University at Buffalo
State University of New York
Office of Research Compliance

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
MINUTES OF THE MEETING
April 18, 2022

****

ATTENDANCE:

****

CALL TO ORDER:

The IACUC Chair commenced the meeting at 12:33 PM.
If any member of the IACUC has submitted a protocol or amendment for review and approval, that member is not present during the discussion of and voting on their protocol or amendment. Quorum is maintained.

PRESENTATION OF THE MINUTES:
The minutes of the 2022 IACUC March meeting were presented. The March meeting minutes were voted on and unanimously approved with the correction of two typos.

ANNUALS

A list of annual renewal submissions are provided in the CLICK system.

For protocols involving Pain Category E procedures, the PIs have been contacted and asked to provide the number of animals used over the past year for these procedures, summarize the monitoring regimen for these animals, indicate whether or not a monitoring chart/record is used, provide a copy of this record, indicate if there were any unexpected occurrences (i.e., problems, deaths), and confirm that these occurrences were reported to the LAF veterinary staff.

BUSINESS:

Category E annual submissions: Submissions are listed under protocol review.

Noncompliance PMY41040Y: The IACUC has accepted the PI's corrective action plan, and no further corrective actions are needed.

Noncompliance PROTO201900203: The IACUC has accepted the PI's corrective action plan, and no further corrective actions are needed.

Noncompliance PROTO201900182: The noncompliance consisted of the lab performing serial IV tail vein injections, which are not described in the protocol. The serial tail vein injections were conducted in August 2021, September 2021, and January 2022.
In addition to the procedure not being listed on the protocol, the procedure resulted in lesions and necrosis of the tail. The laboratory staff also did not observe humane endpoints as outlined in the protocol. During the semiannual inspection (April 13, 2022), the committee noted that two mice had necrotic tumors. The LAF informed the committee that the lab was notified of the necrotic tumors on April 12, 2022. The animals were not euthanized until the following day. The committee has determined that the issue is a noncompliance. The PI must complete the following corrective actions: The PI needs to create an alternate plan to achieve the experimental outcome without using serial tail vein injections. The PI needs to create a plan to provide laboratory staff with appropriate oversight and ensure humane endpoints are followed. Letters will be sent to regulatory agencies.

AMEND202200117: This amendment is associated with PROTO2019182 and the above noncompliance issue. The exact frequency and duration of the tail vein injections are unclear. The committee is recommending replacing the tail vein injections with IV jugular cannulation.

Noncompliance PROTO201800154: The noncompliance incident consisted of tail biopsies performed on mice more than 21 days old. The mice were not treated with topical anesthetic, nor were they administered general anesthesia or systemic analgesics as per the LAF SOP 2.A. 9 for mice greater than 21 days of age. The committee has determined that the issue is a noncompliance. A Clinical Vet also disclosed that there had been multiple issues with the overall management of the breeding colony. The committee strongly recommends that personnel listed under PROTO2018000154 take a breeding colony management course, such as the one offered by Jackson Laboratory. The committee has determined that the issue is a noncompliance. The PI needs to inform the IACUC committee how many mice were involved in the noncompliance. The PI must complete the following corrective actions: The CITI course "Reducing Pain and Distress in Laboratory Mice and Rats" must be retaken. The PI must inform the IACUC committee who was conducting the tail biopsy while the PI was out of the country. The PI must submit paperwork regarding breeding colony management monthly until the IACUC is satisfied that the lab is appropriately managing the breeding colony. Letters will be sent to regulatory agencies.

Noncompliance BME130285Y: Tumors exceeded humane endpoints due to incorrect placement and improper measurement. The lab also had two other noncompliance incidents in 2020 undertook place under different protocols. There seems to be a disconnect between oversight of laboratory staff due to the number of noncompliance issues over the past couple of years. The committee has determined that the following items need to be addressed: The PI needs to reiterate to their staff that working closely with the LAF Clinical Vets will help ensure responsible research. The PI must provide the IACUC with a plan regarding how they will provide the lab staff with appropriate oversight to prevent noncompliance issues from occurring across the PI's protocols. Letters will be sent to regulatory agencies.

Animal Welfare Concern Facilities: During the semiannual inspection, low humidity was noted in several rooms in the Biomedical Education Building. It was determined that issue compromised animal health. As per federal guidelines, the rodent holding room's humidity levels must be maintained at 30%-70%. The humidity levels in numerous rooms have been below 30% from December through April. As a result of the low humidity, health issues were documented in 25 rats. Several rats developed "ringtail" whereby annular constrictions/necrotic tails tips are found along the tail and or feet are dry, and the distal toes tips may slough off. In addition, a litter of 13 pups all had identical crusted skin lesions over the ventral abdomen, which was thought to be due to dry skin caused by low humidity. The LAF called in the low humidity level issue numerous times to UB Customer Service to generate work orders. Over the past few months, per the LAF, these calls have occurred regularly 2-3 times a week. The committee has determined that the issue is an animal welfare concern. The IACUC committee determined that the following action must be completed: UB Facilities must provide a schedule for BEB regular maintenance of the HVAC system/s. Facilities must also provide a letter stating how the issue will be addressed to prevent the animal welfare concern from reoccurring. The IACUC will send a letter to UB Facilities stating the corrective actions. Letters will be sent to regulatory agencies.

APPROVALS:

A list of the submissions approved since the last meeting has been presented to the committee.

PROTOCOL REVIEW:

In addition to IACUC review, Environment, Health & Safety (EHS) has also reviewed all protocols and amendments submitted this month. For protocols involving the use of hazardous agents in live animals, their use will be approved by the appropriate EHS authority and, as appropriate, laboratory SOPs will be placed as recommended by EHS prior to IACUC approval.

1. **AR2022000017 (Annual Review for PROTO20210008)**
   Annual Summary: From PI's annual summary: In this study, we utilize an acute respiratory distress syndrome (ARDS) model in rats induced by intraperitoneal (IP) administration of bacterial lipopolysaccharide (LPS), which mimics the cytokine storm observed in severely ill COVID-19 patients suffering from ARDS. This research aims to evaluate the pharmacokinetics (PK) and pharmacodynamics (PD) of dexamethasone (DEX) in this model of ARDS with expectations of expanding the model to other drugs. Studies in rats include examination of the DEX PK and PD over a range of doses, induction of acute inflammation, assessment of drug effects on concentrations of cytokines, nitric oxide, corticosterone, and blood cell counts (neutrophil and lymphocyte counts), and finally, development of advanced mechanism-based PK/PD models that may aid in selecting optimal dosing schedules of corticosteroids for the treatment of severe COVID-19 and clarify determinants of DEX action in ARDS. So far, two out of four experiments that were specified in the protocol PROTO202100008 are completely finished and one experiment was partially completed. Experiment 1 was a pilot study to assess a dose-response relationship following LPS administration in rats. LPS was administered IP to Wistar rats at two dose levels, i.e. 2 mg/kg (n = 4) and 5 mg/kg (n = 4). One additional control group (n = 4) received saline by IP injection instead of LPS. Therefore, a total of 12 rats were used in this experiment. Plasma samples obtained in this study were analyzed using ELISA to quantify TNF-alpha and IL-6 concentrations. According to our analysis, the LPS dose of 5 mg/kg was sufficient to induce acute inflammation (a considerable increase in TNF-alpha and IL-6 plasma concentrations) and did not cause any symptoms of respiratory failure or mortality in rats. Therefore this dose was selected for subsequent studies. Experiment 2 is a study to

assess a dose-response relationship of DEX administered in rats with experimentally induced ARDS. DEX was administered subcutaneously (SC) at one of eight dose levels ranging from 0.0028 mg/kg to 2.25 mg/kg, followed by IP injection of LPS solution at a dose of 5 mg/kg to male Wistar rats (n = 4 or n = 8 in each study group). Additional group of rats (n = 8) received IP saline instead of DEX, another group (n = 8) IP saline instead of LPS and DEX (n = 8), and an additional group (n = 8) received IP saline instead of LPS and DEX at a SC dose of 0.225 mg/kg. So far, a total of 71 rats were used in this study. Blood and plasma samples obtained in this experiment were used to quantify TNF-alpha and IL-6, nitric oxide, blood cell counts (neutrophil and lymphocyte counts) and corticosterone concentrations. The obtained time courses of the measured biomarkers will be used to design PK/PD models. Experiment 3 involved 8 Wistar rats injected SC with DEX at a dose of 2.25 mg/kg followed by an IP administration of LPS (30 min later). A total of 8 rats were used in this study. Serum samples obtained in this experiment were analyzed using an LC-MS/MS method to quantify DEX and corticosterone concentrations. These results will be used for the assessment of the potential influence of inflammation on PK of DEX and to evaluate a potential impact of disease state and DEX treatment on corticosterone concentrations.
According to the protocol, all animals were euthanized by exsanguination 24 or 48 h post-LPS injection under isoflurane anesthesia. So far, a total of 91 rats were used in the experiments. No unanticipated adverse results involving animal health and well-being were observed with careful monitoring during this series of experiments.
Committee Discussion: The annual was detailed and well written.
Committee Action: The committee unanimously voted to approve the annual submission.

2. **AR202200041 (Annual Review for PROTO201900203)**
Annual Summary: From PI's annual summary: We continue to use our animals toward understanding the neurobiology of addiction. We were recently informed that our protocol in CLICK did not include a section on cannulation implantation. This was an oversight on our end while going from paper to CLICK.
Committee Discussion: The PI stated that no category E procedures were conducted over the past year.
Committee Action: The committee unanimously voted to approve the annual submission.

3. **PROTO202200007**
Protocol Summary: From PI's non-scientific summary: Information pertaining to how the brain responds to inflammation in regards to iron accumulation is lacking. Specific questions remain including if chronic inflammation correlates to an increase in brain iron, and if the blood brain barrier (BBB) is more permeable to iron in a chronic inflammatory state. This protocol will examine brain iron accumulation and BBB permeability in a mouse model of chronic inflammation, using chronic LPS injections. Changes in brain iron accumulation will be assessed by Perl's and Turnbull blue staining and effects on BBB permeability will be measured by Evans Blue extravasation. Ultimately, this research aims to correlate chronic inflammation and brain iron accumulation to develop new therapeutics to treat diseases involving both phenomena.
Committee Discussion: Edits and clarifications need to be made in Click. Pain categories need to be reviewed, and any procedures with LPS need to be labeled as category E. The procedure "Non-Surgical Procedure: LPS administration" needs edits to the procedure, and a monitoring chart needs to be added. A substance administration needs to be added for the LPS experiment. The substance administration for Fatal Plus needs more information. The procedure "Saline administration" is redundant and can be removed. The substance administration "Evan's blue

extravasation" needs edits and an NPG form needs to be added. The euthanasia procedures need to be reviewed and edited. Fatal Plus cannot be used as anesthesia; the procedure "Sodium Pentobarbital (Fatal Plus) Anesthesia" needs to be removed or edited. The procedure "Perfusion" needs edits to the substances being used. The experiment "Euthanasia" needs clarification regarding if this is a stand-alone experiment or a procedure. The experiment "Evan's Blue" needs edits to the experimental description. The LPS administration needs to be added to the experiment, and the pain category needs to be reviewed. The animal numbers, common procedures, and pain categories in the experiment "LPS injection" need to be reviewed. The experiment "Perfusion" needs clarifications regarding if this is a stand-alone experiment. The alternatives section needs to be completed.
Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

4. **PROTO202200017**
Protocol Summary: From PI's non-scientific summary: Work under this protocol will lead to the development of new monoclonal antibodies (mAb) and antibody fragments, with potential utility for treatment of cancer and other diseases.
Committee Discussion: Edits and clarifications need to be made in Click. The procedure "Blood sampling-Saphenous vein" needs edits to the blood collection timeline. The type of anesthesia being used needs to be added. The duplication section needs to be updated.
Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

5. **PROTO202200022**
Protocol Summary: From PI's non-scientific summary: The objective of this research is to investigate the pharmacokinetics and pharmacodynamics of various therapeutics, including anticancer drugs and therapeutics that may enhance anticancer drug activities when used in combination, in xenograft-bearing animal models. The study of in vivo pharmacokinetics of a drug involves determination of drug concentration in plasma in a time dependent manner following a drug administration and provides the information of drug's absorption, distribution, metabolism, and excretion (ADME) properties. The study of biodistribution involves determination of drug concentration in various tissues and organs of interests and provide the information whether a drug is reaching to the site of actions (e.g., tumors) sufficiently for efficacy and/or to any particular tissues causing off-target toxicity. Thus, quantitative understanding drug pharmacokinetics and pharmacodynamic is critical in designing optimal dosing regimens (amount and frequency of dosing) to maximize treatment benefit while minimizing toxicity.
Committee Discussion: Edits and clarifications need to be made in Click. Monitoring charts need to be developed, and endpoints must be clearly described. The procedure "Intraperitoneal Injection" needs to list the range of doses, and details of the IP injections need to be added. The procedure "Oral gavage (HSN)" needs clarifications, a list of the range of doses, and a monitoring program needs to be developed. The procedure "SC tumor cell inoculation (LC-2/ad) needs clarifications to the anesthesia, cell metastasize expectancy, and a monitoring program needs to be developed. The experiment "MTD Study" needs clarifications to the husbandry exceptions. The experiment "PK and Tissue Distribution" and "PK/PD Study" needs clarifications to the pain category. The animal justification needs edits to experimental group

sizes. The housing section needs clarification regarding if animals are taken to the lab for euthanasia. An updated scientific merit form needs to be added.
Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

6. **TR202200009 (Triennial for PHC08128N)**
Protocol Summary: From PI's non-scientific summary: Corticosteroids are important anti-inflammatory and immunosuppressive therapeutic agents, but they have complicated mechanisms of action and numerous side effects. This class of drugs is widely used for the treatment of various conditions like rheumatic diseases, bronchial asthma, cancer, ocular diseases, certain neurological conditions, as well as in organ transplant patients to prevent graft rejection. These drugs are generally used on a long-term basis and high doses are very often associated with significant side effects, some of which include muscle wasting, osteoporosis and diabetes. In addition, corticosteroids often exhibit drug interactions with other therapeutic agents.
Committee Discussion: Edits and clarifications need to be made in Click. The procedure "isoflurane anesthesia" needs edits to the induction rate. The procedure "normal saline administration" needs clarifications to the substances being used. The procedure "VBP6 bolus administration needs clarification regarding to the type of drug and if it is pharmaceutical grade. The procedure Arthritis induction" needs examples of extreme distress that would necessitate early removal from the study. The procedure "collagen injection" needs clarifications to pharmaceutical grade, and any adverse reactions must be listed. The procedure "pump implantation LNG" needs to describe the anesthesia/ analgesia, and the induction rate needs to be edited. Experiment "6" needs clarification on the frequency the catheters will be flushed, and the solution used will need to be added as a substance administration. The procedure "Cannulation" needs the anesthesia and analgesia described. The committee recommends that Marcaine and lidocaine be instilled around the incisions. The procedure "Ketamine/xylazine anesthesia" needs edits to dosage. The animal justification needs clarifications. The housing section needs edits.
Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

7. **TR202200011 (Triennial for BCH08076N)**
Protocol Summary: From PI's non-scientific summary: The objective of this submission is to understand the developmental role of a novel protein, NRMT1, that can add a methyl group to the first amino acid of its target proteins. When addition of the methyl group is misregulated, severe developmental defects and tumor formation occur. Mice that do not express NRMT1 (NRMT1 knockout mice) exhibit many characterized developmental defects, with especially severe phenotypes seen in the stem cells in the brain. We will now use the NRMT1 knockout mice to determine if the phenotypes seen in the early brain translate to characteristics of Alzheimer's disease later in life and determine if NRMT1 plays roles in the development of other stem cell types.
Committee Discussion: Edits and clarifications need to be made in Click. The procedure "Euthanasia: Perfusion Following Anesthesia" needs clarifications to the type of anesthesia used. In the procedure "Euthanasia: CO2 inhalation/cervical dislocation," the committee recommends using a more humane method of euthanasia. The experiment "Behavioral Studies" needs clarifications to the animal number groups. The experiment "Breeding" needs

clarifications to the animal numbers. The experiment "Production of mouse embryonic fibroblasts" needs justification for the number of animals requested for the experiment. The animal justification section needs to be completed, and the housing section must be completed.

Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

8. **TR202200013 (Triennial for PROTO201900039)**

Protocol Summary: From PI's non-scientific summary: Frailty is a condition of poor physiological reserve that is highly prevalent during aging (>50% in those 85 and older), and increases susceptibility to falls, hospitalization, disability, and mortality. Exercise has proven benefits for frailty, yet older adults rarely attain the recommended 150 minutes a week of moderate intensity continuous exercise (MICT), as lack of time and fear of injury are the most common barriers. High intensity interval training (HIIT) is emerging as an alternative as it can safely provide strength and endurance benefits with lower time commitments than MICT. Additionally, exercise can boost nicotinamide adenine dinucleotide (NAD+) levels, an essential co-enzyme for mitochondrial and cellular function that declines with aging. Supplementation with nicotinamide riboside (NR) can also increase NAD+, and both interventions are gaining support as a therapeutic strategy for aging and related conditions such as frailty.

Committee Discussion: Edits and clarifications need to be made in Click. The pain category for the treadmill test needs to be reviewed—behavioral procedure for the overnight wheel usage to the appropriate experiments. The behavioral procedure "Treadmill assessment" needs more information regarding the shock. The procedure "Serum collection" needs clarification regarding the volume of blood collected, and the committee recommends SQ fluids following blood collection. The committee recommends the PI that the procedure "DEXA" needs to have post-opt reports turned into the LAF. The procedure "Tamoxifen injection" needs an NPG form, chemical hazard cage cards, and door signage. The experiment "Delta-ARE-Physical Performance" needs a husbandry exception for food restriction, a substance administration for ketamine and xylazine, and animal group numbers need clarifications. The experiment "NAD replenishment in aging and Alzheimer's mouse models" needs a husbandry exception for a special diet. The animal number and group sizes need clarifications. The experiment "NR Dose," "NR HIIT," NR SIRTKO, VitD- Stem Cells, and NR-SLEEP needs a husbandry exception for food restriction and supplemented water. The experiment "NR HIIT" needs clarifications to the administration of tamoxifen. The animal justification needs clarifications. The alternatives section needs to be updated.

Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

9. **TR202200014 (Triennial for PROTO201900040)**

Protocol Summary: From PI's non-scientific summary: The field of endovascular surgery has grown immensely in recent years and allowed for treatment of many previously inoperable conditions, including ischemic stroke and intracranial aneurysms among others. With treatment of these diseases does come the risk of causing unintended complications such as stroke or endothelial damage. In order for a new device to progress in development toward use in human patients, it must be confirmed that the risk of these complications is not significant which is what we hope to ascertain with this study. These new endovascular devices, although proprietary in nature, are stent-based wire mesh in design and typically remain attached to a

wire which allows for it to be retrieved after deployment. Many also allow for detachment in case of complication, in which case the wire is removed leaving the stent device in place.
Committee Discussion: Edits and clarifications need to be made in Click. The euthanasia procedure "Formalin Perfusion Pig" needs clarifications to the anesthesia procedure. The procedure "Blood for Clot Formation (Swine)" needs clarification regarding the volume of blood collected. The procedure "Autologous Clot Introduction Chronic Delphi (Swine)" needs clarifications to the type of clot, size, and volume of the clot used. The Neuro examination may not be necessary for this procedure and should be removed, and more clarifications are needed. A ventilator is recommended for anesthesia lasting more than 30 minutes. The substance administration "Aspirin/ Plavix Administration (Canine)" needs clarifications regarding if both drugs are administered. The procedure "Substance Administration: Autologous Clot Introduction - Chronic Delphi (Canine)" needs to state the volume of the blood clot. The committee recommends reviewing the SOP used for transporting dogs to the MRI in Kaleida. The procedure "Blood Collection for Testing – Delphi (Canine)" needs edits to the procedure. The volume of the blood being collected needs to be listed. The procedure "Blood collection for clot creation (Canine)" needs clarifications to blood collection, and the clot formation is being collected. The procedure "Substance Administration: Autologous Clot Introduction - Acute Delphi (Canine)" needs to indicate the volume or size of the clot injected into the dog. The procedure "Acute Device Deployment (Swine)" needs clarifications to the neurological examination and to live blood collection, and a substance administration needs to be added for midazolam. The procedure "Chronic Device Deployment (Swine)" needs clarifications to the contrast used in the study, blood collection volume, and why a neurological examination is required.
Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

The IACUC Chair adjourned the meeting at 2:33 PM.