[redacted]

Title: **Thoracic Allograft Tolerance in Non-Human Primates: Application of Mixed Chimerism to Lung** [redacted]

Sponsor Name: [redacted]

PI Name: [redacted]

Protocol #: [redacted]

Type: **Current View**

Species: **MONKEYS_MACAQUE_CYNO**

Of Animals: **38**

Date Received: **April 16, 2008**

Study Staff

Name	Role	Degree	Organization
[redacted]	Co-Investigator		[redacted]
[redacted]	Principal Investigator	MD, MBA	[redacted] > [redacted] > [redacted] Biology ([redacted]
[redacted]	Other		[redacted] > [redacted]
[redacted]	Co-Investigator	DVM	Partners
[redacted]	Co-Investigator	MD, Ph.D	[redacted] > [redacted] > [redacted]
[redacted]	Co-Investigator	DVM, BS, CVT	
[redacted]	Co-Investigator	MD, Ph.D	[redacted] > [redacted] > [redacted] Biology ([redacted]
[redacted]	Co-Investigator		[redacted] > [redacted] > [redacted]
[redacted]	Research Technician	AS, CVT	[redacted] > [redacted]

Non Study Staff

Name	Degree	Organization
[redacted]		[redacted] > [redacted] > [redacted]
[redacted]	DVM, BS	[redacted] > [redacted] > Trauma Unit
[redacted]	Ph.D	[redacted] > [redacted] > [redacted] Biology ([redacted]

Linked Agreements

Record #	Fund	Project Period	PI Name	Sponsor	Record Type	Process	Link Date	Link Status
[redacted]		07/01/05-06/30/11	[redacted] C	[redacted]	RM – Funded Agreement	IR	04/16/08	Approved

[redacted]

Record #	Fund	Project Period	PI Name	Sponsor	Record Type	Process	Link Date	Link Status
[redacted]		07/01/08-06/30/09	[redacted]	[redacted] ECOR	RM – Funded Agreement	IR	04/16/08	Approved
[redacted]		07/01/05-06/30/11	[redacted] C	[redacted]	RM – Funded Agreement	IR	04/16/08	Approved
[redacted]		05/15/09-04/30/14	[redacted] C	[redacted] NHLBI	RM – Funded Agreement	IR	04/16/08	Approved
[redacted]		05/15/09-04/30/14	[redacted]	[redacted]	RM – Funded Agreement	IR	04/16/08	Approved
[redacted]		11/14/14-10/31/19	[redacted]	[redacted]	RM – Funded Agreement	AME30	06/12/14	Approved

Linked Protocols

Protocol #	Relationship	Link Location	Overall Status	PI Name	Title	Process	Link Date	Link Status	Link Direction
[redacted]	Biosafety	Use of Animals	Active	[redacted]	[redacted]	LA4	04/09/19	Approved	One Way
[redacted]	Biosafety	Use of Animals	Inactive	[redacted]	[redacted]	LA16	05/10/17	Approved	One Way

Protocol Overview

Please answer the following questions using language a non-scientist will understand.

[redacted]

1. Study Goals

How would you explain the long term or overall scientific goals of the proposed work to a non-scientist? **[Please limit to 200 words.]**

To develop strategies to induce transplantation tolerance in lung transplantation.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Detailed Research Plan, section A. Goals**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Study Goals** question above. ***Use of this information is optional.***

To develop strategies to induce transplantation tolerance in lung transplantation. We hypothesize that the induction of mixed chimerism will lead to long-term lung engraftment without the need for ongoing immunosuppression.

2. Benefit to be Gained by Animal Research

How would you explain to a non-scientist that the potential benefits of the study, in terms of biomedical advancement, justify the proposed animal use? **[Please limit to 200 words.]**

Lung transplant recipients currently have a post-transplant survival period of about eight years. The three leading causes of death are chronic rejection, infection and cancer. These three occurrences are inescapable consequences of current immunosuppressive therapy. We are attempting to develop a novel approach to organ transplantation, known as transplantation tolerance. The induction of tolerance will allow for the indefinite survival of the graft without the requirement for long-term immunosuppression and its complications.

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Conventional immunosuppression does not eliminate the risk of graft loss due to rejection and at the same time results in unacceptably high incidences of infections and malignancies. For this reason, we are attempting to create a tolerant state in which a recipient's immune system fails to recognize an organ transplanted from another member of the same species. The achievement of this goal would represent a major paradigm shift in the management and results of the transplanted patient.

[redacted]

Many strategies have been used to induce tolerance in a variety of species. However, the mixed chimerism approach has been shown to be the most robust, particularly in phylogenetically higher species. The Department of [redacted] at [redacted] has been a leader in this field for many years and this strategy has been successful in the renal allografting of cynomolgus monkeys in our laboratory. It has also been successful in limited trials in human patients.

The translation of this work into a lung transplant model is important, as there is a severe shortage of donor lungs, which effectively eliminates retransplantation as an option for patients who are rejecting their lung grafts. Also, our experience has shown that different organs respond differently to the same tolerogenic regimen. Thus, while our success in renal allografting serves as a proof of principle, it by no means eliminates the need for similar translational work to be performed in a lung transplant model.

Since the inception of this project in 2005, we have established a validated NHP lung transplantation model and have achieved tolerance in 3 haplo-identical monkeys. We now wish to extend these results to a fully mismatched model using clinically available reagents.

Research Objective: Research Objective 1

INSTRUCTIONS:
Complete a Research Objective form for each discrete aim of the protocol. To add an additional Research Objective, please click the **add New Research Objective** button at the end of this form.

Limit the discussion to activities involving animals. Do not describe *in vitro* procedures beyond collection of tissues, blood, or other biological products.

A. Rationale: [**Please limit to 200 words**]

To develop strategies to induce transplantation tolerance in lung transplantation. We hypothesize that the induction of mixed chimerism will lead to long-term lung engraftment without the need for ongoing immunosuppression.

B. Experimental Design: For this research objective, outline the time-course indicating each activity. Describe each step and how it relates to an animal enrolled in this study. It should be clear what each animal will experience during the full course of this Research Objective.

- Include the length of time an animal is enrolled in an experiment
- Describe experimental endpoints
- Do not include descriptions of surgical and non-surgical procedures in the **Experimental Design**. Include this information in the specific **Procedure** forms.

Overall Research Plan:

Two experimental groups are proposed:

Group 1: MHC-mismatched transplants on Tolerance Induction Protocol #1

[redacted]

Group 2: MHC-mismatched transplants on Tolerance Induction Protocol #2

The experimental protocols are described on the attaced flowchart and diagram. In both experimental groups, a recipient monkey will undergo a single left lung transplant. The recipient will then be maintained with standard immunosuppressive therapy for four months, and undergo a protocol lung biopsy during this time. At Day 120, a bone marrow transplant will be performed using frozen bone marrow obtained post-mortem from the original lung donor, and a conditionaing regimin that involves a variety of immunosuppressants, thimic irradaition (TI), and total body irradiation (TBI). This is expected to induce tolerance of the lung allograft. 28 days after the bone marrow transplant, all immunosuppression is stopped, and the graft is followed with serial lung biopsies. At the conclusion of the experiment, skin grafting is performed as a final confirmation of tolerance.

The flowchart and protocol diagram most clearly depict the exact sequence for all of the animals. Also included on the flowchart are the specifics of all of the immunosuppressive therapies used in the protocol.

The flowchart is written assuming that BMT is on Day 120. In actuality, this may vary a bit, depending on staff an OR availability. Exact timing of biopsies and medications are guided by each animal's clinical status.

A two-week recovery period will occur between major procedures. Major procedures will be performed only if the PI and the veterinarian agree that the performance status of the animal is acceptable. Unless a veterinary exception is made, all animals and will have a maximum of one transplant and five subsequent biopsies.

Lymph node and/or bone marrow biopsies are occasionally needed to assess for thymic, nodal, and bone marrow chimerism and for the detection of post-transplant lymphoproliferative disease. As with major procedures, these minor will be done only if needed and only if both the PI and the veterinarian agree that the performance status of the animal is acceptable.

A skin graft (with weekly biopsies) would only be performed as a final check for tolerance on a recipient who has demonstrated long-term graft survival off of immunosuppression. Acceptance (30 days) or rejection of the skin would be the final event in the experiment for such an animal.

A terminal kidney harvest is included as a possible procedure in order to minimize the use of other monkeys as donor animals for other experiments in our group. A terminal kidney harvest would only occur in an animal that is immunologically naive and that is intended to be sacrificed regardless.

The following procedures and medictions are common to these experiments:

Operative Procedures:

[redacted]

1. Donor pneumonectomy via sternotomy

2. Orthotopic single lung transplant via thoracotomy

3. Open lung biopsy via limited thoracotomy

4. Superficial lymph node biopsy

5. Skin harvesting

6. Skin grafting

7. Skin biopsy

8. Terminal kidney harvest

Nonoperative procedures:

1. Chest radiographs:

2. Blood sampling:

3. Transfusion/bone marrow transplant:

4. Thymic Irradiation (TI)

5. Total Body Irradiation (TBI)

6. Drugs administered IM/IV/SC/PO or by gavage under sedation or general anesthesia

7. Immunosuppression:

Medications:

See flowchart for dosage, route and timing of administration of principal medications.

Immunosuppressive drugs:

1. Cyclosporine will be injected intramuscularly (volume of solution 0.1-0.6 ml using 25 G needle).

2. Tacrolimus will be injected intramuscularly (volume of solution 0.1-1.0 ml using 25 G needle)

3. Mycophenolate mofetil will be administered IM, PO in food, or by gavage under sedation.

4. Humanized anti-CD8 monoclonal antibody is administered IV under sedation or general anesthesia.

[redacted]

5. Equine ATG is administered IV under sedation or general anesthesia.

6. Anti-CD154 monoclonal antibody is administered IV under sedation or general anesthesia.

7. Anti-IL-6 antibody is administered IV under sedation or general anesthesia.

8. Methyprednisolone is given IV or IM. For IV administration, the animal is under sedation or general anesthesia. For IM administration, the volume of solution is 0.1-1.0 ml and a 25 G needle is used.

9. Tofacitinib is given PO in food or by gauvage under sedation

Commonly used antibiotics/antivirals:

1. Enrofloxacin (intramuscular): Enrofloxacin is a fluoroquinolone antibiotic with activity against gram-positive and gram-negative organisms. It is used prophylactic following potentially contaminated procedures at a dose of 5mg/kg for up to 14 days. It is also used at a dose of 10mg/kg for treatment of minor infections including surgical site cellulitis and thrombophlebitis. Failure to respond promptly to treatment with enrofloxacin will prompt a switch to vancomycin/cefepime.

2. Vancomycin (intravenous): Vancomycin is a glycopeptide antibiotic with activity against resistant gram-positive organisms. It will be administred in combination with cefepime for refractory infections. It will also be given as surgical prophylaxis and prophylatically (20mg/kg) every other day for animals with WBC counts below 1500 cells/uL. Animals with positive blood cultures will be treated with daily administration (25mg/kg) for a five-day course or until sterile blood cultures are obtained. During prolonged administration, trough levels will be checked and dosing adjusted to ensure a level of 15-20mcg/mL. The drug is given intravenously mixed with 100cc of normal saline.

3. Cefepime (intramuscular): Cefepime is a fourth-generation cephalosporin antibiotic with extended spectrum against gram-positive and gram-negative bacteria. It will be administred in combination with vancomycin for refractory infections (50mg/kg). It will also be given prophylatically every other day for animals with WBC counts below 1500 cells/uL. Animals with positive blood cultures will be treated with daily administration for a five-day course or until sterile blood cultures are obtained.

4. Ganciclovir (intravenous): Ganciclovir is used for prevention and treatment of cytomegalovirus. Prophylactic 5mg/kg doses are given prior to bone marrow transplantation and at the time of ATG administration. Subsequent doses are given as needed for animals that are at unique risk for CMV infection/re-activation or that develop CMV infections during immunosuppressive treatment.

Miscellaneous

[redacted]

1. Diphenhydramine (IM/IV): Diphenhydramine is an antihistamine given for treatment and prophylaxis of minor allergic responses (eg urticaria) to antibody and transfusion therapy. The dosing is 5mg/kg given IV or IM.

2. Ketorolac (IM/IV): Ketorolac may be given at the time of lung transplantation and prior to bone marrow transplantation to reduce the risk of graft thrombosis. The dosage is 5mg/kg/day IV/IM for up to a week.

4. Heparin (SC): Whenever the risk of graft thrombosis is deemed to be high, a small dose of this anticoagulant (10 units/kg) is given for up to one week.

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Animals will be observed for a maximum of 3 years.

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This field may contain information that has been migrated from **Insight 3.6.4, Tumor Production, question 4. "What are the experimental endpoints used for this tumor study?"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

C. Flow Chart: For this research objective, a schema or flow chart diagramming the overall picture of the study design and treatment groups must be included. The flow chart should include:

- all experimental groups
- the number of animals per group
- the procedures performed on the animal
- the length of time an animal is enrolled in the experiment

The IACUC must be able to understand the experience of each animal on the protocol. See TIPS for Creating Flow Charts in the FAQ pane for detailed information.

D. Health Status:

1. Describe the health status of the animals during this research objective. Include:

- Expected development and progression of clinical signs, including severity and time course
- Potential adverse events caused by the research model and/or experimental manipulations
- If a scoring system will be used to monitor animal health, please attach it to the protocol below.

Thoracotomy is well tolerated in multiple primate models. Animals generally return to normal activity and appetite within 1-3 days after surgery. The thoracotomy incision is essentially healed by 14 days post transplant. Although

[redacted]

the transplanted lung is functional, a single lung is adequate to sustain normal respiratory status and transplant recipients can live comfortably with a completely rejected, nonfunctional graft.

Failure is typically due to infectious complications during periods of immunosuppression. This is minimized by using aseptic surgical technique and prophylactic antibiotics. Possible infectious complications of the procedure and immunosuppressive regimens are as follows. During treatment peripheral leukocyte counts can decrease to less than 300/ mm3. Without antibiotics coverage, the animals may develop bacteremia which is typically susceptible to vancomycin and/or Cefipime. Therefore, vancomycin 20mg/kg iv every other day will be administered whenever WBC counts are less than 1500/ mm3. When animals develop infection (e.g. pneumonia, wound infection), appropriate procedures will be performed for diagnosis and decision on treatments will be made with CCM staff. Although it has been rare in the recipients treated with our regimens, CMV infection can occur in any recipient. Whenever it is suspected (pneumonia, gastrointestinal bleeding, high fever), gancyclovir 5mg/kg IV qD will be administered under sedation until clinical resolution.

Whenever any technical complications (bleeding or thrombosis of the allograft) are suspected, appropriate examinations (including radiography) will be performed for diagnosis and appropriate procedures will be determined with CCM staff (e.g. exploration, euthanasia etc.)

2. What action will be taken should clinical signs manifest?

Bupivacaine (0.5%) will be infiltrated into all surgical wounds at the time of surgery.

Buprenorphine (0.01 mg/kg) will be injected (IV/IM) at the outset of surgery (pre-emptive analgesia) and the every 10-14 hours for 72 hours. Additional buprenorphine q10 - 14 hours will be given if the animal appears to be in discomfort or pain, as judged by splinting, inactivity, anorexia, grimacing, vocalization, and/or protective or stereotypic behaviors.

As an alternative pain medication regimen, We will use buprenorphine-SR to be given once at the time of surgery at a dose of 0.1-0.2 mg/kg SC. Animals that show signs of pain or discomfort (increased respiratory rate, increased heart rate, guarding of incision, inactivity, etc.) will be provided with buprenorphine 0.005-0.01mg/kg IM.

If pain or distress cannot be relieved, the animal will be euthanized according to AVMA guidelines.

[redacted]

Migrated Data

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At the conclusion of all surgical procedures, the animal is allowed to recover from anesthesia in the operating room. When breathing spontaneously, the endotracheal tube is withdrawn and the animal returned to its cage, where it is monitored continuously until fully alert. The animal is then monitored q 10-14 hrs for 72 hours. Subsequently, it is monitored daily unless clinical condition deteriorates.

Daily veterinary supervision will be provided by the [redacted] CCM staff. In addition, the Principal Investigator has been involved for more than 15 years in development of the procedures utilized in this project and has extensive experience with the individual problems and needs of these valuable experimental animals. At nights and weekends, the postoperative care of these animals will be maintained by a rotation of the investigators involved in the project, and will include 24-hour care if determined to be necessary by the principal investigator or veterinarian.

If oral intake is deemed inadequate at any time (fewer than 10 biscuits per day for more than three days, weight loss lasting more than one week, or >10% pre-transplant body mass, or other concerns by veterinary staff) then supplementation with Ensure nutritional supplement and crushed biscuits will be given by gavage under sedation, as frequently as deemed necessary by the veterinary staff. An 8Fr orogastic tube will be used and correct placement of this tube will be confirmed by aspiration of gastric juices.

Animals are supported as needed throughout the protocol with irradiated blood products to maintain the hematocrit greater than 20% and the platelet count greater than 20,000/mm3. Monkeys in the colony may serve as blood donors. Their hematocrit is checked before each phlebotomy. If it is greater than 35%, 25 to 35 cc of blood will be withdrawn for use as a transfusion.

Antibiotics are administered if there is suspicion of infection, and are adjusted on the basis of culture results.

Blood for monitoring of allograft function and hematologic parameters is obtained via venipuncture under sedation. One to ten milliliters is drawn up to 3X weekly.

[redacted]

Attachments

Name	Mode
Tolerance Induction Regimen _1 (Flowchart)	Electronic
Flowchart Tofacitinib 2 and 4 month (Flowchart)	Electronic
Tolerance Induction Regimen _2 (Flowchart)	Electronic

Tolerance Induction Regimen 1

Day	Date	Treatment/Procedure
-2		Equine ATG (50mg/kg/d) IV
-1		Equine ATG (50mg/kg/d) IV
0	Lung Transplantation	Equine ATG (50mg/kg/d) IV Anti-IL6R Ab (10mg/kg) IV FK (0.1mg/kg) BID MP 40mg IV
1		FK (0.1mg/kg) BID MP 40mg IM Mycophenolatmofetil 200mg BID
2		FK (0.1mg/kg) BID MP 20mg IM Mycophenolatmofetil 200mg BID
3		FK (0.1mg/kg) MP 20mg IM Mycophenolatmofetil 200mg BID
4		FK (0.1mg/kg) MP 10mg IM Mycophenolatmofetil 200mg/d
5		Anti-IL6R Ab (10mg/kg) IV FK (0.1mg/kg) MP 10mg IM Mycophenolatmofetil 200mg/d
6		FK (0.1mg/kg) MP 8mg IM Mycophenolatmofetil 200mg/d
7		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 8mg IM Mycophenolatmofetil 200mg/d
8		FK (adjusted to serum target 20-30ng/ml) MP 6mg IM Mycophenolatmofetil 200mg/d
9		FK (adjusted to serum target 20-30ng/ml) MP 6mg IM Mycophenolatmofetil 200mg/d
10		FK (adjusted to serum target 20-30ng/ml) MP 4mg IM Mycophenolatmofetil 200mg/d
11		FK (adjusted to serum target 20-30ng/ml) MP 4mg IM Mycophenolatmofetil 200mg/d
12		FK (adjusted to serum target 20-30ng/ml) MP 2mg IM

Tolerance Induction Regimen 1

		Mycophenolatmofetil 200mg/d
13		FK (adjusted to serum target 20-30ng/ml) MP 2mg IM Mycophenolatmofetil 200mg/d
14		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 2mg IM Mycophenolatmofetil 200mg/d
28		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 1mg IM Mycophenolatmofetil 200mg/d
19 - 112		FK (adjusted to serum target 20-30ng/ml) MP 1mg IM Mycophenolatmofetil 200mg/d
60-100		Lung biopsy
113	TBI (1.25 Gy)	Ganciclovir (5mg/kg) IV Mycophenolatmofetil 200mg/d
114	TBI (1.25 Gy)	Mycophenolatmofetil 200mg/d
117	-2	Thymoglobulin (10mg/kg) IV (Rabbit ATG) Ganciclovir (5mg/kg) IV MP 4mg IM Mycophenolatmofetil 200mg/d
118	TI (7.0 Gy) -1	Thymoglobulin (10mg/kg) IV (Rabbit ATG) MP 4mg IM Mycophenolatmofetil 200mg/d
119	BMT (0)	Belatacept (20mg/kg) IV MP 2mg IM

Day	Day post BMT	Treatment/Procedure
120-147		**CyA** IM aim for trough level of 200-300ng/mL Weekly blood draws to assess therapeutic CyA
121	**2**	**Belatacept (20mg/kg) IV** **Anti-IL6R Ab (10mg/kg) IV**
124	**5**	**Belatacept (20mg/kg) IV**
127		**Anti-IL6R Ab (10mg/kg) IV**
134	**15**	**Belatacept (20mg/kg) IV** **Anti-IL6R Ab (10mg/kg) IV**
141		**Anti-IL6R Ab (10mg/kg) IV**
>180		Lung biopsy

>270		Skin graft
>290		Skin graft biopsy (punch)
≥400		Euthanasia

*Chest radiographs will be performed weekly after lung transplantation.

Donor:

Day 0: Donor lung harvest with post-mortem skin and bone marrow harvest

Tolerance Induction Regimen 3

4 month Delay			2 month Delay		
Day	**Date**	**Treatment/Procedure**	**Day**	**Date**	**Treatment/Procedure**
-2		Equine ATG (50mg/kg/d) IV 5mg/kg Gancyclovir	-2		Equine ATG (50mg/kg/d) IV 5mg/kg Gancyclovir
-1		Equine ATG (50mg/kg/d) IV FK (0.1mg/kg) BID Mycophenolatmofetil 300mg	-1		Equine ATG (50mg/kg/d) IV FK (0.1mg/kg) BID Mycophenolatmofetil 300mg
0	Lung Transplantation	Equine ATG (50mg/kg/d) IV Anti-IL6R Ab (10mg/kg) IV FK (0.1mg/kg) BID MP 40mg IV	0	Lung Transplantation	Equine ATG (50mg/kg/d) IV Anti-IL6R Ab (10mg/kg) IV FK (0.1mg/kg) BID MP 40mg IV
1		FK (0.1mg/kg) BID MP 40mg IM Mycophenolatmofetil 300mg	1		FK (0.1mg/kg) BID MP 40mg IM Mycophenolatmofetil 300mg
2		FK (0.1mg/kg) BID MP 20mg IM Mycophenolatmofetil 300mg	2		FK (0.1mg/kg) BID MP 20mg IM Mycophenolatmofetil 300mg
3		FK (0.1mg/kg) MP 20mg IM Mycophenolatmofetil 300mg	3		FK (0.1mg/kg) MP 20mg IM Mycophenolatmofetil 300mg
4		FK (0.1mg/kg) MP 10mg IM Mycophenolatmofetil 200mg/d	4		FK (0.1mg/kg) MP 10mg IM Mycophenolatmofetil 200mg/d
5		Anti-IL6R Ab (10mg/kg) IV FK (0.1mg/kg) MP 10mg IM Mycophenolatmofetil 200mg/d	5		Anti-IL6R Ab (10mg/kg) IV FK (0.1mg/kg) MP 10mg IM Mycophenolatmofetil 200mg/d
6		FK (0.1mg/kg) MP 8mg IM Mycophenolatmofetil 200mg/d	6		FK (0.1mg/kg) MP 8mg IM Mycophenolatmofetil 200mg/d
7		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 8mg IM Mycophenolatmofetil 200mg/d	7		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 8mg IM Mycophenolatmofetil 200mg/d
8		FK (adjusted to serum target 20-30ng/ml) MP 6mg IM Mycophenolatmofetil 200mg/d	8		FK (adjusted to serum target 20-30ng/ml) MP 6mg IM Mycophenolatmofetil 200mg/d

9		FK (adjusted to serum target 20-30ng/ml) MP 6mg IM Mycophenolatmofetil 200mg/d	9		FK (adjusted to serum target 20-30ng/ml) MP 6mg IM Mycophenolatmofetil 200mg/d
10		FK (adjusted to serum target 20-30ng/ml) MP 4mg IM Mycophenolatmofetil 200mg/d	10		FK (adjusted to serum target 20-30ng/ml) MP 4mg IM Mycophenolatmofetil 200mg/d
11		FK (adjusted to serum target 20-30ng/ml) MP 4mg IM Mycophenolatmofetil 200mg/d	11		FK (adjusted to serum target 20-30ng/ml) MP 4mg IM Mycophenolatmofetil 200mg/d
12		FK (adjusted to serum target 20-30ng/ml) MP 2mg IM Mycophenolatmofetil 200mg/d	12		FK (adjusted to serum target 20-30ng/ml) MP 2mg IM Mycophenolatmofetil 200mg/d
13		FK (adjusted to serum target 20-30ng/ml) MP 2mg IM Mycophenolatmofetil 200mg/d	13		FK (adjusted to serum target 20-30ng/ml) MP 2mg IM Mycophenolatmofetil 200mg/d
14		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 2mg IM Mycophenolatmofetil 200mg/d	14		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 2mg IM Mycophenolatmofetil 200mg/d
28		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 1mg IM Mycophenolatmofetil 200mg/d	28		Anti-IL6R Ab (10mg/kg) IV FK (adjusted to serum target 20-30ng/ml) MP 1mg IM Mycophenolatmofetil 200mg/d
19 - 112		FK (adjusted to serum target 20-30ng/ml) MP 1mg IM Mycophenolatmofetil 200mg/d	19 – 55/56		FK (adjusted to serum target 20-30ng/ml) MP 1mg IM Mycophenolatmofetil 200mg/d
60-110		Lung biopsy	30-53		Lung Biopsy

Tolerance Induction Regimen 3

112-113	TBI (1.25 Gy)	Ganciclovir (5mg/kg) IV Mycophenolatmofetil 200mg/d	56-57	TBI (1.25 Gy)	Ganciclovir (5mg/kg) IV Mycophenolatmofetil 200mg/d
113-114	TBI (1.25 Gy)	Mycophenolatmofetil 200mg/d	57-58	TBI (1.25 Gy)	Mycophenolatmofetil 200mg/d
116-117	-2	Equine ATG (50mg/kg/d) IV Ganciclovir (5mg/kg) IV MP 4mg IM Mycophenolatmofetil 200mg/d	60-61	-2	Equine ATG (50mg/kg/d) IV Ganciclovir (5mg/kg) IV MP 4mg IM Mycophenolatmofetil 200mg/d
117-118	TI (7.0 Gy) -1	Equine ATG (50mg/kg/d) IV MP 4mg IM Mycophenolatmofetil 200mg/d	61-62	TI (7.0 Gy) -1	Equine ATG (50mg/kg/d) IV MP 4mg IM Mycophenolatmofetil 200mg/d
118-119	BMT (0)	Equine ATG (50mg/kg/d) IV MP 2mg IM Anti-CD154 (20mg/kg) Ketorolac 3mg **Anti-IL6R Ab (10mg/kg) IV**	62-63	BMT (0)	Equine ATG (50mg/kg/d) IV MP 2mg IM Anti-CD154 (20mg/kg) Ketorolac 3mg **Anti-IL6R Ab (10mg/kg) IV**

4 month Delay			2 month Delay		
Day	**Day post BMT**	**Treatment/Procedure**	**Day**	**Day post BMT**	**Treatment/Procedure**
119/120-147	**1-27**	**CyA** IM aim for trough level of 200-300ng/mL Weekly blood draws to assess therapeutic CyA	**63/64-89/90**	**1-27**	**CyA** IM aim for trough level of 200-300ng/mL Weekly blood draws to assess therapeutic CyA
119-133	**0-14**	**Tofacitinib 0.1mg/kg BID**	**62/63-76/77**	**0-14**	**Tofacitinib 0.1mg/kg BID**
124	**2**	**Anti-IL6R Ab (10mg/kg) IV,** Anti-CD154 (20mg/kg) Ketorolac 3mg	**64/65**	**2**	**Anti-IL6R Ab (10mg/kg) IV,** Anti-CD154 (20mg/kg) Ketorolac 3mg
125	**5**	Anti-CD154 (10mg/kg) Ketorolac 3mg	**67/68**	**5**	Anti-CD154 (10mg/kg) Ketorolac 3mg
127	**7**	**Anti-IL6R Ab (10mg/kg) IV,** Anti-CD154 (10mg/kg) Ketorolac 3mg	**69/70**	**7**	**Anti-IL6R Ab (10mg/kg) IV,** Anti-CD154 (10mg/kg) Ketorolac 3mg
129	**9**	Anti-CD154 (10mg/kg) Ketorolac 3mg	**71/72**	**9**	Anti-CD154 (10mg/kg) Ketorolac 3mg
131	**12**	Anti-CD154 (10mg/kg) Ketorolac 3mg	**74/75**	**12**	Anti-CD154 (10mg/kg) Ketorolac 3mg
134	**15**	**Anti-IL6R Ab (10mg/kg) IV**	**77/78**	**15**	**Anti-IL6R Ab (10mg/kg) IV**

141	**22**	**Anti-IL6R Ab (10mg/kg) IV**	**84/85**	**22**	**Anti-IL6R Ab (10mg/kg) IV**
>180		Lung biopsy	**>120**		Lung biopsy
>270		Skin graft	**>210**		Skin graft
>290		Skin graft biopsy (punch)	**>230**		Skin graft biopsy (punch)
≥400		Euthanasia	**≥400**		Euthanasia

*Chest radiographs will be performed weekly after lung transplantation.

Following Bone Marrow Transplantation and Conditioning, the monkeys will receive prophylactic antibiotic treatment (20mg/kg Vancomycin and 50mg/kg Cefepime) three times a week as long as the WBC is <2 $*10^3$/µl.

As an antiviral prophylaxis, monkeys will receive 5mg/kg Ganciclovir IM daily following Bone Marrow Transplantation until d50pBMT as soon as the WBC has recovered.

Donor:

Day 0: Donor lung harvest with post-mortem skin and bone marrow harvest

Tolerance Induction Regimen 2

Recipient:

Day	Date	Treatment/Procedure
-1		Rapamycin (0.2 mg/kg) IM (10-15 ng/mL)
0	**Lung Transplantation**	**Anti-CD40 Ab (50mg/kg) IV** **Anti-IL6R Ab (10mg/kg) IV** Rapamycin (0.2 mg/kg) IM MP 40mg IV
1		Rapamycin (0.2 mg/kg) IM MP 40mg IM
2		**Anti-CD40 Ab (20mg/kg) IV** Rapamycin (0.2 mg/kg) IM
3		Rapamycin (0.2 mg/kg) IM
4		Rapamycin (0.1 mg/kg) IM
5		**Anti-CD40 Ab (20mg/kg) IV** **Anti-IL6R Ab (10mg/kg) IV** Rapamycin (0.1 mg/kg) IM
6		Rapamycin (0.04 mg/kg) IM
7		**Anti-CD40 Ab (20mg/kg) IV** Rapamycin IM (10-15 ng/mL)
8		Rapamycin IM (10-15 ng/mL)
9		**Anti-CD40 Ab (20mg/kg) IV** Rapamycin IM (10-15 ng/mL)
10		Rapamycin IM (10-15 ng/mL)
11		Rapamycin IM (10-15 ng/mL)
12		**Anti-CD40 Ab (20mg/kg) IV** Rapamycin IM (10-15 ng/mL)
19 - 112		**Anti-CD40 Ab (10mg/kg) IV; once a week until BMT** Rapamycin IM (10-15 ng/mL)
60-100		Lung biopsy
113	**TBI (1.25 Gy)**	Ganciclovir (5mg/kg) IV Rapamycin IM (10-15 ng/mL)
114	**TBI (1.25 Gy)**	Rapamycin IM (10-15 ng/mL)
117		**Thymoglobulin (10mg/kg) IV** **Anti-CD40 Ab (10mg/kg) IV** Ganciclovir (5mg/kg) IV MP 4mg IM
118	**TI (7.0 Gy)**	**Thymoglobulin (10mg/kg) IV** MP 4mg IM
119	**BMT**	**Belatacept (20mg/kg) IV** MP 2mg IM

Day	Date	Treatment/Procedure
120-147		**CyA** IM aim for trough level of 200-300ng/mL Weekly blood draws to assess therapeutic CyA
121		**Belatacept (20mg/kg) IV**
125		**Belatacept (20mg/kg) IV**
135		**Belatacept (20mg/kg) IV**
>180		Lung biopsy
>270		Skin graft
>290		Skin biopsy
≥400		Euthanasia

*Chest radiographs will be performed weekly after lung transplantation.

Donor:

Day 0: Donor lung harvest with post-mortem skin and bone marrow harvest

[redacted]

Animals

The IACUC restricts protocols to a single species only. If the protocol will require xenografts, identify the donor species, and the applicable protocol number, in the appropriate **Research Objective** section of the protocol.

1. Select a species from the drop down list:
NHP- Macaque, Cyno

---

2. Do any of the animals have a genetic alteration and/or phenotype that is expected to have any impact on animal health and/or requirements for animal care?

O Yes ◉ No

---

3. Animal Source
Select all that apply:

☑ Animals will be acquired from an approved vendor - no quarantine is required. See FAQ for institution-specific approved vendors.
☐ Animals will be acquired through import. See FAQ for institution-specific procedures.
☐ Animals will be bred as part of this protocol
☐ Animals will be transferred from another protocol at this institution
☐ Animals will be acquired from an outside institution

---

4. Sex

◉ Male O Female O Both

If you will be using one sex only, please explain:
Males are more readily available as females are reserved for breeding.

---

5. Indicate the method(s) of identification that will be used to track these animals *(select all that apply)*:

☐ Implant/microchip (See FAQ for SOP)
☐ Ear tag or notch (See FAQ for SOP)
☑ Tattoos (See FAQ for SOP)
☐ Collar
☐ Cage card
☐ Other

[redacted]

6. The species chosen is appropriate because *(select all that apply)*:

☑ The process resembles that in humans
☑ Prior research has been conducted in this species
☑ Tissues and/or other substances needed are best/uniquely provided be this species
☑ Species lower on the phylogenetic scale cannot be used
☑ The size or anatomy of this species is best/uniquely suited to the procedure(s)
☑ Tissues and/or other substances to be harvested require an animal of this size
☐ Other

Potential Pain and Distress

1. Total number of animals requested for this three-year approval
Enter the number of animals in each pain and distress category. Each animal must be assigned to a category based on the most invasive procedure or the procedure that has the greatest potential to cause pain or distress. See FAQ for definitions and examples.

- If an animal will be used in more than one **Research Objective**, count it only once, in the highest pain category that it will experience.
- If animals are bred in-house, include the progeny that may be culled. Progeny used for experiments should be counted in the specific **Research Objectives**. All other animals should be counted in **Other** as follows:

	Category
Breeders	B
Progeny culled without genotyping	B
Progeny culled after genotyping (<21 days old)	C
Progeny culled after genotyping (>21 days old)	D

TOTAL NUMBER OF ANIMALS REQUESTED
USDA Pain & Distress Category (See FAQ for information)

Animals	B	C	D	E	Total
Research Objective 1	0	0	38	0	38
Other (e.g. breeding, training):					
Total requested	0	0	38	0	38
Animals currently in house					
Total approved for purchase	0	0	38	0	38

2. Justification for the number of animals requested (select all that apply):

[redacted]

☐ Power analyses indicated that the proposed sample size, number of groups and/or number of experiments is the lowest required for statistically valid tests of the hypothesis (i.e., 80% power with 0.05 type I error).
☐ Differences from controls are expected to be small, and large sample sizes are necessary to distinguish differences reliably.
☐ Based on previous and/or published data, the numbers of animals requested are the minimum needed to achieve sufficient statistical power.
☐ These animals will be used to produce antibodies or tissues, and numbers are based on yield.
☐ The numbers of animals or group sizes have been established by federal guidelines/requirements.
☐ This is a pilot/feasibility study that uses the minimum number of animals required to provide meaningful, but not statistically significant data (i.e., model development).
☐ This model involves breeding of genetically modified rodents. Based on Mendelian genetics, it is expected that ¼ of all pups will be homozygous and ¼ will be wild type, with the remaining ½ heterozygous. The homozygous and wild type mice will be used to generate data for the experiment, and the heterozygotes will be used to replace the breeding stock or will be euthanized.
☑ Other

Please describe:
Please see attached document - Sample Size Calculation

The following tools can be used to determine minimum sample size:

- Sample Size Calculations in Animal Research (W. W. LaMorte, BUMC)
- ClinCalc Sample Size Calculator
- Jackson Laboratories Breeding Colony Size Planning Worksheet

---

3. Does the number of animals requested include extra animals to cover anticipated failures or to train or familiarize the staff with the procedures described?

O Yes ◉ No

0

Replacement, Reduction, and Refinement

The 3 Rs – replacement, reduction, and refinement – represent a practical strategy for researchers to apply when considering the use of animals in research and in designing humane animal research studies. Government policy and regulatory agencies require the IACUC to assure that researchers consider the 3 Rs when preparing research protocols.

- The Guide for the Care and Use of Laboratory Animals

[redacted]

- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
- USDA Policies 11 and 12

1. Alternatives to Animal Models

☑ Mathematical models are not a suitable alternative to live animals
☑ Computer simulations (in silico models) are not a suitable alternative to live animals
☑ In vitro biological systems are not a suitable alternative to live animals
☐ Other

---

2. Duplication of Research

Unnecessarily duplicative research should be avoided for scientific and ethical reasons. Have the results fulfilling the experimental goals of this study been published in medical, scientific, or veterinary journals?

○ Yes ◉ No

---

3. Search for Alternatives to Painful and/or Distressful Procedures

A literature search for alternative procedures must be performed for each procedure that has the potential to cause pain or distress, including prolonged use of restraint devices. Along with the literature search, consultation with experts in the field and attendance at scientific or professional meetings can be used to identify alternatives to painful and/or distressful procedures.

a. Indicate resources used to search for alternatives to painful and/or distressful procedures. In addition to the selections below, other useful resources can be found at IACUC Central , the [redacted] Office of Laboratory Animal Welfare, and the USDA Animal Welfare Information [redacted]

☐ Medline (http://library.massgeneral.org/)
☑ Pubmed (http://library.massgeneral.org/)
☐ Agricola (https://agricola.nal.usda.gov/)
☐ ALTBIB (https://toxnet.nlm.[redacted]gov/altbib.html)
☐ ALTWEB (http://altweb.jhsph.edu/)
☐ Animal Welfare Institute (https://awionline.org/)
☐ Google Scholar (http://scholar.google.com/)
☐ Other databases (please list):
☐ Consultation with experts with knowledge of alternatives within this specific field. Provide name(s) and qualifications/credentials, date, and content of the consultation.
☐ Scientific/professional meetings attended to remain current with pertinent information regarding alternatives in this specific field. Provide meeting name, date, and relevant topic.

[redacted]

b. Indicate the date the literature search was completed. The search must be conducted within the last 6 months.

Click here to enter a date.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Literature Search, Refine, Question Bii, Date of Literature Search**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Literature Search Date** field above. Literature search dates for field above for all migrated protocols were defaulted to the date of migration. ***Use of this information is optional.***

3/8/17

c. Indicate the time period surveyed in the literature search:

1960-present

d. Indicate the procedure(s) and keyword(s) searched for each potentially painful or distressful procedure or condition described in this protocol.

Procedure	Keywords
Procedure 10: Irradiation	Keywords: Irradiation, monkey
Procedure 2: Donor pnumonectomy via sternotomy	Keywords: pneumonectomy, sternotomy, monkey
Procedure 7: Skin biopsy	Keywords: skin biopsy, monkey
Procedure 1: Orthotopic single lung transplant via thoracotomy	Keywords: lung, transplant, thoracotomy, monkey
Procedure 8: Immunosuppression	Keywords: immunosuppression, monkey
Procedure 4: Superficial lymph node biopsy	Keywords: lymph node, biopsy, monkey
Procedure 6: Skin harvest	Keywords: skin harvest, monkey
Procedure 9: Bone marrow biopsy	Keywords: bone marrow, biopsy, monkey
Procedure 3: Open lung biopsy via limited thoracotomy	Keywords: lung, biposy, thoracotomy, monkey
Procedure 11: Kidney harvest	Keywords: Kidney harvest, laparotomy, monkey
Procedure 5: Skin grafting	Keywords: skin grafting, monkey

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Literature Search, Refine, Question Biv: "Indicate the procedure and keyword(s) used"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Literature Search** Date field above. ***Use of this information is optional.***

Procedure 1: Orthotopic single lung transplant via thoracotomy

[redacted]

Keywords: lung, transplant, thoracotomy, monkey

Procedure 2: Donor pnumonectomy via sternotomy

Keywords: pneumonectomy, sternotomy, monkey

Procedure 3: Open lung biopsy via limited thoracotomy

Keywords: lung, biposy, thoracotomy, monkey

Procedure 4: Superficial lymph node biopsy

Keywords: lymph node, biopsy, monkey

Procedure 5: Skin grafting

Keywords: skin grafting, monkey

Procedure 6: Skin harvest

Keywords: skin harvest, monkey

Procedure 7: Skin biopsy

Keywords: skin biopsy, monkey

Procedure 8: Immunosuppression

Keywords: immunosuppression, monkey

Procedure 9: Bone marrow biopsy

Keywords: bone marrow, biopsy, monkey

Procedure 10: Irradiation

Keywords: Irradiation, monkey

Procedure 11: Kidney harvest

Keywords: Kidney harvest, laparotomy, monkey

e. Results of the Search for Alternatives to Painful and/or Distressful Procedures

☑ The literature search conducted indicates that there are no alternative procedures that would involve less pain or distress.

☐ There are alternative procedures, however, they cannot be used for these experiments.

If there are any relevant citations or other documents that are needed to support this search for alternatives, please attach them to this form.

[redacted]

Humane Endpoint Disposition and Euthanasia

A. Humane Endpoints

Mammals:

- Persistent recumbence; inability to rise; loss of righting reflex
- Pain or distress that cannot be alleviated by analgesics
- Difficulty with ambulation (paralysis, fractures, trauma, etc.)
- Severe central nervous system signs (e.g., circling, rolling, persistent seizures or convulsions)
- Abnormal breathing (dyspnea) and cyanosis
- Body condition score of 2 (out of 5) or less (see FAQ for links to species-specific body condition scoring charts)
- Excessive weight loss (see institution-specific guidelines)
- Vomiting/diarrhea resulting in severe dehydration
- Tumor production specific endpoints (see FAQ for links to institution-specific guidelines)
- Other model-specific endpoints (please describe)

Please choose:

◉ Animals will be removed from the study and euthanized if any of the above clinical signs/conditions are found

O Some or all of the criteria listed above cannot apply to this study. Animals will be euthanized if the following criteria are met.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 5. Endpoints**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

All monkeys in this study will be maintained for long-term survival. (3 years) The endpoint of the experiment is graft failure. If the transplanted lung is rejected the animal will be euthanized. Euthanasia will also be performed if any unalleviated pain or distress occurs. The following conditions will generally be considered to be indications for euthanasia, although each animal will be consided in a broader context in conjunction with the veterinary team:

1) Technical failure without possibility of surgical correction.

2) Other postoperative complications without possibility of recovery.

3) Radiographic or histologic evidence of complete graft rejection.

[redacted]

4) Clinically severe pneumonia not responsive to broad-spectrum antibiotic therapy.

6) Body weight loss > 15%. Animals will be weighed each time they are sedated. We anticipate that this will be at least weekly. Otherwise healthy-appearing animals will not be sedated for the purposes of weighing alone unless directed by CCM staff.The weights and body conditions of all of our animals are discussed weekly with the veterinary staff, and decisions about nutritional support and euthanasia are made on an individual basis based on the veterinarians recommendation. Any weight loss greater than 15% will be relayed immediately to the veterinarian for consideration of euthanasia or additional supportive measures at the veterinarians discretion.

7) Animal found to be moribund.

In addition, the veterinary staff have the authority to euthanize any animal that they feel is experiencing any unalleviated pain/distress.

Migration Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 5. "Please describe other endpoints"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** field above. ***Use of this information is optional.***

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Tumor Form, Question 5. "Indicate other humane endpoints used"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

B. Moribundity and Mortality

The IACUC acknowledges that some studies may require moribundity (a clinically irreversible condition leading inevitably to death) or mortality (a fatal outcome) as an endpoint. The committee recommends that consideration be given to surrogate markers that can be utilized for a more humane endpoint, such as serial imaging or biomarkers that may permit the detection of experimental endpoints that precede the development of significant clinical signs, rather than allowing the animal to proceed to moribundity or mortality.

The use of death as an endpoint is strongly discouraged and requires scientific justification Rationale

[redacted]

1. Will this protocol include models with severe clinical signs expected?

O Yes ◉ No

2. Will this protocol use death as an endpoint?

O Yes ◉ No

C. Animal Transfer and Disposition

Select all that apply:

☑ Euthanasia or Terminal Procedure
☐ Transfer to another protocol at this institution (see FAQ for institution-specific guidelines)
☐ Transfer to another institution
☐ Release (field studies only)
☐ Animals may be considered for adoption.
☐ Animals may be considered for retirement.

D. Euthanasia Method

Euthanasia methods must be consistent with the AVMA Guidelines for the Euthanasia of Animals, 2013 edition. See FAQ for institution-specific guidelines/SOPs.

• A method must be indicated even if the protocol procedures are not terminal, for use in the event of an emergency.
• [redacted] protocols only: A secondary physical method to confirm euthanasia by carbon dioxide overdose or Isoflurane anesthesia overdose is recommended, but not required.

Species:

NHP- Macaque, Cyno

☑ Pentobarbital euthanasia solution (Euthasol, Fatal Plus, etc.); 100 mg/kg IV (0.22 mL/kg IV)
☐ Pentobarbital anesthetic overdose; 100 mg/kg pentobarbital IV
☑ General anesthesia, followed by a non-survival surgery or exsanguination. *Please complete a Procedure form to cover this method of euthanasia.
☐ Other

Will a sedative, tranquilizer, or anesthetic be administered prior to euthanasia?

◉ Yes O No

Provide the requested information for each agent (**Agent, Dose, Route**).

[redacted]

Agent	Dose	Route
Sodium pentobarbital	100mg/kg	IV

Migrated Data for [redacted] Protocols Only

This filed may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoint, Question 6.c. "If you plan to deviate from the approved [redacted] CCM Euthanasia SOPs, describe the euthanasia method"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

Housing [redacted]

I. HOUSING LOCATIONS

A. CCM Centralized Facilities
Select all applicable housing areas.

[redacted]

B. Investigator-Managed Facilities or Satellite/Laboratory Housing Areas

- Please note that permission to house animals in investigator-managed centralized facilities must be obtained from the appropriate satellite facility manager. See FAQ for contact information.
- All new satellite/laboratory housing areas must be inspected and approved by the IACUC and the Center for Comparative Medicine. Research cannot be conducted until the area has been inspected and notification of approval has been received.

Select applicable housing areas.

☐ [redacted]
☐ [redacted]

☐ Other IACUC approved satellite/laboratory housing area

☐ New satellite/laboratory housing area

C. Offsite Housing

[redacted]

All offsite housing locations must be inspected and approved by the IACUC and the Director, Center for Comparative Medicine. Animals may not be housed in a new location until it has been inspected and notification of approval has been received.

☐ [redacted]
☐ Other

II. SPECIAL HANDLING, HUSBANDRY, OR HOUSING REQUIREMENTS

Will the animals on this protocol require any special handling, husbandry, or housing requirements? This includes anything outside of normal routine husbandry/handling services utilized by CCM, as defined in the species specific SOPs (e.g., alterations in bedding types, cage change frequencies, housing densities, special diets/fluids, deviations from currently approved IACUC policies, etc). See Species Specific Social Housing SOPs for more information.

Please discuss all special handling, husbandry, or housing requirements with CCM facility managers and/or veterinarians.

☐ No special housing or husbandry is required

☐ Breeding (i.e., delayed weaning requirements, harem breeding strategies, etc.)

☑ Immunocompromised

Describe the special housing and/or husbandry requirements.
Single housing is required during phases of the study where the recipient is receiving immunosuppression (see each protocol's flowchart for specifics)

☐ Genetically modified animals (includes knock-outs, knock-ins, and transgenics)

☐ Specialized diet or fluid

☐ Alteration of cage / pen change frequency

☐ Alteration of light cycle

☐ Alteration of temperature and/or humidity

☐ Non-standard caging (e.g., metabolic cages, raised floor)

[redacted]

☐ Other

Exemptions from the Environmental Enhancement Program that are defined and approved by the IACUC Policy on Environmental Enrichment, Social housing and Exercise of Laboratory Animals do not need to be described in the protocol. A flow chart detailing the social housing policy is available to assist in the determination if planned single housing is covered by the policy.

☑ Non-social housing of social animals

Identify the periods of time that animals are singly housed (one animal per cage, pen, or tank) and provide scientific justification.

Single housing is required during phases of the study where the recipient is receiving immunosuppression (see each protocol's flowchart for specifics)

☐ Withholding all cage, pen, or tank environmental enrichment

☐ Exemption from canine exercise program

If there are any relevant citations or other documents that are needed to support these special housing, husbandry, or handling requirements, please attach them to this form.

Anesthesia Regimen: Sedation

Please assign a label for this anesthesia regimen (e.g. Isoflurane Option, Surgical – Minor Procedure, Imaging Sedation, etc.). This label will be used in dropdown lists for other forms in this protocol.

Sedation

1. Enter the agents that will be used for this anesthesia regimen. Include sedatives, paralytic agents, and anesthetic reversal agents. Do not include local anesthetics or other drugs used for analgesia. **See FAQ pane for institution-specific formularies.**

Agent	Dose	Route	Frequency
medetomadine	0.03-0.06 mg/kg	IV/IM	PRN
Ketamine	3-5 mg/kg	IV/IM	PRN

2. Are any of the agents listed paralytics?

[redacted]

◉ Yes O No

a. Indicate the agent:
vecuronium 0.01-0.1mg/kg IV q30' prn

b. Provide the justification for using a paralytic agent:
Vecuronium will be used intermittently to control respirations during surgery, and to limit the total dose of isoflurane.

c. Describe the monitoring methods to be used to ensure that an adequate level of anesthesia is maintained during paralysis:
The depth of anesthesia is monitored by observing hemodynamic parameters (heart rate, blood pressure) which are not affected by this agent. At no time will paralytics be used on a conscious animal.

d. How will ventilation be maintained while the animal is under the effects of the paralytic agent?
ventilator

---

3. The IACUC requires that all anesthetics administered to any animal species be of pharmaceutical grade (USP grade), if that agent is available in pharmaceutical grade. Are all agents in this anesthetic regimen of pharmaceutical grade (USP grade)? **See FAQ for definition of pharmaceutical grade.**

◉ Yes O No

Species:
NHP- Macaque, Cyno

4. The adequacy or depth of anesthesia will be monitored by (select all that apply):

☑ Respiratory rate
☐ Heart rate
☐ Temperature
☐ Jaw tone (major procedures)
☐ Toe pinch
☐ Palpebral (blink) reflex
☑ Muscle relaxation
☐ Pulse oximetry (major procedures)
☐ Mucus membrane color (major procedures)
☐ Blood pressure (major procedures)
☐ Other (please describe):

[redacted]

5. How frequently will the depth of anesthesia be assessed?

See the Policy on Anesthesia and Analgesia for documentation guidelines for USDA-regulated and non-regulated species.

q 5 min

6. How will this assessment be documented and with what frequency?

q 15 min

Anesthesia Regimen: General Anesthesia

Please assign a label for this anesthesia regimen (e.g. Isoflurane Option, Surgical – Minor Procedure, Imaging Sedation, etc.). This label will be used in dropdown lists for other forms in this protocol.

General Anesthesia

1. Enter the agents that will be used for this anesthesia regimen. Include sedatives, paralytic agents, and anesthetic reversal agents. Do not include local anesthetics or other drugs used for analgesia. **See FAQ pane for institution-specific formularies.**

Agent	Dose	Route	Frequency
Vecuronium	0.01 - 0.1 mg/kg	IV	q 30in PRN
isoflurane	0.25-5%	inhalation	continuous
Medetomadine	(0.03-0.06 mg/kg	IM/IV	once
Ketamine	3-5 mg/kg	IM/IV	once

2. Are any of the agents listed paralytics?

◉ Yes O No

a. Indicate the agent:

vecuronium 0.01-0.1mg/kg IV q30' prn

b. Provide the justification for using a paralytic agent:

Vecuronium will be used intermittently to control respirations during surgery, and to limit the total dose of isoflurane.

c. Describe the monitoring methods to be used to ensure that an adequate level of anesthesia is maintained during paralysis:

The depth of anesthesia is monitored by observing hemodynamic parameters (heart rate, blood pressure) which are not affected by this agent. At no time will paralytics be used on a conscious animal.

[redacted]

d. How will ventilation be maintained while the animal is under the effects of the paralytic agent?

ventilator

---

3. The IACUC requires that all anesthetics administered to any animal species be of pharmaceutical grade (USP grade), if that agent is available in pharmaceutical grade. Are all agents in this anesthetic regimen of pharmaceutical grade (USP grade)? **See FAQ for definition of pharmaceutical grade.**

◉ Yes O No

Species:

NHP- Macaque, Cyno

4. The adequacy or depth of anesthesia will be monitored by (select all that apply):

- ☑ Respiratory rate
- ☑ Heart rate
- ☐ Temperature
- ☐ Jaw tone (major procedures)
- ☐ Toe pinch
- ☐ Palpebral (blink) reflex
- ☑ Muscle relaxation
- ☑ Pulse oximetry (major procedures)
- ☐ Mucus membrane color (major procedures)
- ☑ Blood pressure (major procedures)
- ☐ Other (please describe):

5. How frequently will the depth of anesthesia be assessed?

See the Policy on Anesthesia and Analgesia for documentation guidelines for USDA-regulated and non-regulated species.

continuous

6. How will this assessment be documented and with what frequency?

q 15 min

Procedures: Immunosuppression

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination

[redacted]

purposes or for treatment of an induced or spontaneous disease or condition. F r c a ity f definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Immunosuppression

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Immunosuppression

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant

[redacted]

☑ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Sedation

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

The following immunosuppressive reagents are used in this study:

1. Cyclosporine will be injected intramuscularly (volume of solution 0.1-0.6 ml using 25 G needle).

2. Tacrolimus will be injected intramuscularly (volume of solution 0.1-1.0 ml using 25 G needle)

3. Mycophenolate mofetil will be administered IM, PO in food, or by gavage under seadtation.

4. Humanized anti-CD8 monoclonal antibody is administered IV under sedation or general anesthesia.

5. Equine ATG is administered IV under sedation or general anesthesia.

6. Anti-CD154 monoclonal antibody is administered IV under sedation or general anesthesia.

[redacted]

7. Anti-IL-6 antibody is administered IV under sedation or general anesthesia.

8. Methyprednisolone is given IV or IM. For IV administration, the animal is under sedation or general anesthesia. For IM administration, the volume of solution is 0.1-1.0 ml and a 25 G needle is used.

The specific immunosuppressive regimen used in this study is described in the atached flowchard and diagram.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animals will be monitored until upright

---

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

[redacted]

1. Indicate the frequency of post-procedural observations

daily

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required ○ Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

○ Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

○ Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes ○ No ○ Not applicable

[redacted]

Procedures: Combined Lung-Kidney Harvest

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms "surgical procedure"' or "non-surgical procedure" to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Combined Lung-Kidney Harvest

A. Procedure Type

1. What is the type of procedure?

◉ Surgical Procedure ○ Non-Surgical Procedure

---

a. Select the type of surgical procedure:

◉ Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness

○ Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral

○ Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or

[redacted]

	vessel cannulation, percutaneous biopsy	involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or physiological impairment)

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Transplantation

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

C. Preoperative procedures

---

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

☑ Clipping/shaving fur around incision site

[redacted]

☑ Surgical soup scrub alcohol rinse (3 cycles), followed of solution over surgical site
☐ Preparation of aquatic species
☑ Draping of the animal
☐ Other

For non-survival surgery, strict adherence to aseptic technique is not required; however, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean. For non-survival procedures of extended length, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome.

---

b. Preparation of the Surgeon

☐ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
☑ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
☐ Preparation for surgery on aquatic species
☐ Other

---

c. Instruments and consumables (e.g., gauze, suture, etc.)

☑ Autoclave
☐ Chemical sterilant
☑ Ethylene oxide sterilizer
☐ Glass bead sterilizer (Only for sterilizing between animals)
☐ Plasma sterilizer
☐ Purchased sterile from vendor
☐ Other

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☑ Withdrawal of food
☑ Other

Describe
Solid food will be withheld for 12 hours prior to the procedure.

[redacted]

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

General Anesthesia

---

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

In the donor, a midline sternotomy/laparotomy is performed and the aorta and pulmonary artery are dissected superiorly to the level of the aortic arch and pulmonary artery bifurcation, respectively. The renal veins and arteries are also dissected. The superior and inferior vena cavae are mobilized and controlled. Heparin is administered intravenously at a dose of 1mg/kg. The kidney to be procured is cannulated for eventual in situ perfusion.PGE1 (1cc; 500mcg/ml) is administered into the pulmonary circulation and caval inflow is occluded. The ascending aorta is then clamped at the level of the innominate artery. Cold pneumoplegia solution (Euro-Collins with PGE1 1cc/liter) is administered into pulmonary artery. The inferior vena cava and left atrium are vented. Cold renal preservation solution is also administered, Iced saline is poured into the chest and abdomen while ventilation continues. After a minimum of 500 cc of pneumoplegia is delivered, the trachea is divided and the heart-lung block is removed from the chest. The kidney is also excised. The donor is thereby sacrificed by exanguination under deep general anesthesia. The donor left lung, or a cut down portion of the lung, is prepared for implantation, as is the kidney.

---

a. Is this a tumor production procedure?

O Yes ◉ No

[redacted]

6. Indicate method of euthanasia

- ☐ Pentobarbital euthanasia solution (e.g. Euthasol, Fatal Plus, etc.)
- ☐ Potassium chloride (KCl) injection
- ☑ Exsanguination
- ☐ Perfusion
- ☐ Bilateral thoracotomy
- ☐ Removal of vital organs
- ☐ Other (please describe)

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Irradiation-thymic and whole body

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Irradiation

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

2. Indicate other preoperative preparation:

☐ Eye lubricant
☑ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

[redacted]

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Sedation

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Thymic Irradiation (TI): Sedation with domitor and ketamine will be administered. While sedated, the animal will be placed in a large plastic box and transported by van to the [redacted] facility, a travel time of about 10-15 minutes. There, it will receive a single treatment local thymic irradiation (TI, 7Gy). A cobalt source with shielding is used. Sedation will be with serial doses of IM ketamine as needed and the animal will be monitored by the investigators throughout. This will not be done on the same day as total body irradiation.

Total Body Irradiation (TBI): Sedation with domitor and ketamine will be administered. While sedated, the animal will be placed in a large plastic box and transported by van to the [redacted] facility, a travel time of about 10-15 minutes. There, it will receive treatments of non-lethal total body irradiation (TBI, 1.5Gy). A cobalt source is used. Sedation will be with serial doses of IM ketamine as needed and the animal will be monitored by the investigators throughout. This will not be done on the same day as thymic irradiation.

TBI and TI are performed several days prior to the bone marrow transplant procedure as described in the flow charts..

---

a. Is this a tumor production procedure?

O Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

[redacted]

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animals will be monitored until upright.

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

daily

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required ○ Painful, but analgesia cannot be used

[redacted]

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Superficial lymph node biopsy

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

[redacted]

A. Procedure Type

1. What is the type of procedure?

◉ Surgical Procedure

O Non-Surgical Procedure

a. Select the type of surgical procedure:

O Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness

◉ Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy

O Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or

[redacted]

physiological impairment)

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Biopsy, Lymph Node

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

C. Preoperative procedures

---

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

☑ Clipping/shaving fur around incision site
☑ Surgical soup scrub alcohol rinse (3 cycles), followed by final application of solution over surgical site
☐ Preparation of aquatic species
☑ Draping of the animal
☐ Other

---

b. Preparation of the Surgeon

☐ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
☑ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
☐ Preparation for surgery on aquatic species
☐ Other

---

c. Instruments and consumables (e.g., gauze, suture, etc.)

☑ Autoclave

[redacted]

- ☐ Chemical sterilant
- ☑ Ethylene oxide sterilizer
- ☐ Glass bead sterilizer (Only for sterilizing between animals)
- ☐ Plasma sterilizer
- ☐ Purchased sterile from vendor
- ☐ Other

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☑ Withdrawal of food
- ☑ Other

Describe

Solid food will be witheld for 12 hours prior to procedure.

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

General Anesthesia

---

2. Will pre-operative/pre-emptive analgesics be used?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Buprenorphine	0.01mg/kg	IV/IM	once	at outset of surgery

---

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

[redacted]

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Lymph node biopsies are performed under general anesthesia prior to each transplant to demonstrate that the donor and recipient are immunologically incompatible. Subsequent lymph node biopsies are performed to obtain tissue to demonstrate immunologic tolerance and occasionally for diagnostic purposes. There are four potential lymph node biopsy sites (bilateral axillae and bilateral groins fossae). Animals may undergo up to three lymph node biopsies. A 1 cm incision is made in the inguinal or axillary skin. The subcutaneous tissues are bluntly retracted. Typically a 2-3 mm lymph node can be removed under direct vision. The skin is then closed with 3-0 nylon and the animal returned to the housing area. Post operative analgesia is Buprenex IM 0.01mg/kg q12 hrs for 24 hours. Where possible, lymph node biopsies will be performed while the animal is under sedation for another procedure such as a lung graft biopsy. The initial lymph node biopsy must be performed as a separate procedure, however. In order to prove that donors and recipients are mismatched prior to transplantation, in vitro testing of lymphoid tissue is performed. If insufficient cells are obtained then the transplant may have to be deferred, and for this reason it is not appropriate to collect lymph nodes for in vitro testing on the same day as lung transplant.

---

a. Is this a tumor production procedure?

O Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animals will be monitored until upright

[redacted]

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

As a stand-alone minor prodedure, q 10-14 hours x 24 hours, then daily. If lymph node biopsy is done in conjunction with a major surgical pocedure, the observation plan will be that specified for the major procedure.

2. Will post-operative/post-procedural analgesics be administered?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Buprenex	0.01mg/kg	IM	q12 hrs	24 hours

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

◉ Yes O No

Provide the requested information for each agent

[redacted]

Agent	Dose	Route	Frequency	Duration
Cefazolin	25mg/kg	IV/IM	x1	once

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Medication administration

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Medication administration

A. Procedure Type

1. What is the type of procedure?

[redacted]

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Injection, Intrathecal

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☑ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Sedation

---

2. Will pre-operative/pre-emptive analgesics be used?

[redacted]

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Please refer to the Detaiked Research Plan and the Flowchart for details about the mediactions to be used in this study.

In gneral, medications are administered by intramuscular or subcutaneous injection, orally, by gavage, and intravenously.

a. Intramuscular administration: The animal is squeezed to the front of the cage and the drug injected into the right or left thigh or upper arm using a 25-gauge needle. No sedative is given for these small-volume (<1ml) injections.

b. Subcutaneous administration: The animal is squeezed to the front of the cage and the drug injected into the subcutaneous space of the right or left flank using a 25-gauge needle. No sedative is given for these injections.

c. Oral administration: Where possible, oral drugs will be given mixed with fruit. In those cases in which animals will not eat food containing medications, doses will be administered by gavage through an 8Fr orogastric tube which will be introduced under sedation.

d. Intravenous administration: The animal will be sedated. A angiocatheter is placed in a suitable peripheral vein and the drug is administered in sterile fashion with 50-100cc normal saline.

---

a. Is this a tumor production procedure?

O Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

[redacted]

Describe other supportive therapy

Animals will be monitored until upright

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

daily

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required ○ Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

[redacted]

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Open lung biopsy via limited thoracotomy

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Open lung biopsy via limited thoracotomy

A. Procedure Type

1. What is the type of procedure?

[redacted]

◉ Surgical Procedure

O Non-Surgical Procedure

---

a. Select the type of surgical procedure:

O Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness

O Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy

◉ Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or physiological impairment)

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)

[redacted]

Biopsy

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted] and [redacted]

C. Preoperative procedures

---

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

- ☑ Clipping/shaving fur around incision site
- ☑ Surgical soup scrub alcohol rinse (3 cycles), followed by final application of solution over surgical site
- ☐ Preparation of aquatic species
- ☑ Draping of the animal
- ☐ Other

---

b. Preparation of the Surgeon

- ☐ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
- ☑ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
- ☐ Preparation for surgery on aquatic species
- ☐ Other

---

c. Instruments and consumables (e.g., gauze, suture, etc.)

- ☑ Autoclave
- ☐ Chemical sterilant
- ☑ Ethylene oxide sterilizer
- ☐ Glass bead sterilizer (Only for sterilizing between animals)
- ☐ Plasma sterilizer
- ☐ Purchased sterile from vendor
- ☐ Other

[redacted]

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☑ Withdrawal of food
- ☑ Other

Describe

Solid food will be withheld for 12 hrs prior to the procedure.

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes ○ No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

General Anesthesia

2. Will pre-operative/pre-emptive analgesics be used?

◉ Yes ○ No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
buprenorphine	0.01mg/kg	IV/IM	once	at outset of surgery

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

○ Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

[redacted]

Open lung biopsy via limited thoracotomy: Biopsies will be performed under general anesthesia on the schedule outlined in the flow charts below, provided that the animal has recovered adequately from transplant by the time of the scheduled biopsy. With the animal positioned on its side, a limited left thoracotomy is performed. A suitable area of lung is identified and resected using a linear cutting stapler. A chest drain is placed through a small incision caudal to the thoracotomy wound. The chest is then closed in layers using Vicryl sutures appropriate to the size of the animal. The incision is closed using a running subcuticular stitch to the skin. The lungs are fully inflated to test for an air leak and the chest drain is withdrawn. A previous incision may be reexplored so as to permit optimal access to the lung for biopsy. Our experience suggests that this does not lead to a greater infection or dehiscence rate when the wound is closed in separate muscle layers as is the routine.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

The animal will be monitored until upright

---

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all

[redacted]

monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

q 10-14h x 72h, then daily

2. Will post-operative/post-procedural analgesics be administered?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
buprenorphine	0.01mg/kg	IV/IM	q10-14h	72h, then q10-14h PRN

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Cefazolin	25mg/kg	IV/IM	x1	once

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

[redacted]

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Nutrition supplemetation inc. gavage

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Nutrition supplemetation inc. gavage

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Gavage, Oral

[redacted]

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted] and [redacted]

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☑ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Sedation

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

If oral intake is deemed inadequate at any time (fewer than 10 biscuits per day for more than three days, weight loss lasting more than one week, or >10% pre-transplant body mass, or other concerns by veterinary staff) then supplementation with Ensure nutritional supplement and crushed biscuits will be given by gavage under sedation, as frequently as deemed necessary by the veterinary staff. An 8Fr orogastic tube will be used and correct placement of

[redacted]

this tube will be confirmed by aspiration of gastric juices.

a. Is this a tumor production procedure?

O Yes ◉ No

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animals will be monitored until upright

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

daily

2. Will post-operative/post-procedural analgesics be administered?

[redacted]

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Skin biopsy

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of

[redacted]

definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Skin biopsy

A. Procedure Type

1. What is the type of procedure?

◉ Surgical Procedure

O Non-Surgical Procedure

---

a. Select the type of surgical procedure:

O Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness

◉ Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy

O Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (e.g.,

[redacted]

laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or physiological impairment)

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Biopsy, Skin

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

C. Preoperative procedures

---

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

☑ Clipping/shaving fur around incision site
☑ Surgical soup scrub alcohol rinse (3 cycles), followed by final application of solution over surgical site
☐ Preparation of aquatic species
☑ Draping of the animal
☐ Other

[redacted]

b. Preparation of the Surgeon

☐ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
☑ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
☐ Preparation for surgery on aquatic species
☐ Other

---

c. Instruments and consumables (e.g., gauze, suture, etc.)

☑ Autoclave
☐ Chemical sterilant
☑ Ethylene oxide sterilizer
☐ Glass bead sterilizer (Only for sterilizing between animals)
☐ Plasma sterilizer
☐ Purchased sterile from vendor
☐ Other

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☑ Withdrawal of food
☑ Other

Describe

See detailed research plan

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Sedation

---

2. Will pre-operative/pre-emptive analgesics be used?

[redacted]

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
buprenorphine	0.01mg/kg	IV/IM	once	at outset of procedure

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

This procedure is performed after skin grafting to assess for the development of immunologic tolerance. If graft necrosis does not develop after three weeks then a punch biopsy will be performed. The animal will be brought to the [redacted] and sedated. A 5mm punch biopsy of the grafted skin will be obtained and the defect closed with a subcutaneous 5-0 vicryl figure-of-eight suture. The animal will be returned to its cage and 24 hours of analgesia administered as described above.

a. Is this a tumor production procedure?

O Yes ◉ No

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

[redacted]

The animal will be monitored until upright.

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

daily

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

◉ Yes O No

[redacted]

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Cefazolin 25mg/kg	25mg/kg	IV/IM	once	once

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Skin grafting

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

Skin grafting

[redacted]

A. Procedure Type

1. What is the type of procedure?

◉ Surgical Procedure

O Non-Surgical Procedure

---

a. Select the type of surgical procedure:

O Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness

◉ Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy

O Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or physiological impairment)

[redacted]

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Transplantation, Tissue

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

C. Preoperative procedures

---

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

- ☑ Clipping/shaving fur around incision site
- ☑ Surgical soup scrub alcohol rinse (3 cycles), followed by final application of solution over surgical site
- ☐ Preparation of aquatic species
- ☑ Draping of the animal
- ☐ Other

---

b. Preparation of the Surgeon

- ☐ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
- ☑ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
- ☐ Preparation for surgery on aquatic species
- ☐ Other

---

c. Instruments and consumables (e.g., gauze, suture, etc.)

- ☑ Autoclave
- ☐ Chemical sterilant
- ☑ Ethylene oxide sterilizer
- ☐ Glass bead sterilizer (Only for sterilizing between animals)
- ☐ Plasma sterilizer

[redacted]

- ☐ Purchased sterile from vendor
- ☐ Other

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☑ Withdrawal of food
- ☑ Other

Describe

Solid food will be withheld for 12 hrs prior to the procedure.

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

General Anesthesia

2. Will pre-operative/pre-emptive analgesics be used?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
buprenorphine	0.01mg/kg	IV/IM	once	at outset of surgery

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

O Yes ◉ No

[redacted]

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Prior to skin grafting, an attempt will be made to accomodate the monkey to a jacket. This has been done with variable success in the past. The purpose of the jacket is to protect the skin graft while allowing the monkey to have full mobility. Jacketed monkeys are not tethered or restrained. For monkeys that will not accept a jacket, skin grafts will be protected by a temporary semi-rigid dressing, as has been done previously.

This procedure is performed under general anesthesia to assess for the development of immunologic tolerance. Because donors will be sacrificed at the time of transplant, frozen skin is used. Each animal will receive three small skin grafts (self, donor and third-party). The graft will be placed on the back or flank of the animal remote from any prior incisions. Under general anesthesia, the skin graft bed is prepared using a Brown dermatome. A 4x4xm split thickness skin graft is placed on the prepared surface and sutured in place using 4-0 vicryl sutures. An occlusive dressing is placed for 3 days. A bandage is securely taped to the animals torso to prevent picking. Grafts are inspected daily for color, warmth and texture to assess viability. After graft necrosis occurs, the area is cleansed daily and a sterile dressing applied until ingrowth of native skin occurs.

We request that monkeys not be pair-housed while they have a healing skin graft in place.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

[redacted]

The animals will be monitored until upright

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

As a stand-alone minor prodedure, q 10-14 h x 24 h, then q D. If done in conjunction with a major procedure, the obesrvation plan will be that specified for the major procedure.

2. Will post-operative/post-procedural analgesics be administered?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
buprenorphine	0.01mg/kg	IV/IM	q10-14h	PRN

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

◉ Yes O No

[redacted]

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Cefazolin	25mg/kg	IV/IM	x1	once

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Jacket training and jacketing

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

Jacket training and jacketing

[redacted]

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Teaching/Training

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required O Painful, but anesthesia

[redacted]

cannot be used

2. Will pre-operative/pre-emptive analgesics be used?

○ Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Jacket training will consist of placing the monkey in a jacket, and observing the skin under the jacket every 3-4 days for approximately one week. Sedation, as previously described will be used as needed to place the jacket and examine the animal. Every effort will be made to perform the jacketing and observartions in conjunction with other procedures, so that sedations are minimized. If the animal tolerates the jacket for one week and has no signs of skin irritation or breakdown, we will consider the training successful, and use the jacket as needed for skin graft procedures. If the jacket training is not successful, we will modify the fit of the jacket and re-attempt training once the skin is healthy. Please note that the monkey is not teathered or otherwised restrained while wearing the jacket. Jacked animals receive daily observations that include a visual inspection with regard to the integrity of the jacked and to ensure the absence of constriction or chafing.

a. Is this a tumor production procedure?

○ Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

q 3-4 days

[redacted]

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

---

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required O Painful, but analgesia cannot be used

---

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

---

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Donor pneumonectomy via sternotomy

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination

[redacted]

purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

Donor pneumonectomy via sternotomy

A. Procedure Type

1. What is the type of procedure?

◉ Surgical Procedure

O Non-Surgical Procedure

---

a. Select the type of surgical procedure:

◉ Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness

O Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy

O Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection

[redacted]

(e.g., laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or physiological impairment)

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Transplantation, Lung

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

C. Preoperative procedures

---

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

☑ Clipping/shaving fur around incision site
☑ Surgical soup scrub alcohol rinse (3 cycles), followed by final application of solution over surgical site
☐ Preparation of aquatic species
☑ Draping of the animal
☐ Other

[redacted]

For non-survival surgery, strict adherence to aseptic technique is not required; however, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean. For non-survival procedures of extended length, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome.

---

b. Preparation of the Surgeon

☐ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
☑ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
☐ Preparation for surgery on aquatic species
☐ Other

---

c. Instruments and consumables (e.g., gauze, suture, etc.)

☑ Autoclave
☐ Chemical sterilant
☑ Ethylene oxide sterilizer
☐ Glass bead sterilizer (Only for sterilizing between animals)
☐ Plasma sterilizer
☐ Purchased sterile from vendor
☐ Other

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☑ Withdrawal of food
☑ Other

Describe

Solid food will be withheld for 12 hours prior to the procedure.

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

[redacted]

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

General Anesthesia

---

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

○ Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Donor pneumonectomy via sternotomy: In the donor under general anesthesia, a midline sternotomy is performed and the aorta and pulmonary artery are dissected superiorly to the level of the aortic arch and pulmonary artery bifurcation respectively. The superior and inferior venae cavae are mobilized and controlled. Heparin is administered intravenously at a dose of 300U/kg. PGE1 (1cc; 500mcg/ml) is administered into the pulmonary circulation and caval inflow is occluded. The ascending aorta is then clamped at the level of the innominate artery. Cold pneumoplegia solution is administered into the pulmonary artery. The inferior vena cava and left atrium are vented. Iced saline is poured into the chest while ventilation continues. After approximately 500cc of pneumoplegia is delivered, the trachea is divided and the heart-lung block is removed from the chest. The donor is thereby sacrificed by exanguination under deep general anesthesia. The donor left lung, or a cut down portion thereof, is prepared for implantation. Tissue from the carcass of the donor, including bone marrow, lymph nodes and spleen, is collected for further processing at this time.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

---

6. Indicate method of euthanasia

- ☐ Pentobarbital euthanasia solution (e.g. Euthasol, Fatal Plus, etc.)
- ☐ Potassium chloride (KCl) injection
- ☑ Exsanguination
- ☐ Perfusion
- ☐ Bilateral thoracotomy

[redacted]

- ☐ Removal of vital organs
- ☐ Other (please describe)

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Chest radiograph

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Chest radiograph

A. Procedure Type

1. What is the type of procedure?

[redacted]

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Imaging, X-Ray

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☑ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Sedation

---

2. Will pre-operative/pre-emptive analgesics be used?

[redacted]

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Chest radiographs: Sedation will be administered as described. The animal will be brought to the [redacted] and chest radiographs obtained using the portable x-ray machine provided by [redacted] This will be done twice weekly for two weeks after operations requiring thoracotomy and once weekly thereafter except when changes in clinical condition (leukocytosis, unexplained weight loss) demand more frequent radiographs.

---

a. Is this a tumor production procedure?

O Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animals will be monitored until upright

---

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

[redacted]

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

daily

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required ○ Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

○ Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

○ Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

[redacted]

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Bone Marrow Biopsy

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Bone Marrow Biopsy

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Biopsy, Bone Marrow

[redacted]

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted] and [redacted]

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☑ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes ○ No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

General Anesthesia

---

2. Will pre-operative/pre-emptive analgesics be used?

◉ Yes ○ No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Buprenorphine	0.01 mg/kg	IM/IV	once	at time of surgery

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

[redacted]

Animal is fasted for 12 hours and anesthetized as described in main protocol. Lower back is shaved and prepped in sterile fashion. Either left of right posterior superior iliac spine is identified by palpation. Using Illinois intraosseous BM needle (18G or larger) flushed with heparin (100-200 I.U.) percutaneous stick is performed and 2-5 ml of bone marrow (BM) is aspirated. Site can be changed if not enough bone marrow is aspirated from one site. After needle is removed pressure is applied with sterile gauze for minor bleeding to stop. Analgesia is covered with Buprenorphine, for 24 hours following the procedure, given in 2 doses @ 0.01 mg/kg IM q12 hours as described in main protocol.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animal will be monitored until upright

---

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

[redacted]

1. Indicate the frequency of post-procedural observations

daily

2. Will post-operative/post-procedural analgesics be administered?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Buprenorphine	0.01 mg/kg	IM	2 doses @ q12 hours	24 hours

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Transfusion including bone marrow infusions

[redacted]

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Transfusion including bone marrow infusions

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Intravenous injection

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

[redacted]

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☑ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes ○ No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Sedation

---

2. Will pre-operative/pre-emptive analgesics be used?

○ Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Donor-matched cells will be administered intravenously under sedation. Infusion is via a 24-gauge angiocatheter placed in the saphenous vein. A hemofilter is used to prevent embolism.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage

[redacted]

- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animals will be monitored until upright.

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

daily

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required ○ Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesi
CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Blood sampling

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Blood Collection, Venous

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

2. Indicate other preoperative preparation:

☐ Eye lubricant
☑ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

[redacted]

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Sedation

---

2. Will pre-operative/pre-emptive analgesics be used?

○ Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Samples will be obtained via percutaneous femoral vein venipuncture following sedation. We propose to sample blood two to three times each week (2-5cc) during the month following bone marrow transplantation. Otherwise, blood samples will be drawn twice weekly (2-5cc) for therapeutic drug monitoring. Our blood sampling practice easily conforms to the [redacted] recommendation of not drawing greater than 15% of a monkeys total blood volume (calculated to be approximately 75cc for a 5kg animal with a presumed blood volume of 500cc) over any two week period. The animals hematocrit will be carefully monitored at each blood sampling.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

☐ Warming pad/blanket
☐ Incubator or ICU chamber/cage
☐ Intravenous fluids
☐ Subcutaneous fluids
☑ Other

Describe other supportive therapy

Animals will be monitored until upright.

[redacted]

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

daily

---

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

---

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required ○ Painful, but analgesia cannot be used

---

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

○ Yes ◉ No

---

4. Will other miscellaneous post-operative/post-procedural medications be administered?

[redacted]

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Thymic Biopsy

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Thymic Biopsy

A. Procedure Type

1. What is the type of procedure?

◉ Surgical Procedure O Non-Surgical Procedure

[redacted]

a. Select the type of surgical procedure:

O Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness	◉ Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy	O Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or physiological impairment)

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Biopsy, Thymus

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted] [redacted]

Indicate the room number(s):
[redacted] and [redacted]

C. Preoperative procedures

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

- ☑ Clipping/shaving fur around incision site
- ☑ Surgical soup scrub alcohol rinse (3 cycles), followed by final application of solution over surgical site
- ☐ Preparation of aquatic species
- ☑ Draping of the animal
- ☐ Other

b. Preparation of the Surgeon

- ☐ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
- ☑ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
- ☐ Preparation for surgery on aquatic species
- ☐ Other

c. Instruments and consumables (e.g., gauze, suture, etc.)

- ☑ Autoclave
- ☐ Chemical sterilant
- ☐ Ethylene oxide sterilizer
- ☐ Glass bead sterilizer (Only for sterilizing between animals)
- ☐ Plasma sterilizer
- ☐ Purchased sterile from vendor
- ☐ Other

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☑ Other

[redacted]

Describe

Solid food will be withheld for 12 hrs prior to the procedure.

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes O No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

General Anesthesia

2. Will pre-operative/pre-emptive analgesics be used?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
buprenorphine	0.01mg/kg	IV/IM	once	at outset of surgery.

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Under general anesthesia, the neck and chest will be prepped and draped in sterile fashion. A small incision will be made at the base of the neck and carried down to the pretracheal fascia with electrocautery. The cervical thymus will be exposed and a small (<5mm) piece will be directly biopsied. The neck will be closed in layers using Vicryl suture.

[redacted]

a. Is this a tumor production procedure?

○ Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animals will be monitored until upright

---

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

As a stand-alone minor prodedure, q 10-14 hours x 24 hours, then daily. If done in conjunction with a major procedure, the obesrvation plan will be that specified for the major procedure.

---

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

[redacted]

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Orthotopic single lung transplantation via thoracotomy

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

[redacted]

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

Orthotopic single lung transplantation via thoracotomy

A. Procedure Type

1. What is the type of procedure?

◉ Surgical Procedure

O Non-Surgical Procedure

---

a. Select the type of surgical procedure:

O Non-survival: euthanasia is performed while the animal is under general anesthesia. The animal never awakens or regains consciousness

O Minor survival surgery: the procedure does not expose a body cavity and causes little or no physical impairment; e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy

◉ Major survival surgery: the procedure penetrates and exposes a body cavity, procedures substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, craniotomy,

[redacted]

joint replacement, or limb amputation, or laparoscopic surgery that produces substantial physical or physiological impairment)

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Transplantation, Lung

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted] and [redacted]

C. Preoperative procedures

---

1. Aseptic technique

Aseptic technique will be maintained by:

a. Preparation of the Animal

☑ Clipping/shaving fur around incision site
☑ Surgical soup scrub alcohol rinse (3 cycles), followed by final application of solution over surgical site
☐ Preparation of aquatic species
☑ Draping of the animal
☐ Other

---

b. Preparation of the Surgeon

[redacted]

☐ Clean lab coat, mask, hair covering, sterile surgical gloves (for non-USDA regulated species)
☑ Sterile gown, hat/cap, shoe covers, mask, sterile surgical gloves (for USDA regulated species)
☐ Preparation for surgery on aquatic species
☐ Other

---

c. Instruments and consumables (e.g., gauze, suture, etc.)

☑ Autoclave
☐ Chemical sterilant
☑ Ethylene oxide sterilizer
☐ Glass bead sterilizer (Only for sterilizing between animals)
☐ Plasma sterilizer
☐ Purchased sterile from vendor
☐ Other

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☑ Withdrawal of food
☑ Other

Describe
Solid food will be withheld for 12 hrs prior to the procedure.

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes ○ No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

General Anesthesia

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2. Will pre-operative/pre-emptive analgesics be used?

◉ Yes ○ No

[redacted]

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
buprenorphine	0.01mg/kg	IV/IM	once	outset of surgery

3. Will other medications (e.g., antibiotics, sedatives) be administered prior to the induction of anesthesia or the start of the procedure?

○ Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Orthotopic single lung transplant via thoracotomy: In the recipient under genral anesthesia, a left posterolateral thoracotomy is performed and the chest is entered through the bed of the resected fifth rib. The hilar structures (pulmonary artery, pulmonary veins and left main bronchus) are dissected and isolated. After systemic heparinization (100U/kg), the hilar vessels are clamped and divided and the left main bronchus is divided. The lung is removed. The open bronchus is occluded with a bronchial blocker. Next, the donor lung is anastomosed into the recipient in the following order: bronchus, pulmonary vein, then pulmonary artery. After venting and reinflation of the lung, the lung is reperfused. A chest drain is placed through a small incision caudal to the thoracotomy wound. The chest is closed in layers using Vicryl sutures appropriate to the size of the animal. The incision is closed using a running subcuticular stitch to the skin. The lungs are fully inflated to test for an air leak and the chest drain is withdrawn. During lung implantation, a muscle relaxant is sometimes employed to prevent spontaneous breathing that may occur during single lung ventilation. Animals are under general anesthesia at all times with continuous physiologic monitoring as described elsewhere. Signs of discomfort would be readily apparent in the animals cardiovascular response to stimuli (e.g., hypertension and tachycardia). Anesthesia is not terminated until the animals are spontaneously breathing, ensuring that muscle relaxant has sufficiently worn off. Likewise, animals are not extubated until good, sustained peripheral muscle strength is demonstrated.

a. Is this a tumor production procedure?

○ Yes ◉ No

[redacted]

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Animals will be monitored continuously until upright.

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☑ Animal can sit upright (NHPs)
- ☐ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

q10-14h x 72 hrs, then daiily

2. Will post-operative/post-procedural analgesics be administered?

- ◉ Yes
- O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
buprenorphine	0.01mg/kg	IV/IM	q10-14h	72 hrs, then q10-14h PRN

[redacted]

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

◉ Yes O No

Provide the requested information for each agent

Agent	Dose	Route	Frequency	Duration
Cefazolin	25mg/kg IV/IM	IV/IM	x1	once
Enrofloxacin	5mg/kg	IV/IM/PO	x1 qD	14 days

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Multiple Survival procedures

Multiple survival surgical procedures on the same animal are generally discouraged; however, it may be necessary to perform more than one surgical procedure to accomplish study goals.

1. Provide the rationale for multiple survival surgical procedures? Please select all that apply:

☑ Create model with first procedure; evaluate / test subsequent procedure(s)
☐ Second surgery only if first procedure is ineffective
☐ Repair / replace as necessary. Veterinarian consultation is required in advance of the procedure.

[redacted]

- ☐ Create access for experiment; disease model
- ☐ Utilize existing surgically created model
- ☐ Multiple simultaneous surgical procedures not possible
- ☐ Baseline reading must be done after first surgery
- ☐ Evaluate / test subsequent procedure
- ☐ Reuse of an animal that is suitable oocyte producer
- ☐ Other (please describe)

2. Provide detailed scientific justification for these multiple survival surgical procedures:

The aim of this study is to develop a protocol to induce tolerance in lung allotransplantation. The major surgical procedure proposed is a single-lung orthotopic transplant followed by additional procedures to obtain biopsy samples of the transplanted lung, superficial lymph nodes, and thymus. Skin grafting will be performed at the conclusion of the experiment as a rigorous test for the induction of immunologic tolerance. A time course for these procedures is included in the flowcharts. In order to obtain longitudinal data, for which a large animal model is ideally suited, serial operations are necessary. Every effort is made to minimize the size of incisions required for biopsy and to use a minimally invasive approach when possible.

3. The multiple survival operations will be:

- O A minor surgical procedure followed by only minor surgical procedure(s)
- O A minor surgical procedure followed by a major surgical procedure
- O A major surgical procedure followed by only minor surgical procedure(s)
- ◉ A major surgical procedure followed by additional major surgical procedure(s)

4. List the sequence of survival surgical procedures and the interval between them. Please ensure that the interval between procedures is consistent with the flow chart.

The first lung allograft biopsy will be performed 60 or more days post-transplant unless clinically warranted and in consultation with the veterinary staff. Subsequent biopsies will be performed at least 60 or more days later.

5. Describe the methods of evaluation (e.g., physical examination, blood test, biomarkers) and specific criteria for determining if/when the animal may proceed to the next surgery.

The flowcharts outline the general time courses for the different experimental groups. Animals must be in good health as judged by both the investigative and vetrinary staff before research procedures can be performed.

Controlled Substance and Non-Pharmaceutical Grade Substance

A. Controlled Substances

1. Are any of the agents (anesthetics, analgesics, test agents, etc.) used in this protocol DEA/Federally Controlled Substances?

[redacted]

◉ Yes O No

a. Indicate where DEA/Federally Controlled Substances will be stored. If your license is pending or if a secure location is not yet identified, please enter "TBD" in the Room field.

Building:

[redacted]

b. Room:

[redacted]

B. Non-Pharmaceutical Grade Substances

Both OLAW and AAALAC provide guidance regarding the use of non-pharmaceutical grade compounds in laboratory animals.

Pharmaceutical-grade substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals and/or interfere with the interpretation of research results. However, it is frequently necessary to use non-pharmaceutical-grade substances such as investigational substances, veterinarian- or pharmacy-compounded substances, and/or Schedule I controlled substances to meet scientific and research goals.

A listing of pharmaceutical-grade drugs and biologics is available through the FDA database.
· The Orange Book is the reference for FDA-approved human drugs.
· The Green Book is the reference for FDA-approved veterinary drugs.

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1. Are all substances to be administered to animals of pharmaceutical grade?

Examples of non-pharmaceutical grade substances include:

- Anesthetics and analgesics (e.g., Avertin)
- Euthanasia compounds (e.g., pentobarbital)
- Diluents and/or vehicles (e.g., DMSO, methyl cellulose)
- Test compounds

◉ Yes O No

Restraint

The Guide for the Care and Use of Laboratory Animals defines physical restraint as the use of manual or mechanical means to limit some or all of an animal's movement for the purpose of examination or experimental manipulation. Sedatives or anesthetics may be used to immobilize animals for the performance of non-painful procedures that might otherwise be painful or distressful to the animal.

Restraint

[redacted]

O Animals will undergo restraint as part of this research

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Anesthesia Regimen, Label**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it contains details useful in answering the **Indicate the anesthesia regimen or sedative that will be used**, question above.

Device Acclimation

O This research includes devices to which animals must be acclimated, e.g., jackets/tethers

Transportation

The transportation of animals must conform to IACUC policy and the Animal Welfare Act, as applicable.

1. Will live animals be transported to facilities outside the institution?

◉ Yes O No

Select the option that applies:

☑ Transport will be coordinated by CCM
☐ Transport will be coordinated by the investigator and a CCM approved vendor will be used
☐ Transportation of animals returning to their home institution will be coordinated by the home institution
☑ Other

[redacted]

Describe the efforts to comply with USDA and/or DOT regulatio
transportation of animals. Include a description of the transport cage and the van/carrier/vendor that will be used.

Transport from the vendor to [redacted] is done per CCM's SOP.

2. Will animals be moved within or between institution facilities? This includes moving animals between housing areas and laboratory or imaging areas.

◉ Yes ○ No

a. Will animals be moved between Biosafety Level 2 (BSL2) housing and laboratory or imaging areas?

○ Yes ◉ No

b. Select the option that applies:

☑ Transfers will be performed by CCM
☐ Transfers will be performed by protocol study staff following institutional SOPs and/or guidelines (see FAQ for links to institution-specific SOPs/guidelines).
☐ Other (including any animal transport not covered by institutional SOPs or guidelines)

Initial Survey

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

In accordance with federal regulations and hospital policies, all animal research conducted at or funded through the [redacted] must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to initiation of the study. This policy applies to any vertebrate animal used for any type of research, teaching, or testing. The IACUC has the sole authority to approve, require modifications (in order to secure approval), or withhold approval of research protocols involving the use of animals at the selected Institution. Protocols can be approved for a maximum of three years, subject to satisfactory annual reviews where required. The IACUC also must review and approve **in advance** any changes or modifications to previously approved protocols.

Principal Investigator Eligibility: Please note that you must meet the eligibility requirements set by your institution's IACUC in order to serve as the principal investigator (PI) for an animal research protocol. See FAQ for links to institution-specific guidelines.

The questions below will help to identify if an IACUC protocol must be submitted to your institution's IACUC for your research project.

[redacted]

Please enter the full title of the study.

Thoracic Allograft Tolerance in Non-Human Primates: Application of Mixed Chimerism to Lung Transplantation

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A. At which Institution will the research be conducted?

◉ [redacted] or [redacted] O [redacted] O [redacted] O Other Institution

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B. The proposed research project will involve the following:

◉ The entire animal research protocol will be conducted at the selected institution.

O Only a portion of the animal research project will be conducted at the selected Institution. This includes, but is not limited to, housing, surgery procedures, behavior assessments, imaging sessions, etc.

If the IACUC grants approval, it will oversee only the research component that is performed at your institution. Any research component(s) conducted at an outside institution will be conducted under the auspices of that institution's IACUC.

For the protocol to remain active, the investigator must submit satisfactory IACUC annual progress reports (if required), as well as provide annual documentation of the relevant outside IACUC approvals.

For more information, please refer to the IACUC website for your institution:

- [redacted]
- [redacted] http:/ [redacted]

C. The proposed study involves the use of:

◉ Any live animal (ie. mouse, rat, rabbit, dog, cat, swine, sheep, nonhuman primate, etc)

O Animals tissues, products, or blood (including whole dead animal), not otherwise approved by the IACUC as part of the investigator's own animal research protocol.

Attachments

[redacted]

Name	Mode
[redacted] Resubmission Prereview (Other)	Electronic
Sample Size Calculation (Other)	Electronic
Response to Req Mod (Point by Point Response)	Electronic
Response to Pre-review (Point by Point Response)	Electronic
Study Staff certification (Staff certification)	Electronic
Study Staff certification (Staff certification)	Electronic
Study Staff certification (Staff certification)	Electronic