

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

EH&S USE ONLY

PROTOCOL: 10816
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	12	CNPRC

Project Title	Effect of imiquimod (Aldara) on SIV pathogenesis		
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.

This study will investigate the effects of Aldara treatment of rhesus macaques on SIV infection. Twelve animals will be infected with SIVmac and monitored for 6 months. Six of the 12 animals will be treated with Aldara topically (intravaginally) once a day for 2 weeks prior to and 2 weeks after intravaginal SIV inoculation. Animals will have blood samples taken for hematological measurements, as well as immunologic and virologic parameters. At approximately week 24 post-SIV inoculation, all surviving animals will be euthanized and

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.

Infectious housing after SIV inoculation

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

Sick Animals	Dead Animals	Pest Control
<input 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):	Simian immunodeficiency virus SIVmac
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? Yes No

Agent(s):

Funding source:	NIAID	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 pcstillman@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.

Objectives: Interferon alpha (IFN- α) is a cytokine rapidly induced after viral infections. IFN- α treatment is commonly used in viral infections to reduce virus replication. This has also been shown in the setting of HIV-1 and SIV infection. Despite the known antiviral activity of IFN- α , IFN- α has not been explored as a therapeutic agent to prevent infection or to reduce transmission. Thus, we propose to treat monkeys topically (vaginally) with imiquimod/ Aldara, a therapeutic drug known to induce interferon alpha and FDA approved for use in humans to treat genital warts to alter the pattern of infection after a subsequent high dose challenge (intravaginally) with SIVmac. **Hypothesis:** Topical pretreatment of rhesus macaques at the vaginal mucosa with Aldara will either completely prevent vaginal transmission of SIVmac, or severely reduce replication of SIVmac. In addition, the adaptive immune responses induced by IFN- α will contribute to control of virus replication. **Experimental Design:** Twelve female juvenile macaques will be infected with SIVmac intravaginally following baseline measurements and monitored for SIV disease progression and levels of viral replication for 6 months. Six of 12 animals will receive daily administrations of Aldara cream at the vaginal mucosa for 2 weeks before, and for 2 weeks after SIVmac inoculation. Peripheral blood and lymph nodes (2x) will be obtained periodically through week 24, at which time all surviving animals will undergo necropsy. Blood and lymphoid tissues will be collected to assess the level of virus replication and host immune responses. **Data analysis and Significance:** Animals will be grouped into Aldara treated and non-treated animals. The main read-out will be the level of virus replication detectable in plasma and lymphoid tissues of the two groups. Further, as a marker of outcome, CD4 T cell counts and anti-SIV immune responses will be compared in both groups. A reduction of viremia in Aldara treated animals will be an indication that Aldara can developed for use in humans to prevent infection and/ or to reduce the rate of transmission. Further, these results will have important implications for the development of drugs that can stimulate innate anti-viral immune response important in the early reduction of virus replication and spread within the host.

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 checked="" 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.)*

A total of 12 animals will be used in this study. 12 female colony bred rhesus macaques will be infected with a total of 6 doses of 10^5 TCID₅₀ (tissue culture infectious dose causing 50% cell death) of SIVmac administered twice daily intravaginally (Monday/ Wednesday/ Friday: 8:00 am and 2 pm) to assure infection of control animals. Note that the animals need to be anaesthetized with ketamine for each inoculation. This protocol for intravaginal infection has been used for the last 6 years and has been approved by the veterinarians at the CNPRC. Blood samples (10 ml) from all animals will be collected on days 1, 3, 7, 14, and 28 p.i., and monthly thereafter (10 ml) for the determination of virus replication levels, peripheral blood lymphocyte (PBL) subset and Chem20 panel analysis. Two blood samples (10 ml) will be collected from each animal to be used as baseline reference values approximately 1-2 months prior to inoculation with SIVmac. Two lymph node biopsies of inguinal lymph nodes will be performed on each animal. One lymph node biopsy will be performed 1 month prior to SIVmac inoculation (baseline), and the second lymph node biopsy will be performed 2 weeks post SIVmac inoculation.

Group A: Before infection with SIVmac251, 6 animals will be treated once a day intravaginally with 1/4 of the human dose of Aldara (5% cream in single use sachets containing 250 mg ALDARA). Aldara treatment will be administered for a total of 2 weeks prior to SIV inoculation, and will be continued throughout the first two weeks post-SIV inoculation. All animals will be inoculated intravaginally with SIVmac (see above). A blood sample (10 ml) will be collected on day 7 and 14 of the treatment and prior to SIVmac inoculation. The primary laboratory measurements to assess toxicity will be complete blood cell counts and the liver transaminase ALT.

Group B: Six animals with no prior treatment will be inoculated intravaginally with 6 doses of 10^5 TCID₅₀ SIVmac (8:00 am and 2:00 pm on Monday, Wednesday, and Friday) to ensure infection. A blood samples (10 ml) will be collected prior to SIVmac inoculation.

All animals will have weekly weight determination according to CNPRC guidelines. Daily CNPRC staff observation of the specially housed, infected animals will be utilized to monitor the animals. All animals will be euthanized 6 months after SIVmac251 inoculation, and blood and lymphoid tissues will be collected. Animals will be euthanized as specified in the CRPRC guidelines "criteria for euthanasia of retrovirus infected macaques". A complete necropsy will be performed for each animal and peripheral and systemic lymphoid tissues will be prepared for histological, immunohistochemical, flow cytometric, bDNA and PCR analysis.

Note:

Animals will be fasted prior to blood collection and lymph node biopsies, and animals will be anaesthetized for the procedure. Ketamine will be used to sedate animals for blood collections, and Telazol for biopsy procedures. The blood volume will be adjusted according to each animal's weight as not to exceed the allowed blood volume of 12.5 ml/kg/month.

Lymph node biopsy: After anesthesia (medetomidine), the surgical site will be prepared and the skin over the node will be incised with a sterile scalpel blade. Once the node is removed by a combination of blunt and sharp dissection, the skin will be closed using suture and/or sterile surgical adhesive. Post-procedure analgesics will be applied at the veterinarian's discretion, generally ketoprofen will be given once a day for a total of 3 days.

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
1 (A)	Animals will be treated topically (intravaginally) daily for 2 weeks prior and for 2 weeks after SIV inoculation with Aldara. All 6 animals will be inoculated intravaginally with 10^5 TCID ₅₀ of SIVmac. Pretreatment and post-treatment phlebotomy to measure status of SIV infection. Lymph node biopsies will be performed at 1 month prior and 2 weeks after SIVmac inoculation. Necropsy at 6 months after SIVmac infection.	6	3
2 (B)	All 6 animals will be inoculated intravaginally with 10^5 TCID ₅₀ of SIVmac. Pretreatment and post-treatment phlebotomy to measure status of SIV infection. Lymph node biopsies will be performed at 1 month prior and 2 weeks after SIVmac inoculation. Necropsy at 6 months after SIVmac infection.	6	3

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?

1)The SIV rhesus macaque model of HIV heterosexual transmission has become the recognized standard for studies on pathogenesis and prevention of HIV vaginal transmission, because SIV is closely related to HIV biologically and genetically.

2) We have decided on six monkeys per group, which will allow us to determine statistically significant differences between groups using plasma viral RNA levels post-challenge (see Parker et al., J. Virol. (2001), 75:11234).

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 macaques	Ketamine HCl	10 mg/kg	IM	Prior to all procedures
Rhesus macaques	buprenorphine	0.1-0.3 mg/kg	IM	BID for 3 days, discretion of CNPRC vets
Rhesus macaques	telazol	5 mg/kg	IM	Prior to lymph node biopsies
Rhesus macaques	Medetomidine	30-35 µg/kg	IM	Prior to lymph node biopsies
Rhesus macaques	Atipamezole	0.15 mg/kg	IM	Right after lymph node biopsies
Rhesus macaques	Ketoprofen	2 mg/kg	IM	Once a day for 3 days after lymph node biopsy

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

Lymph node biopsies in both SIV infected and uninfected animal have potential risk of secondary infection. Animals will be observed for several days post biopsy and treated at the discretion of the CRPRC veterinary staff.

SIV infection of rhesus macaques can result in a fatal immunodeficiency and wasting syndrome. The animals will be euthanized when they experience 3 of the following: weight loss >15% in 2 weeks or >30% in 3 months; persistent hypothermia <96F 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.

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.

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?

July 2003

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
PubMed	1993-present	Primate, SIV, vaginal transmission, interferon alpha
Reference Update	1993-present	Primate, SIV, vaginal transmission, interferon alpha
Current Contents	1993-present	Primate, SIV, vaginal transmission, interferon alpha

What were your findings with respect to alternative methodologies?

There are no alternative methodologies for assessing genital lentiviral transmission.

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?

Animals will be euthanized 6 months after SIVmac infection. If an animal develops SAIDS prior to this time, the animal will be euthanized at that time.

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 macaque	IV	pentobarbital	60 mg/kg	IV

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

Animals will be either euthanized at 6 months after SIVmac infection, or possibly recycled into a therapeutic SIV study.

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.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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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>
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ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL #_10677_**EXPIRES: _____**

RUA#: _____

BUA#: 0477

CCA#: _____

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

SIV is a primate lentivirus that is genetically similar to HIV and causes fatal immunodeficiency (AIDS) in infected rhesus macaques. SIV can infect humans, but it is unknown whether SIV causes human disease.

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: All mucosal secretions can be contaminated.

Describe any human health risk associated with this agent:

SIV can infect humans: thus, it is possible that SIV could cause fatal, AIDS like disease in humans. Infectious virus and SIV antibodies have been detected in SIV-infected humans but there have been no reports of disease in SIV infected people.

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: Describe: Plastic disposable gown/coveralls
 Other: Describe: Plastic disposable gown/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:

Biosafety level 2+ (BSL 2+) precautions must be used at all times.

Date: Tue, 16 Sep 2003 09:46:54 -0700

To

From:

Subject: Fwd: AUC 10816

,
enclosed is the revised protocol AUC 10816. The following revisions have been made:

- 1) The box for special husbandry is included.
- 2) In section 2c the following additions have been made:
 - blood volumes are included
 - the lymph node biopsy procedure is described
 - requirements for fasting and anaesthesia prior to procedures have been added
 - agents listed in 2c are now also listed in section 2 g and vice versa
- 3) In section 2c the inoculation schedule for SIVmac has been clarified.

Date: Tue, 23 Sep 2003 14:59:50 -0700

To: >

From: >

Subject: Re: Fwd: additional committee questions protocols
10815/16/17

Cc:

Hello,

The treatments proposed in the AUC's 10815/16/17 will result in viral load changes in the SIVmac infected monkeys. It has been shown previously that to detect a difference in plasma viral load of at least 0.5-1 log₁₀ at the peak of viremia and at viral setpoint of SIV Infection, 6-8 monkeys are needed.

Please let me know if this answer is acceptable for you.

Thanks.-

, you can answer these via e-mail. No need to revise the protocol again.

Hi,

I have received the following additional comment regarding protocols 10815, 10816 and 10817. Please send the response to: campusvet@ucdavis.edu.

Thanks in advance,

#10815,16,17():

1. Section E (numbers justification) includes a reference as justification for the numbers, but otherwise fails to adequately justify the number of animals. Since we are now asking PIs to remove all reference to other names, I will remove the references and ask that Dr. group provide the statistical justification.