Clark noted that she did not notice the odor during the walk through. Redacted by agreement responded that some days it is strong and other days it is not. Dr. Clark added that Siemen's work on this problem has been delayed due to the pandemic. Dr. Bodine responded that it is still possible that the rat room could be relocated.

   The only outstanding minor deficiency for the Spring walk through is the chemical fume in Redacted by agreement Redacted by agreement that needs to be certified. Redacted by agreement reported this to DOHS.

## NEW BUSINESS

1. One ***new*** **Animal Study Proposal was** reviewed at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

   Redacted by agreement "Understanding Function of Genes Involved in Melanoma Progression by Genetic, Genomic and Phenotyping Approaches in Zebrafish." PI. Redacted by agreement

   The aim of this study is to determine the function and phenotype of novel melanoblast genes previously identified in the mouse. They want to determine what function these genes play in melanocyte development using zebrafish genetics, imaging and phenotype studies. Recently they published that these novel melanoblast genes contribute to human metastatic melanoma and therefore knowing how their expression impacts the melanocytic lineage is critical to understanding how they can take advantage of these genes/pathways to treat melanoma metastasis. Any gene functions retained between zebrafish and mice are more likely to be conserved through to humans and therefore relevant to human melanoma.

   Redacted by agreement presented his protocol. He explained that this is a new study for his group, and they are collaborating with a NCI investigator Redacted by agreement who is studying melanoma using zebrafish. Redacted by agreement explained that they have identified genes involved in melanocyte development in zebrafish. Redacted by agreement

commented that [Redacted by agreement] is an expert in this field. [Redacted by agreement] explained that they will generate fish and mark the cell types involved in melanoma development. They will do imaging and test small molecule treatments. Squeezing and prolonged restraint are the only pain/distress category "D" procedures proposed. Dr. Clark explained that the zebrafish undergoing prolonged restraint would be under anesthesia that could last up to 5 days. [Redacted by agreement] added that this is how long it takes for the cells to develop. There is the potential for the animals to die under anesthesia. [Redacted by agreement] commented that keeping the fish under anesthesia for 5 days and expecting them to live was ambitious. [Redacted by agreement] responded that [Redacted by agreement] had been successful with this procedure in the past. Further discussion took place and it was determined that the procedure would remain pain/distress category "D" and [Redacted by agreement] would report back to the ACUC if 25% or more of the fish die under anesthesia. Dr. Bodine asked if the procedures would be done in [Redacted by agreement] and [Redacted by agreement] responded that she would prefer to do the procedure in [Redacted by agreement] requested a description of the restraint. [Redacted by agreement] responded that the fish would be placed in agarose E3 media with buffered tricaine. As the fish grow over the five-day time period, she would scrape the agarose away from their heads and tails to allow room for them to increase in size. Dr. Bodine asked if [Redacted by agreement] [Redacted by agreement] had questions. She responded that she worked with [Redacted by agreement] before the meeting packets went out to edit Section K. [Redacted by agreement] recommended that the numbers be increased. [Redacted by agreement] [Redacted by agreement] asked if he was referring to embryo numbers and [Redacted by agreement] responded that he was. Dr. Bodine advised that when/if they came close to exceeding the proposed numbers, they should submit an amendment. [Redacted by agreement] added that the study sounded reasonable in its relationship to human disease. Dr. Bodine complimented the literature search. There were no further questions or comments.

**Decision**: Approved by unanimous vote (9-0) without modification.

2. Two **three-year renewals were** reviewed at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

[Redacted by agreement] "Mouse Models for Disorders of the Biogenesis of Lysosomes and Lysosome-Related Organelles." PI.; [Redacted by agreement]

The PI is studying rare genetic disorders of lysosomes and lysosome-related organelles (LROs). These disorders include Hermansky-Pudlak (HPS) and Chediak Higashi (CHS) syndromes, and related (new) disorders that manifest with combinations of albinism, bleeding, neurological symptoms, immunodeficiency, inflammation of the large intestines, or pulmonary fibrosis. The goal of this protocol is to obtain or generate mouse models for LRO and related human disorders to investigate underlying pathology/cell biology and proposed therapies.

Over the years, they have identified genes in human patients that affect the biology of lysosomes and LROs, including *CLCN7*, *TRAPPC6A/B*, and others. Mouse models for *Trappc6A* have been associated with mosaic hypopigmentation phenotype, and as such are of inherent interest to their studies in lysosomal organelles. Given the associated phenotype, they reasoned that the paralog of *Trappc6A*, *Trappc6B*, may also serve a role in pigmentation and lysosomal physiology, making models of the latter of interest as well. As the paralogs may complement each other, a double knockout line is additionally of interest. The human *TRAPPC6A* and *TRAPPC6B* encode trafficking protein particle complex proteins that are subunits of the TRAPPI and TRAPPII complexes.

They propose to use existing mouse models or to generate novel models using available gene editing technologies in collaboration with the NHGRI Transgenic Mouse Core to better understand the mechanism of HPS, CHD, and lysosome-related disorders. They also aim to evaluate therapeutic options including pharmaceutical compounds of interest and gene therapy, and gene editing.

Redacted by agreement presented her protocol. She explained that she is resubmitting her study of mouse models of lysosomes and lysosome-related disorders. She studies multiple genes and multiple human diseases. These disorders cause combinations of albinism, neurological symptoms, immunodeficiency and other symptoms but the most severe is the pulmonary fibrosis. The mouse models do not always have a pulmonary fibrosis phenotype, so they induce it with bleomycin and wait for it to develop. They use drugs or gene therapy to alter the course of the disease or to prevent pulmonary fibrosis. Redacted by agreement asked for a description of the induction of pulmonary fibrosis. Redacted by agreement explained that the mouse is anesthetized, and a tube is placed in its trachea for delivery of the bleomycin. This procedure is USDA pain/distress category "E" because it causes chemical irritation to the breathing system. Redacted by agreement asked if this was similar to intubation in humans and Redacted by agreement responded that the process was similar, but bleomycin is not introduced into humans. Redacted by agreement noted that Redacted by agreement did a great job on the nomenclature. Dr. Bodine asked Redacted by agreement if she had any safety concerns. She responded that they were addressed with Redacted by agreement before the meeting packets went out. Redacted by agreement commented that the study had good relevance to human disease. Redacted by agreement explained the questions/comments provided by the Redacted by agreement facility vet and the Redacted by agreement manager. Need to change "clean" scalpel to "sterile" scalpel section F, page 11. Need to add that behavioral equipment will be sanitized with facility-approved disinfectant in section F, page 13. Need to add that the mice will be weighed three times weekly when endpoints are approaching to section F, page 13. Need to add that mice will be weighed three times weekly and observed daily for respiratory distress post surgically to section G, item #1, page 14. Need to add that iodophor/chlorhexidine and 70% alcohol prep will be alternated 2-3 times. Need to add that the PI places the surgery card on the cage and is responsible for providing the card to OLAM for filing to Section G, item #1. Need to add that handling of animals that have undergone implantation by the scruff should be avoided until the surgical wound is completely healed to Section G, item #4, page 15. Add that the PI will notify the care staff via technical request 48 hours in advance of the animal recovery in order to alert the staff on how to handle the animals to Section G. For mice with no teeth, add that the PI will mark cages for soft foods and notify the care staff via technical request 48 hours in advance to section M. For mice following gavage, add that the PI will notify the care staff via technical request 48 hours in advance of the procedure so that the staff is aware of the procedures. Add that the PI will submit a technical request at least 48 hours in advance of drug trials and gene therapy trials so that the CAF Staff are alerted to the trials and if necessary, provide any specific information that the CAF staff needs to be aware of. Dr. Bodine explained that this ASP included descriptions of procedures that occurred postmortem and other unnecessary information that made it hard to read. He agreed that the ASP should be approved but would like for it to be resubmitted at some point and he would work with the PI to make improvements. There were no further questions or comments.

**Decision**: It was determined that the changes listed above would be made and the ASP would go to DMR with Dr. Bodine as the reviewer. The ASP was approved by DMR on 4/30/20 with no further changes.

Redacted by agreement "Mouse Models of Rare and Undiagnosed Genetic Diseases. PI.; Redacted by agreement

The NIH Undiagnosed Diseases Program (UDP) is a pilot program designed to address the needs of persons with debilitating medical conditions for which no diagnosis has been found despite an extensive workup. The goals of the UDP include finding accurate diagnoses and discovering new diseases that provide insight into human physiology and genetics. The UDP makes use of a diverse set of diagnostic and research techniques both to attempt to find a diagnosis and to generate research projects to advance medical knowledge. It is the goal of this ASP to functionally validate genes identified in patients through next generation whole exome/genome sequencing and genomics analysis using mouse models. Genes and gene variants that are candidates for disease will be deleted or introduced in mice through CRISPR-Cas9 gene editing, to understand the function of potentially disease-causing genes and the *in vivo* consequences of mutations in mice. Indeed, they have been able to generate mouse models of monogenic disorders that helped them solidify the diagnosis of patients seen in the UDP. For the next three years, they aim to continue characterizing the mouse models that they have generated, and to contribute to the diagnosis of UDP patients by understanding the phenotypic spectrum of human disease in mouse models.

Redacted by agreement presented her resubmission. She explained that this ASP supports the Undiagnosed Disease Program (UDP). The UDP sees patients with rare genetic disorders. The diseases are so rare that they don't know if the gene causes the disorder or not. They study these diseases using animal models. They review the literature and if they don't find the mouse model, they generate one. They are currently studying several rare disorders. Finding the gene in an animal can support the diagnosis in humans. Most of the phenotyping described is standard. They will follow the natural history of the mouse models. Dr. Bodine asked how they would determine kidney/liver function. Redacted by agreement responded that they would do tissue phenotyping (glomelular filtration rate). They give labeled inulin to the animals and follow excretion of inulin as it compares to wild-type mice. Dr. Bodine questioned the pain/distress category "D" animals and Redacted by agreement responded that the GFR mice are "D". There were no further questions or comments.

**Decision**: Approved by unanimous vote (8-0, with Redacted by agreement recusing herself from the vote as the PI) without modification.

3. One **Animal Study Proposal was subjected to annual review** at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor):

Redacted by agreement "Mouse Models of Methylmalonic Acidemia and Propionate Metabolic Disorders." P.I.; Redacted by agreement

Since the last review they have continued to characterize and study various mouse models of methylmalonic acidemia (MMA) and propionic acidemia (PA). Current and ongoing work, which is in the process of being prepared for publication, includes new mouse models of cblC (Mmachc deficiency), new knock-in mouse models of MMut deficiency, and new genome editing models of propionic acidemia, which have provided a foundation for a new gene therapy project sponsored by NCATS. The objectives for the next year are to complete studies in the models mentioned above, submit for publication when the studies are completed, and begin a new phase of exploration of post translation modification biology in MMA and PA using the mouse models they have developed. Of note, a major publication appeared in Scientific Advances (Pavuluri K, Manoli I, Pass A, Li Y, Vernon HJ, Venditti CP, McMahon MT. (2019) Noninvasive monitoring of chronic kidney disease using pH and perfusion imaging. Sci Adv.14;5(8):eaaw8357. PMCID:PMC6693904) where they used their mouse models to enable a new method to measure kidney function. The NHGRI mouse models have also further facilitated the award of an R01 grant to Redacted by agreement as part of a continued collaboration with our team. Dr. Bodine was the primary reviewer for this annual review.

Dr. Bodine presented this annual review. He explained that Redacted by agreement studies deficiencies in enzymes that process amino acids for energy. Toxins build up with deficiencies of these enzymes. The goal is to test different approaches in gene therapy, and that while gene therapy works in principal, making gene therapy safer is an ongoing process. Dr. Bodine added that Redacted by agreement is a prolific animal user and has many co-investigators working with him. He also complimented the literature search. There were no further questions or comments. Dr. Bodine recommended that this protocol be continued.

**Decision**: Approved by unanimous vote (9-0) without modification.

4. Five **protocol amendments** were submitted at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement "Genetic and Embryological Studies of Murine Mouse Models for Neural Crest Disorders." P.I.; Redacted by agreement Redacted by agreement

The PI would like to remove the skin graft surgery from sections F and G of his Animal Study Proposal. They will no longer be performing this procedure. This will change the USDA Pain/Distress Category "D" numbers from 144 to 100 per year.

Dr. Bodine presented this amendment. There were no questions or comments.

**Decision**: Approved by unanimous vote (9-0) without modification.

(b)(5)

(b)(5)

(b)(5) It was decided to table the amendment until the June meeting so this ASP could be added to the amendment. There were no further questions or comments.

**Decision**: Unanimous vote to table the amendment (7-0, with Redacted by agreement recusing themselves from the vote as PIs).

Redacted by agreement "Analysis of Murine Models of Gaucher Disease and *GBA* associated Parkinsonism." PI.; Redacted by agreement

The PI would like to add a new project exploring mouse models of Gaucher disease (GD) type 3c. The PI would like to add the B6;129-*Gba1*$^{D409H}$ strain, imported from the University of Cincinnati. This strain is intended to model Gaucher disease type 3c (GD3c), a rare human phenotype associated with this genotype.

In humans, GD3c is caused by homozygosity for a *GBA1* point mutation, D409H. In patients the major findings in GD3c are cardiovascular calcifications of the aortic and mitral valves and the ascending aorta leading to cardiac failure and early death, which is not seen in other types of GD. To better understand the mutation-specific pathogenesis of GD3c, accurate disease models are needed. The previously developed B6;129-*Gba1*$^{D409H}$ mice have a relatively mild phenotype, including a few storage cells in the spleen and small accumulations of glucosylceramide in visceral organs, but histopathological and ultrastructural analyses of the cardiac valve were not performed. The imported B6;129-*Gba1*$^{D409H}$ homozygous mice will be rederived in the NHGRI Transgenic Mouse Core to generate the GD3c mouse models. The mutant mice will be euthanized as described in the ASP and tissues will be collected for micro CT imaging to the Mouse Image Facility (MIF) in Redacted by agreement to assess calcification of aortic valve.

Animal Numbers: B6;129-*Gba1*$^{D409H}$ knock-in mice were imported in early 2020. Once re-derived, they plan 4BU/line (8 mice) x 6 litters/year x 6 pups/litter = 144+8 =152 additional mice per year to maintain the line for the needed evaluations. Mice will be weighed and then euthanized for tissue collection every two months, between age 4 months and 20 months as per Section J. No adverse phenotype is expected.

Redacted by agreement presented her amendment. She explained that she is adding a new mouse line whose phenotype models a rare subgroup of patients with Gaucher disease. These patients suffer from progressive

cardiovascular calcification. This mouse model was made by another group, but they did not study the cardiovascular problems. She will import the mice and she may try a high fat diet in the future to induce the cardiovascular phenotype. Dr. Bodine added that if she has interesting findings, she will be back with another amendment to add mice and procedures. There were no further questions or comments.

**Decision**: Approved by unanimous vote (8-0, with Redacted by agreement recusing herself from the vote as the PI) without modification.

Redacted by agreement "Mouse Models for Disorders of Sialylation." P.I.; Redacted by agreement

The PI would like to make the following changes to her Animal Study Proposal:

1. Add two new lines, B6;129S-*Slc17a5*$^{em1mal}$ (ΔSlc17a5$^{Ex1}$B6;129S) and B6;129S- *Slc17a5*$^{em2mal}$ (Slc17a5$^{R39C}$B6;129S). These lines were generated in collaboration with Transgenic Mouse Core facility. In humans, mutations in SLC17A5 cause sialic acid storage disease (SASD). These mutations cause a deficiency in the lysosomal membrane transporter sialin and leads to a spectrum of disorders from severe neurodevelopmental defects that rapidly deteriorate and become fatal (infantile sialic acid storage disease or ISSD due to SLC17A5 nonsense mutations), to psychomotor and cognitive disability in young adults. Mutant mice that lack Slc17a5 show characteristics of ISSD with neurobehavioral defects, abnormal brain morphology, hypomyelination, and lysosomal vacuolization of central nervous tissue as well as peripheral organs that start after birth; complete deletion of this gene leads to early postnatal lethality (~P20). They expect a knockin of R39C in mice will result in a milder phenotype and allow longer survival, as R39C in humans is a hypomorph mutation that results in a slightly delayed onset and progression of neurological symptoms. Methods for phenotyping this line are already in the existing protocol.

2. Define humane endpoints for these new lines. They anticipate that the ΔSlc17a5$^{Ex1}$B6;129S will manifest with runting and organomegaly, hence they will use the body conditioning score to assess the welfare of the mice. Body condition scores of 1 (emaciation) or 5 (obesity) will require humane euthanasia.

3. For the Slc17a5$^{R39C}$B6;129S, they expect that these mice will manifest with neurologic phenotype, including varying degrees of ataxia and locomotor abnormalities resulting from severe abnormality in central and peripheral myelin formation. The humane endpoint for this line will be euthanasia upon worsening of the ataxia beyond the moderate level and/or advanced age-related illness. Assessment of the welfare of the animal in terms of the ataxia will be by the Locomotor Activity Scale:

   <u>Scale 0</u> (normal) Locomotor activity: normal. No intervention needed
   <u>Scale 1</u> (mild) Locomotor activity: wobbly, lurching, gait, mild ataxia, and shorter stride. Suggested intervention: food on the floor (FOF)
   <u>Scale 2</u> (moderate) Locomotor activity: staggered gait (increased unsteady side to side movement), effort required to align themselves, moderate ataxia, and splayed legs. Suggested intervention: supportive care (SC) and FOF.
   <u>Scale 3</u> (severe) Locomotor activity: loss of motor coordination, pronounced hindlimb incoordination, severe ataxia, frequent falls, and inability to align themselves. Intervention: requires euthanasia of these animals.

   If the mutants cannot feed through the wire bar lid, normal chow, fruit and/or mush will be placed on the floor of the cage. When these endpoints are reached, and they will likely expect that severe mice will be scored as Scale 3 as noted above, mice will be euthanized as per section J. Regardless of the phenotype, any mouse that is noted to be in pain or distress that cannot be medically managed will be euthanized.

4. Add X-ray analyses: for direct measurements of anthropomorphic parameters, indirect measurement of organomegaly, and study of bone formation and skeletal abnormalities. Mutant and control mice will be anesthetized or euthanized (as per sections I and J). Around 30 mice per genotype will be imaged (total of ~60 mice per year). X-rays (Faxitron) will be performed in the Redacted by agreement animal facility Redacted by agreement as per the NHGRI Faxitron Transport and Use SOPs. After the procedure, mice may be euthanized, transported to DVR Pathology for euthanasia/necropsy while still anesthetized or returned to their animal room in Redacted by agreement

5. Add Redacted by agreement to Redacted by agreement Since they anticipate that this mouse will have a lysosomal storage phenotype, they anticipate that they will need to perform x-ray analysis.

This line will not significantly increase the total number of mice per year in the above protocol. They anticipate that there will be 4 breeding pairs/line for the initial identification of mutant lines (~ 100 mice; 2 lines x 4 breeding pairs x 10 pups per pair + 16 breeders = 96), and another 4 breeding pairs per line for phenotyping (~100 mice; 2 lines x 4 breeding pairs x 10 pups per pair + 16 breeders = 96) for a total of 200 mice/year.

Redacted by agreement presented her amendment. She explained that she is adding two new mouse lines made by the Transgenic Mouse Core. SLC17A5 functions in lysosomal transport of sugar. Without the transport, the sugar accumulates in the lysosomes. This condition causes postnatal lethality around P20 due to neurological deficits. They are hoping the knockin of R39C will produce a model with a milder phenotype that survives longer for a natural history study. She noted that she will do behavioral analysis on the mice because they believe they will develop behavioral deficits. Redacted by agreement asked if the pain/distress category changed as the mice go through the described the locomotor activity scale. Redacted by agreement responded that she does not know if the mice experience any pain. Dr. Bodine asked if the human patients feel pain and Redacted by agreement responded that the patients generally can't say they are in pain because they have a neurological deficit. Dr. Bodine asked if the patients are treated for pain and Redacted by agreement responded that they are not. Dr. Clark added that we also have to consider possible distress in addition to pain. In this case, for scale 1 the mice are wobbly, so they get fed food on the floor. They become wobbly gradually, so they are likely not distressed by it. Dr. Clark noted that supplemental care would be provided, and the animals would be closely monitored and euthanized at scale 3. There were no further questions or comments.

**Decision**: Approved by unanimous vote (8-0, with Redacted by agreement recusing herself from the vote as the PI) without modification.

Redacted by agreement "Mouse Models for the Study of Saul-Wilson syndrome and Other Skeletal Dysplasias"." P.I.; Redacted by agreement

The PI would like to adjust the numbers of mice on this protocol. At present, they are expanding their colony (F2s born within the last two weeks, with normal litter sizes), and during the course of the upcoming year they expect to start characterizing the phenotype of the mutant mice, to see if it differs from wild-type, and if recapitulates human disease. Originally, they asked for 226 mice/year; however, for the establishment of appropriate littermate-controlled experimental cohorts, they would request to increase their animal numbers to 400/year. This represents an additional 174 animals per year. They anticipate that they will use the same number of animals in 2020-2021 and 2021-2022.

Dr. Bodine presented this amendment. He explained that the PI is just increasing his animal numbers. Redacted by agreement Redacted by agreement asked if he had enough rack space to support the increase in numbers. Dr. Clark responded that his space was increased from ½ to a full rack and this rack is currently only half full. There were no further questions or comments.

**Decision**: Approved by unanimous vote (9-0) without modification.

5. The following minor amendments, significant amendments approved administratively and VVC amendments were announced for concurrence of the committee. For amendments for the **addition or removal of personnel, location and/or normal strains**, all the new personnel have completed the NIH OACU Animal User training course, registered with AEP and have been specifically trained by OLAM personnel. The additional strains will not increase animal numbers by more than 10% and require no special care beyond that described in the original Animal Study Proposal (Change in the Principal Investigator or change of species requires resubmission of the protocol.) In the case of adding an animal holding location, the signature of the veterinarian and facility manager of the affected facility is required.

Redacted by agreement Remove location
Redacted by agreement Add location

Significant Amendments for Administrative Approval

None

Amendments Approved Via VVC

None

6. **NHGRI Guidelines**

02.2 NHGRI ASP Amendment Guideline

Dr. Clark presented the revised guideline. She explained that we are trying to bring the business of the ACUC in line with the most recent OLAW guidance. The changes included clarification of designated member review (DMR), procedures for conducting ACUC business during a pandemic or disaster were added, the Veterinary Verification Consultation (VVC) procedures were clarified according to suggestions from OLAW and a list of references was added. Dr. Bodine questioned the difference between a regular amendment and VVC. Dr. Clark responded that the VVC is more veterinary/clinical in natures instead of administrative. For example, a change in analgesia or dose could be looked up in a formulary that was previously approved by the ACUC as a reference. VVC is intended to streamline the amendment process. Redacted by agreement asked if this meant there was a choice between full ACUC review and expediting amendments through VVC or DMR? Dr. Bodine responded that it was correct that these were all avenues of the review process but when in doubt, any ACUC member could call for full member review (FMR). Dr. Bodine referred to the example earlier with Redacted by agreement where we discussed changes and then after the meeting the changes would be reviewed and approved by DMR. Dr. Clark added that approval cannot be withheld using DMR, but it can be sent to FMR.

Decision: Approved by unanimous vote (9-0) without further modification.

7. **NHGRI SOPS**

(b)(5)

Dr. Clark presented this SOP. Redacted by agreement had some concerns about changes that had been made.

Decision: Tabled and will be revised and resubmitted.

Teratoma Injection Protocol

Dr. Clark presented this SOP. There were no questions or comments.

Decision: Approved by unanimous vote (9-0) without further modification

Vasectomy

Dr. Clark presented this SOP. There were no questions or comments.

Decision: Approved by unanimous vote (9-0) without further modification

Retro-orbital Injection

Dr. Clark presented this SOP. There were no questions or comments.

Decision: Approved by unanimous vote (9-0) without further modification

Rodent Routes of Administration

Dr. Clark presented this SOP. There were no questions or comments.

Decision: Approved by unanimous vote (9-0) without further modification

Post Approval Monitoring

Dr. Clark presented this SOP. There were no questions or comments.

Decision: Approved by unanimous vote (9-0) without further modification.

8. Other new business:

- Hot Topic – Compassion Fatigue

  Dr. Clark discussed the handouts and noted that Redacted by agreement contacted the Employee Assistance Program (EAP) and they are prepared to assist any employee or contractor with symptoms of compassion fatigue.

- Disaster Plan – discussed as part of the semiannual program review below.

- Lewis & Clark College FOIA

  Dr. Clark explained that multiple institutes at NIH had all received the same FOIA request from Lewis & Clark College to provide information on all animal welfare concerns that had been documented since January 2019. NHGRI had no concerns to report. Redacted by agreement asked if the ACUC meeting minutes were subject to FOIA and Redacted by agreement responded that they were. He also wanted to know if the WebEx meetings were recorded and saved. Redacted by agreement responded that she had contacted IT and she was told that the meetings are not recorded unless you want them recorded.

* Spring Semiannual Program Review

Dr. Clark explained that we only needed one ACUC member to visit each area because mice and zebrafish are not USDA covered species. Dr. Clark visited some areas, Redacted by agreement visited some areas and Redacted by agreement was able to participate in two walkthroughs via FaceTime Redacted by agreement

Redacted by agreement As a result of the pandemic it was also possible for us to take a waiver, but this did not turn out to be necessary. All areas were either visited by one of our ACUC members or by ad hoc consultants. Dr. Clark also explained that walk throughs of laboratories are only required annually and all of our labs were visited in the Fall. All of our zebrafish satellite incubators were visited, and all were empty. Redacted by agreement asked why we had to use ad hoc consultants and Dr. Clark explained that in the case of Redacted by agreement there were several COVID-19 positive animal care personnel so it was not advisable for us to enter. In the case of the Mouse Imaging Facility and Redacted by agreement we do not have access afterhours. Redacted by agreement questioned the mouse cage lid and cage parts observed on the floor in one of the animal rooms during the Redacted by agreement walk through. Redacted by agreement responded that she spoke to the floor leader and he believes that a cage set up was just accidentally dropped and not well cleaned up.

Approval of Ad hoc Consultants – Redacted by agreement proposed a list of ad hoc consultants for approval by the ACUC.

Decision: Approved by unanimous vote (9-0).

Semiannual Assessment Checklist

Dr. Bodine reviewed the semiannual program review checklist and discussed each item. The Disaster and Pandemic Plans were combined, and the revised document was included in the meeting packet and discussed. Dr. Bodine noted that cryopreservation is the major component of the plan. Both the Transgenic Mouse and Zebrafish Cores have multiple freezers in multiple locations.

- OLAM Announcements

AAALAC Site Visit Update – Postponed until October 2020.
Pandemic Update – All animal facilities housing NHGRI animals continue to be fully staffed.

***Post Approval Monitoring Report***

Post Approval Monitoring was performed on the following protocols:

Redacted by agreement

Redacted by agreement reviewed the ASP for humane and study endpoints. She noted that next review cycle, they should make the endpoints more specific.

Redacted by agreement

Redacted by agreement reviewed the study remotely with Redacted by agreement She answered questions related to the ASP and had to be reminded of the proper the method of euthanasia for after hours. A procedure room needed to be added to the ASP and this amendment has been submitted.

Redacted by agreement

Redacted by agreement reviewed the study remotely with their new lab member Redacted by . He properly answered questions related to the ASP and there were no revisions or amendments needed.

Redacted by agreement

Redacted by agreement reviewed the rack for space allocation and also saw that the pups needed fruit and mush. No deficiencies were noted.

The next meeting will be held on May 26, 2020 at 1:00 pm in the Redacted by agreement conference room.

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David Bodine, Ph.D.
Chairperson NHGRI ACUC

**NHGRI ACUC MINUTES**
**26 May 2020**
**1:00 pm**
**WebEx Meeting due to Covid-19**

**Members present:** Drs. Bodine, Clark, [redacted]
**Members absent**: None
**OACU Observer**: [redacted]
**Visitors:** [redacted]
Note: The Facility Veterinarian and Facility Manager of the facilities where protocols are performed are given the opportunity to review all new Animal Study Proposals, resubmissions and significant amendments prior to the meeting. Their comments are provided in the text below, when applicable.

## OLD BUSINESS

1. The minutes of the 27 April 2020 meeting were approved without modification.

2. OACU Refresher Course update – No one was due to complete the refresher course in April. [redacted] were due to complete the refresher course in May. [redacted] completed the course on 5/8/20. [redacted] completed the course on 5/31/20. [redacted] are due to complete the refresher course in June.

3. Update on Fall and Spring Semiannual Report Deficiencies

   The only outstanding minor deficiency from the Fall Semiannual Walk Through is the strong rat odor that remains in [redacted] The rat odor is NEI's responsibility. Dr. Clark added that Siemen's work on this problem has been delayed due to the pandemic and [redacted] deployment. Dr. Bodine noted that in recent visits to the Transgenic Mouse Core, he has not noticed the rat odor. [redacted] agreed that the odor had been much less noticeable the last few weeks.

   The only outstanding minor deficiency for the Spring Semiannual Walk Through is the chemical fume in [redacted] that needs to be repaired/certified. [redacted] submitted a work request to have the air flow adjusted in response to the failed certification, but the work has not been done yet.

## NEW BUSINESS

1. No ***new*** **Animal Study Proposals were** reviewed at this meeting.

2. Two **three-year renewals were** reviewed at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

[redacted] "Analysis of Murine Models of Gaucher Disease and *GBA* associated Parkinsonism." PI.; [redacted]

The goals of this study are to better understand aspects of Gaucher disease, to study the link between Gaucher disease and Parkinson disease and to develop and test new therapies for patients. This work is helping to advance understanding of the basic processes involved in Gaucher disease and facilitates the development of treatments. Furthermore, these studies are helping to elucidate aspects of the pathophysiology and treatment of Parkinson disease.

**Progress of the last three years:**

1) **The modeling of glucocerebrosidase–associated parkinsonism in the mouse:** The lab has crossed transgenic human A53T α-synuclein (*SNCA*$^{A53T}$) mice with heterozygous null *gba* mice (*gba*$^{+/}$). Mice were followed for up to two years and survival analysis showed the symptom onset was significantly earlier with exacerbated disease progression in the crossed mice. This is now published. Behavior testing of these mice and the corresponding controls was performed and the data are now being analyzed.
2) **Development of new therapeutics for Gaucher disease:** The lab has evaluated a new murine line with mutation D409V, generated an immortalized neuronal cell line and have evaluated peritoneal macrophages to enhance their evaluation of new chaperone drugs for Gaucher and Parkinson disease.
3) **New models to evaluate acute neuronopathic Gaucher disease:** The lab has crossed the lethal null allele Gaucher disease mouse with point mutation mice and have evaluated the phenotype.

**Ongoing Goals:**

1) They will assess the new type 3c Gaucher disease model (name of mouse line: D409H/D409H).
2) They will continue to cross Gaucher and Parkinson mouse models as a means to better understand the connection between glucocerebrosidase and parkinsonism. Single cell RNAseq and further protein evaluations using mouse brain samples are in progress (name of mouse lines: L444/L444P//TJaSYN, D409H/D409H//TJaSYN, ΔMGC$^{tm}$//TJaSYN).
3) They will continue to use murine peritoneal macrophages and murine models for drug screening and validation (name of mouse line: L444/L444P, D409V/D409V).

Redacted by agreement presented her protocol. She explained that she studies Gaucher and Gaucher related Parkinson disease. Gaucher Disease is caused by an inherited deficiency of an enzyme called glucocerebrosidase. Different mutations can cause Gaucher babies to die at birth while other mutations are found in adults that live into their 70's without knowing that they have the disease. They are studying the relationship between the genotype and the resulting variety of phenotypes. Patients that carry the mutations for Gaucher disease are more apt to develop Parkinson disease. In the mouse models, the knockout mice die at birth which has helped them to identify a human phenotype that also results in death at birth. When they crossed transgenic human A53T α-synuclein (*SNCA*$^{A53T}$) mice with heterozygous null *gba* mice (*gba*$^{+/}$), the resulting mice died earlier, and the progression of the symptoms was much faster. Redacted by agreement Redacted by agreement noted that they inject sodium thioglycolate in the peritoneum of the mice to collect macrophages which they use in the screening of new drugs. Dr. Bodine explained that the injection of the sodium thioglycolate was evaluated several years ago in a pilot study through which data was collected that showed that this procedure results in achy, flu-like symptoms that last three days. Because the distress was transient, it was determined to be USDA Pain Category "C". The only USDA Pain Category "E" procedure on this ASP is the mice with severe neurological phenotype (limb paralysis). These mice generally get euthanized around 24 hours after they develop the severe phenotype. Dr. Clark explained that since mice are prey animals that being paralyzed is very distressful. Redacted by agreement asked if the estimate of 40-60 mice for pain category "E" was an estimate based on previous experience and Redacted by agreement responded that this was correct. Redacted by agreement asked for confirmation that the hind leg paralysis was more distressful than painful, and Dr. Bodine responded that this was correct. Dr. Clark added that the lab was careful to euthanize the mice as soon as endpoints are reached. Redacted by agreement commented that the numbers were appropriate, and Dr. Bodine added that good rationale for animal use was provided. Redacted by agreement noted that the Redacted by agreement Facility Manager had requested that the ASP state that technical requests would be submitted for handling the ΔMGC mutation mice that die within first few hours after birth, for handling the mice being weighed weekly and monitored for the onset of neurological symptoms, for handling the symptomatic mice with associated neurological symptoms that can be so severe that they are considered USDA Pain Category "E," for increased monitoring of mice injected with thioglycolate that may need an approved source of moisture (e.g., cold fruits or Clear Water products) in each cage to encourage the mice

to eat during the inflammatory response process, and to alert the staff to feed cold fruit and/or Clearwater products to aging mice or mice with other clinical ramifications of the phenotype and/or treatments.

**Decision**: Approved by unanimous vote (9-0, with Redacted by agreement recusing herself from the vote as the PI) with the modifications listed above which were made as per ACUC Guideline 02.2 under "Unconditional Administrative Process".

Redacted by agreement "Mouse Models of Glycosphingolipid Storage Disorders: Understanding Disease Pathogenesis Provides Clues to Therapeutic Options." PI.; Redacted by agreement

GM1 and GM2 gangliosidosis (Tay-Sachs and Sandhoff diseases) are fatal neurodegenerative lysosomal storage disorders (LSDs) with no effective therapy. The study objectives of this protocol are to complete phenotyping and characterizing of their murine models by determining endpoints, behavioral testing, and pathology and then test therapies.

GM1 gangliosidosis**:** affects multiple organ systems, primarily the central nervous system (CNS), including multiple functional and structural anomalies. They have generated a mouse model targeting exon 2 and 6, common sites for mutations in human patients for the evaluation of treatment modalities. They have confirmed the neurologic progression of disease and are in the process of analyzing results to address potential therapeutics and optimize testing timepoints across the lifespan. In addition, they are using this model to evaluate noninvasive functional testing (MRI and CT) and comparing biomarkers, bone pathology between mouse models and human patients. They plan to study the possible biomarkers and investigate agents that will lead to amelioration of the neurodegenerative phenotype.

GM2 gangliosidosis (Tay Sachs and Sandhoff): They found that, in addition to storing GM2 ganglioside in neurons, there was an inflammatory response contributing to neurodegeneration and death. This demonstrated a more general phenomenon: inflammation in the CNS is part of the pathogenic process in most LSDs and likely more common neurodegenerative disorders involving the brain. They subsequently demonstrated that BMT (bone marrow transplant) using normal donor mice could mitigate this inflammatory component and increase the lifespan of the animals. Under this protocol, Sandhoff mouse, was bred to a pure C57BL/6J background in order to have a mouse line that could be compared to an isogenic wild-type line to more closely compare the disease model to non-diseased animals.

Glycosphingolipid Storage Disease: They have included the $Tsc2^{+/-}$mouse strain in their protocol for studying autophagy and inflammation. $Tsc2^{+/-}$mice exhibit mTOR hyperactivation that causes impaired basal neuronal autophagy and will help better understand the pathogenesis.

Redacted by agreement presented her resubmission. She explained that she is interested in the pathogenesis of neurodegenerative diseases GM1 and GM2. There are currently no effective therapies for these diseases. Most of the patients have the GM1 (juvenile) form of the disease. In 2009, they started a clinical protocol to do a natural history study of the diseases. Several mouse models are being studied. The Tay Sachs mouse model has little to no phenotype. The Sandhoff mouse is being used for both Sandhoff and Tay Sachs studies. One of the first strains made by the Transgenic Mouse Core, the B6J-GE(Glb1)$^{\text{del exon2,6/tif}}$ mouse, is currently being studied and they are characterizing this mouse using lipid analysis, histology and MRI spectroscopy. They were breeding the mice prior to the COVID-19 outbreak and they were just recently given an exemption to begin imaging procedures on these mice. These mice show a sex difference in terms of longevity. The females die approximately a month earlier than the males. This is not true for the children that have the disease so they are not sure why it occurs in the mice. They are approximately 2/3 of the way done with characterization of this mouse model. Dr. Bodine questioned the procedures done on live mice. Redacted by agreement responded that they do urine collection, imaging, blood collection and behavioral studies in the NHLBI Phenotyping Core. Some animals are housed in metabolic cages. Redacted by agreement questioned the low animal numbers in years 1 and 3. Redacted by agreement responded that they have a large number of mice bred from the previous protocol year and have not been able to proceed with studies due to the COVID-19 pandemic. They will spend the first year of this resubmission characterizing these mice. In

year two they will breed more mice and then use year 3 for studies of these mice. Redacted by agreement asked for a description of a metabolic cage. Redacted by agreement explained and will send her some pictures of this type of caging. Redacted by agreement noted that the Redacted by agreement facility manager had questioned the cleaning procedures for the Morris Water Maze. The equipment used in the NHGRI Phenotyping Core is disinfected per their SOP. This will be added to the ASP. They use MB-10 specifically for the Morris Water Maze. Redacted by agreement asked if Redacted by agreement had any problems transferring animals from the holding facility in Redacted by agreement to Redacted by agreement Redacted by agreement and the MIF. Redacted by agreement responded that there are procedures in place, and she has not had problems. Dr. Bodine asked if all the procedures were USDA Pain/Distress Category "C" and Redacted by agreement confirmed that this was correct. Redacted by agreement added that they have been able to obtain brain tissue from two human fetuses. Their hypothesis is that the disease starts in the second trimester so the treatment may need to start much sooner than they had originally thought. They will study fetal brains from mice, calves and sheep. Redacted by agreement noted that Tay Sachs disease should be listed under "GM2 gangliosidosis" instead of GM1 and the Redacted by agreement veterinarian asked that the "BMT" acronym be defined. There were no further questions or comments.

**Decision**: Approved by unanimous vote (10-0) with the modifications listed above which were made as per ACUC Guideline 02.2 under "Unconditional Administrative Process".

3. No **Animal Study Proposals were subjected to annual review** at this meeting.

4. Four **protocol amendments** were submitted at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement **"**Genetic and Embryological Studies of Murine Mouse Models for Neural Crest Disorders." P.I.; Redacted by agreement Redacted by agreement

Measure mouse coat and tail skin color using a handheld spectrophotometer:
The Redacted by lab needs to quantitate the coat color difference between mouse pigment gene mutants. The differences are subtle, and a quantitative measure of the hair and skin color will allow them to more accurately report the mutant phenotype. These colorimetry measurements are non-invasive and routinely used in the literature for both mouse and human dermatology studies.

Mouse restraint, samples/mouse, sampling time, body parts sampled:
Each mouse will be held in a standard hand restraint for a maximum of 1-2 minutes. During that time, a handheld visible light spectrophotometer (X-Rite Ci62) will be held against the mouse coat or tail, up to 12 separate times, to get one second readings of the color. The spec will be held gently against the mouse's body, just close enough to get an accurate reading of the light reflected from the spec's visible wavelength light flash. Because the light is flashed for less than a second through a 4mm aperture, the mouse will not see the light. They will sample the mouse fur color at any of the following areas: the tail skin, the mid ventral surface, or the upper, mid, or lower dorsal surface. They will not shave the mouse for this procedure. The spectrophotometer shoe bottom and target window will be wiped with 70% ethanol or 70% isopropanol before opening each cage of mice. No adverse effects are expected.

They estimate 10 groups of 10 mice each for a total of 100 mice sampled. They will not increase the numbers of mice produced in their colony for this work.

Dr. Bodine presented this amendment. He explained that they will measure how much light can pass through the skin in order to measure pigment. They will restrain the mouse and put the instrument against the hair/skin of the mice. Dr. Bodine asked if Dr. Clark had observed the instrument in use. Dr. Clark responded that she had not seen the equipment but has reviewed the user's manual. She added that the lab will be purchasing the equipment soon. Dr. Bodine asked Dr. Clark if she would observe the procedure the

first time it was done, and she responded that she or another OLAM staff member would. Redacted by agreement noted that he had no questions or comments. There were no further questions or comments.

**Decision**: Approved by unanimous vote (10-0) without modification.

Redacted by agreement Various ASP titles.

PIs.: Redacted by agreement

Redacted by agreement

The PIs would like to add weighing and measuring zebrafish to the Animal Study Proposals listed above. Weighing and measuring zebrafish may be done without anesthesia or the fish may be anesthetized as described in Section I. of the Animal Study Proposal. The zebrafish will be weighed and/or measured as described in OLAM SOP "General Practices for Weighing and Measuring Zebrafish."

Dr. Bodine presented this amendment. He explained that this amendment had been tabled at the April ACUC meeting because Redacted by agreement had been inadvertently excluded from the amendment. This ASP was added to the amendment. There were no further questions or comments.

**Decision**: Approved by unanimous vote (8-0, with Redacted by agreement recusing themselves from the vote as PIs) without modification.

Redacted by agreement "Mouse Models of Methylmalonic Acidemia and Propionate Metabolic Disorders." P.I.; Redacted by agreement

The PI is adding a new strain, B6.SJL-Ptprca Pep3b/BoyJ (CD45.1). This C57BL/6 congenic strain is used widely in transplant studies because it carries the differential *Ptprc*[a] pan leukocyte marker commonly known as CD45.1 or Ly5.1. This strain of mice has no abnormal phenotype/physiology and no special care will be needed. An additional 525 mice will be needed for breeding and experimental studies.

Blood Collection to assess alloantigen expression:
As per the ASP, they will collect 50 µL of blood from the various MMA mice equally (n=40) to analyze the expression of CD45/Ly-5 alloantigen by flow cytometry. is observed, C57BL/6J (B6) (CD45.2) or B6.SJL-Ptprca Pep3b/BoyJ (CD45.1) will serve as donors/recipients to track the percentage of donor cells based on alloantigen expression. For every 1 donor mouse, there will be 5 recipient mice used. If the alloantigen expression is not suitable for tracking the donor/recipient chimerism, isolated bone marrow cells from euthanized WT B6 mice obtained from the mouse core (n=20, 10 M and 10 F) will be transduced with lentivirus vectors expressing florescent protein such as GFP for determining chimerism and lineage analysis.

Irradiation:
To improve engraftment, they will use the standard method of whole-body irradiation by irradiating mice at 2.0-10 Gy (lethal) with a starting dose of 7Gy from a $^{137}$Cesium source as per the building 49. For strains more susceptible to radiation toxicity, the dose will be titrated down (5 or 2 Gy). They will use 8 to 10 week old mice (20 males and 20 females) from MMA mice that can live to adulthood. These are from the colony stock. An additional 40 mice from C57BL/6J (B6) or 40 mice from B6.SJL-Ptprca Pep3b/BoyJ are irradiated for the HSCs transplantation from the MMA mice.

Bone Marrow Transplantation
Recipient mice will be irradiated 5 to 12 hours prior to bone marrow transplantation. Whole bone marrow from euthanized donor mice will be isolated prior to the procedure. Cells isolated from the donor mice will be injected into the recipient mice via retro-orbitally or tail vein post irradiation. Once the mice are transplanted, they will be bled via the retro-orbital route (as per the ASP) every 4 weeks to determine the chimerism by flow cytometry in peripheral blood. Blood will also be analyzed by CBC and peripheral

blood smear. Biochemical studies from blood will be performed at 4 weeks after transplantation initially and with each subsequent blood collection per ASP.

Other transplant methods:
They will also attempt transplants with two different approaches in Mut $^{-/-}$ mice, which display neonatal lethality. 1) As per the ASP, they will inject the livers of mice at birth with AAV expressing functional *Mmut*. When these mice reach eight to ten weeks, normal bone marrow from euthanized C57BL/6J (B6) (CD45.2) or B6.SJL-Ptprca Pep3b/BoyJ (CD45.1) mice will be transplanted using the bone marrow transplantation procedure described above. They expect to study n=5-10 mice of each sex and matched controls. A total of 40 mice will be studied. They will isolate cells from donor mice and transplant bone marrow derived cells into the newborn MMA mice by directly injecting their liver.

Bone Marrow Aspiration:
Chimerism in the bone marrow of the 40 MMA, B6.SJL-Ptprca Pep3b/BoyJ, and/or C57BL/6 transplanted mice will also be evaluated by bone marrow aspirate performed on these mice at 6 weeks and at 12-18 weeks post-transplantation without euthanizing the mice. Biochemical analysis from cells aspirated from bone marrow will also be performed concurrently at 6 weeks and at 12-18 weeks post-transplant.

They will carry out modified BM aspiration procedures following the protocol established by investigators from Memorial Sloan-Kettering Cancer Center.
(https://www.jove.com/video/51660/femoral-bone-marrow-aspiration-in-live-mice)
A total of 40 animals will be accounted for USDA Pain/Distress Category "D".

To evaluate the long-term hematopoietic stem/progenitor cells from the MMA mice, serial bone marrow transplants will occur. Irradiation and bone marrow transplantation procedure is the same as described above. Eight to twelve week old irradiated C57BL/6J (B6) (CD45.2) or B6.SJL-Ptprca Pep3b/BoyJ (CD45.1) will serve as recipients to bone marrow cells from the MMA mice. The transplanted mice will be euthanized at approximately 16 weeks after the initial transplantation and bone marrow cells will be harvested. These cells will be transplanted to the secondary irradiated recipient. This process will be repeated at 16 weeks on the mice receiving secondary transplant to produce tertiary transplant. At 16 weeks post tertiary transplant, these mice will be harvested for quaternary transplant. They will perform a maximum of four serial transplants per original donor, and transplant 5-8 recipient mice per donor per step. This will require 5 male and 5 female MMA donors, then 10 donors x 8 recipients x 5 transplants, including the donors, or an additional 400 mice.

Serial transplantation from the bone marrow cells obtained from C57BL/6J (B6) (CD45.2) or B6.SJL-Ptprca Pep3b/BoyJ (CD45.1) mice while using the eight to twelve week old MMA mice as recipients will also be performed concurrently as described above. This is the reciprocal arm where "normal" bone marrow is transplanted into MMA mice and will likewise require the same number of mice. They will perform a maximum of five serial transplants per original donor. In total, they will add 800 more mice for these studies.

Hematopoietic cells from fetal liver:
For those MMA mice that have a shorter lifespan (i.e.: *mut*$^{0}$ mice), hematopoietic stem/progenitor cells will be isolated from the fetal liver. Five to ten breeding pairs will be set up for timed mating. The adult female will be euthanized as per the ASP between E12.5 and E18.5 days for the collection of fetal livers from the embryos. Isolated HSCs from fetal livers will be transplanted into recipient 5 male and 5 female MMA mice. 0.5-2x$10^{6}$ cells resuspended in sterile injectable saline will be injected via a retro-orbital injection or via tail vein. For these mice, blood collection and bone marrow aspirate will occur as described above.

A literature search and humane endpoints were provided.

Dr. Bodine presented this amendment. He explained that the metabolite MMA builds up in the blood of mutant mice. The only available treatment in humans is a liver transplant. The PI is looking at other options like bone marrow transplants. The hypothesis is that normal cells expressing the missing enzyme will reduce the toxic level of MMA in the mouse models. A marker present on the normal blood cells is used to distinguish them from the donor blood cells. They will irradiate the mice to kill off some of the marrow to make space for the donor cells. They will then collect blood and look at the effects on metabolites. Bone marrow aspirations have been done routinely in the past as a terminal procedure. In this study, they will do the bone marrow aspirations on live animals. Serial bone marrow transplants will be done on the MMA mice. Redacted by agreement questioned the use of the term "chimera". Dr. Bodine responded that a chimera is a mouse with a mixture of donor and recipient bone marrow. In this case, they want to see what percent of the donor it is. Redacted by agreement questioned the use of Buprenex. Dr. Bodine explained that it is a long acting anesthetic used for pain relief. Redacted by agreement asked if the liver injections were painful. Dr. Bodine responded that the animals are under anesthesia and the investigators are very skilled at the technique. Redacted by agreement had no questions or comments. Redacted by agreement asked if this procedure could be described in a SOP and Dr. Bodine responded that this was a good idea. Redacted by agreement asked if the PI was doing retroviral gene therapy. Dr. Bodine responded that he was. Redacted by agreement added that the procedure described in this amendment was a different approach which was simpler and safer than using adeno-associated virus (AAV). Dr. Bodine asked if the procedures would be performed in Redacted by agreement and Dr. Clark confirmed that they would. Redacted by agreement noted that the Redacted by agreement facility manager requested that the PI notify the care staff via technical request 48 hours in advance of the animal recovery in order to alert the staff and also provide instructions on how to handle the animals after radiation procedures. He also asked that a technical request will be submitted at least 48 hours in advance so that the CAF Staff are alerted of the special requirements for food and clear water supplements. She also noted that the Radiation Safety Specialist requested that when an irradiator is to be used, all individual users must comply with Division of Radiation Safety requirements for irradiator training, and all individual assessors will comply with applicable security requirements for escorts and proxy card access approval. This will be added to the amendment. There were no further questions or comments

**Decision**: Approved by unanimous vote (10-0) with the modifications listed above which were made as per ACUC Guideline 02.2 under "Unconditional Administrative Process".

Redacted by agreement "Genetic and Functional Studies of Familial Mediterranean Fever and Autoinflammatory Diseases." P.I.; Redacted by agreement

The PI would like to add the new mouse strain, Sharpin deficient mice 'cpdm' (C57BL/KaLawRij-*Sharpin*$^{cpdm}$/RijSunJ. The importance of SHARPIN in immune homeostasis has been emphasized by the inflammatory phenotype of cpdm, a Sharpin-deficient mouse strain. Recently they identified a biallelic frameshift mutation in the *SHARPIN* gene in a patient with early onset recurrent sterile polyarthritis. They would like to use cpdm as a model to examine the background of the pathophysiology of the patient's arthritis at molecular and cellular level. There will be no change in animal numbers.

Cpdm mice are reported to demonstrate arthritis. However, the frequency and the severity of the arthritis in the cpdm mice are known to vary substantially between different facilities. Therefore, they would like to start by observing the spontaneous occurrence of the joint inflammation in cpdm using the severity of an arthritis scale described in the amendment. For spontaneous occurrence evaluation the homozygous mice (n=20) are considered USDA Pain/distress Column E.

If the joint inflammation is not observed, then they will utilize *in vivo* arthritis induction models discussed below.

1) Collagen antibody induced arthritis model (CAIA model)

A rapid arthritic symptom is induced by injecting a cocktail of mouse monoclonal antibodies that are directed toward conserved auto-antigenic epitopes in collagen type II. To induce arthritis, 100 to 400 ul of the mouse monoclonal antibody cocktail (1 to 4 mg of antibody) or the same volume of non-specific immunoglobulin are injected intraperitoneally into 5 to 7-week-old mice (10 homozygotes and 10 wild-type littermates with both sexes). On day 3, intraperitoneal injection of low dose (~50 ug/mouse) LPS is considered to boost the arthritic symptoms, depending on the arthritic symptoms of the mice in their facility. Low dose LPS induces systemic inflammation for 24 hours and most wild-type mice recover. The progress of the arthritis is assessed every 2 to 3 days, by qualitative scoring on a scale of 0 to 4 and quantitative measurement by measuring the ankle at the widest point on all four paws. On day 14, the mice are euthanized as per ASP and the inflammatory changes are evaluated by histological examinations. USDA Pain/Distress Category "E" for the homozygotes (n=10). The wild-type mice are USDA Pain/Distress Category "C".

2) Serum transfer from K/BxN model (K/BxN model)
K/BxN transgenic mice develop severe spontaneous inflammatory arthritis, and the serum from these mice causes a similar arthritis in a wide range of mouse strains due to autoantibodies against glucose-6-phosphate isomerase (GPI). To induce arthritis, 200ul of serum from K/BxN mice or the same volume of sterile injectable saline are injected intraperitoneally into 5 to 7-week-old mice (10 homozygotes and 10 wild-type littermates with both sexes) once daily on day 0 and day 2. The progress of the arthritis is assessed every 2 to 3 days, by qualitative scoring on a scale of 0 to 4 and quantitative measurement by measuring the ankle at the widest point, both on all four paws. On day 14, the mice are euthanized as per ASP and the inflammatory changes are evaluated by histological examinations. USDA Pain/Distress Category "E" for the homozygotes (n=10) and "C" for the heterozygotes

These cpdm mice are characterized by systemic inflammation in skin, lungs, spleen, GI and joints due to spontaneously occurring homozygous frameshift mutation in the *Sharpin* gene. One commonly affected organ is the skin. Skin inflammation may be severe and therefore is monitored closely by following the a dermatologic *Sharpin* severity scoring criteria, and most importantly, these mice are euthanized when the skin score reaches 4. The criteria are established by Redacted by agreement (the Walter and Eliza Hall Institute, Melbourne, Australia).

Dr. Clark presented this amendment. She explained that the PI is looking at inflammatory arthritis and he is adding a new strain with an inflammatory phenotype. If they do not see a spontaneous arthritis phenotype, they will induce it in one of two ways, injecting a monoclonal antibody cocktail or by a serum transfer from K/BxN mice. The wild-type mice will be USDA Pain/Distress Category "C" and the mice that go through arthritic changes without pain meds will be USDA Pain/Distress Category "E". Redacted by Redacted by agreement noted that the Redacted by agreement Vet and Facility Manager had questions on the monitoring and endpoints for the study animals. Redacted by agreement noted that a literature search was needed for alternatives to the USDA Pain/Distress Category "E" phenotype. Dr. Bodine called for a Designated Member Review (DMR) to clarify monitoring procedures and endpoints for the study mice and to revise the literature search to include alternatives to the pain category "E". Dr. Clark was the designated reviewer.

**Decision**: This amendment was sent to Dr. Clark for Designated Member Review (DMR) and was approved on 6/2/20.

5. The following minor amendments, significant amendments approved administratively and VVC amendments were announced for concurrence of the committee. For amendments for the **addition or removal of personnel, location and/or normal strains**, all the new personnel have completed the NIH OACU Animal User training course, registered with AEP and have been specifically trained by OLAM personnel. The additional strains will not increase animal numbers by more than 10% and require no special care beyond that described in the original Animal Study Proposal (Change in the Principal

Investigator or change of species requires resubmission of the protocol.) In the case of adding an animal holding location, the signature of the veterinarian and facility manager of the affected facility is required.

None

Significant Amendments for Administrative Approval

None

Amendments Approved Via VVC

None

6. **NHGRI Guidelines**

98.1 NHGRI Behavior Guideline

Dr. Clark presented the revised guideline. She explained that information had been added concerning the disinfection of the equipment used in behavior testing, quality control of the cleaning process and procedures for disinfecting the behavior room. Dr. Bodine noted that NHGRI does not have an expert in behavior testing so this guideline directs our investigators to reach out to an expert. There were no further questions or comments.

Decision: Approved by unanimous vote (10-0) without further modification.

7. **NHGRI SOPS**

Clear $H_20$ Supplemental Care and Enrichment

Dr. Clark presented this SOP. She explained that it was a new SOP to describe new options for supplemental care that is available for both medical care and enrichment. The information provided can be evaluated by the investigators so they can determine if this type of supplemental care is appropriate for their mice or if it would potentially interfere with their studies. There were no questions or comments.

**Decision**: Approved by unanimous vote (10-0) without further modification

Hepatocyte Collection

Dr. Clark presented this SOP. She explained that protocol Redacted by agreement was removed from the SOP as this lab is no longer performing this procedure. The Redacted by agreement lab reviewed the SOP and verified that the procedures were correct. There were no questions or comments.

**Decision**: Approved by unanimous vote (10-0) without further modification

Procedure for Inoculation of Mice with Influenza (PR8 or X31) Using the Inhalation Exposure Apparatus Redacted by agreement or the Intranasal Route in the Redacted by agreement CAF

Dr. Clark presented this SOP. She explained that the Redacted by agreement lab had reviewed the SOP and verified that the procedures are updated for the new machine that was recently purchased. The room number for the procedure room housing the machine was updated. There were no questions or comments.

**Decision**: Approved by unanimous vote (10-0) without further modification.

8. Other new business:

- Hot Topic – The Role of the ACUC in Responsible Animal Research

Dr. Clark discussed the handout and explained that it outlined the role of the ACUC in harm-benefit analysis, scientific merit, husbandry, veterinary care, ethics, animal welfare, experimental design, training, safety and non-compliance. Dr. Clark noted that the best message from the article was "A well-run IACUC will enable researchers to navigate the regulatory environment through a science-based, flexible program while still placing the humane care of animals as its number one priority and can assist the PI in developing and reporting high quality, reproducible studies." Dr. Bodine questioned the ACUC's involvement in scientific merit. Dr. Clark responded that there are no regulations that require the ACUC to look at scientific merit, but it does go hand in hand with the responsible use of animals. For NHGRI, the Branch Chief signs the ASP and this signature confirms the scientific merit of the ASP. Redacted by agreement added that the Board of Scientific Review is responsible for the scientific merit, not the ACUC. Dr. Bodine asked each member to comment on the article.

- Parabiosis Update

  Dr. Clark explained no surgeries have been done since the COVID-19 pandemic began and zero animals have been used.

- OLAM Announcements

  AAALAC Site Visit Update – No new updates but likely postponed until October 2020.
  Pandemic Update – All animal facilities housing NHGRI animals continue to be fully staffed and operate as essential services supporting colony maintenance. A new return to work plan should be in place next week. June 15 is the target date for the return of Group A personnel.

  ***Post Approval Monitoring Report***

  Post Approval Monitoring was performed on the following protocols:

  Redacted by agreement

  Redacted by agreement explained that an incident occurred where there were four female and one male mice housed in breeding units found in several cages. The Animal Study Proposal is limited to trio breeding. The mice in the cages were separated immediately in order to be in compliance with the ASP.

  Redacted by agreement

  Redacted by agreement reviewed the Animal Study Proposal and the new anesthesia for tail snips. No deficiencies were noted.

The next meeting will be held on June 30, 2020 at 10:30 am.

Redacted by agreement

_____
David Bodine, Ph.D.
Chairperson NHGRI ACUC

**NHGRI ACUC MINUTES**
**6 August 2020**
**1:00 pm**
**WebEx Meeting due to Covid-19**

**Members present:** Drs. Bodine, Clark, [redacted]
**Members absent**: None
**OACU Observer**: [redacted]
**Visitors:** [redacted]
Note: The Facility Veterinarian and Facility Manager of the facilities where protocols are performed are given the opportunity to review all new Animal Study Proposals, resubmissions and significant amendments prior to the meeting. Their comments are provided in the text below, when applicable.

**OLD BUSINESS**

1. The minutes of the 26 May 2020 meeting were approved without modification.

2. OACU Refresher Course update – [redacted] were due to complete the refresher course in June. [redacted] completed the course on 6/5/20, [redacted] completed the course on 6/22/20, [redacted] completed the course on 6/23/20 and [redacted] completed the course on 7/1/20. [redacted] were due to complete the refresher course in July. [redacted] completed the course on 7/8/20, [redacted] completed the course on 7/10/20 and [redacted] completed the course on 7/28/20. [redacted] were [redacted] were due to complete the course in August. [redacted] has left the Institute and [redacted] completed the course on 8/28/20. [redacted] is due to take the refresher course in September.

3. Update on Fall and Spring Semiannual Report Deficiencies

The only outstanding minor deficiency from the Fall Semiannual Walk Through is the strong rat odor that remains in [redacted] The rat odor is NEI's responsibility. Siemen's work on this problem has been delayed due to the pandemic and [redacted] deployment. [redacted] noted that the odor had been much less noticeable the last few weeks.

The only outstanding minor deficiency for the Spring Semiannual Walk Through is the chemical fume in [redacted] that needs to be repaired/certified. [redacted] submitted a work request to have the air flow adjusted in response to the failed certification, but the work has not been done yet.

4. Designated Member Review

[redacted] "Genetic and Embryological Studies of Murine Mouse Models for Neural Crest Disorders." P.I.; [redacted] Amendment dated 6/5/20 to add photography and video to document phenotypes.

**Disposition: Approved 07/01/2020 (DMR – Reviewer:** [redacted]

[redacted] "Genetic and Functional Studies of Familial Mediterranean Fever and Autoinflammatory Diseases." P.I.; [redacted] Annual review dated 6/1/20.

**Disposition: Approved 06/26/2020 (DMR – Reviewer: David Bodine)**

Redacted by agreement "Zebrafish Models of Human Disease." P.I.; Redacted by agreement Annual Review dated 5/21/20.
**Disposition: Approved 06/29/2020 (DMR – Reviewer: Tannia Clark)**

**NEW BUSINESS**

1. No ***new*** **Animal Study Proposals were** reviewed at this meeting.

2. Three **three-year renewals were** reviewed at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement "Generation of Genetically Engineered Mice Using Targeted and Conventional Transgenics and Embryo Rederivation in the NHGRI Mouse Transgenic and Gene Editing Core." PI.; Redacted by agreement

The mission of the Mouse Transgenic and Gene Editing Core is to develop mouse models for human genetic diseases. Mice can be genetically engineered by several methods: direct microinjection of DNA or RNA into fertilized eggs or microinjection of modified embryonic stem (ES) cells into 2.5-3.5 day embryos. Primarily, the Core uses site-specific nucleases and CRISPR/Cas technology to easily target genes by direct microinjection into fertilized eggs. The Core generates ~80% of the stock animals from various genetic backgrounds for embryo donation and surgical transfer (recipients). These mice are superior to vendor-supplied animals, due to their consistent weight, age, and reproductive characteristics. Once founder animals have been generated by the Core, the lines will be tested for germline transmission by subsequent breeding to the F1 generation. Following genotyping of the F1 progeny, the mice are transferred to the investigator's protocol, except in special cases where there is an adverse phenotype. All newly generated transgenic/gene-edited mice are cryopreserved as they are created. Once F1 animals are genotypically screened, the Core will freeze one male for disaster purposes.

The Core anticipates 40-50 transgenic projects (primarily by gene editing) per year with a range of 1-10 (founder animals) new strains per construct. This translates to ~ 500 new mouse strains per year. The Core may perform timed pregnancies, embryo harvest and dissection when there are embryonic lethal phenotypes presenting before the F1/F2 generation.

The Core rederives in all imported mice by *in vitro* fertilization followed by transfer of 2-cell embryos. Any surplus embryos and sperm are cryopreserved for disaster purposes. The Core estimates that they perform 10-20 rederivations per year.

The NHGRI Transgenic Core also generates *de novo* embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells).

Redacted by agreement presented her protocol. Dr. Bodine noted that this was the very first ASP reviewed by the NHGRI ACUC. She explained that the procedures performed on live mice under this ASP included embryo transfer, rederivation, vasectomy, tail clips, ear notching, IP injection and retro-orbital blood collection. Dr. Bodine asked how many transgenic projects had been done in the past year and Redacted by agreement responded that 39 were done. Dr. Bodine asked how many had been done since March and Redacted by agreement responded that they had not had any since March, but that they had started back up last week. They have maintained the colonies since the pandemic started but have not initiated new projects. Redacted by agreement explained that the Core personnel are rotating in to do microinjection, Redacted by agreement and Redacted by agreement come in every other day and the cryopreservation technician comes in 2-3 days a week. Dr. Bodine added that this ASP generates approximately 75% of the mice we use on ASPs in NHGRI. Redacted by agreement asked if the pain category D mice were anesthetized. Dr. Bodine responded that these mice undergo surgery and they were definitely anesthetized. Redacted by agreement added that the mice are given analgesics and anesthetics. The 3,800 mice listed as pain category D undergo rederivation, embryo transfer or vasectomy. Redacted by agreement

questioned the practice of mating the vasectomized males. Redacted by agreement responded that they want the males to go through the motions and make the female mice think they are pregnant so they can do embryo transfer with the pseudopregnant females. Redacted by agreement questioned the term "dirty mouse". Redacted by agreement responded that a dirty mouse was one that potentially was infected with a mouse virus. Dirty mice are not allowed in Redacted by agreement but can be imported into Redacted by agreement for rederivation. Redacted by agreement explained the rederivation procedure to Redacted by agreement and noted that good science needs clean mice because viruses could confound research. Dr. Bodine added that Redacted by agreement should take a tour of the Mouse Core once COVID-19 restrictions are relaxed. Redacted by agreement asked how the animals could be euthanized without $CO_2$ for IVF. Redacted by agreement responded that they had published a paper showing that eggs from animals euthanized by cervical dislocation gave superior results to eggs collected from animals euthanized by $CO_2$ asphyxiation. They showed that it is very important that they remove the oocytes as quickly as possible and that the decreased pH associated with $CO_2$ asphyxiation and the longer time to harvest causes the eggs to harden over time reducing fertilization. Dr. Clark added that this is an approved method according to the AVMA and the Core SOPs have been reviewed by the ACUC. Dr. Bodine added that the literature search was good. There were no further questions or comments.

**Disposition**: Approved by unanimous vote (9-0, with Redacted by agreement recusing herself from the vote as the PI) without modification.

Redacted by agreement "Understanding Human Genetic Disorders by Genetic, Genomic and Phenotyping Approaches in Zebrafish." P.I.; Redacted by agreement

The protocol was established in 2005 to provide Core services to NHGRI researchers interested in establishing a zebrafish model to investigate gene function. After identification of novel genes responsible for human genetic diseases through genomic approaches, animal models are generated with loss or gain of function of the given gene or with exact point mutations as seen in patients for validation and further understanding of disease mechanisms. Zebrafish make a powerful vertebrate model system due to evolutionary conservation of gene function. In the past three years, they have provided services to investigators from many NHGRI laboratories. These services included generation of knock-in and knockout mutants using CRISPR/Cas9, generation of transgenic zebrafish lines, and manipulation of gene dosage using microinjections of morpholinos and mRNA In the coming years, they will continue to provide the existing services and adopt new protocols developed by the zebrafish community for use by NHGRI investigators.

Redacted by agreement presented her resubmission. She explained that she the Zebrafish Core is functioning with everyone there going to work with a staggered schedule of one person in the lab at a time. Redacted by agreement is teleworking and not participating in the rotation. Redacted by agreement are doing genotyping and Redacted by agreement has started back to work on her project. Redacted by agreement noted that this ASP has been in place for 15 years. Their main purpose is to generate mutant and transgenic fish for investigators. This time around she removed many procedures they have done in the past that have now been transferred to individual investigator's ASPs. Procedures performed on live fish under this ASP include breeding, anesthesia, fin clips, imaging, weighing and measuring and squeezing (USDA Pain and Distress Category "D"). Redacted by agreement explained that there are three reasons they may need to squeeze. They need to squeeze females that are egg bound, important females that are not breeding or to collect sperm for cryopreservation. They have never lost any fish as a result of squeezing. They grow embryos in PTU to increase staining and they image them in agarose. The temperature is maintained at 28°C and they are anesthetized with tricaine. Images are taken every ten minutes. Some embryos are exposed to chemicals, fixed, imaged and euthanized. Fish larvae and juveniles are imaged, weighed and measured.

Redacted by agreement asked why the fish squeezed were USDA pain and distress category "D" when they were anesthetized for the procedure. Dr. Clark responded that squeezing could damage the skin and cause distress afterwards. Redacted by agreement added that when animals are anesthetized for the purpose of pain relief,

they are accounted for in pain category "D". Redacted by agreement questioned the size of the fish undergoing squeezing and Dr. Clark responded they were adults around an inch long. Redacted by agreement asked if the squeezing was done using your fingers and Redacted by agreement responded that it was. Redacted by agreement commented that he could see that there was the potential for distress due to the possibility of skin damage as well as distress from the squeezing procedure itself. Dr. Clark asked Redacted by agreement if the squeezing procedure could be done without anesthesia. Redacted by agreement responded that the procedure is very quick. He added that the fish squirm but mainly because they are out of the water. Dr. Bodine asked about the number of fish that may undergo squeezing in a given year and Redacted by agreement responded that 100 fish were estimated per year. Redacted by agreement noted that there were no questions or comments from the facility vet or manager. Dr. Bodine asked if two years were long enough to maintain the study animals. Redacted by agreement responded that fish do not breed after two years and if an investigator wants to age the fish, they should be transferred to their protocol. Redacted by agreement commented that the animal numbers were great. He noted that the numbers are large but appropriate. Dr. Bodine added that the numbers are just estimates based on available data. Dr. Bodine noted that the literature search was good. Redacted by agreement commended Redacted by agreement for turning the ASP in early, answering all the questions in a timely fashion and for removing all the procedures not being actively performed. There were no further questions or comments.

**Disposition**: Approved by unanimous vote (10-0) without modification.

Redacted by agreement "Mouse Models for Disorders of Sialylation." P.I.; Redacted by agreement

The PI is studying sialic-acid related disorders and will focus on two genetic human disorders:

**GNE Myopathy**, characterized by progressive muscle weakness and is due to mutations in the *GNE* gene, which codes for a key protein in the synthesis of sialic acid. Their mouse models have shown that giving sialic acid either orally or through the circulation can correct the muscle and kidney phenotypes. They currently have three ongoing clinical trials to provide sialylation-increasing therapies in human patients.

**Sialic acid storage disease (SASD)**, characterized by a spectrum of disorders from severe neurodevelopmental defects that rapidly deteriorate and become fatal, to psychomotor and cognitive disability in young adults. SASD is due to mutations in *SLC17A5* gene causing a deficiency in the lysosomal membrane transporter sialin, which increases storage of sialic acid in lysosomes. Mutant mice that lack Slc17a5 show characteristics of ISSD with neurobehavioral defects. Complete deletion of this gene leads to early postnatal lethality (~P20). They expect that their knockin R39C mice will have a milder phenotype and allow longer survival, similar to R39C in humans, a hypomorph mutation that results in a slightly delayed onset and progression of neurological symptoms.

Over the next three years they plan to continue their data analysis, outcome measures and biomarker discovery of ongoing human trials involving sialylation-increasing therapies (muscle and kidney), with the goal to establish/show efficient delivery to all organs. They plan to explore defects due to hyposialylation in organs other than muscle and kidney; they have indications of liver, heart and brain phenotypes due to hyposialylation in their mice, and possibly in some human diseases. They will attempt to correlate new mouse tissue findings with possibly associated human diseases by testing biopsies of human samples for sialylation status. Lastly, they plan to explore the consequences of sialin deficiencies in mice, and possibly to correct these defects by increasing lysosomal release of sialic acid or by providing sialin in the form of gene therapy or gene editing.

Redacted by agreement presented her resubmission. She explained that this ASP supports ongoing clinical trials. She is studying two genetic diseases, GNE myopathy and sialic acid storage disease. GNE myopathy is caused by a deficit of sialic acid. The body does not make enough and the muscles get weak, which leads to myopathy. In sialic acid storage disease, there is an overabundance of sialic acid. The mouse model of this disease was generated by the Transgenic Core and closely replicates the human disease. The mice are weak and develop kidney disease. Most of her studies are geared towards describing what happens in the mouse model and what happens if they try to replace the sialic acid. Redacted by agreement noted that they do

imaging with the Mouse Imaging Facility (MIF). She added that she removed all the USDA Pain and Distress Category "E" procedures. Redacted by agreement questioned the experimental endpoint scale. He asked if the body condition score 1-5 went from emaciated to obese and Dr. Clark confirmed that it did. He asked if ataxia was the endpoint in the locomotor activity scale 0-3 and if the pain category changed at a scale of 3. Dr. Clark explained that in ASP Redacted by agreement the ataxia progresses until the mice are paralyzed and this would be a USDA Pain Category "E" if they animals were allowed to go through the entire progression. In this case, the animals are euthanized before they become paralyzed, so the pain category is "C". Dr. Bodine asked Redacted by agreement if ataxia was painful in people and she responded that it was only painful if it caused them to fall. Dr. Bodine asked if the human patients received pain relievers and Redacted by agreement responded that they did not. Redacted by agreement noted that at this point she is not sure how the disease will progress so it is possible that some mice would get to level 3 and be USDA pain and distress category "E". She will submit an amendment if that is the case. Redacted by agreement requested that Redacted by agreement report back to the ACUC in a few months concerning pain and distress in this model. Redacted by agreement noted that the box for radiation safety was not checked in regard to the CT scans. Dr. Bodine responded that this is done in the MIF and Dr. Clark added that they are done using the MIF SOPS. Redacted by agreement noted that the ASP had to be signed by radiation safety due to the Faxitron anyway and she would ask Redacted by agreement about the requirements for MIF use. After the meeting, Redacted by agreement confirmed that even if the imaging is done in the MIF, the NHGRI ASP had be reviewed/signed by radiation safety and the ionizing radiation box should be checked in section K. Dr. Bodine asked what was being injected via the retro-orbital route. Redacted by agreement Redacted by agreement responded that she was delivering Lipoplex mixed with sugars to adults and neonates. Dr. Bodine asked that it be added to the table and Dr. Clark noted that it was in table 2 in the third box down, so no change was necessary. Dr. Bodine noted that the literature search was perfect. Redacted by agreement noted that the Redacted by agreement veterinarian, Redacted by agreement had questioned the transport of the animals to pathology. Dr. Clark responded that the tunnel or the door by the Redacted by agreement would be used and Redacted by agreement This will be added to section C. Dr. Portnoy also asked that the acronym "RO" be added to "retro-orbital" on page 4. Dr. Bodine commented that he was glad to see that the ASP was not so lengthy. Redacted by agreement thanked Dr. Clark and Redacted by agreement for their help in that regard. There were no further questions or comments.

**Disposition**: Approved by unanimous vote (9-0, with Redacted by agreement recusing herself from the vote as the PI) with the modifications listed above which were made as per ACUC Guideline 02.2 under "Unconditional Administrative Process".

3. Two **Animal Study Proposals were subjected to annual review** at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement "Tyrosine Kinases Signaling Pathways in Hematopoietic/Lymphoid and Bone Development and Function." P.I.; Redacted by agreement

In the last year they have used a variety of mouse strains to uncover requirements for proper responses to immunization and infection. They have used a mouse model of Activated PI3 Kinase Delta Syndrome that they previously developed to model altered responses to viral infection, providing evidence that PI3 Kinase activity must be tightly regulated to develop immunological memory, i.e. protection against a second infection and antibody-mediated immunity. They have further demonstrated critical roles for the transcription factors TOX, TCF1 and Bach2 in maintaining long-term immunological responses required to keep chronic infections and cancer in check, and provided evidence for a CD8+ stem-like cell that maintains T cell responses during T cell exhaustion and that responds to checkpoint inhibitor therapies. Finally, to reduce animal numbers required to generate new animal models, they have used CRISPR-mediated mutagenesis of primary mouse T cells to uncover genes required for T cell responses *in vivo*. Dr. Bodine was the primary reviewer for this annual review.

Dr. Bodine presented this annual review. He explained that Redacted by agreement studies immunization and infection. He noted that T cells amplify and induce specific B cells to make antibodies. Redacted by agreement studies what causes a T cell to amplify and how T cells interact with B cells. She has knockout mouse models with missing components of these signaling pathways. There were no questions or comments and Dr. Bodine recommended that this study continue.

**Disposition**: Approved by unanimous vote (10-0), without modification.

Redacted by agreement "Characterization of Human Disorders of Intermediate Metabolism Using Zebrafish Models." P.I.; Redacted by agreement Redacted by agreement

Since July 2019, they have characterized the phenotype of the *pcca* and *pccb* models of propionic acidemia; *acad8* and *acadsb* models of deficiency of isobutyryl-CoA dehydrogenase and 2-methylbutiryl-CoA dehydrogenase. Consistent with their hypothesis, piscine models of propionic acidemia, $pcca^{-/-}$ and $pccb^{-/-}$, had a severe phenotype overlapping with that of $mut^{0}$ zebrafish. On the other hand, $acad8^{-/-}$, and $acadsb^{-/-}$ had no discernable phenotype, despite the evidence of biochemical perturbations.

To evaluate a novel therapeutic strategy of substrate deprivation on the growth, development, and survival of $mut^{0}$ and $pccb^{-/-}$, they generated and characterized double and triple knockout lines of *pccb;acad8;acadsb* and $mut^{0}$*;acad8;acadsb*. They have found that double knockout lines $pccb^{-/-}$;$acadsb^{-/-}$, but not $pccb^{-/-}$;$acad8^{-/-}$ have shown improvement in their development and survival. Based on the likely crystal structure of acad8 and acadsb imputed from human ACAD8 and ACADSB, they proposed a mechanism explaining the differential outcomes of $pccb^{-/-}$;$acadsb^{-/-}$ *vs* $pccb^{-/-}$;$acad8^{-/-}$ zebrafish. Additional studies are underway to evaluate whether the improvement in the survival is accompanied by improvements in disease biomarkers.

The transgenic lines Tg(*gfap:GFP*), Tg(*hsp:CRE*), Tg(*HuC:gfp*), Tg(*gad1b:RG*), Tg(*vglut2a:GFP*), Tg(*fabp10a:MUT*), *Tg(β-actin2:loxP-DsRed-STOP-loxP-eGFP), Tg(amhc:CreERT2)* are being used to enable future studies of developmental events in the brain of mut-/- and pccb-/- fish. Redacted by agreement was the primary reviewer for this annual review.

Redacted by agreement presented this annual review. He explained that Redacted by agreement studies human genetic diseases that interfere with the function of mitochondria. He is making zebrafish models equivalent to the kind of diseases that he studies in human patients. The fish get sick and he looks for ways to make them less sick using drugs or other treatments. Redacted by agreement noted that the literature search was good. There were no questions or comments and Redacted by agreement recommended that this study continue.

**Disposition**: Approved by unanimous vote (10-0), without modification.

4. One **protocol amendment was** submitted at this meeting (unless stated otherwise, the pain and distress associated with each protocol was discussed by Dr. Bodine and others and was considered to be either appropriately monitored and relieved, or not expected to be a factor).

Redacted by agreement "Animal Handling and Biomethodology Training for NHGRI Investigators." P.I.; Redacted by agreement

The PI would like to propose adding MIF procedures to this training ASP. It will provide OLAM the ability to train on these modalities and perform tail catherization on mice. MIF approved standard operating procedures will be used to conduct the following procedures:

**Gas anesthesia for induction and maintenance** (section I): For these imaging modalities, the mouse will be placed under general gas anesthesia in an induction chamber with 4-5% isoflurane delivered by oxygen enhanced medical air (as described in the ASP).

**Ultrasound:** performed in the Mouse Imaging Facility (MIF) room Redacted by agreement The Visualsonics high frequency ultrasound is used for imaging as per MIF SOP. Photoacoustic (PA) imaging measures the photoacoustic properties of substances or tissue (i.e. oxygenated or deoxygenated blood flow). This procedure is performed with an ultrasound image for coregistration, which is a technique for mapping the echogenic anatomical structure of tissues and its corresponding optical absorption.

**X-ray micro computed tomography (micro-CT):** is performed in the MIF room Redacted by agreement as per MIF SOP. Total imaging time varies between 2-30 minutes. For survival studies, the animals will be recovered in the MIF with supplemental heat until they are breathing unassisted and ambulatory.

**Magnetic Resonance Imaging (MRI)**: performed in one of the MR imagers located in MIF rooms Redacted by agreement as per MIF SOP. MRI may be performed for up to 3 hours, typical scans are 30-90 minutes. If IV contrast is required, the tail vein is catharized as described below.

**Optical Imaging Procedure:** (bioluminescence, fluorescence) is performed in MIF room Redacted by agreement on a Bruker In-Vivo Xtreme imager as per MIF SOP. The imager can accommodate up to 4 mice and animals are monitored via a camera during the scans. The scans are generally short (30 seconds to 10 minutes) and for training purposes, they will use the shortest scans. For bioluminescence, animals are injected with luciferin substrate (150mg/kg IP, SC, IV) 5-10 minutes prior to imaging. Total volumes of luciferin injections are 0.02-0.12 ml, depending on final concentration of substrate solution. Luciferin has no documented adverse effects, and none are expected. Luciferin is pharmaceutical-grade quality for *in vivo* injection. For bioluminescence, luciferase oxidizes the substrate luciferin in a biochemical reaction that produces light.

**Tail catherization**: For training purposes, only sterile saline will be used instead of actual contrast agents (CT/MRI). The tail vein may be catheterized after the animal is anesthetized. A custom-made catheter is constructed for mice from a 30 gauge needle tip attached to PE-10 tubing. The tail is prepped with . povidone iodine (or chlorhexidine) and alcohol. To help vasodilate the vessels of tail, the tail is warmed with a disposable hand warmer. One of the lateral tail veins is raised by application of gentle pressure with a finger. A prefilled (heparinized saline) custom-made catheter constructed from a 27-30 gauge needle tip attached to PE-10 tubing is inserted into the vein until a good flashback is achieved, then secured to the tail with a drop of tissue adhesive and bandage tape. The tail vein line is prepared with heparinized saline and then the saline ("contrast solution") is attached to the established line.

**Recovery**: the mouse will be recovered in a clean cage or transport box on a circulating warm water heating pad for all the procedures described above. Mice will be visually monitored until that are awake and ambulatory. Animals will be transported back to their home facility Building Redacted by agreement in NIH approved transport boxes or may be housed overnight in the MIF animal room Redacted by agreement All images will be reviewed to ensure that data was captured correctly. Then animals will be euthanized as described within the ASP.

Procedures are USDA Pain Category "C". This amendment will not increase animal numbers.

Redacted by agreement presented her amendment. She explained that this amendment will allow her to do training in the Mouse Imaging Facility (MIF). She needs to train on the ultrasound machine so she went ahead and added all the imaging equipment so training would be covered for other equipment in the future. Redacted by agreement questioned the anesthetic plane monitoring on page 1. Redacted by agreement explained that they start the animals on a high flow rate and the toe pinch confirms that they are in the proper plane of anesthesia for the procedure. There were no further questions or comments.

**Disposition**: Approved by unanimous vote (9-0, with Redacted by agreement recusing herself from the vote as the PI) without modification.

5. The following minor amendments, significant amendments approved administratively and VVC amendments were announced for concurrence of the committee. For amendments for the **addition or removal of personnel, location and/or normal strains**, all the new personnel have completed the NIH OACU Animal User training course, registered with AEP and have been specifically trained by OLAM personnel. The additional strains will not increase animal numbers by more than 10% and require no special care beyond that described in the original Animal Study Proposal (Change in the Principal Investigator or change of species requires resubmission of the protocol.) In the case of adding an animal holding location, the signature of the veterinarian and facility manager of the affected facility is required.

Redacted by agreement Remove four personnel
Redacted by agreement Remove personnel
Add three strains
Add new room for euthanasia
Redacted by agreement Remove personnel
Redacted by agreement Remove two personnel
Redacted by agreement Add two personnel
Remove personnel
Redacted by agreement Remove four personnel
Redacted by agreement Remove personnel
Redacted by agreement Add personnel
Redacted by agreement Remove personnel
Redacted by agreement Remove personnel

Redacted by agreement noted that Redacted by agreement was removed from Redacted by agreement not Redacted by agreement and Redacted by agreement was added to Redacted by agreement not Redacted by agreement These corrections will be made.

Significant Amendments for Administrative Approval

None

Amendments Approved Via VVC

None

6. **NHGRI Guidelines**

20.1 Guideline for Humane Intervention and Endpoints in Laboratory Mice and Zebrafish

Dr. Clark presented this new guideline. She explained that OLAW had commended some Institutes for having an endpoint guideline. This guideline defines humane endpoints, study endpoints, monitoring frequency and addresses adverse phenotypes. Dr. Bodine noted that it was an unusual guideline in that it was more for the ACUC than for the investigators. Dr. Clark responded that this guideline should make the way by which endpoints are reviewed more consistent. There were no further questions or comments.

Decision: Approved by unanimous vote (10-0) without modification.

7. **NHGRI SOPS**

None

8. Other new business:

- Hot Topic – Responsible Science and Research Animal Use

  Dr. Clark discussed the handout and explained that our ACUC is a good reflection of the article. We work hard to build trust with our investigators. Dr. Clark thought it was a good reference for Redacted by agreement Redacted by agreement to read as a new ACUC member. Redacted by agreement also thought it described our ACUC accurately. He looked at the references to see if any of our members were involved with writing it.

- Draft New Mouse ASP Template
  Draft New Zebrafish ASP Template

  Dr. Clark explained that OLAM is trying to streamline the ASP submission process. Check boxes, standard language and hyperlinks were added to reduce the text burden and make things easier on the investigators and reviewers. There are also separate templates now for mice and zebrafish. Dr. Bodine noted that the changes seemed helpful to him. Redacted by agreement was in agreement with the changes and added that other institutes were also adding boiler plate language to their templates. Redacted by agreement suggested changes for the stock/strains for the mouse template and will provide these to Redacted by agreement after the meeting. Redacted by agreement asked that the USDA Pain and Distress Categories be bolded. Redacted by agreement asked if the PI would select all that apply in section C (transportation) and Dr. Clark responded that this was correct. Redacted by agreement noted that page numbers should be added to both templates.

  **Disposition**: Templates approved with the changes listed above by unanimous vote (10-0).

- $CO_2$ Euthanasia Survey

  Dr. Clark explained that given the problems we were having with getting Redacted by agreement hood certified, she wondered why we required a hood for euthanasia. Use of a hood is best practices but if not available, it should not be required. She worked with DOHS and they surveyed the Redacted by agreement hood and confirmed that it was acceptable to euthanize the mice in this hood even if it did not pass certification. Dr. Clark also noted that Redacted by agreement recently added a new hood in Redacted by agreement to facilitate social distancing. DOHS tested this new hood and it passed as well.

- Semiannual Walk Through of the Mouse Imaging Facility (MIF)

  Redacted by agreement and Redacted by agreement (MIF technician serving as an *ad hoc* consultant) did a semiannual walk through of all the procedure areas in the MIF on 8/5/20. Redacted by agreement attended via FaceTime to respond to questions. Dr. Bodine asked who would report to NINDS. Dr. Clark responded that an e-mail was sent to Redacted by agreement noting the minor deficiencies observed. She added that this NHGRI visit will be used as an *ad hoc* walk through by several Institutes, including NINDS.

- OLAM Announcements

  AAALAC Site Visit Update – Tentatively scheduled for June 2021. Dr. Clark noted that the AAALAC Position Description would have to be revisited.

  Pandemic Update – All animal facilities housing NHGRI animals continue to be fully staffed and operate as essential services supporting colony maintenance. Redacted by agreement has expanded his walk throughs to labs and animal rooms. Dr. Clark noted that as we bring in more people, we need to go to shift work but have to be careful not to disrupt the light cycles for the animal rooms.

Fall Semiannual Program Review

Dr. Clark encouraged everyone to volunteer for the facility walk throughs as we need to do all the labs this time around. Dr. Bodine added that it is the ACUC's most important job.

***Post Approval Monitoring Report***

Post Approval Monitoring was performed on the following protocols:

Redacted by agreement

Redacted by agreement explained that she reviewed the protocol and checked with a new animal user to see what procedures she would perform on this ASP. The only finding was that there were drugs listed in section K that were not mentioned in section F.

The next meeting will be held on September 22, 2020 at 10:30 am.

Redacted by agreement

David Bodine, Ph.D.
Chairperson NHGRI ACUC