PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

Investigator

Last Name: Last Name:
First: First:
Middle: Middle:
email: email:
Department: Department:
Phone: Phone:
Fax: Fax:

Contact

Last Name:
First:
Middle:
email:
Department:
Phone:
Fax:

Species (common names):
Rhesus monkey

Number:
120

Source:
Primate Center
(dams+infants)

Project Title
Fetal Monkey Model for Gene Transfer for Sickle Cell Disease

Overnight housing location:
Primate Center
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.

These studies focus on transferring genes into fetal monkeys in vivo. Studies include collection of fetal/maternal samples during gestation, delivering newborns by cesarean-section at term, collection of infant blood and marrow monthly, and necropsy at 3, 6, or 12 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.

None

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

Sick Animals
[x] Call Investigator
[ ] Clinician to treat
[ ] Terminate
[ ] Necropsy

Dead Animals
[x] Call Investigator
[ ] Bag for disposal
[ ] Necropsy

Pest Control
[x] Call Investigator
[ ] OK to use pesticides
[ x] No Pesticides in animal area

Hazardous Materials (only if in the animal room):

Infectious Agents?  [x] Yes  [ ] No  Agent(s): Lentiviral vectors (BAUA 0547)
Radioisotopes?  [ ] Yes  [x] No  Agent(s):
Chemical Carcinogens?  [ ] Yes  [x] No  Agent(s):
Toxic Chemicals?  [ ] Yes  [ ] No  Agent(s):
Is the project already funded? [x] Yes [ ] No
Previously approved? [x] Yes [ ] No

Proposed Funding Source: NIH
Previous protocol number (if any): #8809

What Veterinarian or veterinary clinic will provide care for your animals? (check one)
[ ] Lab Animal Health Clinic (2-0514)
[ ] California Primate Research Center (2-0447)
[ ] VMTH Large Animal Field Service (2-0292)
[ ] Another Veterinarian

If you checked “Another Veterinarian”, please provide:
Veterinarian: 
Address: 

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@ucdavis.edu) for current information about training and record keeping requirements.

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.

The overall goal of this study is to explore fetal gene transfer for sickle cell disease (SCD) in our established fetal rhesus monkey model. Gene therapy strategies that target hematopoietic stem cells (HSC) have been proposed as a long-term treatment for hemoglobinopathies such as SCD. However, traditional ex vivo gene therapy approaches for transducing HSC in humans and large animal models have proven disappointing due to low gene transfer efficiencies, poor expression of the introduced gene, and technical difficulties associated with the transplant procedures. In this study, we propose direct in utero gene transfer because this approach eliminates the problems and limitations associated with removal of HSC, in vitro transduction, and subsequent re-introduction in vivo. Once we have determined the best vector system and approach for the efficient marking of fetal HSC, we will focus on transferring the human B-gamma globin gene into fetal monkey HSC. Thus, we propose to explore methods for safely transferring genes into fetal monkeys that can be persistently expressed in hematopoietic cells. These studies will be crucial for identifying novel gene transfer strategies for treating human fetuses diagnosed in utero with hemoglobinopathies such as SCD.

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 **
[ ] Food or water restriction
[ ] Special diets; food or water treatment.
[ ] Polyclonal Antibody Production **
[ ] Non-recovery surgical procedures
[ ] Induced illness, intoxication, or disease
[ ] LD 50 or ID50 studies.
[ ] Survival surgical procedures
[ ] Death as an endpoint (see h below)
[ x] Catheters, blood collection, intubation
[ ] Multiple survival surgery
[ ] Trapping, banding or marking wild animals
[ ] Prolonged restraint. (8 hrs+)
[ ] Behavioral modification.
[ ] Fasting prior to a procedure.
[ ] 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. Efficiency of lentiviral vector constructs** - Gravid adults will be screened for endogenous viruses (~2 ml blood sample collected from a femoral vessel), then selected for study (N=30). The intent of this study is to compare different lentiviral vector constructs in order to identify the most efficient construct for the transduction of fetal HSC in utero. Three lentiviral vectors will be used (N=10/vector). Gene transfer will be accomplished at ~50 days gestation (fetal intraperitoneal administration via ultrasound guidance; 0.4 ml supernatant). All fetuses will be sonographically monitored approximately every 5-10 days post-transfer until term delivery by cesarean-section. Fetal samples will be collected at approximately 100 (~1 ml fetal blood, ~2 ml amniotic fluid), 120 (1-2 ml fetal blood, ~2 ml amniotic fluid, ~2 mm fetal liver biopsy), and 140 (~2 ml fetal blood, ~2 ml amniotic fluid) using routine ultrasound-guided techniques [1990; 1993]. Samples will also be collected from the dams (~3-12 ml; femoral vessel, volumes will not exceed acceptable limits, based on body weight) prior to fetal transfer, at 1 and 2 wks post-transfer, then every 10 days until delivery. The dams will be administered ketamine hydrochloride (10 mg/kg) for these evaluations. At term, all newborns will be delivered by cesarean-section using established techniques, and cord blood samples collected (~9-12 ml). Standard newborn assessments will be performed (simian Apgar scores similar to the human Apgar at 1, 5, and 10 min of life--heart and respiratory rates, color, state, temperature), then bone marrow aspirates (~2 ml) will be collected from the iliac crest under local lidocaine, using established techniques. Infants will be nursery-reared for postnatal studies. Blood samples (~3-6 ml; dependent upon age) will be collected monthly until necropsy (3, 6, or 12 months--dependent on levels of transduction and gene expression). In addition, bone marrow aspirates (~2 ml) will be collected monthly (alternating sites) until 12 months of age under ketamine (10 mg/kg) and local lidocaine. Infants will be euthanized and a complete tissue harvest performed.

**Study 2. Long-term globin gene expression** - Once we have determined the best lentiviral vector construct for the transduction of fetal HSC (**Study 1**) we will use this viral vector to introduce the human β-γ globin gene into fetal rhesus monkey HSC. We will test three different promoters/enhancers in order to determine the most efficient method for obtaining long-term expression of the globin gene in rhesus HSC and hematopoietic progenitors (N=10/promoter). Gravid adults will be screened for endogenous viruses as noted above (2 ml blood sample collected from a femoral vessel), then selected for study (N=30). All procedures will be the same as noted above for **Study 1**. Fetuses will be transferred, then monitored sonographically until term delivery. Fetal and maternal samples will be collected during gestation, and newborns delivered by cesarean-section at term. Cord blood samples and bone marrow aspirates will be collected at birth. Infants will be nursery-reared for postnatal studies, and blood and marrow collected monthly under ketamine (10 mg/kg) and local lidocaine. Infants will be euthanized at 3, 6, or 12 months of age.

**TOTAL ANIMALS YEARS 1-3 = 120 (60 dams and 60 offspring)**

**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>Fetal gene transfer, maternal/fetal blood samples, infant blood and marrow collection</td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Fetal gene transfer, maternal/fetal blood samples, infant blood and marrow collection</td>
<td>60</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 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 reproductive, developmental,  
 hematologic, and immune system similarities when compared to humans. Based on our experience  
with this model, the number chosen is the minimum required in order to adequately assess group  
differences.

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>dams: ~15x; infants: monthly</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>5-8</td>
<td>IM</td>
<td>Dams, 6-8 times</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Isoflurane</td>
<td>to effect</td>
<td>inhal.</td>
<td>Cesarean-section, 1x</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-monthly</td>
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</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)

We do not anticipate any adverse effects based on our extensive experience with this model and the gene transfer procedures. Minimal discomfort may be associated with blood sample collection, bone marrow aspiration, 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.

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.

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? 9/10/02

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>Fetus, in utero, gene therapy, congenital disease, animal models, sickle cell disease, hemoglobinopathies</td>
</tr>
<tr>
<td>Reference Update®</td>
<td>Most recent publications</td>
<td>Fetus, in utero, gene therapy, congenital disease, animal models, sickle cell disease, hemoglobinopathies</td>
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</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 if potential human application is to be considered.

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?

All offspring will be euthanized at 3, 6, or 12 months postnatal age. The dams will be returned to the breeding colony two weeks post-cesarean-section.

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

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

See comments above.
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>
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<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/ 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/](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>Professor</th>
<th>9/20/02</th>
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<thead>
<tr>
<th>Principal Investigator</th>
<th>Rank / Title</th>
<th>Date</th>
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</table>

<table>
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<tr>
<th>CRPRC Director</th>
<th>Date</th>
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</table>

Committee Use Only Below

** Conditions necessary for Committee Approval:

---

Final Disposition of this protocol:

- [ ] Approved
- [x] 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>
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</table>
ANIMAL ROOM SAFETY INFORMATION
Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

PROTOCOL #________
EXPIRES: ________

RUA#: __________ BUA#: 0547 CCA#: ________

Identity of Hazard: Lentiviral vectors

Provide a short description of the agent:
The 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 (not likely).

This agent / material is hazardous for: [X] Humans and Animals
For which Animal Species? Monkeys

The agent can be spread by:
[ ] Blood
[ ] Feces/urine
[ ] 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. There are no known cases of accidental human infection or recombination, to date. 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.

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:
[ ] The following personal protective equipment must be worn/used in the room:
[ ] Lab Coat/Coveralls
[ ] Disposable Gloves
[ ] NIOSH Certified Dust Mask
[ ] Eye Protection/Face Shield
[ ] Fitted Respirator
[ ] Other: 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.