

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

EH&S USE ONLY

PROTOCOL: 10693
EXPIRES: 08/13/04

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:
cynomolgus	4	CNPRC

Project Title	Effect of of a monoclonal antibody (3E6) on Hematology parameters		
Overnight housing location::	CNPRC	Day use:	CNPRC (workrooms or animal quarters)
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.

Animal will be sedated and a single infusion of a protein (monoclonal antibody) will be infused for 10-15 minutes. The animals will be bled every day for 4 days and 1 week after the infusion. If all the blood values are normal, the animals will be monitored for 3-6 months
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Special Husbandry Requirements: Describe any special requirements your animals have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions of husbandry.

none

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

Sick Animals	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 type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

Hazardous Materials (only if in the animal room):

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
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:	Genentech	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 pctlillman@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.

This study is necessitated by the absence of appropriate non-animal alternatives and is intended to characterize the potential toxicity of a single dose of anti-NCA chimeric, a primate specific monoclonal antibody to the GPI-linked CEACAM, when administered by intravenous injection in cynomolgus monkeys. The information in this study does not unnecessarily duplicate the results of previous studies and could not be obtained by other means. The antigen recognized by the antibody, NCA, is expressed in cancer cells and therefore represents a target for potential therapy. However it is also expressed at lower levels in the cells from bone marrow and therefore may result in toxicity. The aim of this study is (a) to determine the toxicity of the potentially efficacious dose and (b) to determine if the infusion is well tolerated for a subsequent whole body bio-distribution study using PET. A protocol will be submitted for the imaging studies with a labeled monoclonal antibody will be undertaken using non-invasive positron-imaging tomography (PET).

Please note: Mab 3E6 is an monoclonal antibody (anti-NCA) to the cancer specific antigen CEACAM6.

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 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.)

We will use 4 male cynomolgus macaques for this study. For all procedures, animals will be fasted 12 hours prior to immobilization using ketamine (6-10 mg/kg).

Group A- Treatment with 1 mg/kg Mab 3E6 intravenously

Two males cynomolgus monkeys will be given 1 mg/kg Mab 3E6 intravenously on Day 0. Blood will be drawn from a femoral vein(10-20 mls, not to exceed 12 ml/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) on Day 0, 24 hours, 72 hours, 96 hours and 7 days post-treatment.

Group B- Treatment with 10 mg/kg Mab 3E6 intravenously

Two males cynomolgus monkeys will be given 10 mg/kg Mab 3E6 intravenously on Day 0. Blood will be drawn from a femoral vein(10-20 mls, not to exceed 12 ml/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) on Day 0, 24 hours, 72 hours, 96 hours and 7 days post-treatment.

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	1 mg/kg MAb 3E6	2	2
B	10 mg/kg Mab 3E6	2	2

Categories of invasiveness

Category	Description
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?

1. The study antibody, anti-NCA, is directed against an antigen that is expressed only in primates.

Anti-NCA is being evaluated as a potential cancer therapeutic. Because of the limited species range of expression of the target antigen, non-human primates, specifically macaques, will be the required species for use in any non-clinical toxicology program which will be required if full development is undertaken.

2. Four animals are adequate to answer the focused question being asked in this study. This single dose acute pilot study is designed to determine if this monoclonal antibody causes overt acute changes in hematology after administration. The aim of this pilot study is to determine and confirm that the antibody is well tolerated in order to enable a subsequent whole body bio-distribution study using PET (a new protocol will be submitted for the imaging studies with a labeled monoclonal antibody which will be undertaken using non-invasive positron-imaging tomography(PET)).

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?
cynomolgus	ketamine	10 mg/kg	IM	As needed for sample s collection or drug infusion. (days 0, 1, 2, 4, and 7)

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)

None expected, although anaphylaxis is a possibility whenever a protein is infused IV.

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.

CNPRC vets may intervene at anytime to provide support for animals on study based solely on their best clinical judgment.

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?

06/18/03

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
Medline	1966-2003	CEACAM6
Current Contents	1966-2003	CEACAM6

What were your findings with respect to alternative methodologies?

The results confirm that human but not rodents express CEACAM5-CEACAM8. There is no 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 confirms the specificity of CEACAM6 to humans)

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.

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

In the event of an unanticipated reaction (eg. Anaphylaxis) the animals will be euthanized if based on the clinical judgment of the CNPRC staff veterinarians, the animal should no longer be supported.

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
cynomolgus	overdose	phenobarbitol	60 mg/kg	IV

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

Return to the colony at the discretion of appropriate CNPRC staff.

Date: Mon, 21 Jul 2003 16:02:44 -0700
 To:
 From:
 Subject: Fwd: RE: Fwd: pre review questions protocol 10693

Date: 21 Jul 2003 16:04:01 -0700
 From:
 Subject: RE: Fwd: pre review questions protocol 10693
 To:
 CC:

Reply to: RE: Fwd: pre review questions protocol 10693 Hi- ,
 This protocol will be served to perform a contract with GenenTech company to test the antibody involves therapeutic side effects. The GenenTech made this antibody, they want to try in cynomolgus monkeys. The detail about this antibody is proprietary question.

To answer the questions:

1 Mab 3E6 is a monoclonal antibody, generated from hybridoma mice cells. it is a protein and non infectious agents. 3E6 is the identity of this antibody. 2. As our knowlegde so far, non-human primate is the best model for preclinical therapeutic study for human diseases. and cynomologus is the best choose because their blood cells can be characterized by flow cytometry technique and this kind of monkeys are available at CNPRC . 3. Mab3E6 is a mouse igG1 immunoglobulin specific to an antigen. it is a mouse protein, and has no infectious ability. it was purified by chromatograph tecnique and when inject to human may cause an immune response "serum sick" i.e. side effect. That is why they want first to try in non-human primate.
 Hope I answed the questions. thank you !

wrote:
 >Questions for this weeks meeting. Need to get these answered ASAP.
 >

>>Date: Mon, 23 Jun 2003 14:53:58 -0700
 >>To:
 >>From: >
 >>Subject: pre review questions protocol 10693

>>X-RCPT-TO:

>>

>>Hi ,

>>

>>I have received and pre reviewed the following protocol which has >>been assigned accession number 10693 for future reference. I have >>attached a copy of the protocol with the embedded number for ease of >>making revisions to the questions provided below. For this protocol to be >>considered on the July 3rd committee agenda, please forward your revised >>protocol to me on or before noon, tomorrow, Tuesday, June 24th.

>>

>>If you have any questions, feel free to contact me at 2-7077 or via email.

>>

>>Thanks in advance.

>>

>>

>>

>>Protocol 10693 ()

>>1. Section a was very brief and needs additional clarification. What do >>you mean when you state that this Mab may have therapeutic value? Please >>clarify. Also, what does Mab stand for. This is jargon and you are asked >>to target a faculty member from a discipline unrelated to yours. Please >>clarify what you mean by Mab3E6.

>>

>>2. In section e, you are asked two questions. How did you determine the >>species choice was appropriate? This particular question was not >>addressed. Please expand section e to address why you chose a non human >>primate.

>>

>>>3. In section j, you left this section blank and noted that it was not >>>applicable. Since USDA requires this section to be completed as per Title >>>12, and since you are conducting contract work, the company sponsoring >>>your work most likely has conducted the search before creating this >>>agent. Section j does need to be completed, so Genentech may be able to >>>supply you with the necessary information about their >>>products. Note: This protocol cannot be reviewed by the UCD committee >>>unless this section is complete.

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>>>RFC822 header

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> Date: Mon, 21 Jul 2003 11:09:32 -0700

> To:

> From:

> Subject: Fwd: pre review questions protocol 10693 > Mime-Version: 1.0