Institutional Animal Care and Use Committee
10/8/19 Minutes
VCRC - 76D

Meeting Convened: 12:07 PM	Quorum Requirement: 10
Meeting Adjourned: 1:13 PM	Members Present to Vote: 10

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

Non-Voting, Ex-Officio:

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

Institutional Veterinarian:

3	X	(M, S)

Correlates to Version **v2.95** 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. **IACUC-NEW (# Protocols: 3)**

    1. **Protocol Title:** 1909-37444A TPD2 inhibition to improve topoisomerase targeting in cancer
    Species &Pain Class: (B) Mice
    Question the Research Addresses: Novel inhibitors of TPD2, first synthesized at the University of Minnesota in the Center for Drug Design program, will be tested in murine models to determine if these agents enhance or rescue the clinical activity of existing TopoII inhibitors such as etoposide.

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

    - While the Species section requests 478 animals, it is not clear how this number was derived as there are 4 total experiments listed in the Experimental Design (2 in syngeneic model, 2 in xenograft model) with n=40 each. Please update the protocol to provide a chart or other means to clearly outline the total number of mice requested, and if the numbers listed in the Species section are incorrect, please update.

    - Under experiment 1 and 2 for both the syngeneic and xenograft models, please update the protocol to clarify the route that is indicated by "injected into bilateral flanks" including whether this is a subcutaneous or intramuscular injection.

    - Descriptions of experiments provided in the experimental design section include inoculation of LM2 and NSCLC cells, while the tumor induction procedure describes inoculation of 3 cell types - LM2, LLC and KP cells. Please update the experimental design section to clarify if these describe the same cell lines and if not, update the experimental design and/or the procedure to clarify which cells are used. It is unclear in the experimental design section how many mice will receive bilateral vs. unilateral inoculations (it states that cells will be inoculated bilaterally but then that unilateral flank tumors will be treated). Please update the protocol to clarify. Please update the experimental design to indicate where in the experimental timeline the blood collection procedure occurs.

    - The Intraperitoneal injection procedure states that antibodies will be injected IP with a 22g or larger syringe. The maximum recommended needle size for mouse IP injections is 25g. Please justify with associated references anything larger than 25g, or update the protocol to use the recommended gauge. See section "Recommended locations and approximate needle sizes": https://www.researchservices.umn.edu/services-name/research-animal-resources/research-support/guidelines/routes-administration

    - Moribund state is described as an endpoint /time at which animal will be euthanized (in health and monitoring and this section) but the box for question 4 is not checked yes for moribundity. Please check this and provide justification or indicate earlier endpoint that can be used if mice become ill and have not reached tumor endpoint criteria. If allowing animals to reach moribundity is required, please indicate how frequently they will be checked when signs of distress are noted. Currently in the health and monitoring section it states that they will be monitored closely but doesn't give frequency. Note that if moribundity is the planned endpoint, the mice should be re-categorized to pain class C.

    - We are unable to confirm Zhengqiang Wang has completed the Animal Exposure Questionnaire, ROHP Intro training, tetanus requirement, and Animal Use Tutorial. Please follow up with Zhengqiang to ensure these requirements are completed. Directions to complete requirements were sent via email on 10/2. Once requirements are complete, please confirm here in eProtocol.

    - Please submit a toxic hazard class SOP (https://dehs.umn.edu/node/129581/attachment) for Etoposide. You may list any toxic chemicals you are using in your lab in this one SOP.

    Committee Decision: Stipulations must be met

For: 10 Against: 0 Abstain: 0

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2. **Protocol Title:** 1905-37099A 1. Subcutaneous/intravenous injections of human/mouse OS cell lines and MSCs with CRISPR/Cas9 library 2: Calcaneal injections of human/mouse OS cell lines with and without cDNA and CRISPR/Cas9 injections 3: Treating osteosarcoma mouse models with checkpoint inhibitors PD-1/CTLA-4 and SEMA4D/ZNF217 pathway drugs 4: Flank injection of mice with human osteoblast precursors derived from human induced pluripotent stem cells that are wild-type genotype or have been genetically modified to disrupt specific candidate genes linked to metastasis. 5: Engraftment of human ovarian tumor cells in mice will be treated with modified human NK cell for tumor burden reduction or complete loss of tumor 6: Production of IDUA from human B cells 7: Determine the efficacy of BE4- and Cas9-generated CAR-T cells in xenograft mouse models of multiple myeloma – pilot study 8: Determine the efficacy of BE4- and Cas9-generated CAR-T cells in xenograft mouse models of multiple myeloma 9: Determine the efficacy of BE4- and Cas9-generated CAR-T cells in PBMC culture in xenograft mouse models of multiple myeloma 10: Determine the efficacy of multi-KO CAR-T cells in mouse models of B cell lymphoma - pilot study 11: Determine the efficacy of multi-KO CAR-T cells in mouse models of B cell lymphoma
Species &Pain Class: (A,B,C) Mice
Question the Research Addresses: In an attempt to further study our candidate cancer genes (CCGs), we are interested in understanding which gene mutations can lead to/cause osteosarcoma and metastasis. In an attempt to further study the use of NK cells for ovarian cancer therapy, we are interested in understanding what NK modification are most effective in reducing tumor burden. In an attempt to further study the safety and efficacy of genetically engineered T cells in patients with blood cancers, we are interested in the most effective edits we can make in human T cells to allow them to target and destroy human tumors in an in vivo model.

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

- In experiment SA3 the protocol states that Anti-Pd-1/Ctla-4 will be administered i.p. in water. Please confirm that these antibodies are designed to use plain water as a vehicle and the final osmolarity is physiologically appropriate, or update the protocol if another vehicle will be used.

- In this section it states that one of the tumor endpoints is size at 2cm3. This is an excessively large tumor for that region in the bone and would undoubtedly cause mobility issue before it got to that size. Please update the procedure to further describe this size and whether or not the hock area will really get to that size before endpoint.

- Please update the Health and Monitoring section to describe in more detail the growth of the tumor in the calcaneus/tibal regions. Include what signs are monitored for to determine an endpoint. Depending on the tumor type (osteolytic or -blastic) there can be significant pain associated with its growth in addition to functional problems. Please include in H/M the steps that are taken to identify and alleviate these issues.

- We are unable to confirm the following personnel have completed requirements to work with animals on the protocol: -[redacted] (overdue Animal Exposure Questionnaire) -[redacted] (ROHP Intro training, tetanus requirement, and Animal Use Tutorial) -Nicole Maeser (Animal Exposure Questionnaire, tetanus requirement) Please follow up with personnel to ensure these requirements are completed. On 10/4 they received directions regarding how to complete requirements. Once requirements are completed, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0

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3. **Protocol Title:** 1909-37420A Functional Characterization of Anergic Helper T Cells Adenosine Modulation of Autoimmune Arthritis
Species &Pain Class: (A,C) Mice
Question the Research Addresses: What is the best approach to re-induce immune self tolerance in an individual who has developed autoimmune disease.

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

- 1. The protocol includes an EAE procedure. Please update the Experimental Design to include this. 2. Under bullet point 6, the protocol states that neonatal pups will be placed directly on wet ice for anesthesia. As this is inconsistent with RAR and IACUC guidelines on hypothermia as anesthesia in neonatal rodents, please either request an exception to these guidelines (with scientific justification) or review and edit the protocol as needed. Per RAR's website: - Animals should not be placed directly on ice (use latex glove or another substance between the animal and the underlying ice bath). - Animals have reached the proper plane of anesthesia when pedal reflex is lost. - Do not use incandescent light during the procedure, as it can warm the surgical field and cause animals to awaken from a surgical plane of anesthesia. - Following anesthesia, the animal should be re-warmed slowly. Rapid warming can cause tissue damage. Patients can be re-warmed on a circulating water blanket, heating pad (40°C), or in an incubator (33°C). - Pups can be returned to dam once they are able to move without direct physical stimulation. - Refer to the IACUC guidelines on using hypothermia as anesthesia for neonatal rodents.

- Please indicate how doses of drugs being administered were decided and if they are based on previous studies, or established literature. Note that including a statement that the doses are sufficient to cause the effect needed without causing undue pain and stress to the animals would be helpful and could be included in the refinements section of the Alternatives Search.

- Section 5 of the Experimental Design describes adoptive transfer from one adult mouse to another, but then goes on to mention injection via the parietal vein using ice anesthesia. If this specific route/method will be done in adult mice, please add a procedure to describe it. If the parietal vein injections will only be done in neonates, please update the protocol to clarify that.

- Please describe update the protocol for RO injection technique to include supportive agents (ophthalmic antibiotic ointment and topical ophthalmic anesthetic).

- The Experimental Design section states that some mice may require injections every 3 days until they are euthanized, but the procedure currently reads that injection will occur once. Please update the procedure to include additional doses here and give an approximate length of time mice will be kept on study. For instance, "....repeated injections every 3 days at 25 µg/kg until the endpoint is reached (up to 3 months)".

- Since mice will be on antibiotic water for 2 weeks as part of the bone marrow chimera procedure, please add a "Modified Diet/Fluid" procedure to capture IACUC specific questions surrounding special water.

- Please note that the term "aseptic" has been administratively removed from the description of this procedure, since the text describes using 1 needle/cage and giving multiple injections to each mouse in the cage. Please confirm that you use a clean needle each time you puncture the bottle/draw up more solution.

- Please update the Health and Monitoring section to describe supportive care options for arthritic mice. Consider adding soft bedding and moistened food on the cage floor to allow for easier access. It looks as though mice with EAE get ascending paralysis -- please indicate whether bladder function is checked on these mice. Do they lose bladder function once they've reached stage 4 (completely hind limb paralysis)? To determine bladder function, you should be checking that the mouse urinates

on it's own when you pick it up and you should palpate the abdomen for a large, tense bladder. If the mouse is not able to urinate normally, you should be expressing the bladder 1-2X daily until they reach their endpoint at stage 5. Notify RAR if this is seen.

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 2)

1. **Protocol Title:** 1901-36724A Preclinical development of antimicrobial peptide DGL13K.
Species &Pain Class: (A,B,C) Mice
Question the Research Addresses: The goal of this project is to optimize peptide treatment and minimize toxicity in burn wound infections in a mouse model.

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

   - If additional animals are needed to optimize the new burn wound procedure (i.e. will Task 1 be repeated), please update the Species section to include the additional animals needed and update the Experimental Design section to describe the pilot work to optimize the new burn protocol.

   - Please clarify why hydrogel is used as a vehicle in Task 4, as previously in Task 2 HPC was used as a the vehicle for the peptide.

   - Please update the protocol to describe how a satisfactory burn condition will be defined, including the following: -What is meant by partial thickness for the burn depth and what is meant by going deeper? Please include the targeted depth parameters. -The protocol also indicates that surface area involvement will be increased from 2-3% to 5%. Please clarify whether this is in a single location, and whether the bolt head size is going to match the targeted surface area for the burn. -The new burn protocol gives a wide time window for rod application (2-20 seconds) but it is not clear how one will determine whether to burn for 2 or 20 seconds, please clarify. -Please include how the time by temperature calculation will be used to result in desired burn level.

   Committee Decision: Stipulations must be met
   For: 10 Against: 0 Abstain: 0

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2. **Protocol Title:** 1610-34255A A pilot study to determine therapeutic effect of Zika virus on glial tumors in rats and mice
Species &Pain Class: (B,C) Rat; (B,C) Mice
Question the Research Addresses: Does presentation of Zika virus provide therapeutic effects when used to induce apoptosis to target tumors (glioma) in the brain?

   Committee Decision: Approved as submitted
   For: 10 Against: 0 Abstain: 0

Institutional Animal Care and Use Committee
11/05/19 Minutes
VCRC - 76D

Meeting Convened: 12:18 PM	Quorum Requirement: 10
Meeting Adjourned: 2:15 PM	Members Present to Vote: 13

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

Non-Voting, Ex-Officio:

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

Institutional Veterinarian:

3	X	(M, S)

Correlates to Version **v2.96** 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 discussed a self-report outlining a concern regarding a recent compliance issue in which the lab neglected to complete a mouse wellness check. A wellness check was completed on the following day and all mice were in good health. As a result of the failure to conduct the wellness check on 10/17/19, the PI has conducted retraining with the personnel on the protocol. The committee considers the matter closed.

2. The committee discussed a report of concern regarding calves on pasture that had not received supplemental feed. The animal care staff provided the animals with additional feed and the IACUC chair has reached out to the PI. There is also an HR component to this report. The IACUC leadership will continue to collect additional information and the compliance group will visit the site on November 22, 2019. The IACUC will continue to be updated at upcoming meetings on the issue.

3. The committee discussed a UReport that was submitted regarding RAR daily checks on rodents. While the report was vague, it was assumed that it referred to a recent change in policy where RAR will no longer pull out rodent cages during daily health checks as there is evidence that the disruption causes significant stress to animals. Instead, animals will be observed without moving cages. Cages will only be moved if there appears to be an animal welfare concern. The committee discussed the concern and will receive an additional training session on the new policy change at an upcoming meeting. The committee had no additional concerns and considers the matter closed.

4. The committee discussed a self-report in which a group of animals were not recovered properly from an anesthetic event after inoculation with virus. RAR staff identified the animals and worked to recover them from anesthesia. One of the animals, however, died over the course of the evening. A necropsy is currently being done to help determine the cause of death. The PI has voluntarily ceased animal work until the lab can receive additional training and more information is collected on the events. The committee will be updated on the additional training and the results of the necropsy at upcoming meetings.

5. The committee discussed a recent self-report in which two mice in a treatment group died following treatment. The lab hypothesizes that the cause of death was inflammation following rapid fungal die-off during disease, and proposes addition of dexamethasone treatment daily during the treatment period of the study. An amendment has been submitted outlining the preventative dexamethasone treatment and the committee considers the matter closed unless further complications arise.

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

    1. **Protocol Title:** 1910-37489A Efficacy of analgesic treatments on limb function in a canine urate crystal synovitis model
    Species &Pain Class: (B) Dog
    Question the Research Addresses: The objective of this masked, randomized, controlled, prospective study is to evaluate the anti-inflammatory and analgesic efficacy of drug 2433P as compared to a negative and positive control in a canine urate-crystal synovitis model.

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

    - Consider requesting 1-2 extra animals in the event animals need to be removed from study early for any reason (study related or spontaneous illness or injury that confounds use with this model, animals who fail to learn to walk on leash for gail analysis, etc.)

    - It appears that all animals should fall under pain class C, non alleviated pain and distress, since all dogs will be assigned to the placebo (no analgesia) group in this study. Please update the Species table accordingly.

    - Under study objective part d, the protocol mentions the use of "healthy mixed breed hound dogs" but the experimental design states that beagle dogs will be used. Please update the protocol to reconcile.

- Please update the Health and Monitoring to describe what intervention (if any) will occur for animals that are painful before the 24h time point. Please confirm that dogs with signs suggestive of infection (or any other illness or injury) will be reported to RAR before instituting treatment. Please also contact RAR if pain exceeds what is expected for model (unusually severe or chronic), or if pain is not managed with the extended NSAID treatment described here.

- Please provide a narrative in the attached alternative search on whether and how the information in the 8 sources identified in the alternative search were incorporated into the ultimate study design. Alternative Search guidelines, for reference: "Please include this search strategy as an attachment to the protocol. The search strategy should include a summary of the combinations of search terms used for each search along with results and a narrative addressing whether the results were applicable and if not applicable, why."

- Please update the Health and Monitoring section to address whether there is concern for long term pain or injury with repeat injection of urate crystals. Please also specify whether the same joint will receive the urate crystal injection each time or if it is possible to alternate the leg being treated.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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2. **Protocol Title:** 1904-36936A Therapeutic Development of Non-Opioid Strategically Substituted Agmatines for Chronic Pain Management
Species &Pain Class: (A,B,C) Rat; (A) Dog
Question the Research Addresses: Our objective in this application is to develop an orally bioavailable strategically-substituted agmatine (SSA) compounds to treat neuropathic pain. The central hypothesis of this application is that SSAs can be successfully developed for the safe and effective treatment of chronic pain. To pursue this program we will select four candidate SSAs for advancement. The lead SSA will be further developed through IND-enabling studies to be performed by the ISO, Center for Translational Medicine for the completion of the IND package. We intend to accomplish these objectives through three Specific Aims which are articulated in Section D below.

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

   - It appears that there may be a discrepancy in the number of animals requested in the attachments and those listed in the species (746 rats in the attachments versus 686 listed on the species table and 64 versus 68 dogs). Please review and update as needed.

   - Please update the rationale for species selection to elaborate on why the dog is an appropriate model and was chosen vs other possible non-rodent species commonly used (e.g. rabbits, pigs, etc).

   - The post-op analgesic table says that buprenorphine will be administered once daily for 3 days following surgery, but regular buprenorphine only provides analgesia for at most 12 hours, so should be given twice daily. Alternatively, an NSAID could be used (such as carprofen) that would only need to be dosed once daily or extended release buprenorphine which is only dosed once every 72 hours (as listed in the prophylactic/intra-procedural table). Please update the procedure to clarify the analgesic plan.

   - Please update this procedure to describe doses and routes of administration for the proposed drugs that will be injected for this part of the study.

   - Please update this procedure to clarify the frequency and duration of blood collection. The procedure states that blood will be collected 6 times, and once throughout the study. Please indicate whether this is 6 times in one day or what the specific timepoints are.

- Please update the protocol to provide more information about how drug dosing will be converted between rats and dogs.

- For the MTD and NOAEL portions of the study - as the toxicities are currently unknown, please update this section to include some additional monitoring details regarding observations (e.g. for a half hour after administration, 2 hours later, 6 hours later, then daily). A statement about updating RAR/IACUC and the protocol with any observed toxicities/side effects should also be included in the protocol.

- The barbiturate overdose section for the dog just says acepromazine 0.05 mg/kg SQ. This is not a method of euthanasia. Please update with the barbiturate that will be used as well. Note that if the form does not allow selection of more than one agent, you can use "other" and write in.

- Due to the potential for toxicity, please increase the pain class of the dogs to B, and fill out the Alternatives Search accordingly (keyword search within the form as well as USDA Alternatives Search supplement attachment). Please see the links on this section of eprotocol for instructions and examples

- The ESS roster protocol is referenced in the Experimental Design and ESS staff are listed in the Personnel section. If ESS will be involved in this study, please confirm that they will be contacted well in advance to coordinate. If this was carried over from another study, please delete the references.

- We are unable to confirm the following personnel have completed all necessary requirements: - Carolyn Fairbanks (rabies surveillance) - [redacted] (rabies surveillance) - [redacted] (parasitic/rabies ROHP trainings, rabies surveillance) - George Wilcox (parasitic/rabies ROHP trainings, rabies surveillance) - [redacted] (parasitic/rabies ROHP trainings, rabies surveillance) - Craig Flory (parasitic/rabies ROHP trainings, rabies surveillance) - Lia Coicou (parasitic/rabies ROHP trainings, rabies surveillance) - Kelley Kitto (animal exposure questionnaire expired) All personnel have been sent directions via email regarding their incomplete requirements. Please follow up with personnel to ensure all requirements are completed. Once complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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3. **Protocol Title:** 1910-37482A Tissue regeneration and maintenance. Genetic regulation of progenitor cells in appendicular skeletal development Role of novel human POMC promoter in HPA axis
Species &Pain Class: (A,B,C) Mice
Question the Research Addresses: Is BMP signaling involved in the maintenance of articular cartilage? Is Sall4 required for digit regeneration? Is POMC promoter 2 active in vivo to maintain POMC gene expression?

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

   - In the Class B and C attachments, many of the mice are euthanized without any surgical procedures (as a part of the breeding). These animals should be identified as Class A; please update the Species table accordingly.

   - Please update the study objectives section to be readable by a layperson, including defining acronyms. Please also update the aims so that they are not an exact copy of the questions.

   - Since this is a 3-year renewal protocol, please fill out the new question (e) on the Rationale page to provide a brief summary of results from the previous 3 year period.

- 1. Please update the procedure to include use of artificial tears or other sterile eye lubrication during anesthesia. 2. Please update the exception request (click on "avertin" in the anesthetic agents table) to provide additional scientific justification for the use of avertin versus other, pharmaceutical-grade anesthetics. Also confirm that pH and color is checked before each use (please add to SOP - Dosage - Use 1.) It is recommended that alternatives be re-considered. Pharmaceutical products such as ketamine and xylazine can be used effectively and have animals recover within 45 minutes, especially if a reversal agent for xylazine is used (Xylazine can be reversed with atipamizole (Antisedan®, 1-2 mg/kg SQ or IP), or yohimbine (Yobine®, 0.5-1 mg/kg IP). Also isoflurane will give the best induction and recovery time and is a very safe anesthetic to use with mice. Respiration can be monitored while using this anesthetic. Also, [redacted] has ventilated hoods in all procedure rooms that will allow for adequate ventilation. Additionally, charcoal cartridges can be used for surgery as well. If you have concerns, please contact DEHS to discuss monitoring for excess exposure. If you have additional concerns, please contact your area vet to discuss these anesthetic considerations

- Based on collection times it seems that the pups will have digit amputation on the day of (or day after) birth. Please confirm this and update the protocol accordingly, or at least confirm it will be less than or equal to 7 days post birth. Due to possible detrimental effects of hypothermia in young pups and the minimally invasive distal phalanx biopsy, it is recommended that clipping be done without this type of anesthesia. If signs of distress are noted on post procedure checks (pups outside of nest, no milk spots), then euthanasia would be required as is already mentioned in your protocol. Also, it is recommended to lower the pain class from C to A, since pain is expected to be momentary.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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4. **Protocol Title:** 1910-37491A A pilot study to determine therapeutic effect of Zika virus on glial tumors in rats and mice
Species &Pain Class: (B,C) Rat; (B,C) Mice
Question the Research Addresses: Does presentation of Zika virus provide therapeutic effects when used to induce apoptosis to target tumors (glioma) in the brain?

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

   - As this is a renewal, please update the protocol under the "Rationale Section question e." to include a brief summary of the research results obtained during the prior approval period. In addition, update other sections including the title in the Rationale section and the Justification for Animal Numbers in the Experimental Design section if the project has evolved beyond the pilot phase.

   - There appears to be some inconsistencies with the total animal numbers listed on the species table and throughout the rest of the protocol, with 688 mice and 174 rats listed throughout the experimental design and procedures sections but only 544 mice and 96 rats requested in Species Section. Please review the Experimental Design section and ensure each experiment includes how many animals/group. Consider adding a table in the protocol that simply shows the number of mice or rats per experimental group with a grand total. This total should match what you have in the Species section.

   - 1. In the experimental design section the protocol refers to vaccinating with GMCSF. Please include the SQ injections and provide a maximum volume you will inject. 2. Please add a "Tumor Induction" specific procedure for each species to capture IACUC specific questions regarding tumor models. 3. SR-Bup needs to be given 2-4 hours prior to the painful procedure for mice and rats. Please update this everywhere in the protocol where SR-Bup is listed.

   - 1. Because rats will undergo this surgery multiple times, please include the duration of the

procedure. For rats that are anesthetized multiple days in a row, consider administering 2-3 cc saline SQ to help them maintain hydration. This can be added as a supportive agent. 2. Consider trimming nails while they are anesthetized to avoid overgrooming lesions along surgical site. 3. Please note that there is a recent article describing SR-Bup in nude rats (Lack of Absorption of a Sustained-release Buprenorphine Formulation Administered Subcutaneously to Athymic Nude Rats. JAALAS J Am Assoc Lab Anim Sci. 2019 Sep 1;58(5):597-600) that sows SR-Bup is not absorbed. Therefore, it should not be used an an analgesic in nude rats as it is not currently clear whether or not it reaches therapeutic levels. Please state that you will use BupHCl in nude rats.

- 1. Because mice will undergo this surgery multiple times, please include the duration of the procedure. For mice that are anesthetized multiple days in a row, consider administering 0.5 - 1.0 cc saline SQ to help them maintain hydration. This can be added as a supportive agent. 2. Consider trimming nails while they are anesthetized to avoid overgrooming lesions along surgical site.

- For both the procedures "Luciferase imaging, rats" and "Luciferase imaging, mice" Please add ophthalmic ointment as a supportive agent.

- Please include a maximum volume that will be injected IP.

- 1. If CNS signs such as ataxia or seizures associated with the induced brain tumors are expected, please list these in the Health and Monitoring section. 2. Protocol states that infection is possible, please clarify what will be done for treatment if evidence of infection (redness, swelling, pus at surgical site) is observed. (You can simply state that you will notify RAR to determine treatment plan if evidence of infection is noted.) 3. Please confirm that lab staff plan to check on animals daily, including weekends. Please update to address the following: How do you determine, cage-side, when animals need twice daily monitoring? Is it based on activity level? Weight loss? Aka - how are you determining an animal is distressed? Do you notify RAR once an animal has reached this point but is not yet moribund? 4. As it may be difficult to accurately monitor food/water intake by looking at the levels in the cage, once animals become distressed, consider monitoring body weight 2-3X/week and hydration via skin tent test. Animals that lose 10-15% body weight compared to starting weight prior to brain tumor surgery should be given moist feed. Animals that have a prolonged skin tent should be given SQ fluids.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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5. **Protocol Title:** 1910-37495A Investigating the therapeutic effects of ultrasound on glial tumors in mice
Species &Pain Class: (B,C) Mice
Question the Research Addresses: Does focused ultrasound provide therapeutic effects when used to target tumors (glioma) in the brain?

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

- Please update the following in the "Induction of brain tumor cell line" section: -In the Pre-op prep section the second sentence references rat instead of mouse, please correct. - In the analgesics table and post-operative care section - The dose for sustained release buprenorphine should be 2 mg/kg given 2-4 hours prior to the procedure. The dosing schedule for standard buprenorphine currently states that it will be given after procedure and once daily. Dosing recommendations are to give every 4-6 hours for the first 12 hours, and every 8-12 hours afterward. Dosing frequency may be decreased if using multi-modal analgesia. Please update accordingly.

- Please update the analgesia table to match the revised analgesia information listed in surgical procedure description.

- Administration of analgesics is mentioned but no detail on drug, dose, frequency, etc. Please update the protocol to add this information. The procedure description indicates that isoflurane anesthesia will be induced in a bell jar or other container and then fur is shaved/cream applied before connecting to the anesthetic vaporizer. I recommend if possible that mice are connected to the vaporizer immediately after induction to reduce chance that they will start to wake up.

- Please address the following in Health and Monitoring: - Please update information about analgesics to match revised information in surgical procedure section. - Please update to provide the criteria for initially identifying distressed animals. - Extra fluids at time of surgery is noted but there wasn't a description of that in the surgery section. Please add this information in the procedure details. - Please clarify whether urine scalding and autophagia are expected outcomes for these mice or if this was included in error. - Recommend a monitoring frequency of more than 2 time/day (3x/day?) for animals that are showing severe clinical signs in order to ensure euthanasia occurs as quickly as possible.

- Please provide additional references regarding previous use of ultrasound for tumor treatment in rodent models and address whether there are specific considerations for excessive tissue damage of non-tumor tissue with ultra sound treatments.

- For response to Questions 1 and 2, do you think there would be differences in what needs to be monitored based on surgery, tumor growth, or the ultrasound treatment?

- The response to #3 indicates you will use the items a-f to determine further action such as wet food and/or pain relief. However, items a-f refer to euthanasia criteria. Please clarify if you are asking for an exemption from the euthanasia criteria with the exception of the moribund state and if so provide additional justification.

- In the DEHS section PI mentions use of activated charcoal canisters scavenging for isoflurane in [redacted]. The procedures mention the use of a bell jar for knocking out mice with isoflurane in [redacted] Will the bell jar be used in a fume hood? Please clarify.

- Please clarify if the ketamine administered in [redacted] provided by RAR or if it is being transported from the main lab [redacted].

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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6. **Protocol Title:** 1910-37539A Genesis Project for Organ, Tissue, and Cell Engineering; Interrogating Human Stem Cells for Engineering Human Organs/Cells via Blastocyst Complementation; Creating Human Dopamine Neurons in Human-Pig Chimeras for Parkinson's Disease; Creating Young Blood to Rejuvenate Old Brains; Production of Exogenic Human Dopamine Neurons; Creating Humanized Mouse and Pig Chimeras to Study HIV Pathogenesis and Develop Novel Therapeutic Interventions to Treat HIV Infection and AIDS Dementia; Generating Human Hematopoietic Cells in the Pig as a Biological Incubator; A Novel Stem Cell-Based Approach for Generating Non-Human Primate Livers in Pigs
Species &Pain Class: (A,B) Mice; (A) Pig (Biomedical)
Question the Research Addresses: To identify gene knockouts that will generate specific human cell systems and organs of interest

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

- IBC and SCRO review will be needed for the studies on this protocol. While there may be existing SCRO protocols for the human-pig chimera, the SCRO office indicated that they do not have a protocol for the human -mouse chimera studies. Please contact both IBC and SCRO to ensure that all necessary forms and protocols have been completed to account for this work.

- Since this is a 3-year renewal protocol, please fill out the new question (e) on the Rationale page to provide a brief summary of results from the previous 3 year period.

- If animals will be transferred from the [redacted], please include these in the Internal Transfer column. The [redacted] IACUC protocol number is 1702-34602A.

- Regarding the end point for this portion of the OTX2 Experiment 1: " -Conduct live cell imaging every 12 hours up to E8.5 to quantify RFP+ cell location and numbers. Euthanize at E8.5 by decapitation." It's not clear if pups will undergo live imaging for RFP or if they will be euthanized and cells are imaged. Please provide more detail so that the end of the experiment for these pups is understood. Consider updating the Experimental Endpoints to add the E8.5 time point.

- Please attempt to give ranges for the numbers of animals required on the basis of the literature or past experience. Right now, there's a reference to "the expectation that we will generate sufficient chimeric embryos to carry out this study." Please elaborate the basis for that expectation.

- 1. Please update this section to reflect which animals will undergo ABR, and include the timeline by which they will be evaluated relative to other experimental manipulations. 2. The protocol states swine will be monitored for gestation and parturition. Please update this section to confirm that the intent is to perform terminal harvest before parturition. Is there a need to develop a plan for unanticipated parturition on site (e.g. when attempting to collect late term fetuses)? 3. Please include in the protocol how long swine are expected to be housed in RAR before terminal harvest. (there is no right or wrong duration, but you are encouraged to consider which delivery, housing, and terminal procedure would be expected to cause the least stress to the animal while still meeting experimental needs). - Suggestion, not necessary for approval. 4. The mouse numbers described here do not match numbers in the species section. Please review and update either or both sections accordingly. 5. The protocol states pig procedures will be performed under supervision of UMN veterinarians. It is unclear if this refers to veterinarians listed on the protocol or other clinicians (if other, please add to protocol). 6. Consider simplifying this section. The aim of this section is to provide a high level overview of what any given animal will experience so the reviewers can identify welfare risks or concerns. Specific animal procedure descriptions should be include in the Procedure section. Descriptions of work with tissues, cells, etc. need not be included. Suggestion - not necessary for approval.

- 1. Use of non-pharmaceutical grade anesthetics requires scientific justification. Please update the protocol to clarify why Avertin is required versus pharmaceutical grade options (click Avertin in the anesthetic agents table and and change the response to the exception question to Yes, then fill in the resulting text box). You can find more information about mouse anesthesia options here: https://www.researchservices.umn.edu/services-name/research-animal-resources/research-support/guidelines/anesthesia-mice If Avertin is necessary for scientific reasons, please familiarize yourself with the IACUC Policies on the use of Avertin (https://drive.google.com/file/d/0B4clNGOYSdMYOFM5Q214RE9qV2c/view) and use of non-pharmaceutical grade compounds (https://drive.google.com/file/d/1Kdh9-7IxYv0GKt7NzTYpkzA7WtEnMFeM/view). 2. Please update the protocol to confirm heat will be provided during all stages of anesthesia (shaving/betadine, surgery as well as recovery). Heating pads, warm water circulating blankets, and commercial devices that can be activated or warmed in a microwave are all preferred over heat lamps. 3. Please update the analgesic section to clarify the intended schedule of buprenorphine administration. Daily doses of regular formulation buprenorphine are generally not necessary when SR buprenorphine is used. If SR buprenorphine is not used, once daily administration of regular formulation is not sufficient (a dose is only active for ~4-8 hours). You are encouraged to contact your RAR veterinarian to discuss your specific anesthesia and analgesia needs.

- If pigs will be euthanized in [redacted] as described, transport from primary housing to [redacted] must be described in this section (change the response for "will you be moving animals" to Yes then fill out

the resulting text boxes).

- This section states involvement from U of MN veterinarians, RAR veterinary staff, and [redacted] employees. It is unclear which veterinary staff will be performing these procedures. Since these are procedures on your protocol (and not medical care for welfare purposes) please provide the name of the staff who will perform these procedures and ensure they are listed in the Personnel section.

- Please update the responses to questions 1-3 to with regard to mouse procedures other than euthanasia of pregnant dams. Namely: superovulation, tamoxifen injection, embryo transfer surgery, ABR, homozygous Neurog1 lethal phenotype. The protocol states mice and pigs are monitored daily. Please update this section to clarify this refers to lab personnel (not RAR daily health checks).

- Are there concerns with pregnant sows or mice aborting fetuses early, or giving birth early? If so, please update the health and monitoring sections to include these signs or symptoms, and any action that may be taken e.g. increased monitoring if these symptoms are seen.

- Telazol is listed in this section but its use is not described in the protocol. Please remove, or update the appropriate section(s) of the protocol if it will be used (presumably the pig euthanasia and embryo harvest).

- Is the sodium pentobarbital used administered by RAR? If not please update your controlled substances protocol to include it. Ketamine and Buprenorphine usage is listed in the procedure but not the controlled substances section of the IACUC protocol. Please add.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 2)

1. **Protocol Title:** 1807-36193A Linking neuronal, metabolic, and hemodynamic responses across scales
Species &Pain Class: (B) Nonhuman Primate (Macaques)
Question the Research Addresses: While functional magnetic resonance (fMRI) has proved invaluable for identifying where in the brain activation is occurring during a particular task, it has had less to say about how the dynamics of that activation actually contribute to task performance. Indeed, because of the belief that fMRI signals are sluggish and temporally imprecise, fMRI experimental paradigms traditionally have used sustained block designs which deliberately preclude measuring the rapid changes in sensory and motor signals that underlie everyday actions. Recent evidence, however, suggests that there is considerable temporal information present in the blood oxygen level dependent (BOLD) signal, opening the possibility that fast neuronal dynamics can be revealed by fMRI. In this proposal, we will examine this possibility with a series of multimodal experiments in which a consistent experimental paradigm is applied across spatial and temporal scales to quantify responses to transient inputs.

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

   - Please include the maximum number of MRIs a monkey will undergo and when these will happen in relation to the other procedures on the protocol.

   - Temperature should be assessed every 15 minutes in addition to HR, RR, and SP02. Please update the protocol to include temperature monitoring for the animal during the procedures.

   - Please note that antipamazole does not reverse ketamine, it reverses the dexmedetomidine. Dexmedetomidine can be reversed with the same volume of atipamezole as the volume of dexmedetomidine used; it is not equivalent in terms of mg/kg dosing.

- We are unable to confirm [redacted] has completed the tetanus and tuberculosis requirements. Please follow up with [redacted] to ensure these requirements are completed. Once complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0
Members 12, W, and N out

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2. **Protocol Title:** 1901-36681A Modulating attention and decision making with closed loop control of low frequency oscillations
Species &Pain Class: (B) Nonhuman Primate (Macaques)
Question the Research Addresses: Synchronous low-frequency brain activity, as measured by human EEG, has been implicated in both normal cognition and in disease states such as schizophrenia. However, we still do not know whether changes in such rhythms directly alter neuronal information processing, or are merely epiphenomenal. To address this issue, we will measure how single and multi unit activity linked to task performance in non-human primates is altered by endogenous alpha rhythms, and how that activity is changed when alpha rhythms are directly modulated via closed-loop electrical stimulation.

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

- Please include the maximum number of MRIs a monkey will undergo and when these will happen in relation to the other procedures on the protocol.

- Temperature should be assessed every 15 minutes in addition to HR, RR, and SP02. Please update the protocol to include temperature monitoring for the animal during the procedures.

- Please note that antipamazole does not reverse ketamine, it reverses the dexmedetomidine. Dexmedetomidine can be reversed with the same volume of atipamezole as the volume of dexmedetomidine used; it is not equivalent in terms of mg/kg dosing.

- We are unable to confirm [redacted] has completed the tetanus and tuberculosis requirements. Please follow up with [redacted] to ensure these requirements are completed. Once complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0
Members 12, W, and N out

Institutional Animal Care and Use Committee
11/19/19 Minutes
VCRC - 76D

Meeting Convened: 12:20PM			Quorum Requirement: 10		
Meeting Adjourned: 2:40PM			Members Present to Vote: 12		
Voting Members			Alternates		
1	X	(Chair - M, S)			
2		(Vice-Chair - M, S)			
3	X	(M, S)	A		(A, S)
			B		(A, S)
			C		(A, S)
			D		(A, S)
			E		(A, S)
			F		(A, S)
			G		(A, S)
4		(M, S)	H		(A, S)
5	X	(A, U)	I		(A, U)
			J		(A, U)
			K		(A, U)
			L		(A, U)
			M		(A, U)
6		(M, S)	N	X	(A, S)
7	X	(M, V)	O		(A, S)
8	X	(M, S)	P		(A, S)
9	X	(M, S)	Q		(A, S)
10		(M, S)	—		
11	X	(M, S)	R		(A, S)
12		(M, S)	S	X	(A, S)
13	X	(M - NA, NS)	T		(A - NA, NS)
14		(M, S)	U	X	(A, S)
15		(M, S)	V		(A, S)
16		(M, S)	W		(A, S)
17		(M - NA, NS)	—		
18	X	(M – St)	X		(A, St)

Non-Voting, Ex-Officio:

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

Institutional Veterinarian:

3	X	(M, S)

Correlates to Version **v2.97** 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 October 2019 Inspection Findings – Notes to File- Veterinary Recommendations report.

2. The committee was updated on a self-report in which a group of animals were not recovered properly from an anesthetic event after being inoculated with virus. The lab has ceased activity until additional training can be arranged with RAR, Controlled Substances, and the IACUC office.

3. The committee was updated on a report of concern regarding calves on pasture that had not received supplemental feed. The animal care staff provided the animals with additional feed and the IACUC chair has reached out to the PI. A new SOP is being written to cover these animals and their feed and will be submitted to the IACUC for discussion at a future meeting.

4. The committee discussed a proposal to process requests for using animals allotted on a study for training personnel on procedures currently approved on the protocol. The committee has approved the proposal.

5. The committee discussed a self-report in which a group of animals died unexpectedly following tamoxifen injections. Staff has stopped further injections of tamoxifen until they have a better idea if the issue was due to contamination. PI will be submitting sample of tamoxifen to VDL for culturing to determine if this was the issue. The committee will be updated at upcoming meetings regarding test results.

6. The committee approved a proposal to allow reduced veterinary consults for the [redacted] IMHA based on good performance.

7. The committee reviewed and approved the final edition of the Fall 2019 Program Review Packet. The finalized packet will be sent on to the IO.

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

1. Protocol Title: 1911-37578A Evaluation of an In Vivo Engineered Pulmonary Heart Valve Scaffold in the Adult Sheep Model
Species &Pain Class: (B) Sheep (Biomedical)
Question the Research Addresses: This purpose of this study is to evaluate an in vivo engineered heart valve. Will the construct implanted in a subcutaneous pocket yield a heart valve suitable for implant. - Will the autologous valve have good functionality and biocompatibility when implanted in the pulmonary position.

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

- In the paragraph that begins with "Experiment 3, this protocol..." you state that all valves are harvested and evaluated in the 2nd surgery. In the paragraph that begins with "Animals will be induced for a second surgery..." you state that up to three valves will be

removed and evaluated. Please clarify whether all will be removed or not. If some may be left in place, please address whether and what kinds of issues this may present in the Heath and Monitoring section. As the Experiment 3 study design is updated based on results from experiments 1 and 2, please also update the health and monitoring to reflect new adverse outcomes, monitoring strategies, etc. that were discovered or developed in the first 2 experiments.

- Is the valve scaffold sterilized before implantation? If the composition of construct precludes standard sterilization procedures, please describe if/how the construct is treated to minimize infection risk. ROVT and interpositional surgeries you lists carprofen administration as "once or twice every 24 hours." Recommend changing to "once or twice every 24 hours as needed post-operatively" or "once or twice every 24 hours per veterinary discretion." As it reads now it is unclear when and for how long animals will receive this drug relative to surgery.

- Animal fasting was described for the valve removal/replacement surgical procedures and the terminal angiography surgical procedure, but was not described in the description of the initial subcutaneous pocket surgical procedure. Please update to include the fasting the animals will receive in preparation for this procedure in the procedure's description.

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0
Members 11 and S out

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2. **Protocol Title:** 1910-37527A Prostate Cancer Studies
Species &Pain Class: (A,C) Mice
Question the Research Addresses: To genetically determine whether HK2-mediated glycolysis plays a crucial role in Pten/p53 deficiency-driven CRPC. To test whether the chemical compounds can effectively inhibit Pten/p53 deficiency-driven CRPC.

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

- Please work with your area veterinarian, Dr. Nate Koewler (koew0004@umn.edu) to establish a more robust plan for Experimental Endpoints and Health and Monitoring

- While the Species section requests 250 Class C, other portions of the protocol suggest that 290 Class C animals will be needed. Please review the numbers and update the protocol to reconcile any discrepancies.

- Please add a procedure for drug administration and include volumes for drugs that will be given by oral gavage and IP injection in that procedure.

- In the experimental design and endpoint sections, it says that mice will be euthanized if there is failure to gain weight appropriately over a 3 week period. Please describe what measure is used to determine appropriate weight gain, and clarify what would constitute inappropriate gain (e.g. growth chart and if they are >20% under target weight for that age)

- Under "other support agents used", please add that you will give eye lubricant before the procedure to protect the eyes from drying out. Additionally, a heating pad or other heat support should be used during surgery to keep the mice from becoming too cold. It says that heart rate will be monitored - please include how this will be accomplished. Pulses are typically difficult to feel in mice, so these should not be relied on for information about anesthetic stability. It is also typically quite challenging to see the pupil in mice, so a widely dilated pupil should not be counted on to give information about anesthetic depth. (number 1 under parameters measured) If these and other parameters will not be used to measure anesthetic depth, please remove from the protocol. As there are no catheters or devices mentioned in this protocol, please remove reference in post-op care section.

- The use of wound clips to close a mouse castration is not typical given the location and nature of the tissue. Recommend adding suture and tissue glue for closure as at least options as these are they typical closure methods.

- In response to the question about inherent problems associated with the genetically modified phenotype, answer is currently no. However, the study is based on these mice developing prostate cancer, so please include a description of the effects of the tumor on the animals (e.g. difficulty urinating, lethal at a certain age, etc).

- Toe-clipping must be described and justified in the Animal Care and Use Protocol and approved by the IACUC. Toe-clipping should be used only when no other individual identification method is feasible and only on altricial pre-weaning rodents (mice and rats, but not guinea pigs) after the toes are no longer webbed and before they reach 8 days of age. If possible, it is preferable to remove toes from a hind paw rather than a forepaw. In neonatal mice before 8 days of age it appears to have few adverse effects on behavior and well-being. When possible toe clipping and genotyping should be combined. Under all circumstances aseptic practices should be followed. Please update the protocol to clarify age that toe clipping will be done, justify its use, and confirm that aseptic practices will be followed.

- Please update the following in the Health and Monitoring section: - This section says mice that are hunched and breathing rapidly for >1 hr will be euthanized, but experimental design says they'll only be euthanized if breathing like this AND weight loss. Please reconcile. - Please describe general health conditions for conditional knockout out Pten, p53 and HK2. Otherwise provide statement of normal health of these lines. Note: consultation with the area veterinarian is required to address this section completely.

- Please update the following in the Experimental Endpoints section: - In the experimental design the endpoints are described as needing to meet at least 1 of the first two criteria and 1 of the last 3 criteria, but in the response to question 1 of this section, this need to meet two of the criteria is not mentioned. Additionally, the first criteria listed in the experimental design is not listed here. Please clarify and keep these endpoints consistent throughout the protocol. - Please clarify how often mice are palpated to check for abdominal tumor progression. - Please provide more robust explanation of "Moribund conditions" such as lack of mobility, vocalization, etc. Note: consultation with the area veterinarian is required to address this section completely.

- Please update the following under the Alternative Search section: - Please provide more robust justification for following animals up to moribund conditions. Citations will be helpful. - Under the refinement section, it says ibuprofen will be given if needed after surgery, but in the procedure and health and monitoring sections it says only SR-buprenorphine will be used because other pain medications could interfere with tumor development. Please reconcile. - This section mentions ibuprofen for post-op pain, but this is not mentioned elsewhere in the protocol. Please clarify if there is intention to use ibuprofen, otherwise remove.

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

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3. **Protocol Title:** 1910-37528A Prostate cancer models of bone metastasis
Species &Pain Class: (B,C) Mice
Question the Research Addresses: To study prostate cancer cell lines' genetic and biologic changes involved in tumorigenesis and metastasis in bone.

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

- Please work with your area veterinarian, Dr. Nate Koewler (koew0004@umn.edu) to establish a more robust plan for Experimental Endpoints and Health and Monitoring.

- As there are human cell lines that have been genetically modified, please contact IBC 612-626-5654 or ibc@umn.edu

- Please update the Rationale section to provide a stronger rationale supported by previous data and/or citations to justify survival studies.

- As the age of the mice will help determine whether criteria 1 and 2 for weight loss are reasonable, please clarify the age of the animals on study.

- It would be helpful for understanding the design if you gave a fuller description of the mice involved. Please update the protocol to clarify the sex of the animals, if the will be post-pubertal, and if they will be castrated. Note, protocol mentions experience with castration in surgical experience but no castration procedure listed, so if animals will be castrated and additional procedure outlining this surgery will be needed.

- Below the table with 16 groups of 15 mice, protocol states "Total 250", however 16x15 is 240. Please clarify if you are requesting an extra 10 mice in to be used in case of injection failure or loss from another cause or is this an arithmetical error, and update the protocol accordingly.

- Please update the following in the "Intratibial Injection" procedure: - Please update the anesthetic parameters, to those that will be used during anesthesia. For example, parameters such as capillary refill time are difficult to assess in mice and would not be a useful for monitoring. Measuring body temperature is great with the appropriate equipment. Is that equipment available and is this something that is regularly done during

surgeries? Things like toe pinch (and other stimuli), respiratory rate/pattern, temperature (if possible) are all good parameters to use. - Please update the protocol to list that both eye lubricant and a heat pad are used during the surgery. - Both ketamine and atipamezole are mentioned in this section, however injectable anesthetics are not requested. Please remove these from the anesthesia text or request their use in the table. - In the surgery description it states that after 3 days and pain is still noted, that an additional dose of SR-buprenorphine will be used. In the Health and Monitoring it states that after the initial dose, no additional analgesics will be used. Please amend to be consistent on when pain control will end during the experimental timeline.

- Please update to clarify at what time(s) of day transport will occur e.g. 7 am-6 pm

- Procedure details: In the cell line group injection endpoints and mice number section states 50 mice per group however, shouldn't this be 60 mice since each consists of four groups of 15 mice/group. Please update accordingly.

- Please update the protocol to provide responses to the parameters measured rather than listing (N/A). Note this can include a description that the procedure is short and that toe pinch will be used for the initial induction before imaging begins.

- Please update the Procedure Details section to include a "Tumor Induction" procedure.

- Under Health and Monitoring 3. steps taken to alleviate pain: protocol states that only the initial dose of buprenorphine will be given as further doses may affect tumor growth, but in procedure description for intratibial injection, 12. you state "If animals continue to show signs of distress or pain after 3 days we will inject additional long efficacy Buprenorphine." please update the protocol to reconcile. Note: consultation with the area veterinarian is required to address this section completely.

- Alternatives, refinement – should include statement that animals will be monitored and when show signs of moribundity they will be euthanased as will all members of the experimental group – this reduces burden on the group. (assuming this is the intent of earlier statement)

- Please update the Alternative Search section to provide more citations and rationale for survival studies.

- Please update the following in the Experimental Endpoints section: - There is a wide range of endpoints listed in this protocol, some of which contradict each other. Please consolidate all humane endpoints to make one cohesive list that can be easily followed by reviewers and staff using them during the project. Areas to focus on and consolidate include experimental design, health and monitoring, and experimental endpoint pages. In particular, a more robust loss of cortical bone (imaging) and loss of limb function (visual monitoring) should be well described. - Endpoints: there are some minor inconsistencies in different areas of the protocol that address euthanasia. Specifically please add to the endpoint section language from the intratibial procedure description that "Once several mice develop lesions that begin to break through the cortical bone, become paraplegic, or lose between 10-20% of body weight, sacrifice all mice in study using IACUC approved method." This is taken to mean that all mice in that experimental group will be euthanased at that time regardless of whether they are moribund or reach tumor criteria. -

Please describe in greater detail the monitoring that will take place in regard to tumor growth. In several areas it states that one endpoint will be when the tumor reaches 2cm. However, in the Health and Monitoring (#1) it states that as soon as the tumor breaks through the cortical bone, all mice in the study will be euthanized. Please amend the protocol to match the latter statement of the endpoint being at cortical bone loss. - in general, please provide more robust endpoint criteria (for example lack of mobility, vocalization, ect) which can be clearly followed by external evaluator as the current criteria is largely based on strong personal experience. Note: consultation with the area veterinarian is required to address this section completely.

Committee Decision: Deferred
For: 11 Against: 0 Abstain: 0
Member S out

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4. **Protocol Title:** 1910-37502A Methamphetamine, mitochondria, and neurodegeneration
Species &Pain Class: (B,C) Mice
Question the Research Addresses: The primary question: to what neuronal populations, if any, is chronic.

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

- In its current format, it was hard to understand the different experiments being performed in Aim 1 and Aim 2. Please update the protocol to clarify the individual experiments being performed for Aim 1 and Aim 2, including any surgical treatments (e.g. adeno-associated virus, guide RNA), medical treatments (e.g. methamphetamine or saline control, phenelzine, isradipine, Bay K8644), timeline for the mice including specific endpoints, and the total number of animals needed per experiment. Recommend using a format similar to what is listed in the answer to question 2 for the Justification for Number of Animals (i.e. Aim 1: Step 1.1, 1.2 etc.).

- In the description of estimation of how many animals are needed (which was written in a very informative way), please update to provide the basis for the estimate of the study requiring a final sample size of 15 brain slices per group, if possible.

- In justification for number of animals, it is stated that 2070 mice are requested to "account for the number of breeders needed to generate experimental mice", but in the species section of the protocol, all animals are listed as coming from internal transfer from another IACUC protocol (as opposed to "produced in house") and there is no breeding procedure listed in the procedures portion of the protocol or mentioned in the experimental design. Please clarify whether animals are bred on this experimental protocol and update the protocol accordingly (i.e. classification in the Species table, Breeding procedure)

- Both surgical procedures indicate the time maintained after surgery as up to one month, but it appears that a longer time may be needed (i.e. 28 days of methamphetamine + 28 days of abstinence). Please update if needed.

- If any mice will undergo multiple surgeries (i.e. AAV and osmotic pump), please update

the multiple surgery question within the surgical procedures to Yes and provide the requested information.

- If breeding of mice is being performed on this protocol, please use a 'Breeding' procedure instead of a 'Biopsy' procedure as the tail biopsy procedure for genotyping will be captured in the breeding procedure. If no breeding is being performed, please confirm the age of mice that the tail biopsy procedure will be performed on. If animals are transferred from a breeding protocol to the experimental protocol, please remove the reference. Based on age, please confirm that the procedure will follow the IACUC Guideline on Rodent Tail Biopsy (https://docs.google.com/document/d/14RZQyVYCrM_sCqqKojilTTBF_nfCKPkafnIlPqn5KJ0/edit).

- Both surgical procedures are listed as Pain Class C despite analgesics (e.g. EMLA cream, NSAID) being used. Please clarify the reasoning for the classification of these procedures. Since a class C procedure is needed by the form in order for the mice to be categorized in class C, it might be more appropriate to designate the methamphetamine administration as C (and the surgeries as B).

- Repeated intraperitoneal injections can be associated with increased stress and adverse effects such as inflammation or infection of the peritoneal cavity. Please confirm that less invasive alternatives have been considered (e.g. vascular catheter, osmotic minipump) for the administration of methamphetamine and phenelzine. Injection of irritating materials (e.g. low pH, high pH, clumped material) can result in pain during and following injection and peritonitis. Please confirm the vehicle that is being used for these injections and how the methamphetamine and phenelzine will be prepared in solution to minimize these adverse effects.

- Please update the Stereotaxic Surgery procedure to address the following: In the description of the aseptic technique being used, the rinse with 70% ethanol is not necessary after sterilization with the hot bead sterilizer. Just make sure that the instrument cools down before being used to manipulate tissue. - Recommendation In the Anesthetic Agent table, recommend increasing the dose of isoflurane to 1-5% as induction may be more rapid when higher concentrations of isoflurane are used. The maintenance rate will be lower and should be based on the anesthetic depth. - Recommendation In the Post-Operative Care section, the RAR recommended dose of meloxicam is 1-2 mg/kg SC or PO, not IP. (https://www.researchservices.umn.edu/services-name/research-animal-resources/research-support/guidelines/analgesia). Please revise meloxicam dose to follow these guidelines. Also, carprofen and meloxicam are dosed every 24 hours per RAR guidelines. Please revise the number of doses over the 72 hour procedure to not exceed this guideline. - Stipulation In the Post-Operative Care section, please define the clinical signs that constitute 'distress'. Please also confirm that "weight <75% of starting weight" means 25% weight loss, which would be consistent with the IACUC Euthanasia Guidelines. - Stipulation In the Post-Operative Analgesic table, the dose of meloxicam is listed as 2 mg/kg. Please clarify dose and make consistent with the Post-Operative Care section that lists the dose as 1 mg/kg.

- In the description of the surgical procedure, it states that absorbable sutures are being used whereas in the aseptic techniques section it says that non-absorbable sutures are used. Please confirm the type of suture being used. - Stipulation In the description of the

aseptic technique being used, the rinse with 70% ethanol is not necessary after sterilization with the hot bead sterilizer. Just make sure that the instrument cools down before being used to manipulate tissue. - Recommendation In the Anesthetic Agent table, recommend increasing the dose of isoflurane to 1-5% as induction may be more rapid when higher concentrations of isoflurane are used. The maintenance rate will be lower and should be based on the anesthetic depth.

- Please update the responses to questions 1 - 3 to include any clinical signs associated with the stereotaxic surgery procedure (e.g. dehiscence, intracerebral bleeding), osmotic pump implantation (e.g. dehiscence, infection), medical treatments (e.g. phenelzine, isradipine), and repeated intraperitoneal injections (e.g. infection, irritation).

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

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5. **Protocol Title:** 1910-37493A Preclinical evaluation of new wearable ultrasound device and its efficacy in treating inflammation
Species &Pain Class: (A,B,C) Mice; (B) Rat
Question the Research Addresses: Does our wearable ultrasound device prototype reduce clinical signs of arthritis and endotoxemia in animal models?

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

- Please contact Dr. Nate Koewler (koew0004@umn.edu) and Dr. Jen Hubbard (hubba082@umn.edu) for additional assistance addressing these comments.

- Please add a contact phone number for the PI in this section.

- The goal of the Experimental Design section is to understand what happens to all animals enrolled in the study. We should be able to understand the cumulative experimental burden for all animals. All of the procedures on the protocol should be described here, and there should be a narrative of what order the procedures happen in, how much time is between procedures, which procedures different groups undergo, etc. Stage 1: It is not clear to me from this description what happens to mice on study. On day 0 they are given an IP injection to induce arthritis... then what happens? When does the probe procedure occur? How often do they undergo this procedure? How often do they undergo the ultrasound stimulation procedure? Are they anesthetized daily? Or are they wearing a device? What are the 4 experimental groups? Based on the current text, it sounds like mice are injected with serum and then euthanized 7-14 days later, but in the IP injection procedure it makes it sound as though mice may be maintained for up to 8 weeks. Please clarify. Stage 2: Please describe when the LPS injection happens in relation to other procedures on the protocol. Are they all on the same day? Do they happened within hours? Under the same anesthetic event? Please also confirm that all procedures for the rats are terminal.

- The number of rats needed appears to be based on results from previous electrophysiology studies, which will not be performed on all rats in this study. Please

clarify whether 12 rats per experiment is the minimum number needed for the endotoxemia experiments and how many rats will be used for the electrophysiology recordings.

- The protocol title and experimental design section mention wearable ultrasound devices, but I do not see a procedure or description anywhere in the protocol or mice/rats wearing anything. Will animals be wearing a device?

- In the Experimental Design section you state that "7-14 days post serum injection, animals will be euthanized, and blood and/or tissues may be collected." In this procedure you state "Each mouse will not be used beyond 8 weeks of first induction" How long will mice be maintained for? Please review both sections and make sure it is clear to reviewers what is happening.

- Currently you have a citation to reference the procedure you plan to do, but you must actually write and describe the procedure in the protocol. At what point after the injections do you anesthetize/euthanize the rats? Is there a reason why they cannot be anesthetized and then receive the injection? Please clarify when (e.g. relative to induction of arthritis), how frequently and the maximum number of times mice will undergo infrared imaging to visualize inflammation in arthritic mice.

- How long will the imaging procedure take? How many times do mice undergo this procedure? Please include ophthalmic ointment as a supportive agent.

- In the Experimental Design section you state " 7-14 days post serum injection, animals will be euthanized, and blood and/or tissues may be collected." In the procedure you state that blood may be collected twice weekly in chronic studies. Please review both sections and ensure that they accurately reflect what you intend to do on this protocol.

- In the Experimental Design section you state " Acute, anesthetized experiments will be performed in rats." and elsewhere in the protocol it seems as though rats are used in a single terminal procedure. However, in the blood collection procedure you state that blood may be collected twice weekly in chronic studies. Please review both sections and ensure that they accurately reflect what you intend to do on this protocol.

- Because animals have to be covered while they are transported, it is inappropriate to transport anesthetized animals which you cannot visualize. If they are stressed from transport, consider bringing them to the lab sooner and letting them re-acclimate in the room prior to handling/anesthesia. You can also use an induction chamber for rats being administered isoflurane, so all you need to do is scoop up the rat in a glove box and place them in the induction chamber. Ketamine/Xylazine is an inappropriate anesthetic choice for animals anesthetized for 10-24 hrs and where you can only monitor them every 30 minutes. Since isoflurane is an option, it should always be used. Please include language in your protocol that isoflurane will alway be used; if you plan to use Ket+Xyl, please include under what circumstances this would happen. Please state how you will confirm that an animal is appropriately anesthetized prior to administering paralytics. Also, a ventilator must be used in rats receiving vecuronium. For all drugs, please include dose, route, and maximum volume. You mention administering yohimbine to help distinguish between dopamine and norepinephrine -- would you administer this with Ket+Xyl anesthesia? This is contraindicated as Yohimbine can reverse the effects of xylazine, and

thus cause the rats to wake up.

- Please include a maximum amount of time this procedure will last. How often will mice undergo this procedure? Is this a terminal procedure? In the recordkeeping section you state " At the end of the experiment, we record the euthanasia procedure." Animals must be monitored continuously and anesthetic depth should be checked/recorded every 10-15 minutes; not 15-20. Include ophthalmic ointment as a supportive agent.

- In the Experimental Design section you state that rats are used acutely and it is implied that they undergo a single, terminal procedure. I assume that this non-invasive ultrasound procedure it happening under the same anesthetic event as the LPS injection and surgery, although as the protocol is currently written it is not yet clear what you actually intend to do. However, in this Ultrasound procedure you state "After providing the stimulation paradigm, we will allow the animal to recover similar to the steps provided for our survival surgeries." Please review all sections and make sure it is clear to reviewers what will happen to these animals

- Please clarify when (e.g. relative to induction of arthritis), how frequently and the maximum number of times mice will undergo infrared imaging to visualize inflammation in arthritic mice

- You mention a surgical mouse model here, but there are no mouse surgeries listed on the protocol. Please review and edit as needed. You mention using a rodent arthritis index (0-12 scale) but it is not clear how this is used to determine when mice need treatment/supportive care. Do certain scores trigger you to provide supportive care? Increase monitoring? Euthanasia? Please address the supportive care involved in maintained arthritic mice. Have you considered switching to soft bedding such as care fresh? Do you plan to place moistened food in the cage to help them access food/water and maintain body weight? These are both refinements which could be added to your Alternative Search section.

Committee Decision: Deferred
For: 11 Against: 0 Abstain: 0
Member S out

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6. **Protocol Title:** 1908-37334A Pacing-Mediated left ventricular remodeling reduced myocardial stiffness
Species &Pain Class: (B) Pig (Biomedical)
Question the Research Addresses: Can Pacing-Mediated left ventricular remodeling reduce myocardial stiffness e.g. by reducing collagen levels, and define the pacing exposure required to mediate these changes?

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

- Please add a contact phone number for the PI in this section.

- For all surgical procedures, ceftriaxone is listed as being delivered by IV in the sections of other support agents and then by IM in post op care but the dose level is the same,

please update the protocol to clarify.

- For all procedures, transport was only listed as occurring in the morning. Please clarify whether the animals are not being transported back to primary housing for 24 hours.

- Please update the protocol to confirm the route of examination for the echocardiogram - will this be transthoracic or intracardiac echo (ICE) or other?

- Please update the Induction and surgical prep procedure to address the following: Please describe the closing procedures for the femoral artery cut down for blood pressure recording. In the Induction and Surgical prep for all procedures, it states that the femoral artery will be placed for all procedures to measure blood pressure. In other procedure sections it appears as though the carotid artery may be used for blood pressure evaluation. Please clarify if both or only one vessel will be accessed at the time of implant and at the follow up time points. Will blood pressure be monitored from the same artery for the 3 week, 5 week and 8 week procedures or will alternate vessels be used?

- In general, procedure/surgical descriptions do not provide much detail. Please update to include more detailed descriptions of the procedures. SR buprenorphine is usually given before prep since it takes a couple hours to be effective. Consider giving 2 hours before procedures, especially shorter ones.

- For the question regarding sampling frequency, the response is "throughout the procedure". Please note that this does not provide the IACUC reviewer the ability to discern the amount of blood volume that would be removed during a single procedure. In cases like these you may determine what the maximal number of times a sample would be collected and then write the response to reflect the maximal volume of blood removed per procedure. Please update the blood collection procedure to indicate the maximum frequency and volume that would be collected. (e.g. my procedure is 2 hours long, the most we would collect a sample is once every 15 min, therefore we would not collect more than 8 samples and would not exceed 16 ml per procedure).

- Please update the protocol to provide a more complete description of this procedure. Include closure of all incisions.

- Please update the Health and Monitoring section to include whether there are any concerns with unalleviated bleeding from biopsy site sampling or concerns with arrhythmia/fibrillation from sample collection, or any concerns with fluid/infection in the pacemaker pocket.

- Please update the protocol to confirm/clarify whether the control group receives all the same procedures as the experimental group with the only difference being pacing/not pacing, or if there are any other differences.

- We are unable to confirm all personnel requirements have been completed. Missing requirements are noted below: -Dr. Markus Meyer (tetanus requirement, ROHP Salmonella training, and Animal Use Tutorial) -Danielle Burroughs (Animal Exposure Questionnaire, all ROHP trainings, tetanus requirement, and Animal Use Tutorial). Personnel have been sent directions via the emails provided in the protocol. Once all

requirements are completed, please confirm here in eProtocol.

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

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2. IACUC-AMENDMENT (# Protocols: 1)

1. **Protocol Title:** 1905-37094A MPPA: Chemical pancreatectomy for chronic pancreatitis and pancreatic cancer **Species &Pain Class:** (B) Nonhuman Primate (Macaques)
   Question the Research Addresses: Can an innovative surgical approach using ethanol or acetic acid ablate the exocrine function of the pancreas, but preserve the endocrine function and significantly reduce morbidity related to manipulation of the pancreas?

   Committee Decision: Approved as submitted
   For: 12 Against: 0 Abstain: 0

Institutional Animal Care and Use Committee
12/17/19 Minutes
VCRC - 76D

Meeting Convened: 12:30PM	Quorum Requirement: 10
Meeting Adjourned: 2:50PM	Members Present to Vote: 16

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

Non-Voting, Ex-Officio:

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

Institutional Veterinarian:

3	X	(M, S)

Correlates to Version **v2.97** 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 November 2019 Inspection Findings – Notes to File- Veterinary Recommendations report.

2. The committee was updated on the NCROC Fall and Spring Pasture Grazing Management and Supplemental Feeding SOP. This SOP was in response to a recent issue regarding calves on pasture that had not received supplemental feed where there appeared to be a communication issue. The SOP is posted on the IACUC member website under the discussion materials. The committee had no additional concerns at this time.

3. The committee discussed requirements outlining RAR staff hired for technical services by labs and if they should be listed as personnel on IACUC protocols. In the past, the committee had determined that RAR staff would not be listed on research protocols in these cases and a roster protocol is currently in place for RAR staff to monitor their training requirements. The roster protocol is similar those that are currently used by larger labs such as ESS and PCRC with a large number of protocols. There will be a subcommittee created to investigate discrepancies between requirements and processes between RAR and researchers.

4. The committee received a recommendation and update from a subcommittee collecting information on how to provide oversight with labs using cephalopods for research as there are currently no regulatory requirements placed on the IACUC for cephalopod research. The subcommittee provided the following recommendation:

    - Partner with the investigator as a consultant. IACUC would assist in creating a document or protocol that outlines the experiments and husbandry and provides the investigator with feedback or suggestions on the content as opposed to stipulations that would require formal IACUC approval.

    - The IACUC has asked that the subcommittee collect additional information as to how other institutions are managing cephalopod research. Subcommittee representatives will also visit the PI's current lab and housing. The IACUC will continue to receive updates from the subcommittee.

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 IACUC leadership contacted the lab and they have voluntarily stopped all animal activity while the leadership group collects additional information. The committee will be updated at upcoming meetings.

1. **IACUC-NEW (# Protocols: 4)**

    1. **Protocol Title:** 1910-37493A Preclinical evaluation of new wearable ultrasound device and its efficacy in treating inflammation
    Species &Pain Class: (A,B,C) Mice; (B) Rat
    Question the Research Addresses: Does our wearable ultrasound device prototype reduce clinical signs of arthritis and endotoxemia in animal models?

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

    - Please contact Dr. Nate Koewler (koew0004@umn.edu) and Dr. Jen Hubbard (hubba082@umn.edu) for additional assistance addressing these comments.

    - Please add a contact phone number for the PI in this section.

    - The goal of the Experimental Design section is to understand what happens to all animals enrolled in the study. We should be able to understand the cumulative experimental burden for all animals. All of the procedures on the protocol should be described here, and there should be a narrative of what

order the procedures happen in, how much time is between procedures, which procedures different groups undergo, etc. Stage 1: It is not clear to me from this description what happens to mice on study. On day 0 they are given an IP injection to induce arthritis... then what happens? When does the probe procedure occur? How often do they undergo this procedure? How often do they undergo the ultrasound stimulation procedure? Are they anesthetized daily? Or are they wearing a device? What are the 4 experimental groups? Based on the current text, it sounds like mice are injected with serum and then euthanized 7-14 days later, but in the IP injection procedure it makes it sound as though mice may be maintained for up to 8 weeks. Please clarify. Stage 2: Please describe when the LPS injection happens in relation to other procedures on the protocol. Are they all on the same day? Do they happened within hours? Under the same anesthetic event? Please also confirm that all procedures for the rats are terminal.

- The number of rats needed appears to be based on results from previous electrophysiology studies, which will not be performed on all rats in this study. Please clarify whether 12 rats per experiment is the minimum number needed for the endotoxemia experiments and how many rats will be used for the electrophysiology recordings.

- The protocol title and experimental design section mention wearable ultrasound devices, but I do not see a procedure or description anywhere in the protocol or mice/rats wearing anything. Will animals be wearing a device?

- In the Experimental Design section you state that "7-14 days post serum injection, animals will be euthanized, and blood and/or tissues may be collected." In this procedure you state "Each mouse will not be used beyond 8 weeks of first induction" How long will mice be maintained for? Please review both sections and make sure it is clear to reviewers what is happening.

- Currently you have a citation to reference the procedure you plan to do, but you must actually write and describe the procedure in the protocol. At what point after the injections do you anesthetize/euthanize the rats? Is there a reason why they cannot be anesthetized and then receive the injection? Please clarify when (e.g. relative to induction of arthritis), how frequently and the maximum number of times mice will undergo infrared imaging to visualize inflammation in arthritic mice.

- How long will the imaging procedure take? How many times do mice undergo this procedure? Please include ophthalmic ointment as a supportive agent.

- In the Experimental Design section you state " 7-14 days post serum injection, animals will be euthanized, and blood and/or tissues may be collected." In the procedure you state that blood may be collected twice weekly in chronic studies. Please review both sections and ensure that they accurately reflect what you intend to do on this protocol.

- In the Experimental Design section you state " Acute, anesthetized experiments will be performed in rats." and elsewhere in the protocol it seems as though rats are used in a single terminal procedure. However, in the blood collection procedure you state that blood may be collected twice weekly in chronic studies. Please review both sections and ensure that they accurately reflect what you intend to do on this protocol.

- Because animals have to be covered while they are transported, it is inappropriate to transport anesthetized animals which you cannot visualize. If they are stressed from transport, consider bringing them to the lab sooner and letting them re-acclimate in the room prior to handling/anesthesia. You can also use an induction chamber for rats being administered isoflurane, so all you need to do is scoop up the rat in a glove box and place them in the induction chamber. Ketamine/Xylazine is an inappropriate anesthetic choice for animals anesthetized for 10-24 hrs and where you can only monitor them every 30 minutes. Since isoflurane is an option, it should always be used. Please include language in your protocol that isoflurane will alway be used; if you plan to use Ket+Xyl, please include under what circumstances this would happen. Please state how you will

confirm that an animal is appropriately anesthetized prior to administering paralytics. Also, a ventilator must be used in rats receiving vecuronium. For all drugs, please include dose, route, and maximum volume. You mention administering yohimbine to help distinguish between dopamine and norepinephrine -- would you administer this with Ket+Xyl anesthesia? This is contraindicated as Yohimbine can reverse the effects of xylazine, and thus cause the rats to wake up.

- Please include a maximum amount of time this procedure will last. How often will mice undergo this procedure? Is this a terminal procedure? In the recordkeeping section you state " At the end of the experiment, we record the euthanasia procedure." Animals must be monitored continuously and anesthetic depth should be checked/recorded every 10-15 minutes; not 15-20. Include ophthalmic ointment as a supportive agent.

- In the Experimental Design section you state that rats are used acutely and it is implied that they undergo a single, terminal procedure. I assume that this non-invasive ultrasound procedure it happening under the same anesthetic event as the LPS injection and surgery, although as the protocol is currently written it is not yet clear what you actually intend to do. However, in this Ultrasound procedure you state "After providing the stimulation paradigm, we will allow the animal to recover similar to the steps provided for our survival surgeries." Please review all sections and make sure it is clear to reviewers what will happen to these animals.

- Please clarify when (e.g. relative to induction of arthritis), how frequently and the maximum number of times mice will undergo infrared imaging to visualize inflammation in arthritic mice.

- You mention a surgical mouse model here, but there are no mouse surgeries listed on the protocol. Please review and edit as needed. You mention using a rodent arthritis index (0-12 scale) but it is not clear how this is used to determine when mice need treatment/supportive care. Do certain scores trigger you to provide supportive care? Increase monitoring? Euthanasia? Please address the supportive care involved in maintained arthritic mice. Have you considered switching to soft bedding such as care fresh? Do you plan to place moistened food in the cage to help them access food/water and maintain body weight? These are both refinements which could be added to your Alternative Search section.

Committee Decision: Stipulations must be met
For: 16 Against: 0 Abstain: 0

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2. **Protocol Title:** 1911-37621A Immune response during demyelinating disease and the role of microglia during demyelinating disease: Developing new therapies for neuropathic pain during multiple sclerosis
Species &Pain Class: (A,C) Mice
Question the Research Addresses: We are determining the role of the innate immune response in development and progression of demyelinating disease, MS. We are also determining how the immune response may be promoting the excitation of neurons leading to neuropathic pain.

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

   - Please update the protocol to indicate when animals will undergo the behavioral testing that is outlined in the procedures section. Will each animal undergo behavior testing once, or at multiple points throughout the study? Will each animal undergo all the tests that are indicated (Von Frey, locomotor, activity)?

   - Use of diphtheria toxin is mentioned in Aim 1E. Please include the dose/route/schedule of this administration, and add diphtheria toxin to the IBC tab (and IBC protocol, if needed).

   - For some of the procedures, it states that 4 animals will be placed in a isoflurane chamber at the same time for procedures that will then happen one at a time. Animals should be anesthetized one at

a time to allow for monitoring as well as to limit the amount of time under anesthesia. Please justify this process scientifically or change it to allow for one animal to be anesthetized at a time.

- In this breeding section, it states that the mice are congenic, suggesting that they are genetically modified, however the protocol states that you are not breeding such animals in the below questions. Please change the response to this question and provide any information on any phenotypic problems they may have.

- In this section, it states that animals will be returned to their cages once they regain consciousness. However, there is no listed anesthesia. Please remove this statement as anesthesia would not be necessary for an IP injection, or update to describe anesthesia if it will be used.

- Please add a Diet/Fluid modification procedure in order to fully describe the antibiotic treatments in the water bottle. Please make sure to state the dose that will be given to them mice (approximate mg/kg or mg each mouse will drink). Also please state the duration the antibiotic water will be used.

- Please update question #2 in the behavior procedure section. It currently states that these animals will not undergo a battery of tests, however there are three distinct tests listed above that question. Please outline when a mouse will get each of these tests, the frequency, duration, and time between each.

- Neonatal mice are decapitated without anesthesia. If no other procedures are performed it appears that these mice could be categorized as pain class A rather than B. Please clarify.

- In the Health and monitoring there is no mention of animals undergoing the irradiation and bone marrow reconstitution process. Please describe the potential health implications of mice that receive this procedure, monitoring that is needed, and interventions (supportive or euthanasia) that are necessary when complications arise.

- Since TMEV is an [redacted] agent please verify and update the Procedure Location section to confirm that animals and agents will only be handled in [redacted] areas. Currently, in this procedure sections it only states that they will be handled in room [redacted] which is not [redacted].

- Since the cranium is being punctured and is a possible site of infection it is recommended that animals have hair shaved and the skin disinfected similar to a surgical preparation. This will help prevent the introduction of skin flora into the brain tissue/CSF. Please update the procedure to either confirm that this will be done or explain why not.

Committee Decision: Stipulations must be met
For: 16 Against: 0 Abstain: 0

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3. **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: (A,B,C) Mice
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 can be induced function 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 subsequent full committee review once the following stipulations are addressed by the PI:

- For question E: Please provide a brief summary of the research results that have been obtained from animals over the past renewal period (e.g. We have used 1000 mice and completed experiments one and two and have started work on project three). Particularly, if experiments using death as an

endpoint have been conducted, provide updates on numbers already used and any outcomes on death vs survival.

- The numbers listed here apportioned to transfer from another protocol vs purchased do not appear to match the apportionment in the attachment (table). Please reconcile once you have confirmed numbers in the attachment (see attachment comments).

- In the experimental design section under "Determine how self-specific autoimmune CD8 T cells cause pathology in the intestine" mice are described as needing to go to death as an endpoint (The pathology induced with our model will lead to mouse death, but we have found that certain interventions will rescue mice, even animals that are moribund). It does not seem that this particular experiment is described any further in the procedures and it is unclear what interventions are used to rescue the mice. Please elaborate on this within the Experimental Design and provide a procedure if applicable, as this is crucial to reviewers understanding of why death as endpoint is needed.

- In the section "Understand what types of T cells are affected by current clinical immuno-therapeutics and how to potentiate effectiveness of these drugs", irradiation is described as being used in concert with cancer immunotherapy drugs. Although x-ray irradiation is described in the Procedures section, this is not included in the attachment that describes the procedures for each experimental group. Please confirm that irradiation is being confirmed and update the attachment as necessary.

- Please update this section to provide more background to assist in understanding the rationale for specific aspects of the experiments. It would be useful to explain the mouse strains and why each is important (what they bring to the experiment/why they are used, including in direct comparisons). Likewise some other aspects of the experiment need better introduction before the reader needs to wade through it. Each section in experimental design could also be expanded for clarity, currently it is a very broad overview, there is information/explanation missing that would help interpret what is going on between the experimental design prose and the table attachment. e.g. in the table what is the difference between line 3 and line 4 – Different mouse strain (IFABP-Ova vs B6) but please explain what the purpose of each strain is. Tattoo of peptides not well explained until we reach the procedure description but having a brief outline in the experimental design section would help make the research understandable from the start (what it is and superiority over other routes/techniques). Which mice get multiple infections? Which mice get polyoma virus? Please provide information in experimental design section to fill in these understanding gaps.

- Regarding the use of avertin: Is there any indication or physiologic systemic effect from other commonly used pharmaceutical grade anesthetics that you are concerned would alter the results of the experiment? Particularly, if the mice are previously anesthetized with iso, it seems like this could also be used for this procedure. Or if an injectable route is needed, explain why something like ketamine/xylazine (used in other procedures as well) would be expected to interfere with previous data. If it is determined that Avertin is still needed, please answer the second part of the question which asks to detail the storage and preparation of the compound. Click on the entry for Avertin in the anesthetic table to update this.

- For procedures using isoflurane in a drop jar, please confirm the jar has a barrier that prevents the mice from coming into direct contact with the isoflurane soaked cotton ball. Also, it seems that for some surgical procedures, an isoflurane machine is available. Please clarify why the drop jar is used for some procedures versus an isoflurane machine.

- 1.) In multiple procedures (Infection with pathogens, Adoptive transfer of lymphocytes, Monoclonal antibody injection, Leukemic cell infusion), the IV volume is listed as 200 - 500 uL. The maximum dose per RAR recommendations is 5 ml/kg (i.e. for a 25g mouse, 125 uL). Please confirm the volume being administered IV and provide a justification if it falls outside the RAR recommendations. 2.) LINAC radiation and x-ray irradiation are both listed as procedures, but neither is in the attachment describing the experimental groups and associated procedures. Please confirm that these procedures will be performed and update the attachment as necessary.

- MCMV, Polyoma virus, and influenza are listed as being used, but these pathogens are not listed in the attachment describing the experimental groups and treatments. Please confirm that these pathogens will be used in this protocol and update the attachment as necessary.

- LCMV, adenovirus, MCMV, Polyoma virus, and influenza are listed as being used, but these pathogens are not listed in the attachment describing the experimental groups and treatments. Please confirm that these pathogens will be used in this protocol for class C mice and update the attachment as necessary.

- It appears that this procedure is identical to the class A infection with pathogens procedure and could be removed. If this procedure exists to account for the non-DSS intestinal pathology animals, please update the procedure to provide details specific to that experiment.

- The antibodies listed in the attachment do not include anti-CD4, anti-CD8, anti-Thy1.1, anti-CD45.1 or anti-CD45.2 which are listed in this procedure. Please confirm that these antibodies are being administered and update the attachment as necessary.

- Please update the procedure to include the volume that will be administered subcutaneously or intradermally.

- Please update this procedure to confirm whether mice are anesthetized; description states mice will be restrained but Avertin is listed in the table. Under anesthesia steps taken if parameters outside range state: “Anesthetic depth will be ensured by inhalation until the animal is unresponsive to reflexes.” which sounds like isoflurane will be used but only avertin is listed. Please update with the relevant steps that will be taken for the anesthetic used.

- Because this procedure involves fluid modification, please add a dietary or fluid modification procedure to cover DSS treatment, and fill out the questions that will be generated

- This procedure states: “All mice receiving Xray irradiation will receive Bone Marrow transfer outlined in Bone Marrow Transfer procedure.” There is no bone marrow transfer procedure listed. Does this refer to the leukemic cell infusion (cells sourced from bone marrow) and will irradiation also be performed prior to adoptive transfer of lymphocytes procedure? Please update the protocol to clarify.

- Weight loss is indicated as a criterion for euthanasia; please update the protocol to include how this will be assessed. Twice a week weighing is suggested for those with intestinal issues (antigen, DSS).

- The response to question 3 is not just directed at anesthetics used, but should also include other interventions that may be used. Some of these were mentioned previously in question 2 and can be referenced here as well as any other potential interventions that may be used especially in the case of sick/moribund animals. Please include other interventions (e.g. warming pads, fluids, soft bedding) that may be used either by the lab or by RAR to minimize pain, distress and discomfort.

- The mice with intestinal pathology as listed as needing death as an endpoint, but in the DSS procedure it states "Animals that lose 20-25% of their original body weight and have continuous diarrhea or blood in their stool will be euthanized, as per IACUC stipulations." Please clarify if death is indeed being used as an endpoint for these animals. The mice that are infected with VSV, Listeria, and Vaccinia (lines 19-21 in attachment) require 40 class B and 20 class C mice. In the justification for death as an endpoint, it states "This is because rescuing animals from death is a very high bar for efficacy." However, in the attachment it looks like no treatments are being performed on these mice and only blood is being collected. Please clarify if death is indeed being used as an endpoint and if no treatments are being performed why death is a necessary endpoint for these animals, or provide more information on the treatments being tested.

- Please update the DEHS section to add isoflurane via nose cone, and LINAC for irradiation.

- Please double check the following in the attached table and update as needed; if this changes the numbers in the Species table please update there as well: Line 1 number in experimental box doesn't match number given in total # (348 vs 500) – IACUC is this within reasonable allowance? Line 7 number in experimental box appears to be in error. You provide a calculation: mouse #= 1 mice per group x 2 tumor types (B16 or B16-OVA) x 4 timepoints x 2 iv treatments (+/- i.v) x 2 assays (IHC or flow) x 3 replicates= 480 Whereas this math 1x2x4x2x2x3 = 96 Please explain how you get 480 mice here and then your number in the total numbers column also doesn't match (960). Presumptively the 1 mouse per group listed is part of the error? Line 9 – similar math discrepancy to line 7 – you come up with 480 mice but the equation you give appears to only equal 96 mice Line 17 – it would be helpful to include all factors in your numbers equation, presumptively it is missing the 3 infection types (which would get it to 270) If giving all infections to the same mice (i.e the same mouse gets all infections) then it would only be 90 mice Line 18 – same comment as 17 Line 19 – To make the math work it appears all mice get all 3 infections, if this is correct ignore this comment. If not then do you need to request more mice to account for the 3 different infections listed? Line 20 – same comment as line 19 Line 21 – same comment as line 19 Line 25 – as written it appears these particular cells are only used in line 27 (they are not mentioned in lines 28,29 or 30; is their omission in these lines an error or do you really only need approx. 7 of these donor mice (since they are only donating to the 135 in line 27)? Line 30 – minor but I think your number of mice here is 81 not 84

- The table describes 204 category C mice but the final total requested is 244. Please explain this discrepancy and update the protocol as needed to reconcile these numbers.

Committee Decision: Deferred
For: 16 Against: 0 Abstain: 0
Member 11 out (alternate present)

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4. **Protocol Title:** 1912-37672A In vivo investigation of tumor reduction using novel aminopeptidase inhibitors and nucleoside analogs in a murine model of solid tumor formation
Species &Pain Class: (B,C) Mice
Question the Research Addresses: Can aminopeptidase inhibitors or nucleoside analogs reduce tumor size, or inhibit growth in a subcutaneous tumor mouse model?

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

   - It is stated that mice will be euthanized by overdose of ketamine/xylazine followed by euthanasia. Please note that your doses for ketamine/xylazine would not necessarily lead to death so I recommend either increasing the doses to at least 300 mg/kg ketamine and 30-40 mg/kg xylazine or you may amend to state that the mice will be deeply anesthetized with ketamine/xylazine at your current doses followed by exsanguination as the primary euthanasia method.

   - It might not be necessary to classify the mice as C since they are monitored frequently and euthanized if showing signs of pain/distress. Please provide explanation why for tumor induction by SC injection is listed as category C.

   - Please update the response to Question 1 to provide a brief statement about potential adverse effects of the compounds used.

   - Since human cancer cells will be used for experiments please check " Yes" and check the box for Human blood, body fluids, normal or neoplastic tissue (including human cell lines), and confirm bloodborne pathogen training/immunization have been completed.

   Committee Decision: Stipulations must be met

For: 16 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 1)

1. **Protocol Title:** 1708-35065A Cell Signaling and Neurodegeneration Molecular Genetics SCA1 UNS: Developing Serial Optical Coherence Scanning to Reveal White Matter Changes in SCA1 Development of an ASO-based therapy for spinocerebellar ataxia type 1 Effects of PGM031074 on cerebellar neurochemistry in SCA1 Defining Biomarkers for Spinocerebellar Ataxia Type 3 Intraperitoneal (i.p.) injection of CCK receptor agonists
   Species &Pain Class: (A,B,C) Mice
   Question the Research Addresses: There is no effective treatment for SCA1 and our goal is to examine specific elements in the pathway leading from the genetic mutation to development of the disease causing protein and to investigate steps ultimately responsible for causing the disease and thus test potential therapeutics for the disease.

   Committee Decision: Approved as submitted
   For: 16 Against: 0 Abstain: 0
   Member 11 out (alternate present)
