PROTOCOL FOR ANIMAL USE AND CARE

CRPRC

Investigator Contact

Last Name: | Last Name: |
First: | First: |
Middle: | Middle: |
email: | email: |
Department: | Department: |
Phone / Fax: | Phone: | After hrs. #: |

Species (common names): | Number: | Source: |
Rhesus macaques | 80 | Primate Center |
(dams+infants) |

Project Title: Intramarrow Gene Transfer in Neonatal Rhesus Monkeys

Overnight housing location: | Day use only: |
Primate Center | |
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.

These studies focus on transferring genes into newborn rhesus monkey bone marrow in vivo. Studies include collection of fetal/maternal samples during gestation, administration of cytokines to fetal monkeys in utero, delivery of newborns by cesarean-section, collection of infant blood and marrow at birth then monthly, and necropsy for collection of tissues at ~6 months postnatal age.

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.

Protective clothing when handling uninfected infants during the first week of life. BSL2+ housing for SIV-infected infants.

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

Hazardous Materials (only if in the animal room):

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>Radioisotopes?</th>
<th>Chemical Carcinogens?</th>
<th>Toxic Chemicals?</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ X ] Yes</td>
<td>[ ] Yes</td>
<td>[ X ] Yes</td>
<td>[ X ] Yes</td>
</tr>
<tr>
<td>[ ] No</td>
<td>[ X ] No</td>
<td>[ ] No</td>
<td>[ X ] No</td>
</tr>
</tbody>
</table>

Agent(s): Self-inactivating lentiviral vectors; SIVmac251
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.

Highly active antiretroviral therapy (HAART) has resulted in dramatic reductions in viral loads in HIV-1 seropositive patients. However, despite these improvements in drug treatment, HIV proviral DNA is still present in lymphoid cell populations, and patients that have terminated treatment, either because of intolerance or non-compliance, experience a rapid resurgence of viral burden to pretreatment levels. Moreover, even in long-term compliant patients, the risk of emergence of resistant mutant virus is a major concern. Thus, in spite of improved treatment of HIV-infected adults and children, there has been limited progress made in identifying methods for long-term treatment of disease. Many synthetic genes have been developed which can inhibit infection or replication of HIV-1 using a gene therapy approach. Because the hematopoietic abnormalities associated with HIV infection are multilineage, pluripotent hematopoietic stem cells (HSC) that generate all cells of lymphoid, erythroid, and myeloid origin are ideal candidates for use in HIV-1 gene therapy. With even a moderate percentage of gene-protected mature cell populations, selective survival could lead to increased immune function, with diminished production of HIV-1. This proposal focuses on the use of HSC as a target for the delivery of antiviral gene therapy in newborn rhesus monkeys in vivo. As an alternative to ex vivo transduction, we are exploring the in vivo transduction of HSC in their native environment, the milieu of the bone marrow. Initial studies focus on uninfected infant rhesus monkeys to explore the effects of cytokines on the in vivo transduction efficiency of HSC, and the number of infusions necessary for the most efficient marking of HSC and their progeny. We then propose to deliver anti-SIV genes to the HSC of SIV-infected rhesus neonates in order to explore the efficacy of this in vivo approach to inhibit SIV replication. Thus, our goal is to explore a safe and efficacious approach for pediatric gene therapy for HIV-1 infection targeting HSC using in vivo transduction for the delivery of antiviral genes.

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
- [ ] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [ ] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] Behavioral modification.
- [ ] Special diets; food or water treatment.
- [ ] Induced illness, intoxication, or disease
- [ ] Death as an endpoint (see i below)
- [ ] Trapping, banding or marking wild animals
- [ ] Prolonged restraint. (8 hrs+)
- [ ] 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.)

| Study 1. Uninfected infants. Time-mated gravid animals (N=20) will be selected for these studies. They will be screened for endogenous viruses (5 ml blood sample collected under ketamine from a femoral vessel). Fetuses will be assessed sonographically using established techniques every 7-10 days during the duration of pregnancy. The dams will be administered ketamine for these evaluations. Maternal (3-12 ml; femoral vessel) and fetal blood samples (1-2 ml using established ultrasound-guided cardiocentesis [, 1990]) will be collected during gestation (~90, 120, 140 days gestation) and from the dams prior to cesarean-section (~12 ml). Half of the fetuses (N=10) will receive stem cell factor (SCF) intraperitoneal under ultrasound-guidance within 2 days of delivery (0.3 ml; 10 µg/fetus) under ketamine, using established techniques [, 1999] (see Table 1). SCF is a hematopoietic cytokine which is active during early stages of hematopoiesis, and may provide more essential hematopoietic cell types for gene transfer at the time of birth. Newborns will be delivered by cesarean-section using established techniques [, 1989]. Upon delivery, cord blood samples will collected (~6-8 ml; CBCs, chemistry panels, immunophenotyping, plasma and serum). Simian Apgars scores (1, 5, 10 min of life) will be assessed; the simian Apgar is a scoring system similar to the human Apgar which includes the following observations: respiratory effort (respirations/min), heart rate (beats/min), muscle tone, color, state, and body temperature [, 1989]. A bone marrow aspirate will be collected (~2 ml) from the iliac crest or humerus under local lidocaine, then ~0.2 ml vector producer cells will be injected (marrow collected, syringe removed, new syringe attached with 5x10⁶ irradiated producer cells for injection). All infants will be nursery reared for postnatal studies. Post-transfer specimens (1-3 ml of blood collected from a femoral vessel; volume dependent upon age) will be collected 2 weeks post-transfer, then monthly until necropsy. Bone marrow will also be collected under ketamine and local lidocaine (2-3 ml) from alternating sites until necropsy. This is a standard protocol we have used for many years, with no evidence of adverse effects. Half of the animals in each group (N=10; 5 with prenatal SCF administration) will receive a second transfer at 1 month postnatal age (see Table 1). All animals will be euthanized for tissue harvest at approximately 6 months postnatal age.

Table 1. Study 1: Uninfected animals

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Fetal SCF (N)</th>
<th>2nd transfer (N)</th>
<th>Blood/marrow</th>
<th>Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (10)</td>
<td>none</td>
<td>5</td>
<td>birth, 2 wks, monthly</td>
<td>6 months</td>
</tr>
<tr>
<td>2 (10)</td>
<td>10</td>
<td>5</td>
<td>birth, 2 wks, monthly</td>
<td>6 months</td>
</tr>
</tbody>
</table>

Study 2. SIV-infected infants. Time-mated gravid animals (N=20) will be selected for these studies. They will be screened for endogenous viruses as noted above (5 ml blood sample). Fetuses will be assessed sonographically, then inoculated with SIVmac251 (10⁶ TCID₅₀/fetus; 0.3 ml intraperitoneal) using established techniques at 65 days gestation [ et al., 1993]. Fetuses will be monitored sonographically weekly during the duration of pregnancy. The dams will be administered ketamine for these evaluations. Tenofovir (PMPA) will be administered to the dams once daily beginning on day 80 of gestation (subcutaneous [SQ] injection; 10 mg/kg). Although not anticipated, if any of the fetuses show evidence of compromise (growth restriction, low amniotic fluid volumes), then the dams will be administered a higher dose of tenofovir (30 mg/kg/day) until parameters normalize. Fetal inoculation will result in the delivery of newborns with an established infection and detectable viral loads [ et al., 1999; Submitted]. Maternal (3-12 ml; femoral vessel) and fetal blood (1-2 ml) will be collected during gestation (~90, 120, 140 days gestation). Maternal blood samples will also be collected on the day scheduled for cesarean-section. Newborns will be delivered as described above and cord blood samples collected (~6-8 ml; CBCs, chemistry panels, immunophenotyping, plasma and serum, samples for quantitative SIV). Simian Apgars will also be performed. Marrow will be aspirated and 5x10⁶ irradiated producer cells will be injected (0.3 ml) intramarrow as described above. If Study 1 suggests that a second transfer at one month enhances gene expression, then this approach will be included. All infants will be nursery reared for postnatal studies (see Table 2). Post-transfer specimens (1-3 ml of blood from a femoral vessel; volume dependent upon age) will be collected 2 weeks post-transfer, then monthly until necropsy. Bone marrow will also be collected under ketamine and local lidocaine (2-3 ml) from alternating sites until necropsy. We will monitor the infants to assess whether there is a
significant reduction in viral burden post-gene transfer relative to the measure of viral titers at birth (samples collected at 2 wks, then monthly post-transfer). If a significant reduction in viral titers is shown (i.e., levels decline below the detection of our current assays at 3 months of age), then we will terminate tenofovir administration in individual animals and monitor progress (hematology, immunology, viral titers; additional blood samples may be collected weekly or every other week, depending on outcome but will not exceed acceptable limits for animals in this age group). We have an extensive database from SIV-infected infants with and without tenofovir administration for comparison. If it is determined that re-initiating treatment would be advantageous for short periods of time (based on hematology and viral titers), then treatment will be re-initiated. This approach is feasible because it has been shown that terminating tenofovir treatment for short periods does not compromise SIV-infected infants nor result in the development of tenofovir resistant viral mutants [et al., 1999]. All supportive and nutritional measures will be included to maintain SIV-infected infants in a healthy state. Food intake and physical signs will be assessed each daily. Experiments will be conducted in a step-wise manner to ensure that adverse effects do not occur. Our objective is to maintain healthy animals; any infants showing evidence of disease or compromise (such as significant weight loss) will be euthanized.

Table 2. Study 2: SIV-infected

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Fetal SCF (N)</th>
<th>2nd transfer (N)</th>
<th>Blood/marrow</th>
<th>Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (10)</td>
<td>none</td>
<td>tbd</td>
<td>birth, 2 wks, monthly</td>
<td>12 months</td>
</tr>
<tr>
<td>4 (10)</td>
<td>5</td>
<td>tbd</td>
<td>birth, 2 wks, monthly</td>
<td>12 months</td>
</tr>
</tbody>
</table>

*tbd=to be determined, based on Study 1*

**YEAR 1:** N=15 dams and offspring = 30 animals

**YEAR 2:** N=15 dams and offspring = 30 animals

**YEAR 3:** N=10 dams and offspring = 20 animals
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>10 dams and their offspring, fetal/maternal samples and sonographic monitoring, c-section at term, blood and marrow at birth, vector transfer intramarrow at birth with half with 2nd transfer at 1 month, blood and marrow at 2 wks and monthly, necropsy at ~ 6 months</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>10 dams and their offspring, fetal/maternal samples and sonographic monitoring, half receive SCF IP within 2 days of delivery, c-section at term, blood and marrow at birth, vector transfer intramarrow at birth with half with 2nd transfer at 1 month, blood and marrow at 2 wks and monthly, necropsy at ~ 6 months</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>10 dams and their offspring, fetal/maternal samples and sonographic monitoring, fetal SIV infection at 65 days gestation, maternal and newborn tenofovir administration (SQ; 10 mg/kg/day), c-section at term, blood and marrow at birth, vector transfer intramarrow, blood and marrow at 2 wks and monthly, necropsy at ~ 12 months</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>10 dams and their offspring, fetal/maternal samples and sonographic monitoring, fetal SIV infection at 65 days gestation, maternal and newborn tenofovir administration (SQ; 10 mg/kg/day), half receive SCF IP within 2 days of delivery, c-section at term, blood and marrow at birth, vector transfer intramarrow, blood and marrow at 2 wks and monthly, necropsy at ~ 12 months</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Little or no discomfort or stress</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>2</td>
<td>Minor stress or pain of short duration</td>
</tr>
<tr>
<td></td>
<td>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</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe distress</td>
</tr>
<tr>
<td></td>
<td>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</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain near, at or above the pain tolerance threshold</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
</tbody>
</table>

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?

Monkeys are the only appropriate model for these studies because of physiologic similarities when compared to humans. Based on our experience with this model, the number chosen is the minimum required in order to adequately assess the efficiency of the proposed techniques.

f) Surgery: If the project involves survival surgery, where will the surgery be conducted?
Building: CRPRC animal quarters Room: Surgery suite

Who will be the surgeon? CRPRC veterinarians

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>Rhesus</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>10-12 times</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>5-8</td>
<td>IM</td>
<td>4-5 times</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Isoflurane</td>
<td>to effect</td>
<td>inhal.</td>
<td>Once, for c-section</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Oxymorphone</td>
<td>0.15</td>
<td>IM</td>
<td>Post-surgery for dams</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Lidocaine</td>
<td>0.1 ml</td>
<td>SQ</td>
<td>Marrow aspirates</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)

All possible measures will be taken to minimize discomfort and adverse effects. Minimal discomfort may be associated with blood sample collection, bone marrow aspirates, and cesarean-section. Oxymorphone will be administered to the dams for 2 days post-cesarean-section, and lidocaine administered prior to bone marrow aspirates. Tenofovir will be administered at a dose that has been shown to prevent disease and illness in dams, fetuses, and infants, as well as bone-related toxicity [et al., 1999; 2002; Submitted].

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.

See comments above. There are no other adverse effects anticipated or procedures planned that would require administration of analgesics or anesthetics other than those described above. The decision to administer additional pharmacologic agents or euthanize animals will be made by the investigator in consultation with a senior CRPRC veterinarian, using standard SIV guidelines. The objective is to maintain animals in a healthy state.

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:

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

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit http://trc.ucdavis.edu/jawelsh/Databases/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu) or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwwood@ucdavis.edu)

What was the date on which you conducted this search? [2/1/02]

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>PubMed</td>
<td>1980 to current</td>
<td>Gene therapy, HIV, SIV, gene transfer, gene therapy, HSC, monkeys, antiretrovirals, HAART</td>
</tr>
<tr>
<td>Reference Update®</td>
<td>Most recent publications</td>
<td>Gene therapy, HIV, SIV, gene transfer, gene therapy, HSC, monkeys, antiretrovirals, HAART</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There are none that would allow us to investigate the questions we propose to address. A primate model is essential for these investigations in order to obtain relevant information for potential human application.

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.

The studies outlined are novel and have never been conducted in the manner we propose.

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

All animals will be euthanized as noted above. The dams will be re-bred each year to obtain infants for study. Although not anticipated based on many years of experience, if any of the dams become debilitated, they will be euthanized.

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>Rhesus</td>
<td>Overdose</td>
<td>Pentobarbital</td>
<td>60</td>
<td>IV</td>
</tr>
</tbody>
</table>

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

All animals will be euthanized at the conclusion of each of the studies.
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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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/ 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/.
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.

<table>
<thead>
<tr>
<th>Assoc. Adj. Prof</th>
<th>3/15/02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal Investigator</td>
<td>Rank / Title</td>
</tr>
<tr>
<td>CRPRC Director</td>
<td>Date</td>
</tr>
</tbody>
</table>

Committee Use Only Below

** 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.

<table>
<thead>
<tr>
<th>Campus Veterinarian</th>
<th>Date</th>
</tr>
</thead>
</table>
**ANIMAL ROOM SAFETY INFORMATION**

Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

**PROTOCOL # __10030__**  
**EXPIRES: __________**

**Identity of Hazard:** Retroviral vectors (HIV-1-derived lentivirus), SIVmac251

**Provide a short description of the agent:**

The lentiviral vectors are self-inactivating and replication-defective and the only potential infection risk is if recombination occurs between vectors of the packaging sequences, which could lead to emergence of replication-competent viruses. This is highly unlikely because the vectors are self-inactivating. SIV is a primate lentivirus that is genetically and serologically related to HIV-1 and HIV-2. SIV isolates appear to be nonpathogenic in their indigenous hosts, including African green monkeys and sooty mangabeys. However, when experimentally inoculated into rhesus monkeys, many strains of SIV (including SIVmac251) cause fatal immunodeficiency very similar to human HIV infection and AIDS.

**This agent / material is hazardous for:**  
[ ] Humans only  
[ ] Animals only  
[ X ] Humans and Animals

**For which Animal Species?** Monkeys

**The agent can be spread by:**  
[ X ] Blood  
[ X ] Feces/urine  
[ X ] Saliva/nasal droplets  
[ ] Does not leave animal  
[ ] Other:

**Describe any human health risk associated with this agent:**

Vectors have all viral genes removed and thus are replication-defective. The generation of self-inactivating (SIN) vectors enhances the safety features of these vectors by reducing the possibility of recombination to generate replication-competent virus because there is no complete U3 in the virus production system. SIV has the potential for causing immune deficiency disease. The potential for infection of humans with SIV has been demonstrated through two accidental exposures of lab personnel [ 1992; et al., 1994]. However, the potential for disease in SIV-infected humans has not been shown.

**The precautions checked below apply to this experiment:**  
**Standard CRPRC conditions for handling and housing applies.**

[ ] The researcher or his/her technicians are responsible for the feeding and care of these animals.  
[ ] The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians.

[ ] Cage  
[ ] Stall  
[ ] Water Bottle  
[ ] Animal Carcasses  
[ ] Bedding  
[ ] Other:

[ ] Cages must be autoclaved before cleaning.  
[ ] Label cages and remove label after decontamination.  
[ ] Animal carcasses must be labeled and disposed of as follows:

[ ] Incineration  
[ ] Bag and Autoclave  
[ ] Biohazardous Waste Container  
[ ] EH&S will pick-up (2-1493).

[ ] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:

[ ] Incineration  
[ ] Bag and Autoclave  
[ ] Biohazardous Waste Container  
[ ] EH&S will pick-up (2-1493).

**Personal Protective Equipment Required:**

[ X ] The following personal protective equipment must be worn/used in the room:

[ X ] Lab Coat/Coveralls  
[ X ] Disposable Gloves  
[ ] NIOSH Certified Dust Mask  
[ X ] Eye Protection/Face Shield  
[ ] Fitted Respirator  
[ ] Other:  

Type:  

Describe:

[ ] Personal protective equipment must be removed before leaving the room.  
[ ] Personal protective equipment must be discarded or decontaminated at the end of the project  
[ ] Hands, arms, and face must be thoroughly washed upon leaving the room  
[ ] Full shower, including washing of hair, must be taken upon leaving the room.
Decontaminate Room (Inform ARS area supervisor when cage and/or room can be returned to general use).

Provide any other information needed to safely work in this room: