**PROTOCOL FOR ANIMAL USE AND CARE**

**Handwritten forms are not accepted**

**CRPRC**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
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<tbody>
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<td>Last Name:</td>
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<td>After hrs. #:</td>
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**Species** (common names): **Rhesus monkeys**  Number: **8**  Source: **CRPC or Charles River BRF**

**Project Title**: Tolerance Induction by MHC mismatched DST in Monkeys

**Overnight housing location**: CRPC  **Day use only**: [ ]

Animals will be maintained by: [X ] Vivarium  [ ] Investigator  *(If investigator maintained, attach husbandry SOP's.)*

**Procedures**: Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Monkeys will receive a kidney transplant from an unrelated donor animal. The donor will later serve as a recipient. The animals will receive immunosuppressive agents and blood transfusions to prevent rejection. Rejection will be monitored by clinical blood tests and biopsies of the graft. At the end of 150 days, all animals will be euthanized.

**Special Husbandry Requirements**: Describe any special requirements your animals have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions of husbandry.

These animals will be immunosuppressed and so must be housed in areas free of exposure to infectious agents.

**Other instructions for animal care staff**: (check applicable entries)

<table>
<thead>
<tr>
<th>Sick Animals</th>
<th>Dead Animals</th>
<th>Pest Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>[X ] Call Investigator</td>
<td>[X ] Call Investigator</td>
<td>[ ] Call Investigator</td>
</tr>
<tr>
<td>[X ] Clinician to treat</td>
<td>[ ] Save for Investigator</td>
<td>[X ] OK to use pesticides</td>
</tr>
<tr>
<td>[ ] Terminate</td>
<td>[ ] Bag for disposal</td>
<td>[ ] No Pesticides in animal area</td>
</tr>
<tr>
<td>[ ] Necropsy</td>
<td>[X ] Necropsy</td>
<td></td>
</tr>
</tbody>
</table>

**Hazardous Materials** *(only if in the animal room)*:

- Infectious Agents? [ ] Yes [X ] No  Agent(s):
- Radioisotopes? [ ] Yes [X ] No  Agent(s):
- Chemical Carcinogens? [ ] Yes [X ] No  Agent(s):
- Toxic Chemicals? [ ] Yes [X ] No  Agent(s):
Summary of Procedures:

a) Briefly describe the overall intent of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

This study will evaluate the effectiveness of donor specific blood transfusions and partial histocompatibility matching for inducing tolerance to kidney allografts. With tolerance, all immunosuppressive agents can be withdrawn from the patient. Our center will study unmatched monkeys. Dr. group in the Netherlands will study partially matched monkeys.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- [ ] Monoclonal Antibody Production
- [ ] Polyclonal Antibody Production
- [ ] LD 50 or ID50 studies.
- [ ] catheters, blood collection, intubation
- [ ] Prolonged restraint (8 hrs+)
- [ ] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [ ] Survival surgical procedures
- [X ] Multiple survival surgery
- [ ] Induced illness, intoxication, or disease
- [ ] Death as an endpoint (see i below)
- [ ] Trapping, banding or marking wild animals
- [ ] Special diets; food or water treatment.
- [ ] Behavioral modification.
- [ ] Aversive conditioning.

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.
c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

Animals in one experimental group (n=8) will receive a T-cell ablative antibody, costimulation blocker, rapamycin and donor specific blood transfusions in an attempt to induce tolerance for a renal allograft. After the renal transplantation surgery, the animals will be followed either until they show terminal rejection or until postoperative day 150. Kidney function will be observed by monitoring renal function through general chemistry. Terminal rejection is defined clinically to be present in animals that have a serum creatinine of more than 7 mg/dl for more than two days at least 6 days post surgery. This is the standard that we have used for our protocols at Stanford University. Graft histology will be monitored by serial biopsies that are performed with a 20 g biopsy needle on postoperative days 7, 28 and 100. Final histology will be performed following necropsy (after terminal rejection or postoperative day 150) using multiple samples from the allograft. Each donor/recipient pair will be blood group compatible and MHC incompatible as determined by a mixed lymphocyte response test.

For the transplant procedure, a naïve kidney donor and a recipient monkey are anesthetized at the same time. A midline laparotomy is performed first in the donor animal. The left kidney is removed from the peritoneum and the kidney is handled by two stay sutures placed on the lateral edge of the peritoneal fold. These stay sutures allow the surgeons to manipulate the kidney without actually touching the kidney with forceps. The ureter is prepared down to its entry into the bladder, taking care to avoid denudation or interruption of blood supply. The hilar vessels are next isolated from the surrounding tissue and a gauze pad soaked with papaverine is placed around the vessels. The team of surgeons then moves to the recipient animal. Again, a midline laparotomy is performed and the infrarenal aorta and cava are isolated from the surrounding peritoneum, ureter and lymphatic structures. The lumbar vessels are not interrupted. The pressure lead of an arterial line is inserted into the aorta proximal to the site where the anastomosis will be performed. This allows continuous monitoring of the recipients blood pressure during the period the aorta is clamped for the anastomosis. The team then moves back to the donor animal. The ureter is tied and separated as far distal as possible. A ligature is placed around the renal vein and the aorta is clamped with a Satinsky clamp. The renal vein is ligated and separated. Twenty milliliters of Euro-Collins solution at 4°C is injected through a 25 gauge needle place into the clamped aorta. The preservative solution only perfuses the kidney. Upon completion of the in-situ perfusion the renal artery is cut from the aorta, leaving a small cuff on the renal artery side of the separation. The kidney is placed in iced preservation solution. The aorta is closed with a 7-0 prolene suture and the clamp is removed. The laparotomy is closed in layers and the animal is recovered. The team moves back to the recipient animal. The graft is implanted end-to-side to the infrarenal aorta using 7-0 suture. Upon completion of the venous and arterial anastomosis the graft is reperfused. The ureter is anastomosed to the bladder. Finally, the right kidney is excised and the abdomen is closed. All animals are recovered and monitored in the CRPC intensive care unit. Following at least one month of rest, the donor animal will serve as a recipient in a future procedure. At that time, the remaining native kidney will be excised and submitted for pathologic examination.

Postoperative care will follow CRPC protocols for major invasive surgery. Body temperature, hematocrit, chemistry profile, electrolyte and acid base, status, and urine output will be monitored as required. Intravenous fluids, balanced for electrolyte and acid/base status will be administered until the animal is fully recovered. Antibiotics will be administered perioperatively, cefazolin (22mg/kg/IV q 6 hours). During the survival period, monitoring for infections will consist of observation of the incision line and general activity of the animal, monitoring food and water intake, palpation of the abdomen, monitoring body temperature, and analysis of the complete blood count.
Renal biopsies will be performed percutaneously through a 2mm skin incision using a 20g biopsy needle. Ketamine will be used for sedation. Biopsies will be performed at 7,28 and 100 days, and at the time of a suspected rejection episode.

Starting two weeks prior to surgery, either an anti CD-3 (T cell) monoclonal antibody (1mg/kg/IV/SID/14 days) or Antilymphocyte Serum (Thymoglobulin, Sangstat, 1.5 mg/kg/SID/7 days) will be given to deplete the native T cell population. Costimulation blockaid will be achieved using antiCD40L monoclonal antibody (25mg/kg/IV/day -14, 20 mg/KG/Day -12, 15 mg/kg day -10 and -8, 10 mg/kg/Day -6 and -4, and 10 mg/kg weekly until postoperative day day 50). Rapamycin (1 mg/KG/SID PO) will be given starting two weeks prior to surgery and then for 70 days postoperatively. Rapamycin aids in preventing lymphocyte proliferation, graft rejection, and has been shown to allow the induction of tolerance in rodent models. To aid in the induction of tolerance, 3 donor specific blood transfusions will be given, over two weeks, prior to surgery.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; you may add as many rows as you require. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Renal transplantation, renal donation, renal biopsies; monoclonal antibody, polyclonal antibody, rapamycin</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1        | Little or no discomfort or stress  
Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral. |
| 2        | Minor stress or pain of short duration  
Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress |
| 3        | Moderate to severe distress  
Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation |
| 4        | Severe pain near, at or above the pain tolerance threshold  
Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness. |

Further descriptions of these categories are included in the instructions following this document.
e) Rationale for species and numbers: How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

Primates are the only species of animal that truly predict the usefulness of an immuno-suppressive agent in human beings. We chose 8 animals per group as the least number that could show us statistical significance with the possibility of early graft dysfunction. It is not unusual, using the non-human primate model, to have one or two grafts fail to function resulting in early euthanasia of an animal. We hope to have a minimum of 6 animals for the final evaluation.

f) Surgery: If the project involves survival surgery, where will the surgery be conducted?

Building: CRPC Room: Operating suite

Who will be the surgeon?


g) Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>primate</td>
<td>ketamine</td>
<td>10</td>
<td>IM</td>
<td>For sedation</td>
</tr>
<tr>
<td>primate</td>
<td>midazolam</td>
<td>.01</td>
<td>IV</td>
<td>Induction</td>
</tr>
<tr>
<td>primate</td>
<td>propofol</td>
<td>10</td>
<td>IV</td>
<td>Induction/intubation</td>
</tr>
<tr>
<td>primate</td>
<td>midazolam</td>
<td>.35ug/kg/min</td>
<td>IV</td>
<td>maintenance</td>
</tr>
<tr>
<td>primate</td>
<td>Propofol</td>
<td>.1/kg/min</td>
<td>IV</td>
<td>Maintenance</td>
</tr>
<tr>
<td>primate</td>
<td>buprenorphine</td>
<td>.01-.03</td>
<td>IV,IM</td>
<td>Post op pain relief</td>
</tr>
</tbody>
</table>

h) Neuromuscular blocking agents can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

Ileus (lack of motility and dilation of the bowel with gas caused by pain), uroabdomen (urine in the abdomen caused by leakage from the site where the ureter is sutured to the bladder), abdominal bleeding, postoperative pain, renal failure, infections. Antibody administration: infection, fever, chills, tachycardia. Rapamycin: thrombocytopenia.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

buprenorphine as needed. Renal failure: euthanasia if creatinine is 7mg/dl for 2 days, fluid therapy, acid/base correction if creatinine is less than 7 mg/dl. Infections will be treated with antibiotics (based on sensitivity testing) and/or local therapy depending on the location. Infection will be controlled using proper aseptic techniques and antibiotics as necessary. The chills and fever associated with antibody therapy are rare and resolve shortly after administration. Ibuprofen can be administered as needed. Thrombocytopenia is controlled by reducing the dose of rapamycin.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure?  [ ] Yes  [X] No

(Note: “Death as an endpoint” refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. “Alternative methodologies” refers to reduction, replacement, and refinement (the three R’s) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search?  7/12/01

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medline/healthstar</td>
<td>all</td>
<td>Renal transplantation, primate, alternative models</td>
</tr>
<tr>
<td>Biosis</td>
<td>all</td>
<td>same</td>
</tr>
<tr>
<td>History of Science, technology and medicine</td>
<td>all</td>
<td>same</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There were no alternative technologies that could predict the effect of immunosuppressive strategies on the rejection of organ allografts in the primate.

Has this study been previously conducted?  [ ] Yes  [X] No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

k) Disposition of animals: At what point in the study, if any, will the animals be euthanized?

At postoperative day 150, if the serum creatinine is 7mg/dl or greater for two consecutive days at least 6 days following surgery.
l) Methods of euthanasia: Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
</tr>
</thead>
<tbody>
<tr>
<td>primate</td>
<td>injection</td>
<td>ketamine</td>
<td>10</td>
<td>IM</td>
</tr>
<tr>
<td>primate</td>
<td>injection</td>
<td>pentobarbital</td>
<td>60</td>
<td>IV</td>
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Ketamine will be given prior to the pentobarbital to provide restraint for the IV injection of pentobarbital.

m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project?

Any primates not used in the study will be returned to the CRPC.
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don't need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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Occupational Health Program:

Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://clueless.ucdavis.edu/health/](http://clueless.ucdavis.edu/health/) or read the UC Davis Policy & Procedure Manual 290-25.

Training:

Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at [http://clueless.ucdavis.edu/](http://clueless.ucdavis.edu/).
Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the UC Davis Policy and Procedure Manual section 290-30 (Animal Use and Care). This project will be conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals, and the UC Davis Animal Welfare Assurance on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at http://ehs.ucdavis.edu/). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

__________________________  __________________________  _______________________
Principal Investigator            Rank / Title                   Date

** Conditions necessary for Committee Approval:


Final Disposition of this protocol:

_________ Approved
_________ Not Approved
_________ Withdrawn by Investigator

Date of Action: _____ / _____ / _____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

__________________________  _______________________
Campus Veterinarian            Date