**CNPRC**

**PROTOCOL FOR ANIMAL USE AND CARE**

**Email to:** campusvet@ucdavis.edu

<table>
<thead>
<tr>
<th>PROTOCOL: 10778</th>
<th>EXPIRES: 8/27/04</th>
</tr>
</thead>
</table>

**Species (common names):**

<table>
<thead>
<tr>
<th>Cynomolgus macaques</th>
<th>Number: 2</th>
<th>Source: CNPRC</th>
</tr>
</thead>
</table>

**Project Title:** MicroPET imaging of Cu64-tagged antibody in non-human primates

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

**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.

Animals will be anesthetized and administered a monoclonal antibody tagged with a radioisotope (Cu-64; half-life: 12 hrs). They will be imaged on a microPET scanner then allowed to recover. They will be reanesthetized and rescanned 24 and 48 hr. later, and at day 5 or 6. Radioactivity will have decayed to background levels after 12 half-lives (6 days). Animals can then be returned to their home cages.

**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 will be housed in metabolism cages while radioactive. Feeding schedules will be unaltered except for pre-anesthesia fasting. Animals will have access to potable water ad lib.

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

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

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

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>[ ] Yes</th>
<th>[ ] No</th>
<th>Agent(s):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioisotopes?</td>
<td>[ X ] Yes</td>
<td>[ ] No</td>
<td>Agent(s): Cu-64</td>
</tr>
<tr>
<td>Chemical Carcinogens?</td>
<td>[ ] Yes</td>
<td>[ ] No</td>
<td>Agent(s):</td>
</tr>
<tr>
<td>Toxic Chemicals?</td>
<td>[ ] Yes</td>
<td>[ ] No</td>
<td>Agent(s):</td>
</tr>
</tbody>
</table>
This is a pilot study to investigate the potential application of PET imaging to determination of pharmacokinetics (PK) and pharmacodynamics (PD) of an anti-NCA (monoclonal antibody to CEACAM6 that is selectively expressed in some tumors) antibody in non-human primates. Based on a previous PET study in athymic nude mice, it is likely that performing successive scans after single administration of a Cu-64-tagged antibody will allow us to characterize PK/PD in a non-human primate model. If successful, this can lead to a more clinically relevant characterization of potential new anti-cancer therapies. The target antigen for this study is CEACAM6 and although CAECAM1-3 is expressed in rodent the specific target for our study is CAECAM6 and only expressed in primates. We are hoping that PET imaging will dramatically reduce the number of animals required for pharmacokinetic studies that are required for development of this molecule for the treatment of cancer.
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.)

Animals will be fasted for 8-12 hours as per CNPRC SOP. Animals will be given 10 mg/kg ketamine IM, intubated and started on inhalational anesthesia with isoflorane (also per CNPRC SOP). An intravenous catheter will be placed (either in the arm or leg) and animals will be placed on plastic-backed absorbent paper on the scanning bed and administered the Cu64-tagged antibody intravenously (1 mg/kg in 1 ml) via the catheter. They will be scanned in 2 or 3 body regions (thorax, abdomen) for approximately 0.5 hr each. At the completion of the scanning procedure, they will be recovered from anesthesia and maintained in metabolism cages for radioactivity monitoring.

Animals will be reanesthetized (using the above procedures and fasted before each procedure) and rescanned at approximately 24 and 48 hours, and again at 5 or 6 days after injection. Scan times at 48 hr and at 5/6 days are expected to be approximately 1 hr/region and 2 hr/region, respectively. (Scanning on day 5 or 6 will depend on the overall scanner schedule and how quickly the radioactivity is cleared (monitored in urine collected from metabolism cages).

Animals will be maintained in metabolism cages and radioactivity will be monitored via wipe tests and urine evaluation (collected from metabolism cages) until decayed to background levels and then will be returned to their home cages.

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>Anti-NCA monoclonal antibody</td>
<td>2</td>
<td>2</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 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?

Preliminary microPET imaging studies with this antibody in mice with NCA- and non-NCA expressing tumors indicated that this antibody preferentially bound to NCA expressing tumors. It is important now to study the biodistribution of the antibody in a non-human primate to begin to assess its possible eventual use in the human clinical setting. This is a pilot study and we anticipate that it will require no more than 2 animals to establish the relative distribution to thoracic and abdominal organs.

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

<table>
<thead>
<tr>
<th>Building</th>
<th>Room</th>
</tr>
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<tbody>
<tr>
<td></td>
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Who will be the surgeon?

<table>
<thead>
<tr>
<th>Surgeon</th>
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</table>

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>Cynomolgus macaques</td>
<td>Ketamine</td>
<td>10 mg/kg</td>
<td>IM</td>
<td>Once for each of three anesthesias</td>
</tr>
<tr>
<td>Cynomolgus macaques</td>
<td>Isoflurane</td>
<td>1-2 %</td>
<td>inhaled</td>
<td>Once for each of three anesthesias (2-6 hours each)</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?

| N/A |
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)

Minimal pain from initial ketamine injection will be expected. It is unlikely, but a potential for anaphylactic reaction to the antibody is a possibility. Long-term effects of radioactivity are unlikely at the proposed doses but include mutagenesis leading to tumor formation.

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.

Every attempt will be made to minimize any discomfort. Only trained primate center personnel will be administering the ketamine. The animals will be monitored throughout the injection and scanning procedures by the CNPRC Primate Medicine staff. Adverse reactions will be treated as they deem appropriate (steroids, diphenhydramine, etc). Animals will be returned to the colony after the project but will be monitored though routine colony monitoring for any long-term adverse health effects (tumor formation).

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? 6/20/2003

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>1996 - 2003</td>
<td>CEACAM6</td>
</tr>
<tr>
<td>Science Citation</td>
<td>1996 - 2003</td>
<td>CEACAM6</td>
</tr>
</tbody>
</table>
What were your findings with respect to alternative methodologies?

The results confirm that human but not rodents express CEACAM5-CEACAM8. There is no known expression of CEACAM6 in any rodent model. No report of CEACAM6 in rodents was identified from the above search. (please see J. Immunol 2002 May 15;168 (10) 5139-46 conforms the specificity of CEACAM6 to humans)

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?

Animals will not be euthanized as part of this 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>
</tr>
</thead>
<tbody>
<tr>
<td>Cynomolgus macaques</td>
<td>Anesthetic overdose</td>
<td>Pentobarbitol</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?

Animals will be returned to the CNPRC colony.
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don't need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
</tr>
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<tbody>
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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://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.

| Principal Investigator | Rank / Title | Date |

** 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.

| Campus Veterinarian | Date |

**ANIMAL ROOM SAFETY INFORMATION**

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

**PROTOCOL #10778_**

<table>
<thead>
<tr>
<th>RUA#</th>
<th>BUA#</th>
<th>CCA#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1458</td>
<td></td>
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</table>

Identity of Hazard: **Radioisotope Cu-64**

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

The PET radioisotope, Cu-64, attached to a monoclonal antibody will be utilized. The half-life of the isotope is 12 hr.

**This agent / material is hazardous for:**

- [ ] Humans only
- [ ] Animals only
- [x] Humans and Animals

**The agent can be spread by:**

- [x] Blood
- [x] Feces/urine
- [ ] Saliva/nasal droplets
- [ ] Does not leave animal

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

Radiation exposure. Animals will be maintained in metabolism cages and radiation levels will be monitored until radiation levels have returned to background (approximately 6 days) before they are returned to their home cages.

**The precautions checked below apply to this experiment:**

- [ ] 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.
  - [x] Cage
  - [ ] Stall
  - [ ] Water Bottle
  - [x] Animal Carcasses
  - [ ] Bedding
- [ ] Cages must be autoclaved before cleaning.
- [ ] Label cages and remove label after decontamination.
- [x] Animal carcasses must be labeled and disposed of as follows:
  - [x] Incineration
  - [ ] Biohazardous Waste Container
  - [ ] Bag and Autoclave
  - [x] 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:**

- [x] The following personal protective equipment must be worn/used in the room:
  - [x] Lab Coat/Coveralls
  - [x] Disposable Gloves
  - [x] NIOSH Certified Dust Mask
  - [x] Eye Protection/Face Shield
  - [x] Fitted Respirator
  - [ ] Shoe Covers/Booties
  - [x] Head Cover
  - [ ] Disinfectant footbath
  - [ ] Other: Describe:

- [x] 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:**
To: 
From: Julie Willis <jawillis@ucdavis.edu>
Subject: Fwd: Re: Fwd: pre review questions protocol 10778

Date: Fri, 15 Aug 2003 07:14:16 -0700
To: -Sender

, Answers to questions....

1. Yes- animals will be fasted prior to each anesthesia.

2. Scanning on day 5 or 6 will depend on the overall scanner schedule and how quickly the radioactivity is cleared (monitored in urine collected from metabolism cages).

3. There will be no blood collection as the primate center is unable to process radioactive samples.

Just let me know if there are any further questions,

At 12:28 PM 7/31/2003, you wrote:

X-Sender:
Date: Thu, 31 Jul 2003 10:59:25 -0700
To:
From:
Subject: pre review questions protocol 10778

Hi ,

I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10778 for future reference. For this protocol to be considered on the August 14th committee agenda, please respond to me on or before noon, Tuesday, August 5th. I will make the appropriate changes for you at that time.

If you have any questions, feel free to contact me via phone or email.

Thank you in advance,

Protocol 10778 ( )

1. In section c, second paragraph, it is not clear if the animals are fasted each time before they are scanned. Please clarify.

2. In section c, second paragraph, you state that the animals will be rescanned 5 or 6 days. What determines the timing difference between 5 and 6 days? Please clarify.

3. There is no mention of blood collection. Will you be collecting blood at any time during the study?
08/25/03
Committee Questions Protocol 10778

Date: Mon, 25 Aug 2003 07:02:28 -0700
To:

From:
Subject: Re: Protocol #10778

Hi , I obviously didn't review this closely enough. Yes please make the proposed changes, in addition please add as the contact. Thanks very much,       At 04:52 PM 8/24/2003,       wrote:

A few things:
1. CNPRC designation should be added to the first page
2. Please use correct spelling of cynomolgus macaque (incorrectly spelled on pages 1, 4, 6).
3. Suggest that for i. Adverse effects, state "...minimize any discomfort." rather than "...minimize the stress and pain induced by the ketamine injection."
4. j. literature search -- was this meant to be 6/20/03 and the month and day reversed? or?

Thanks
--

Proposed Changes:

We would like to add one more animal to this protocol to be able to do a preliminary monkey study on the microPET scanner. We will inject F18-FDG rather than the Cu64-antibody that we will use in the actual study. The animals will be prepped as specified in the submitted protocol, anesthetized and injected with isotope through a venous catheter, before being scanned in the thoracic and abdominal region for 0.5 hr. in each of 2 or 3 bed positions within the scanner. It may be possible to use one of the animals that has been designated for the proposed study, since radioactivity levels will return to background levels after FDG administration in about 22 hours.

Justification:

The submitted protocol requests two study animals, but we feel it would be advisable to do all the procedures we will use on a test animal in order to identify any problems or issues which we have not foreseen, before we do the actual experimental animals, particularly since we will be conducting a microPET scanning protocol on a monkey for the first time. It will also be helpful to determine whether we will need to use 2 or 3 bed positions to image enough of the animal to assess biodistribution. This study will not require the euthanization of an animal.

Potential Adverse Effects

No adverse effects are anticipated. FDG is routinely used for primate PET scans.
Note: This is a resubmission of an amendment request that was submitted earlier this morning, but was not in this format. The request for this amendment has been discussed with .