PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last Name:</td>
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<td>First:</td>
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<td>Fax:</td>
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</tbody>
</table>

Species (common names): Rhesus monkey
Number: 36
Source: Primate Center

Project Title: Lentiviral vectors for gene transfer into monkey HSC

Overnight housing location: Primate Center
Day use only: [x] Vivarium [ ] Investigator (If investigator maintained, attach husbandry SOP’s.)

Animals will be maintained by: [x] Investigator

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.

Bone marrow will be collected for transduction with lentiviral vectors, then the cells will be injected back into the animals after cytoreduction with busulfan. Blood and marrow will be collected post-transplant, and animals will be necropsied approximately 6 months post-transplant. Studies will be conducted with uninfected and SHIV-infected animals.

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.

BSL2+ housing.

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>[x] Yes</td>
<td>[x] Yes</td>
<td>[x] Yes</td>
</tr>
<tr>
<td>[ ] No</td>
<td>[x] No</td>
<td>[x] No</td>
<td>[x] No</td>
</tr>
</tbody>
</table>

Agent(s): Lentiviral vectors, SHIV162P3

University of California, Davis
Version 7/21/2004 2:59:57 PM Page 1
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 noncompliance, 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. Using a well-established rhesus monkey model our goal is to explore gene transduction/engraftment of autologous HSC transduced by lentiviral vectors with anti-HIV-1 genes. In these studies, we will explore whether conditioning therapy (busulfan) is necessary for transplant of autologous transduced HSC. Busulfan produces a specific loss of early stem cells and has been used extensively as a stem cell cytoreductive conditioning agent prior to bone marrow transplantation. We have previously assessed the kinetics and safety of busulfan in monkeys, and found that a 4-day course of treatment (32 mg/kg) is safe and well-tolerated. We will focus on the effects of pre-transplant cytoreductive conditioning using busulfan in order to “make space” in the marrow for transduced bone marrow-derived HSC. We will also test novel lentiviral vector constructs with anti-HIV genes by transplant of ex vivo transduced HSC from infant monkeys with and without pathogenic simian/human immunodeficiency virus (SHIV) infection. These studies will assess the current “best” conditions for transduction and transfer of primate HSC with anti-HIV genes, and subsequent expression in myeloid and lymphoid cells in vivo. The results of these preclinical studies will be essential prior to proposed clinical trials in HIV-infected humans.

b) Procedures employed in this project: Please check the appropriate boxes if any of these procedures will be employed

- [ ] Monoclonal Antibody Production
- [ ] Polyclonal Antibody Production
- [ ] LD 50 or ID50 studies.
- [x] Catheters, blood collection, intubation
- [ ] Prolonged restraint (8 hrs+)
- [x] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [ ] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] Special diets; food or water treatment.
- [ ] Induced illness, intoxication, or disease
- [ ] Death as an endpoint (see h below)
- [ ] Trapping, banding or marking wild animals
- [ ] Behavioral modification.
- [ ] Aversive conditioning.
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. Cytoreduction with busulfan (Year 1 and 2).** Assessing the use of busulfan in these studies is essential because it has been shown that engraftment of HSC is a directly competitive process, therefore "space" for the engrafting cells must be made in order to achieve high levels of engraftment. We are using a cytoreductive (rather than ablative) protocol, which requires a much lower dose and steady-state concentration to achieve high levels of engraftment.

**Study 1.1. Uninfected animals:** Twelve 3-month-old monkeys will be used for this study. These animals will be approximately 1 kg at the initiation of treatment, and pre-screened to ensure they are negative for endogenous viruses such as SRV and STLV (1 ml of blood collected under ketamine from a peripheral vessel). The following busulfan doses will be assessed: 0, 1, 3, and 6 mg/kg. We will first assess the kinetics of a single dose at 1 mg/kg (N=3). Busulfan will be administered using an IV formulation every 6 hours over a 24-hr period. An indwelling catheter will be placed at the time of the first dose which will be removed after the last dose is administered. Treatments will be given under ketamine (first dose) or with the animals placed on a restraint board designed for animals in this age group. We will collect blood samples from a femoral vessel in hand-restrained animals after the first dose (0, 0.5, 1, 2, 4 hrs, with the last sample collected prior to the second dose; 800 µl/sample for busulfan levels, 200 µl samples for CBCs at 0, 6, and 24 hrs post-treatment) in order to determine the steady state concentration. All infants will be housed in relative isolation and monitored closely. No adverse effects are anticipated based on our prior studies using much higher doses (32 mg/kg). We will increase the dose to 3 mg/kg (N=3) then to 6 mg/kg (N=3) in order to compare outcome, both in steady state concentrations (Css) and the potential for adverse effects. In all cases, animals will receive an autologous transplant 24 hrs after the last dose of busulfan to assess marking *in vivo* (see below). Although not anticipated, treatment-related toxicity will be carefully monitored in each animal daily throughout the duration of the study. Each dose group will be carefully evaluated for 1 month before initiating studies with the next higher dose. Animals will be assessed for a 6 month duration post-treatment and transplant (blood and marrow collection, growth, health), then euthanized for a complete tissue harvest (see below).

**Study 1. Cytoreduction with busulfan**

<table>
<thead>
<tr>
<th>Animals (N)</th>
<th>Busulfan (N)</th>
<th>Blood</th>
<th>Marrow</th>
<th>Necropsy</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. Uninfected (12)</td>
<td>0 (3) 1 mg/kg (3) 3 mg/kg (3) 6 mg/kg (3)</td>
<td>0, 1 and 2 wks post-transplant, then monthly</td>
<td>0, 2 wks post-transplant, then monthly</td>
<td>9 months (6 months post-transplant)</td>
<td>1</td>
</tr>
<tr>
<td>1.2. SHIV-infected (8)</td>
<td>Based on results, above</td>
<td>0, 1 and 2 wks post-transplant, then monthly</td>
<td>0, 2 wks post-transplant, then monthly</td>
<td>9 months (6 months post-transplant)</td>
<td>2</td>
</tr>
</tbody>
</table>

HSC transplant studies will be performed in all animals administered busulfan as noted above (dose selection; N=9), and compared to animals that will not be administered busulfan (controls; N=3). Animals will be administered ketamine (10 mg/kg) and local lidocaine for marrow aspiration prior to the administration of busulfan. A 1 ml blood sample (CBC, chemistry panel) and ~10 ml bone marrow aspirate will be collected under ketamine and local lidocaine from the iliac crest, using established techniques. Busulfan administration will begin immediately after marrow collection (once every 6 hrs; 4 doses over one day), with one day clearance prior to transplant. Transplant of autologous CD34⁺ HSC will be performed IV in an approximate 1 ml volume within 48 hrs of marrow collection. Blood samples will be collected from all animals, as noted above, to monitor busulfan levels. Post-transplant specimens
(~2-3 ml blood from a peripheral vessel) will be collected at 5-7 days and 2 weeks post-transplant (CBCs, chemistry panels; 1 ml), then monthly until necropsy (9 months postnatal age; 6 months post-transplant) under ketamine.

Study 1.2. SHIV-infected animals: Time-mated gravid animals (N=8) will be selected for these studies. They will be screened for endogenous viruses (1 ml blood sample collected under ketamine from a peripheral vessel). Fetuses will be assessed sonographically using established techniques [, 1988], then inoculated with pathogenic SHIV162P3 (1-200 TCID\textsubscript{50}/fetus; 0.4 ml intraperitoneal) using established techniques at 65 days gestation [, 1993].

Fetuses will be monitored sonographically weekly during the duration of pregnancy (dams 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 infection will result in the delivery of newborns with an established infection with detectable viral loads [, et al., 1999 and unpublished]. Maternal (3-10 ml; peripheral vessel) and fetal (1-2 ml using established ultrasound-guided techniques; cardiolentesis [, 1990]) will be collected during gestation (approximately 90, 120, 140 days gestation and at birth). Newborns will be delivered by cesarean-section using established techniques [, 1989]. Upon delivery by cesarean-section, cord blood samples will collected (~6 ml; CBCs, chemistry panels, plasma and serum, and samples for quantitative SHIV). Infants will be collected for CBCs, chemistry panels, and quantitative SHIV levels (~3 ml per month). At three months of age, infants will be immobilized with ketamine, and ~10 ml bone marrow will be aspirated under local lidocaine from the iliac crest, as noted above in Study 1.1. Busulfan will be initiated on the day of marrow collection and administered over a 24-hr period (once every 6 hrs) using the best dose identified in Study 1.1. Post-transplant specimens (~2-3 ml blood from a peripheral vessel) will be collected at 5-7 days then 2 weeks post-transplant, then monthly until necropsy, as noted above (9 months postnatal age; 6 months post-transplant). In addition, bone marrow aspirates (~2 ml) will be collected monthly from alternating sites (right and left iliac crest) under ketamine (10 mg/kg) and local lidocaine. All of our standard supportive and nutritional measures will be included to maintain SHIV-infected animals in a healthy state. Experiments will be conducted in a step-wise manner to ensure that adverse effects do not occur.

Study 2. Novel lentiviral vector constructs (Year 3). Eight animals (1 vector construct) will be included in these studies. The dams from Study 1.2 will be bred to obtain the infants for these studies, using established CRPRC techniques. As noted above, fetuses will be directly infected with SHIV162P3, monitored during gestation with maternal (3-10 ml) and fetal (1-2 ml) blood samples collected at approximately 90, 120, and 140 days gestation., and tenofovir will be administered to the dams beginning at 80 days gestation (10 mg/kg). Newborns will be delivered by cesarean-section at term, cord blood samples collected, and infants raised in the nursery for postnatal studies. Tenofovir will be administered to infants once daily (10 mg/kg), and blood samples collected monthly (~3 ml). At 3 months of age, a marrow aspirate will be collected, busulfan administered, and a transplant performed within 48 hrs of marrow collection, as described above. Blood and marrow will be collected as noted above, and necropsy performed at 9 months postnatal age (6 months post-transfer).

YEAR 1: N=12 animals
YEAR 2: N=8 dams+8 offspring = 16 animals
YEAR 3: N=8 offspring (dams from Year 2 rebred) = 8 animals
d) 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>12 - 3 month old infants - Blood and marrow collection, busulfan administration and cellular transplant at 3 mos, necropsy at 9 mos</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>8 dams and their 8 respective offspring - Fetal infection with SHIV, maternal/fetal blood sample collection, c-section, tenofovir, infant blood and marrow transplant at 3 mos, necropsy at 9 mos.</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Fetal infection with SHIV, maternal/fetal blood sample collection, c-section, tenofovir, infant blood and marrow collection, busulfan and cellular transplant at 3 mos, necropsy at 9 mos.</td>
<td>8</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 skilful 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 the species choice was appropriate and the number of animals in the groups above 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. In order to determine if this protocol will be appropriate for human use, studies in monkeys are essential. Based on our experience with this model, the number chosen is the minimum required in order to adequately assess engraftment and gene transfer efficiency. Statistical significance is not required for these studies.

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 aspires</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. All possible measures will be taken to minimize discomfort from these procedures. Oxymorphone will be given 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, and bone-related toxicity [et al., 1999; Submitted]. We have previously administered busulfan to young rhesus monkeys for a 4-day duration (32 mg/kg total dose over 4 days), and observed the expected decrease in neutrophils, platelets, and erythrocytes post-therapy, and no significant change in absolute lymphocyte counts. There was also no abnormality detected in liver function tests for up to 2 months post-therapy. Since the dosages proposed for study are much lower and for a shorter treatment period (1 day) we do not anticipate any adverse effects. We will be rigorously monitoring the animals throughout the day of treatment and daily post-treatment, and should any unanticipated adverse effects arise, they will be addressed immediately. Any animals that display adverse findings will be treated in consultation with the CRPRC senior veterinary staff, and any that are unresponsive to treatment will be euthanized. In a study in children where busulfan (14-20 mg/kg) was administered with cyclophosphamide (200-240 mg/kg), of the 38 patients assessed (6 months-18 yrs), 36/38 experienced mild GI toxicity (diarrhea), 30/38 mild hepatic toxicity, 14/38 mild renal toxicity, 4 patients had seizures, and 2 had hemorrhagic cystitis. It is important to note that treatment was for a 4-day duration in this study, and that busulfan was administered at higher doses than those proposed, and with cyclophosphamide, which enhances the potential for toxicity. In addition, as noted above, we have not observed adverse findings in our prior studies in monkeys with busulfan and, thus, do not anticipate significant adverse effects in the studies proposed.

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.

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?  8/1/01

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>PubMed</td>
<td>1980 to current</td>
<td>Gene therapy, HIV, SHIV, gene transfer, monkeys, busulfan</td>
</tr>
<tr>
<td>Reference Update®</td>
<td>Most recent publications</td>
<td>Gene therapy, HIV, SHIV, gene transfer, monkeys, busulfan</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 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>
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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.

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**Conditions necessary for Committee Approval:**

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Final Disposition of this protocol:

- [ ] Approved
- [ ] Not Approved
- [ ] Withdrawn by Investigator

Date of Action: _____ / _____ / _____

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

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Campus Veterinarian

Date
# ANIMAL ROOM SAFETY INFORMATION

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

- **Identity of Hazard:** Lentiviral vectors (HIV-1-derived lentivirus), SHIV
- **Investigator Last Name:**
- **First Name:**
- **Phone:**
- **Email:**
- **Fax:**

### 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. SHIVs are chimeric viruses comprised of HIV-1 and SIV genes (HIV-1 env, tat, rev, and vpu genes, with the remaining genes derived from SIV).

### 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] Saliva/nasal droplets
- [ ] Does not leave animal

### 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. SHIVs have the potential for causing immune deficiency disease.

### 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 as biohazardous and for investigator
  - [ ] 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
  - [X] NIOSH Certified Dust Mask
  - [X] Eye Protection/Face Shield
  - [X] Fitted Respirator
  - [ ] Other:

**Type:**

- [ ] Head Cover
- [ ] Shoe Covers/Booties
- [ ] Disinfectant footbath

**Type:**

- [ ] Description:

- [ ] 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.