

PROTOCOL FOR ANIMAL USE AND CAREEmail to: campusvet@ucdavis.edu**CNPRC**

EH&S USE ONLY

**PROTOCOL: 10855
EXPIRES:**

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

Species (common names):	Number:	Source:
Rhesus Macaques	30	CNPRC

Project Title	Affordable AIDS Therapies (Cansoon project)		
Overnight housing location::	CNPRC, UC-Davis	Day use:	CNPRC, UC-Davis
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> 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.

Groups of SIV-or SHIV infected or uninfected rhesus macaques will be treated with a mixture of vitamin and steroid supplements to assess its therapeutic effects on the disease course.

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.

Animals are housed in standard CNPRC BSL2+ housing.

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

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input checked="" type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

Hazardous Materials (only if in the animal room):

Infectious Agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	SIV, SHIV
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	City of Hope	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email ptillman@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 advent of highly active anti-retroviral therapy (HAART) has dramatically influenced the AIDS epidemic in developed countries such as the United States. However, the price of these drugs is beyond the reach of the majority of HIV-infected people who live in developing countries. In addition, because these drugs are targeted to viral enzymes, the emergence of drug-resistant viral mutants is a major problem. Thus, immunomodulatory compounds that strengthen the immune system to control virus replication and/or limit the immune damage and/or would be very useful.

Certain natural compounds have been found to have anti-HIV activity in vitro, including pyrodoxal phosphate (PLP; a form of vitamin B6), and 3 β -acetoxyandrost-5-ene-7,17-dione (7-oxo-DHEA-acetate, or further abbreviated to 7-ODA).

Vitamin B6 is known to have many physiologic effects, including on the immune system, and is often deficient under conditions of extreme stress, injury or disease. HIV-infected people have decreased vitamin B6 levels that correlate with reduced CD4+/CD8 ratios. Vitamin B6 binds magnesium, and high doses of vitamin B6 are therefore given together with magnesium. Relatively large doses of vitamin B6 have been taken by many children and adult, without evidence of adverse effects. Vitamin B6 supplementation benefits children with autism. From a vast clinical experience, upper daily oral doses for adults are 1.5 gram vit B6 and 600 mg Mg. Children have received doses of 1 gram vit B6 without adverse effects.

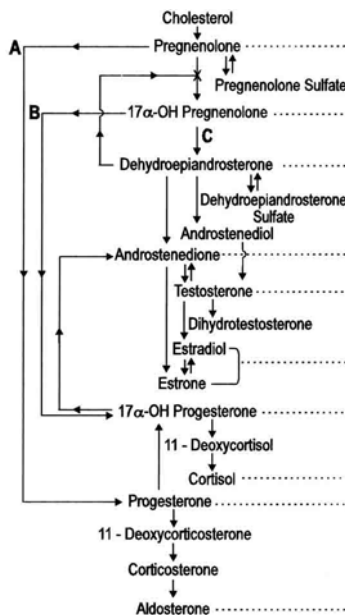
7-ODA is produced from cholesterol via DHEA. 7-ODA has a history as an immune-boosting agent. Human trials have found 7-ODA to be safe at doses of 200 mg orally, daily for 28 days (Clin. Invest. Med, 2000, V. 23, p. 300-310). Doses up to 2.4 grams per day have been given to adults without any reported toxicity. In studies with rhesus macaques, daily oral administration of doses up to 500 mg/kg body weight did not result in detectable clinical effects or macroscopic or microscopic lesions, while at higher doses of 1000 mg/kg some vomiting was observed (Biochem & Biophys. Research Communications, 1999, v. 254, p 124-126).

In unpublished data, 7-ODA has previously shown some promising results in a few SIV-infected monkeys with late stage disease at the Wisconsin National Primate Research Center. Oral 7-ODA treatment for periods of 28 days, at a total regimen of 0.5 g, resulted in an increase in CD4+ cell counts and increase in weight gain. However, a lack of funding forced termination of that study.

DHEA is known to antagonize some of the unwanted immunosuppressive effects of cortisol. A number of studies have reported that DHEA levels decrease during the progression of HIV disease (while cortisol levels are increased), and some pilot studies have suggested that DHEA treatment of HIV-infected patients leads to some moderate reduction in virus levels. A DHEA derivative was found to have beneficial effects on feline immunodeficiency virus-infected cats (Vet. Immunology & Immunopathology, 2003; v. 94, p 133-148).

As indicated in the figure below, pregnenolone is an intermediate precursor for all these steroid compounds

(sex steroids, glucocorticoids, and mineralocorticoids). Due to tissue-specific differences in metabolism, it can go to different pathways. Pregnenolone's formation and subsequent metabolism is rate-limiting for the formation of several steroid hormones necessary to maintain the body homeostasis. The therapeutic use of pregnenolone was mainly investigated in the 1940's to 1950's, and it was shown to have beneficial effects in a number of autoimmune diseases (SLE, rheumatoid arthritis). It was later replaced by glucocorticosteroids, at a time when the side-effects of long-term glucocorticosteroids were not fully appreciated. However, because pregnenolone is also a precursor for the other pathways, it may provide a better balance, and thus supplementation also warrants further investigation. More detailed information is available upon request.



Infection of macaques with SIV (simian immunodeficiency virus) or SHIV (a simian-human chimeric virus) is a good animal model to test the potential therapeutic benefits of such compounds. The purpose of this project is to use SIV- and SHIV-infected macaques that are currently part of ongoing projects by several investigators, and that are no longer needed. Instead of euthanizing such animals, useful preliminary data can be gathered by starting these animals on treatment with a mixture of these compounds, and monitoring viral markers (virus levels), immunological markers (CD4 and CD8 cell counts) and clinical parameters (weight, etc.). If uninfected animals become available, they would be useful to gather data on the effects of these compounds on an uninfected hosts. Such preliminary data can then be the basis to seek funding for doing more detailed and controlled studies.

Because the purpose of this study is to demonstrate the feasibility of this approach, we propose to first test a mixture of these compounds (VitB6/Mg, 7-ODA, DHEA and pregnenolone). It is not anticipated that these compounds would have antagonistic effects. If beneficial effects are observed in the first animals, then subsequent animals would be given fewer compounds and/or lower amounts.

b) Procedures employed in this project:

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

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

** 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.)

TABLE 1

Procedure	Frequency
Oral drug administration	Once daily
Blood collection,urine	Every week
Lymphnode biopsy	Every 3 months (or less frequently)
Radiographs, bone marrow, CSF taps	Monthly (or less frequently)
Pharmacokinetics	Every 3 months (or less frequently)

Housing, samples and surgical procedures:

Animals are housed in standard CNPRC BSL2+ housing. Animals are from previous experiments (including SIV and SHIV infections) that are no longer needed by their investigators. The schedule of samples is summarized in table 1 above. Blood samples (total volume not to exceed 12 ml/kg/month) will be collected from each animal by femoral venipuncture under ketamine (10 mg/kg) anesthesia (after overnight fasting) at weekly time points following virus inoculation. Blood samples will be used to monitor hematological, viral and immunological parameters, and standard chemistry panels. Urine will be collected via cystocentesis (see below) to monitor toxicity and measure metabolites of the administered compounds.

To monitor immunocompetence, animals will be given immunizations with commercial DTP (diphtheria—tetanus-pertussis toxoid; 0.5 ml intramuscularly), and commercial hepatitis B vaccine (Recombivax HB; Merck & Co), 1 ml IM) and with cholera toxin subunit B (CTB; 0.1 mg subcutaneously) to determine immune responses to non-viral test antigens. Booster immunizations with these antigens will be given every 3 months to monitor immunocompetence. These antigens have been used previously in our macaque studies and have been found to be safe. Peripheral lymph node biopsies (axillary or inguinal) will be performed under ketamine anesthesia with topical analgesia as per CNPRC SOP described below. To monitor toxicity of long-term administration, radiographs, cerebrospinal fluid (CSF) taps, and bone marrow aspirates will initially be performed at monthly (and subsequently less frequent) time intervals under metedomidine (5-8 mg/kg) + ketamine (5 mg/kg) anesthesia, or under telazol (30-50 microgram/kg) anesthesia, and will coincide with times of blood sampling. Local lidocaine will be used for collection of the bone marrow aspirates as per SOP

described below. These procedures will be scheduled to coincide with the collection of blood samples, to minimize the number of days on which animals receive anesthesia and overnight fasting.

To determine the pharmacokinetics of the dosage regimens of these compounds (+ their metabolites), blood samples will be collected by venipuncture at 0, 15 min, 30 min, 1, 1.5, 2, 4, 6, 8, 10 and 24 hours after drug administration under ketamine anesthesia as per SOP. Blood volumes do not exceed the maximum allowable volume of 12ml/kg body weight per month. As outlined in table 1, these 24-h pharmacokinetic studies may be repeated at 3-monthly (or less-frequent) intervals to monitor if chronic drug administration affects the pharmacokinetics. The purpose of these pharmacokinetics studies is so that, if a positive clinical effect is seen, we can determine which compounds (or their metabolites) had the biggest impact on increasing their natural levels and/or the levels of other hormones (many of which have a diurnal pattern, as has been determined by other investigators), so that this information can be used to try to determine if fewer than 4 compounds can be given and/or at lower concentrations, and therapeutic benefits be maintained.

As requested, the procedures are described in more detail here (these excerpts are copied directly from the current CNPRC SOP's):

Lymph Node Biopsy - Procedure:

- The following procedure is performed AFTER chemical restraint of the animal. See CRPRC SOP# FF-1: Restraint Procedures: Chemical.
- Topical analgesia is attained via a subcutaneous infiltration of Bupivacaine (0.1 - 0.2 ml of 0.25% solution) proximal and medial to the lymph node to be biopsied. Care must be taken not to inject any of the anaesthetic intravascularly.
- The site is surgically prepared. The skin over the node is incised with a scalpel blade. The node is exposed by blunt dissection. The node can either be removed in its entirety by a combination of blunt and sharp dissection, or the node can be clamped with hemostats and a portion removed by sharp dissection.
- The skin is then closed using suture and/or sterile surgical adhesive.

Bone Marrow Aspiration- Procedure

1. Wear protective clothing and equipment per current CRPRC Infection Control Policy (or SOP # AA-7: Animal Area Protective Clothing). Check the animal's tattoo number to verify that it is the correct animal, then immobilize the animal with ketamine HCl according to SOP FF-1 (Restraint Procedures: Chemical).
2. The area over the iliac crest or head of the humerus is shaved and surgically prepped according to SOP II-1 (Surgery Preparation). Sterile gloves must be worn during this procedure, a sterile drape can be used at the discretion of the veterinarian.
3. Topical analgesia is recommended via a subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 2% solution). Care must be taken not to inject any of the anesthetic intravascularly.
4. The anterior dorsal rim of the iliac crest or head of the humerus is identified by palpation. An 18 or 20 gauge 1-1 1/2 inch bone marrow aspiration needle is advanced in a rotating manner 5-15 mm depending on the size of the animal.
5. After the stylet is removed a syringe (usually heparinized) is attached to the aspiration needle.
6. Suction is applied by a steady full extension of the plunger to create back pressure. After each pull the needle should be rotated 90 degrees to redirect the direction of aspiration. If marrow does not appear, the stylet is replaced and placement of the needle is changed.

7. The amount of marrow taken from all aspiration sites should not exceed the guidelines for a blood draw.
8. Post-procedure analgesics may be administered, per SOP II-5 (post procedure analgesia), at the discretion of the veterinarian.

CSF Aspiration- Procedure.

1. Using the clippers, shave the area over the intended CSF tap site (cerebellomedullary cistern or lumbar).
2. Surgically scrub and prep the intended area (see SOP # F-1: Surgery Preparation).
3. Cover the surrounding area with a sterile drape.
4. Using sterile gloves, palpate in between the vertebrae at the intended CSF tap site.
5. Insert the sterile needle into the site and gently aspirate while slowly advancing the needle until fluid starts to accumulate in the needle's hub. Another method would be to advance the sterile needle until fluid accumulates in the needle's hub, then attach the syringe and gently aspirate.
6. Gently withdraw the necessary amount of CSF (.5cc/kg to a maximum of 2cc per animal). Withdraw the syringe and needle, and wipe the site with alcohol.

Urine collection via cystocentesis- Procedure

1. Place the animal on its back so its midline is centered. Palpate the pubic symphysis. The site is disinfected with alcohol.
2. Introduce the needle through the skin and abdominal wall with a clean thrust just anterior to the pubic bone on the midline and angled posteriorly approximately 15°.
3. Once the needle is in place aspirate urine. Stop aspiration once sufficient sample is obtained and remove the needle cleanly, using one smooth motion from the abdominal wall.
4. Place the urine in a sterile redtop vacutainer or other sterile container.

Drug administration:

The compounds will be given by oral administration, by mixing them with food supplements. Based on our ongoing experience with oral dosing, animals will be provided with supplements that they are most eager to drink or eat. Preferably, mixing the compounds with Tang or Ensure allows oral dosing with a syringe (and provides positive reinforcement, so that animals often don't need to be squeezed for restraint). Animals that don't like the syringe-dosing will be offered sandwiches (with peanut-butter jelly) to which the compounds are added, and the food intake will be recorded (by recording the portion of sandwich which wasn't eaten). The starting regimens are, to the best of the available knowledge, pharmacokinetically equivalent to be within the range of doses that have previously been studied and were found to be safe in animal and human studies. However, if clinical monitoring suggests the possibility of toxicity, then the dosage regimen will be reduced. If therapeutic benefits are seen (such as clinical improvement), then the dosage regimen may also get reduced gradually to get preliminary data on the lowest regimen required to maintain such benefits. As discussed above, for the first 10 animals that will become available we will all administer 4 compounds. If clinical benefits are observed, then subsequent animals in groups of 10 (depending on availability) will receive fewer compounds in an attempt to find even simpler and cheaper regimens, and try to delineate the role of each of them. The choice of compounds will then be determined based on the findings in the first animals, and on the available scientific knowledge at that time.

As indicated in the table below, the following starting regimens are proposed for animals that are in the ~3-6 kg weight range. For animals above 6 kg, the dosage will be increased 25%. For animals less than 3 kg, the dose will be decreased 25%. If no effects are seen at this regimen, the dose may be increased to maximally double the regimen listed in the table below.

Compound	Daily dose
Pyridoxal phosphate (Vit.B6)/ magnesium	50 mg
7-ODA	250 mg
DHEA	250 mg
Pregnonolone	250 mg

Groups and numbers of animals:

We propose to use a maximum of 30 animals under this protocol, in groups of 10 animals. Group A (n=10) will get a mixture of all 4 compounds at the dosage regimens listed above, orally once daily. If beneficial effects are seen, then subsequently group B (n=10) and C (n=10) will receive either lower doses of these 4 compounds (group B) and/or fewer compounds (group C). The exact choice for groups B and C will depend on the results of group A, including analysis of immune responses, and analysis of concentrations of hormones and steroid metabolites in the blood of group A. The total number of animals for the whole project (maximum 30) will depend on the availability. As discussed above, these are animals that are already on existing experiments and are infected with SIV or SHIV, and would otherwise be euthanized. Thus, these animals are salvaged for this project. In other words, no new animals are requested from the breeding colony of the CNPRC for this study.

Experimental endpoint:

Animals that are clinically stable can be maintained indefinitely for years to continue gaining information, or they may be euthanized if no further useful information can be collected or no money is available to further support them.

If they develop progressive disease, animals will be euthanized when necessary as determined by the CNPRC veterinary staff following consultation with the investigators, according to the CNPRC guidelines, "Criteria for euthanasia of retrovirus-infected macaques". Animals may be euthanized to determine virus distribution and antiviral immune responses in the different lymphoid tissues or they may be continued indefinitely while clinically stable.

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.

Group	Procedures / Drugs	Number of Animals	Category
A	Venipuncture, Subcutaneous lymph node biopsies, oral drug administration (4 compounds), subcutaneous and intramuscular immunizations (CTB, TT, hep B), bone marrow and CSF aspiration, urine collection (cystocentesis), radiographs.	10	3
B	Same (except fewer compounds and/or lower dose than group A)	10	3

C	Same (except fewer compounds and/or lower dose than group A)	10	3
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Categories of invasiveness

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

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?

Infection of rhesus macaques with SIV/SHIV is currently the best non-human primate model for human AIDS. Specific SIV and SHIV isolates inoculated in macaques are the only primate lentiviruses that reliably produce fatal immunodeficiency in non-human primates. Because this is a study with animals that become available from other studies, the animals will mainly be compared to their base-line data, and thus groups of 10 are considered to be minimal to generate useful data.

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

Building:

Room:

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.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus macaque	Ketamine	10 mg/kg	IM	Every 1-2 weeks (at time of blood draws or lymph node biopsies)
"	Metomidine	5-8 mg/kg	IM	≤ once per month, at time of X-rays or bone marrow aspirates
"	Telazol	30-50 µg per kg	IM	"

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)

Rhesus macaques infected with SIV or SHIV experience moderate discomfort due to the disease and associated opportunistic infections, similar to that observed in HIV-infected human patients. Minimal discomfort is associated with venipuncture, subcutaneous drug administration, and lymph node biopsies. Peripheral lymph node biopsies will be performed under ketamine anesthesia with topical analgesia (0.1-0.2 ml of 0.25% Bupivacaine solution given subcutaneously at the biopsy site) as per CRPRC SOP. All possible measures are taken by the CNPRC veterinary staff to minimize discomfort from surgical and nonsurgical procedures.

The dose regimens of the individual compounds are based on the best available knowledge of studies with primates or humans and other animal species. Animals will be monitored for adverse effects by parameters described in section c.

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.

If, in the opinion of the CNPRC veterinary staff, analgesics and anesthetics would be useful in alleviating the signs, they will be used. In SIV-infected macaques, much of the discomfort is associated with opportunistic infections; supportive therapy with appropriate antimicrobials will be used. Animals that become seriously ill and that fail to respond to supportive and specific therapy will be euthanized.

If animals show signs that may be related to drug toxicity, then the drug dosage regimen will be reduced, or drug treatment withdrawn completely

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 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_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?

8 / 30 / 03

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

Database Name	Years Covered	Keywords / Search Strategy
PubMedline	until 8/03	HIV, SIV, SHIV, DHEA, 7-ODA, steroids, Vitamin B6, pyridoxal phosphate
Current Contents	until 8/03	"

What were your findings with respect to alternative methodologies?

We found no biologically appropriate, alternative methodologies for the study of antiretroviral drugs or immune-therapies in vivo, or for assessing levels of virus replication, antiviral immune responses and disease pathogenesis. Thus, the only way to perform these experiments is by using an animal model. The techniques and procedures used in this study are designed to be minimally invasive and to be as similar as possible to those used for treatment and evaluation of HIV-infected patients.

Has this study been previously conducted?

Yes No

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

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

All animals will be euthanized at the conclusion of the study or when necessary as determined by the CNPRC veterinary staff following consultation with the investigators, according to the CRPRC guidelines, "Criteria for euthanasia of retrovirus-infected macaques". See also section i) above).

Animals that remain healthy may be monitored for years.

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.

Species	Method	Drug	Dose (mg/kg)	route
Rhesus	Overdose	Pentobarbital	60mg/kg	IV

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

Not applicable

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.

Last Name	First Name	Middle Name	UC ID Number or SSN	Email Address

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://ehs.ucdavis.edu/animal/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. Information is available on the world wide web at <http://ehs.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.

	Associate Research Virologist	8/30/03
<i>Principal Investigator</i>	<i>Rank / Title</i>	<i>Date</i>

Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> 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.

<i>Campus Veterinarian</i>	<i>Date</i>

ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL # 10855**EXPIRES:** _____

RUA#: _____

BUA#: _____

CCA#: _____

Identity of Hazard: Investigator Last Name: Department: Investigator First Name: Phone: Investigator Email: Fax: **Provide a short description of the agent:**

This agent / material is hazardous for: Humans only Animals only Humans and Animals
 For which Animal Species?

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

Describe any human health risk associated with this agent:**The precautions checked below apply to this experiment:**

- 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 Biohazardous Waste Container
 Bag and Autoclave 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 Biohazardous Waste Container
 Bag and Autoclave 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 Shoe Covers/Booties
 Disposable Gloves Head Cover
 NIOSH Certified Dust Mask Disinfectant footbath
 Eye Protection/Face Shield
 Fitted Respirator Type: _____
 Other: Describe: Plastic disposable gowns/coveralls
- 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:

Date: Mon, 15 Sep 2003 21:39:59 -0700
To:
From:
Subject: Re: Fwd: pre reveiw questions protocol 10855
Cc

Dear,
Thanks very much for pre-reviewing the protocol. I addressed all the questions directly in the revised protocol, which I'm enclosing.
Many thanks and regards,

Questions from.

Date: Fri, 12 Sep 2003 12:49:29 -0700
To
From
Subject: pre reveiw questions protocol 10855

Hi,

I have received and pre reviewed your recently submitted protocol which has been assigned accession number 10855 for future reference. I have attached a copy of your protocol for ease of making revisions in response to the questions that follow.

For this protocol to be considered on the Sept 25th committee agenda, please return the revised protocol to me on or before noon, Tuesday, Sept 16th.

Thank you in advance,

Protocol 10555 ()

This is a very complex study or at least the way it is presented. There is a lot of additional information now needed, so you will find more details requested per committee deliberations from the past few weeks.

1. On page 1, Special Husbandry Requirements, you stated N/A when you have checked "yes" for hazardous materials. The Special Husbandry section should reflect the specialized housing for the infectious animals.
2. In section a, you discuss "pilot study", but have listed one group of 30 animals. 30 animals does not constitute a pilot study. If you have different groups, they are not listed in sections c or d, so please break out the different groups if there are such. It appears you will be doing a pharmacokinetics study first to determine the doses? Please clarify.
3. In section c, second paragraph, you mention procedures, but have not described them. Please describe the bone marrow aspirate procedure and lymph node biopsy procedure. Will the animals be fasted?
4. In paragraph 3, you discuss blood collection, but do not mention how this is performed. Are the animals anesthetized, sedated or just an arm pull. Please clarify.
5. In the antiviral drug administration section, you mention mixing the compounds with food supplements. How do you know the animals will take the compounds this way? Please clarify.
6. You go on to state that for the first 8 animals, you will administer 4 compounds. Please clarify how this will be conducted. Will all 8 receive each of the 4 compounds, with a wash out period? Please describe how the groups will be set up and timing.

7. In section c, number of animals, you state there will be no new animals for this study. However, you will be administering compounds to animals, so you will be using animals. I am not clear as to what you mean by this statement.

8. In the experimental endpoint, you state that "at the conclusion of the study"..... How long will the animals be on study? It is not clear what your endpoints are. Please clarify.

9. In section d, you list procedures for the 30 animals, but these procedures are not described in section c. Please make sure that you describe all procedures performed on the animals in section c. Also, you list compounds in section c, which appear to be groups, but you have only one group in section d. Please clarify who gets what and when.

10. In section e, you emphasize that this is a "pilot study", but it is unclear why a pilot study needs 30 animals. Please clarify this issue.

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