**PROTOCOL FOR ANIMAL USE AND CARE**

**CNPRC**

**PROTOCOL:** 10357

**EXPIRES:**

<table>
<thead>
<tr>
<th>Last Name:</th>
<th>First:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last Name:</td>
<td>First:</td>
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<tr>
<td>Middle:</td>
<td>Middle:</td>
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<tr>
<td>email:</td>
<td>email:</td>
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<tr>
<td>Department:</td>
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</tr>
<tr>
<td>Phone / Fax:</td>
<td>Phone:</td>
</tr>
<tr>
<td>After hrs. #:</td>
<td>After hrs. #:</td>
</tr>
</tbody>
</table>

**Species** (common names): rhesus  
**Number:** 24  
**Source:** CNPRC

**Project Title:** CpGs as adjuvants in therapeutic HIV vaccines: non-human primate studies

**Overnight housing location:** CNPRC  
**Day use only:**

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

**Procedures:**

Animals will be infected with SIV, put on anti-retroviral therapy (FTC + PMPA) and immunized every two months for six months with either inactivated SIV + CpG ODN or CpG ODN alone. Samples (blood, lymph node biopsies) will be obtained to assess immune responses. Two months after the last immunization, animals will be taken off anti-retroviral therapy and monitored to determine if the immunizations improve the clinical course of infection.

**Special Husbandry Requirements:** none

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

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

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>[X] Yes  [ ] No</th>
<th>Agent(s): SIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioisotopes?</td>
<td>[ ] Yes  [X] No</td>
<td>Agent(s):</td>
</tr>
<tr>
<td>Chemical Carcinogens?</td>
<td>[ ] Yes  [X] No</td>
<td>Agent(s):</td>
</tr>
<tr>
<td>Toxic Chemicals?</td>
<td>[ ] Yes  [X] No</td>
<td>Agent(s):</td>
</tr>
</tbody>
</table>
Summary of Procedures:

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

This project involves immunizing SIV infected, ART (anti-retroviral therapy) treated, rhesus monkeys with a whole-inactivated SIV vaccine in CpG (cytosine and guanine linked by a phosphate, which mimics bacterial DNA) ODN (oligodeoxynucleotide) adjuvant. The ART therapy will consist of PMPA (9-(2R-[2-(R)-(phosphonomethoxypropyl) adendine] and FTC (cis(-) amino - 5-flouro-1-[2R,5S-(hydroxymethyl)-1,3 oxathiolan-5-y]-2(1H)-pyrimidinone). PMPA and FTC block SIV/HIV replication by inhibiting intracellular enzymes the virus needs to replicate. We hypothesize immunizing animals while on ART will generate SIV specific immune responses and boost innate anti-viral defenses. Additionally, the project is designed to determine if therapeutic vaccination during ART with inactivated SIV in CpG ODN adjuvant can provide sustained control of viral replication after ART is discontinued.

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
- [X] Induced illness, intoxication, or disease
- [ ] LD 50 or ID50 studies.
- [ ] Survival surgical procedures
- [ ] Death as an endpoint (see i below)
- [X] catheters, blood collection, intubation
- [ ] Multiple survival surgery
- [ ] Trapping, banding or marking wild animals
- [ ] Prolonged restraint. (8 hrs+)
- [ ] Behavioral modification.
- [X] 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.)*

**Immunization timeline:**

<table>
<thead>
<tr>
<th>2 months</th>
<th>2 months</th>
<th>2 months</th>
<th>2 months</th>
<th>2 months</th>
<th>2 months</th>
<th>2 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIV</td>
<td>Start ART</td>
<td>Immunization #1</td>
<td>Immunization #2</td>
<td>Immunization #3</td>
<td>Stop ART</td>
<td>Necropsy</td>
</tr>
</tbody>
</table>

**Study outline: Groups A-C**

<table>
<thead>
<tr>
<th>6 months prior to infection</th>
<th>Monthly baseline bleeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection w/SIVmac 251 (day 0)</td>
<td>10 ml bleed</td>
</tr>
<tr>
<td>Post-infection (pi)</td>
<td>2 months of weekly bleeds</td>
</tr>
<tr>
<td>ART initiated (2 months pi)</td>
<td>LN biopsy on day initiated 2 months of bleeds every other week,, toxicity monitoring</td>
</tr>
<tr>
<td>Immunization phase(see above) (2 months after ART initiated)</td>
<td>Blood drawn on the day of, and 1,3,10,14, 28, and 52 days after each immunization. ART tx continued, toxicity monitoring continued. LN biopsy within 7 days of last immunization.</td>
</tr>
<tr>
<td>ART continued for 2 months after the last immunization</td>
<td>Blood drawn as stated above. Toxicity monitoring continued.</td>
</tr>
<tr>
<td>Animals taken off ART two months after the last immunization.</td>
<td>LN biopsy taken within 7 days after ART stopped. Blood collected the day ART stops.</td>
</tr>
<tr>
<td>Monitor for two months after ART stopped</td>
<td>Blood collected every other week for two months</td>
</tr>
<tr>
<td>Two months after ART stopped</td>
<td>Animals euthanized and necropsied, lymphoid tissues collected</td>
</tr>
</tbody>
</table>

**Note:** Group D will have monthly bleeds for six months prior to inoculation, then weekly bleeds for 2 months to monitor infection, and then bleeds every other week starting the day ART is initiated.

**GROUP A- Immunization of macaques on ART with inactivated SIV in CpG ODN adjuvant will prevent viral rebound after cessation of ART.**

Male juvenile rhesus macaques will be bled monthly for 6 months prior to SIV infection (20–30 ml volume, not to exceed 12 ml/kg/month). This blood will be used for in-vitro assays prior to the start of the study to determine baseline values, and the ability to respond to CpG ODN.
After six months, animals will be infected intravenously with 1 ml (10^5 TCID₅₀) SIVmac251. Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected weekly to monitor the course of infection.

Two months post-infection, animals will be started on ART. This therapy consists of a once daily subcutaneous dose of 30 mg/kg PMPA and 50 mg/kg FTC. This treatment has already been shown to reduce plasma viremia to undetectable levels in chronically infected rhesus macaques. CBCs, chemistry panels and urinalysis will be performed every other week to monitor for potential toxicity. On the day ART is initiated, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution). Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected every two weeks to monitor viral load for two months.

After two months of ART, the animals will be immunized with a combination of 20 µg SIV p27 capsid protein and 500 µg CpG ODN 10103 in 1 ml sterile saline. Half will be administered intramuscularly and half will be administered intradermally. Monkeys will be immunized three times, once every two months. The animals will remain on ART during the immunization phase of the experiment. Blood (5-10 ml, not to exceed 12 ml/kg/month) will be drawn on the day of immunization, and 1, 3, 10, and 14, 28, and 52 days post-immunization. Within seven days after the third immunization, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the last immunization, animals will be taken off ART. Blood will be collected the day animals are taken off ART, and every other week thereafter (5-10 mls, not to exceed 12 ml/kg/month). Within seven days after the cessation of ART, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the cessation of ART, animals will be euthanized, necropsied and lymphoid tissues will be collected for further analysis.

**GROUP B - Immunization of macaques on ART with CpG ODN will prevent viral rebound after cessation of ART.**

Male juvenile rhesus macaques will be bled monthly for 6 months prior to SIV infection (20-30 ml volume, not to exceed 12 ml/kg/month). This blood will be used for in-vitro assays prior to the start of the study to determine baseline values, and the ability to respond to CpG ODN.

After six months, animals will be infected intravenously with 1 ml (10^5 TCID₅₀) SIVmac251. Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected weekly to monitor the course of infection.

Two months post-infection, animals will be started on ART. This therapy consists of a once daily subcutaneous dose of 30 mg/kg PMPA and 50 mg/kg FTC. This treatment has already been shown to reduce plasma viremia to undetectable levels in chronically infected rhesus macaques. CBCs, chemistry panels and urinalysis will be performed every other week to monitor for potential toxicity. On the day ART is initiated, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution). Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected every two weeks to monitor viral load for two months.

After two months of ART, the animals will be immunized with 500 µg CpG ODN 10103 in 1 ml sterile saline. Half will be administered intramuscularly and
half will be administered intradermally. Monkeys will be immunized three times, once every two months. The animals will remain on ART during the immunization phase of the experiment. Blood (5-10 ml, not to exceed 12 ml/kg/month) will be drawn on the day of immunization, and 1, 3, 10, and 14, 28, and 52 days post-immunization. Within seven days after the third immunization, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anaesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the last immunization, animals will be taken off ART. Blood will be collected the day animals are taken off ART, and every other week thereafter (5-10 ml, not to exceed 12 ml/kg/month). Within seven days after the cessation of ART, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anaesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the cessation of ART, animals will be euthanized, necropsied and lymphoid tissues will be collected for further analysis.

GROUP C - Immunization of macaques on ART with inactivated SIV in alum will prevent viral rebound after cessation of ART.

One month prior to SIV infection, blood will be collected to establish baseline cytokine RNA levels. (20-30 ml, not to exceed 12 ml/kg/month). Male juvenile rhesus macaques will be infected intravenously with 1 ml \(10^5\) TCID\(_{50}\) SIVmac251. Blood (5-10 ml, not to exceed 12 ml/kg/month) will be collected weekly to monitor the course of infection.

Two months post-infection, animals will be started on ART. This therapy consists of a once daily subcutaneous dose of 30 mg/kg PMPA and 50 mg/kg FTC. This treatment has already been shown to reduce plasma viremia to undetectable levels in chronically infected rhesus macaques. CBCs, chemistry panels and urinalysis will be performed every other week to monitor for potential toxicity. On the day ART is initiated, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anaesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution). Blood (5-10 ml, not to exceed 12 ml/kg/month) will be collected every two weeks to monitor viral load for two months.

After two months of ART, the animals will be immunized with 20 ug p27 SIV capsid protein in 1ml alum. Half will be administered intramuscularly and half will be administered intradermally. Monkeys will be immunized three times, once every two months. The animals will remain on ART during the immunization phase of the experiment. Blood (5-10 ml, not to exceed 12 ml/kg/month) will be drawn on the day of immunization, and 1, 3, 10, and 14, 28, and 52 days post-immunization. Within seven days after the third immunization, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anaesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the cessation of ART, animals will be taken off ART. Blood will be collected the day animals are taken off ART, and every other week thereafter (5-10 ml, not to exceed 12 ml/kg/month). Within seven days after the cessation of ART, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anaesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the cessation of ART, animals will be euthanized, necropsied and lymphoid tissues will be collected for further analysis.
GROUP D- SIV infected animals given ART treatment followed by treatment interruption will experience viral rebound.

One month prior to SIV infection, blood will be collected to establish baseline cytokine RNA levels (20-30 mls, not to exceed 12 ml/kg/month).

Male juvenile rhesus macaques will be infected intravenously with 1 ml (10^5 TCID_{50}) SIVmac251. Blood (5-10 mls, not to exceed 12ml/kg/month) will be collected weekly to monitor the course of infection.

Two months post-infection, animals will be started on ART. This therapy consists of a once daily subcutaneous dose of 30 mg/kg PMPA and 50 mg/kg FTC. This treatment has already been shown to reduce plasma viremia to undetectable levels in chronically infected rhesus macaques. CBCs, chemistry panels and urinalysis will be performed every other week to monitor for potential toxicity. On the day ART is initiated, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution) . Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected on the day ART is initiated, and every other week from that date.

After six months, the animals will be taken off ART. Blood will be collected the day animals are taken off ART, and every other week thereafter (5-10 mls, not to exceed 12 ml/kg/month). Within seven days after the cessation of ART, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution) .

Two months after the cessation of ART, animals will be euthanized, necropsied and lymphoid tissues will be collected for further analysis.

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>A</td>
<td>SIV/ART/CpG ODN+inactivated SIV</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>SIV/ART/CpG ODN</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>SIV/ART/inactivated SIV in alum</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>SIV/ART</td>
<td>6</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 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. |
| 2        | Minor stress or pain of short duration  
**Examples:** cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress |
| 3        | Moderate to severe distress  
**Examples:** major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation |
| 4        | Severe pain near, at or above the pain tolerance threshold  
**Examples:** exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress: Surgical experiments which have a high degree of invasiveness. |

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

There is no alternative to the use of rhesus macaques for this study. The SIV rhesus macaque model is the most generally accepted model of HIV pathogenesis and pre-clinical vaccine studies. We have decided on six monkeys per group, which will allow us to determine statistically significant differences between groups (using a student T test).

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.

<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 HCl</td>
<td>10 mg/kg</td>
<td>IM</td>
<td>Prior to all procedures</td>
</tr>
<tr>
<td>rhesus</td>
<td>buprenorphine</td>
<td>0.01-0.03mg/kg</td>
<td>IM</td>
<td>As needed in judgement of CRPRC vets</td>
</tr>
<tr>
<td>rhesus</td>
<td>midazolam</td>
<td>10 mg/kg</td>
<td>IM</td>
<td>Prior to LN biopsies</td>
</tr>
<tr>
<td>rhesus</td>
<td>ketoprofen</td>
<td>20 mg/kg</td>
<td>IM</td>
<td>For three days after LN biopsy</td>
</tr>
<tr>
<td>rhesus</td>
<td>lidocaine</td>
<td>0.01 mg/kg</td>
<td>Sub-q</td>
<td>Prior to LN biopsies</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)

Any injection or venipuncture has the potential to cause minor pain or discomfort, but the animals are immobilized for the procedure and should not experience pain.

Toxicity from ART should not be a problem at the proposed dosage and duration, however there is a possibility PMPA treatment will decrease the availability of zinc and phosphorus as well as cause kidney dysfunction. To prevent nutrient loss, animals will be given a daily multivitamin when ART is initiated. In addition, animals will be monitored closely via CBC, chemistry panels and urinalysis. If phosphorus levels drop, nutraphos supplements will be given. If kidney problems arise, the PMPA dosage will be dropped to 10 mg/kg. FTC has been linked to a decrease in liver enzyme production. If animals show signs of liver dysfunction, the FTC dosage will be dropped to 30 mg/kg. In our experience using ART for longer durations, these measures alleviated any problems associated with drug toxicity.

SIV infection of rhesus macaques results in a fatal immunodeficiency and wasting syndrome. The animals will be euthanized before, or when, they experience 3 of the following: weight loss >15% in two weeks or >30% in 3 months; persistent hypothermia <96°F even with heat supplementation; leukopenia (total WBC<3,000); lymphopenia (lymphocytes <800); anemia (hemoglobin <10); dehydration >10%; nonresponsive to therapy for opportunistic infections; persistent anorexia (>3 days); animal significantly obtunded. These criteria are based on CRPRC guidelines. In addition, the lymph node biopsies will result in some post-procedure pain.

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.

All possible efforts will be made to minimize animal pain and discomfort. Analgesics have no effect on the proposed studies and they will be administered at the discretion of the CRPRC veterinary staff. Infected animals will be euthanized prior to or at the time they develop clinical signs of AIDS. The decision to euthanize will be based on the judgement of the CRPRC veterinarians.

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

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

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

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

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit http://trc.ucdavis.edu/jawelsh/Databases_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?  

What was the date on which you conducted this search? 

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>1990-present</td>
<td>CpG, SIV, anti-retroviral treatment, STI, therapeutic vaccine</td>
</tr>
<tr>
<td>Reference Update</td>
<td>1999-present</td>
<td>CpG, SIV, anti-retroviral treatment, STI, therapeutic vaccine</td>
</tr>
<tr>
<td>Current Contents</td>
<td>1990-present</td>
<td>CpG, SIV, anti-retroviral treatment, STI, therapeutic vaccine</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

CpG oligodinucleotides have been shown to induce immune responses in tissue culture systems, rodent models, and cynomolgous monkeys. Thus far, data has not been published using the rhesus macaque model describing CpGs as an adjuvant to a therapeutic vaccine in SIV infection.

Has this study been previously conducted?  

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

Previously, using control animals from a vaccine study, we conducted a pilot project using CpG ODN obtained from another source. The results were inconclusive but hopeful. The new CpG ODN proposed in this study have proven efficacy in other primate models of allergy and infectious disease. Thus, we are repeating and expanding the study to obtain definitive results.

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

Animals will be euthanized and necropsied at the onset of clinical SAIDS or at the end of the study.

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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<tr>
<td>rhesus</td>
<td>IV</td>
<td>pentobarbital</td>
<td>60 mg/kg</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?

All animals will be euthanized at the end of the project.
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).


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/](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.

__________________________  ____________________  __________________
Principal Investigator       Rank / Title           Date

Committee Use Only Below

** Conditions necessary for Committee Approval:

Final Disposition of this protocol:

_________ Approved

_________ Not Approved

_________ Withdrawn by Investigator

Date of Action: ______/_____/______

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

__________________________________________
ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL # 10357
EXPIRES: ________

RUA#: ________ BUA#: 0447 CCA#: ________

Identity of Hazard: SIV

Investigator Last Name: [Name]
First Name: [Name]
Department: [Department]
Phone: [Phone]
Email: [Email]
Fax: [Fax]

Provide a short description of the agent:
SIV is a simian retrovirus. This virus can infect human cells and potentially humans.

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

For which Animal Species?
[X] For animals
[ ] For humans

The agent can be spread by:
[X] Blood
[X] Saliva/nasal droplets
[ ] Feces/urine
[ ] Does not leave animal
[ ] Other: All mucosal secretions can be contaminated.

Describe any human health risk associated with this agent:
No human disease related to SIV has ever been described. However, there is a potential for SIV to infect humans.

The precautions checked below apply to this experiment:
[X] The researcher or his/her technicians are responsible for the feeding and care of these animals.
[X] 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:

[X] Cages must be autoclaved before cleaning.
[ ] Label cages and remove label after decontamination.
[X] Animal carcasses must be labeled and disposed of as follows:
[ ] Incineration
[ ] Bag and Autoclave
[ ] Biohazardous Waste Container
[ ] EH&S will pick-up (2-1493).

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

[X] Personal protective equipment must be removed before leaving the room.
[X] Personal protective equipment must be discarded or decontaminated at the end of the project
[X] 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:
BSL 2 (BSL2+) precautions must be used at all times.