Institutional Animal Care and Use Committee
1/14/2020 Minutes
VCRC - 76D

Meeting Convened: 12:11PM	Quorum Requirement: 10
Meeting Adjourned: 3:30PM	Members Present to Vote: 11

Voting Members			Alternates		
1	X	(Chair - M, S)			
2		(Vice-Chair - M, S)			
3	X		A		(A, S)
			B		(A, S)
			C		(A, S)
			D		(A, S)
			E		(A, S)
			F		(A, S)
			G		(A, S)
			H		(A, S)
4		(M, S)	I		(A, S)
5	X	(A, U)	J		(A, U)
			K		(A, U)
			L		(A, U)
			M	X	(A, U)
			N		(A, U)
6	X	(M, S)	O		(A, S)
7	X	(M, V)	P		(A, S)
8		(M, S)	—		
9	X	(M, S)	Q		(A, S)
10		(M, S)	—		
11	X	(M, S)	R		(A, S)
12		(M, S)	S		(A, S)
13		(M - NA, NS)	T	X	(A - NA, NS)
14	X	(M, S)	U	X	(A, S)
			V		(A, S)
15		(M, S)	W		(A, S)
16	X	(M, S)	X		(A, S)
17	X	(M – St)	Y		(A, St)
			Z		(A, St)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii		(O, U)
iv		(O, U)
v		(O, S)

Institutional Veterinarian:

3	X	(M, S)

Correlates to Version **v2.98** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee reviewed the December 2019 Inspection Findings – Notes to File- Veterinary Recommendations report.

2. The committee was updated on a lab that had been the focus of an ethics report over the summer. The lab received its normally scheduled post approval monitoring inspection and there were no significant findings. The committee had no further comments and considers the matter closed.

3. The committee met with a PI to discuss potential adoption of an instrumented animal that is going off study. A subgroup of committee members will consult general counsel on this matter and report back to the committee at a future meeting.

4. The committee was updated on the retraining efforts for a lab that recently had an issue with recovering animals from an anesthetic event. Lab personnel worked with RAR veterinarians and received additional instruction on animal handling, anesthetic induction, monitoring, injection techniques, record keeping practices, and proper recovery of animals. An RAR veterinarian will supervise the next set of procedures done by the lab to validate the training and provide additional tips. The committee will be updated on these efforts at a future meeting.

5. The committee discussed an issue where a lab with a history of compliance issues had staff that was not sufficiently trained managing an IMHA and conducting training surgery with minimal oversight from the PI. The investigator submitted a plan to address these issues which included additional training for lab staff and increased oversight from the PI. The committee had the following additional requirements for the lab:

    - Lab staff must complete Controlled Substances online training module and Laboratory safety training in addition to the RAR training courses listed in the plan submitted to the IACUC.
    - The PI must also enroll in the following RAR training courses to ensure that oversight is consistent with the current expectations of the UMN Animal Care Program.
        - "Rat handling and Injections"
        - "Aseptic Techniques in Rodents"
        - "Anesthesia of Mice and Rats"
    - Subsequent surgeries conducted by the lab can only occur with RAR supervision until the IACUC clears you to conduct independent, unsupervised surgeries.
    - The opportunity to house animals in an Investigator Managed Housing Area has been revoked until further notice.
    - IACUC Office compliance staff will conduct unannounced lab visits to ensure adherence to the lab's approved animal use protocol(s) and UMN animal use standards.

6. The committee discussed an incident in which a lesion was found on an animal. It was proposed that the lesion occurred due to an unidentified microchip that was heated during an imaging session in the 10.5T magnet. The animal did not show signs of pain and the veterinary team is working with the lab to treat the lesion. The committee will continue to follow this case and work on implementing practices to prevent any future incidents in this and other animals.

7. The committee was updated on a study in which a subset of animals are displaying potential reperfusion injuries following surgery. The investigator and the UMN veterinarians are working with other institutions to determine potential causes for this incident and treatments. The veterinary team will continue to update the committee on this incident.

8. The committee introduced and briefly discussed a protocol review scenario that was recently published in Lab Animal regarding a NHP study in which a veterinary recommendation for pain management was not included in the final approved protocol. The committee will discuss the scenario in more detail at the next IACUC meeting as part of ongoing member training.

1. **IACUC-NEW (# Protocols: 6)**

    1. **Protocol Title:** 2001-37739A A nonGLP Evaluation of Bovine Pericardial TAVR Valve in the Sheep Model
    Species &Pain Class: Sheep (Biomedical) (B)
    Question the Research Addresses: This is evaluation of new technology. This is a single piece of bovine pericardium that has undergone proprietary tissue processing to reduce calcification. The pericardial tissue is attached to a low profile stent frame.

    Committee Decision: Approved submitted
    For: 10 Against: 0 Abstain: 0
    Member 11 out

---

    2. **Protocol Title:** 2001-37750A MATI-2: Tolerogenic Efficacy and Safety of Apoptotic Donor Leukocytes and Transient Immunosuppression with ASKP1240, Rapamycin, Etanercept, and Tocilizumab in a Preclinical Islet Allotransplant Model in Primates
    Species &Pain Class: Nonhuman Primate (Macaques) (B)
    Question the Research Addresses: Administration of apoptotic donor splenocytes effectively induced antigen-specific tolerance to allografts in murine studies. We recently translated these findings to a preclinical model in nonhuman primates (NHPs) and showed that two peritransplant infusions of apoptotic donor leukocytes (ADLs) under short-term immunotherapy with antagonistic anti-CD40 antibody, rapamycin, soluble tumor necrosis factor receptor, and anti-interleukin 6 receptor antibody induced long-term (≥1 year) tolerance to islet allografts in all nonsensitized, MHC class I-disparate, and 1 MHC class II DRB allele- matched rhesus macaques (A. Singh et al., 2019). These findings, obtained in a stringent preclinical allotransplant model in NHPs, are unique and point to the first clinically applicable path toward nonchimeric transplantation tolerance in humans. This study is designed as the final pivotal safety and efficacy study to support initiation of clinical trials.

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    - The author has stated that a similar study has been performed (A. Singh et al., 2019) using a related macaque species (Rhesus). The aim of the current study is stated as, "to demonstrate long-term transplantation tolerance in a second macaque species to corroborate the previous findings in rhesus macaques and strengthen the likelihood of success in first clinical translation of a cellular, non-chimeric tolerance regimen". Please update the rationale to explain why there is a need for a duplication of a previous study using similar NHP species.

    - The experimental design suggests that this is a replication of studies that have been performed on rhesus, but all of the rationale refers to NHP and macaques, as a single group. There is a phrase that this is a pivotal study: please update the protocol to clarify how the use of cynos (as opposed to or in addition to) rhesus, helps with the pre-clinical goals.

    - In the Rationale, the anti-CD40 listed is 2C10R4. However, in subsequent sections, the antibody listed is ASKP1240. Please clarify and update as needed.

    - The experimental design focuses on a specific vaccine (ASKP1240). Please include in the rationale why that particular vaccine is significant and worth controlling for.

    - The author mentions the use of "smart" blood sampling in which the sampling frequency and the volume of blood collected will be kept to an absolutely minimum necessary to accomplish experimental requirements. However, even through the use of a vascular access port, there is still a considerable number of sampling points, especially at the beginning of the study. A number of those points appear to overlap. Please consider combining time points if possible.

    Committee Decision: Stipulations must be met

For: 10 Against: 0 Abstain: 0
Member 17 out

---

3. **Protocol Title:** 1911-37613A Reprogramming astrocytes into neurons in canine stroke model
Species &Pain Class: Dog (B)
Question the Research Addresses: Can astrocytes reactive after stroke in canine be reprogrammed into neurons?

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Please clarify if this protocol is intended to replace the previous submission, 1812-36598A "Surgical canine Stroke Model". If so, 1812-36598A will be withdrawn.

- Please consult with RAR and VMC to determine the best housing locations for these animals following the "Canine stroke surgical procedure".

- Protocol indicates that surgery will take place in [redacted] but no[redacted] personnel are listed. Please list any/all [redacted] personnel that are expected to handle animals on this study. Please list any personnel that may assist in surgery as surgeons in the surgery procedure sections.

- Include cerebral angiography in this section. You state that animals will undergo CSCARS during 7 day acclimation period. This should be performed at the end of the acclimation period (day 6 or 7) to allow animal the longest duration possible before undergoing examination.

- Please add a section to describe gait analysis (referenced in experimental design and in surgery attachment). Please include frequency of gait assessment, location of assessment, and whether/how assessment is conducted in paretic animals. Please add a procedure for cerebral angiography (referenced in surgery attachment) making sure to describe closure, post operative care, etc. if performed in a separate anesthetic event from surgery.

- Acepromazine alone may not be sufficient premedication. Please contact your RAR area veterinarian to discuss your anesthetic regimen. If a boarded anesthesiologist will be providing anesthesia services during the surgery, please consult with him or her and ensure their preferred drugs/doses are listed on the protocol. Unless contraindicated for study purposes, analgesics should be administered before the onset of painful stimuli. Please discuss your analgesia plan with your RAR veterinarian, and/or consult with your anesthesiologist and update this section per his/her recommendations. Please provide clarity on intended duration of buprenorphine and carprofen administration and this can be a specific number of days, or per RAR veterinary instruction. Please also indicate whether buprenorphine is administered pre or intra-operatively Consider adding injectable carprofen as an option in the event animals are unable to take medications by mouth after surgery. Consider option for sustained release (SR buprenorphine) to minimize the number of injections. This provides up to 72 hours of analgesia with a single injection. Ensure all likely support drugs are listed. Other large animals undergoing craniotomy are administered supportive agents/treatments including mannitol or steroids. Please discuss likely support agents required with your anesthesiologist and or your RAR veterinarian. Protocol indicates that blood pressure is monitored but does not indicate direct versus indirect, what normal values are expected, or how you will intervene if values are out of range. Please amend responses to include this information. Please also monitor oxygen saturation, or explain why it will not be monitored. Clarify duration of phenoparbital administration as well as tapering schedule (unless animals will receive for duration of study). Please list any [redacted] personnel as surgeons that may assist in surgery. Note: Other stipulations/questions pertaining to surgical information in the attachment are listed under "attachments."

- Please update the following in the MRI Imaging Procedure: - The Procedure is listed as class A but animals are under anesthesia – please update to class B. - Please provide monitored parameters (e.g

HR, BP) with acceptable ranges

- All comments in the surgery procedure section regarding anesthesia (drugs, doses, monitoring, etc.) apply to MRI as well. Must aimals be trasnferred [redacted] Transport is stressful and shoule be avoinded and minimzed wherever possible. Consult with your RAR veterinarian before scheduling MRI and anesthesia (with or without transport) to ensure dogs are good candidates for anesthesia. This is most important for animals undergoing their first MRI in the first 1-3 days after surgery. UNless contradindicated for study purposes, oxygen saturation, body temperature, and blood pressure should be monitored in addition to EKG during MRI. Please update your monitoring plan to include these parameters, or justify withholding. Please update your responses to how you will intervene if values are out of range. Please confirm that the second and final MRI sill be terminal.

- Based on attachment female dogs may get a sample for hormone analysis, presumptively there is pre anesthetic blood work taken. Please clarify if there will be sampling for blood gas under anesthesia as part of cardiopulmonary monitoring (health and monitoring section states oxygen saturation is monitored - is this by blood gas or other means). If so, this may require a separate blood sampling procedure to adequately describe frequency and volume. Please state any baseline sampling in addition to samples requested by veterinarian.

- Please update this section and or the blood collection schedule in the surgery attachment so that they agree and reflect your intended collection.

- All comments in the stroke surgery section regarding anesthesia, analgesia, monitoring, etc. apply here as well.

- Clarify route and schedule of BrdU administration. Procedure and Experimental Design sections do not agree. 100mg/kg dose of a 10 mg/ml solution to an 11 kg dog is a 110 ml volume to administer. This volume it too great to bolus and will require IV catheter and controlled drip (recommended rate of 2-4 ml/kg/hr). Consider placing a vascular access port (VAP) or other chronic indwelling catheter at time if surgery to allow for repeated, prolonged IV administration. Please contact your RAR veterinarian to discuss

- Because there is no attempt to close the dura and no bone replacement, depressed intracranial pressure seems likely. Please consider using a dural substitute or sealant, or justify why that would compromise the experimental design.

- Please update your responses to questions 1-3 to include potential adverse events for all procedures on the protocol (BrdU administration, anesthesia and imaging, blood draw, etc.). Please provide a description of anticipated clinical condition of dogs based on previous work (attached). This will presumably include seizure since pentobarbital is being administered. Clarify whether the 6 hours of intensive post op monitoring will be provided by lab staff or RAR. Note that RAR staff will require advanced planning (via RAR technical services), treaining, and will incur tech time costs. Please describe how paretic or paralyzed animals will be managed in response 3. This should include things like bedding, turning/re positioning, whether or not animals are expected to access food and water without assistance, and frequency of monitoring/support. You state that animals will be evaluated for pain every 2-4 hours for the first 24-48 hours. Please confirm this includes overnight/weekends. Please describe what intervention criteria are (a score of X) and what interventions will be initiated if/when animals reach that score. Confrim or update your response to question 5 which currently says you do NOT want an exception to social housing.

- Please clarify who will be performing the 1-to-1 monitoring postsurgically, and who will be administering the Colorado Pain Scale assessment. Also clarify how the pain assessment will inform pain treatment, and whether it will be used in euthanasia decisions.

- Please indicate acceptable ranges for blood pressure.

- The procedure involves relocation of the temporalis muscle and removal of the zygomatic arch. Is mastication and ability to eat a concern?

- Else where in the protocol you indicate that dogs may be kept for 30- 60 days, but that does not agree with endpoints listed in this section. Please update all relevant sections so that they agree and indicate the intended end point.

- Fluoroscopy and angiogram are mentioned as methods to determine appropriate vessel occlusion, radiation sources should be listed in this section (e.g. fluoroscope).

- BrdU must be added to the chemicals section of the DEHS tab and a current hazard SOP must be attached.

- Section lists ketamine which is not described as being used elewhere in the protocol. Please update all relevant sections so that they agree and reflect intended use.

- Canine Stroke Surgical Procedure–2019_v1 - "If female animals are used, we measure blood levels of estrogen, progesterone, FSH and LH levels and perform a vaginal swab." Please add a procedure if this will be performed. Since seminal work was performed in dogs, consider whether or not females should be used in this proof of concept arm of study. (Suggestion, response not required for approval) - THis document suggests surgery is being performed in [redacted] but procedure section indicates [redacted]. Please clarify where surgical procedures will take place and update all relevant sections accordingly. (Ensure appropriate personnel are also listed if [redacted] and or [redacted] team members will assist with surgery, including perform cut downs for angio). - Please clarify timing of angiogram (same anesthetic event or separate event). If separate, when may it occur relative to surgery? This timing, and evaluation of dog for anesthesia should occur with RAR vet.

- We are unable to confirm that the PI, Andrew Grande, has completed the rabies surveillance requirement (expired 9/13/19); that Nikolas Toman has completed the rabies surveillance and dog ROHP modules (rabies training and parasitic diseases); and that Aleta Steevens has completed the rabies surveillance and dog ROHP module (rabies training). Please follow up with personnel to ensure these requirements are completed. Once complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 11 Against: 0 Abstain: 0

---

4. **Protocol Title:** 1911-37642A Fish health, growth, and nutrient excretions in an aquaponics system
Species &Pain Class: Blue Tilapia (A)
Question the Research Addresses:

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Question 8 - Please indicate the maximum size of the fish that will be in the 5 inch cubed cages. Question 14 - Please provide more details on the type of feed, the nutrient content, and how and where it will be stored. Does each individual cage get fed, or how is the food spread throughout the tank? Question 22 says water quality will be measured weekly, while Question 35 says daily. Please reconcile. Question 27 - Please provide a veterinarian rather than "fish supply professionals will be consulted for ill fish." RAR can assist in identifying a suitable candidate if you would like assistance. Question 31 - It says that the director of facilities "may" be able to provide generator for heat if power goes out. If no generator can be provided, please clarify how the temperature will be maintained in [redacted]. Question 35a - It currently says that "optimal concentrations of ammonia/nitrate are less than 1mg/L." Ammonia and nitrite should be as close to zero as possible. Although they are considered toxic over 1mg/L, any detectable level can cause stress and adverse

health effects. Nitrate is less toxic, and should be kept between 5-150 mg/L. 35a - Please include the acceptable temperature range. Question 35g - It says that water temperature, nitrogen, and pH will be measured daily. Will dissolved oxygen also be measured?

- Please clarify how the cubes are arranged within the tub.

- Please provide a description of how this procedure will be performed. Gloves should be worn during handling to protect the slime coating of the fish.

- We are unable to confirm [redacted] has completed the Animal Exposure Questionnaire, ROHP intro training, tetanus requirement, ROHP aquatic pathogens training, and Animal Use Tutorial. Please follow up with personnel to ensure these requirements are completed. Once complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 11 Against: 0 Abstain: 0

---

5. **Protocol Title:** 1912-37667A T Cell Responses to Intestinal Protein; Vaccination to reverse established CD8 T cell tolerance to melanoma; Reversing CD8 T cell tolerance in vivo
Species &Pain Class: Mice (A,B,C)
Question the Research Addresses: We will determine how immune cell (T cell) differentiation, migration, and maintenance is affected in animals with cancer, chronic infection or in animals bearing a known self-antigen. We will also test how the T cells induced function can be better through vaccination. We also study how the intestinal environment influences the development of pathogenic or tolerant immune cells.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Note that information provided in the response to question a has been administratively moved to under question e. Additionally, please update the response to be specific to data that was obtained in the 1611-34309 protocol. I do not think that that the 2014 paper you reference here is a direct result of the protocol that was created in 2016. Essentially the committee wants to know what parts of the protocol have already been completed and how those animals did. Were you able to generate meaningful results? Did you lose more animals from health concerns than expected?

- With movement of experiments the line links in the headings to the table attachment seem to have gotten off by 1; these have been administratively corrected. "Determine how self-specific autoimmune CD8 T cells cause pathology in the intestine. Line 18-22" is now 18-23 “Determine whether therapeutic vaccination can reduce viral load in chronic infections in mice and understand the mechanism behind this. Line 23-24” is now lines 24 and 25. Please update this section of the Experimental Design to include a brief explanation of why you are using that strain (CD4CreERT2) for this experiment. "Study the movement of antigen-specific CD8 T cells in vivo. Line 25-30" is now 26-31

- It is still not clear to me when treatment interventions are happening for mice enrolled in survival curves. I realize that you do not know what the compounds will be, but when are they administered? When DSS is administered? When mice are found moribund? At birth? Please update the Experimental Design to elaborate as reviewers need to understand what is happening to animals on the protocol and what their experience will be.

- In regard to the Avertin compounding in the use of non-survival surgical procedures, please make sure follow the IACUC guideline for its preparation and use as it is more detailed than what is in your description. For example, it needs to be filtered for sterility per the guidelines but this is not mentioned in the protocol. To make this easier, you can add that the IACUC guideline

will be followed. Please note, isoflurane should not cause muscle contraction, if this has been a problem, it is possible that the animals was too light under anesthesia. In survival procedure, "Skin/tumor rechallenge with peptide/virus (tattoo)", please add isoflurane to the anesthetic table and further justify why avertin needs to be used in this procedure. Since isoflurane is already being used in the first part of the procedure it makes sense to use it again and prevent prolonged anesthesia for the tattoo part of the procedure. Recovery will be much faster and the anesthetic event can be tailored to the exact need per the individual tatooing. Also, isoflurane should not cause muscle contraction, if this has been a problem, it is possible that the animals was too light under anesthesia.

- In the following procedures there is still a IV volume of 200-500 ul listed and not changed per the response to the previous stipulations. Please update within each of the following procedures to comply with your response, also stating the approximate volume range to replace it. 200-500 ul is an appropriate volume to give IP. 125ul is a more appropriate volume for IV injections. -Infection with pathogens -Adoptive transfer of lymphocytes -Monoclonal antibody injection -Leukemic cell infusion -Bone Marrow Transplant (for this procedure in addition to the volume please include the number/range of cells and the route)

- Please update the description of surgeon training for Emily to include her training specific to the intestine, as the current information is specific to lymph node.

- Please update this procedure to include more detail on the expected clinical effects and range of manifestations in this model (in addition to the existing information on the signs that will be criteria for euthanasia).

- Please update the Health and Monitoring section to address the following. Do you provide moistened food to mice as soon as they begin the DSS/colitis trial? Do you ever assess hydration (skin tent) or give SQ fluids when dehydration is noted? At what point of weight loss do you intervene. I see that you euthanize at 20-25%, but what do you do when they've love 15%? Is that when you provide moist food +/- SQ fluids to correct for weight loss and anorexia secondary to dehydration?

- Please note that in the Health and Monitoring it states that experimental related health concerns should primarily be managed by lab staff and euthanized per protocol outlined endpoints (e.g. weight loss, diarrhea, etc.). RAR technical staff does do daily health checks and monitors for spontaneous health concerns but they should not be responsible for managing when your animals meet endpoints. If you need help in determining these contact the area veterinarian for further training. Also if the supportive measures you listed in the health and monitoring are acceptable for animals that are losing weight or are otherwise ill, they can be incorporated into the protocol without veterinary intervention so your staff can add these and manage the experimental related illnesses.

- The response to the Cycle 1 stipulations requesting death as an endpoint justification did not adequately provide this information. Based on the experiments being run, all of those that are listed Class C (and also assumed to be death as an endpoint) are for blood collection only after injection with VSV-ova, Vaccinia-ovaeven, or Listeria-ova. What value is gained by allowing these mice to proceed to death? Please update the response to Endpoints Question 4 to justify this further as at this time it does not seem to be useful. If in the future, novel therapies need to be tested for death as an endpoint, it can be added as part of those experiments.

- Please clarify the following points with respect to the attached table: -Line 6 – Are the 3 different tattoo types accounted for in your numbers (there does not seem to be a 3 fold multiplier for this - there is a 3 fold multiplier but it is for replicates). As is your multipliers for factors here are the same as line 7 where no tattooing occurs). Please explain the IV vs no IV – what is given IV? Is it a check point inhibitor and if so is it only 1 inhibitor (as the numbers indicate) and is it only given once or is it given multiple times (based on timeline it is

presumably only given once). That IV explanation would also be useful in lines 7 and 9 - presumptively the same. -Line 12 – Please resolve discrepancy between number of mice calculated in experiment column (180) vs number in Total number column (260). Is there a difference in infection type that accounts for this or is it just a carryover error? -Lines 15 and 16 - Confirm you only need 5 antibody combinations vs the 15 combinations in lines 13 and 14 (it isn't clear why the difference – or is this an error and antibodies used here are as for line 17). If you need 15 total combinations you will need to adjust numbers for these experiments.

- Please confirm the math in the attached table or update (and update the Species table to match) if needed . -Class B transferred from 1905-37057A I get 3,349 vs table 3389 (and this is without changing line 12 to 180 from 260) -For purchased class B I get 4338 vs table listed 4298

Committee Decision: Stipulations must be met
For: 11 Against: 0 Abstain: 0

---

6. **Protocol Title:** 2001-37737A Assessment of physiologic responses under thiafentanil-xylazine anesthesia of wild moose
Species &Pain Class: Moose (B)
Question the Research Addresses: What are the physiological responses by moose to an established thiafentanil-xylazine anesthetic protocol? Can supplemental oxygenation and/or intervention with pharmaceuticals that can reduce the peripheral narcotic effects improve anesthetic parameters.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - As previous researchers have had difficulty with calf survival following moose capture, please consider how these calves will be managed to ensure that they are not deserted or lost in response to capture.

   - A previously approve moose protocol number is referenced in the health and monitoring section, question 1 - "Moose will be chemically restrained for ongoing research (see eprotocol 1812-36635A)". Please confirm that procedures approved on that protocol are not being done under this protocol.

   - Response appears to be a copy/paste or clone error as it includes collared deer and continuation to mortality. Please review the response and revise so that the endpoints listed cover only this study's endpoint rather than the endpoints of other studies animals are associated with due to collaring.

   Committee Decision: Stipulations must be met
   For: 11 Against: 0 Abstain: 0

---

2. IACUC-AMENDMENT (# Protocols: 1)

1. **Protocol Title:** 1909-37389A Macrophage inflammasome activation and the mechanism of lipolysis resistance in aged adipose
Species &Pain Class: Mice (A,B,C)
Question the Research Addresses: How do adipose tissue immune cells promote inflammation and metabolic dysfunction?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - It seems that the change in body temperature from 35 deg C to 30 deg C is quite extreme and would constitute more than "mild" hypothermia. Please provide references or data that these core body

temperatures are compatible with life for a mouse.

- Mice that receive the non-lethal dose are still expected to experience discomfort that is unrelieved and should not be considered category A. Please update the pain class to class C, if pain will not be relieved, or to class B if treatment will be provided to manage pain and discomfort.

- Please elaborate more on the alternative search answers, particularly for the added LPS, in addition to stating that there are no alternatives.

- The rationale for needing death as an endpoint is not clear. If a mouse is moribund following these injections, it is likely very close to death, and if this criteria is used for all groups, it is unclear why this would change the outcome of the results. As another benefit, mice sacrificed at this point would likely result in obtaining much fresher tissue better for analysis. If a mouse is checked on, found to be moribund, and is left to go another 2 hours, it could very easily die within after a few minutes. If is then left to sit for another two hours, it may have significant tissue autolysis. All moribund animals must be euthanized unless death as an endpoint is well-justified. Please update the protocol to provide a clear rationale and justification in this description about why measures cannot be taken to relieve pain and distress, and why animals cannot be euthanized at an earlier state such as moribundity which is defined as “the clinically irreversible condition leading inevitably to death.”

Committee Decision: Stipulations must be met
For: 11 Against: 0 Abstain: 0
