Notice of Award

RESEARCH **Issue Date:** 08/27/2014

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 1R01AI113893-01 REVISED
FAIN: R01AI113893

Principal Investigator(s):
Phillip Wayne Berman, PHD

Project Title: Re-engineering gp120 to include glycan-dependent epitopes

Deirdre Beach
Contract & Grant Officer
University of California, Santa Cruz
1156 High Street
Santa Cruz, CA 950641077

Award e-mailed to: ospdocs@ucsc.edu

Budget Period: 07/18/2014 – 06/30/2015
Project Period: 07/18/2014 – 06/30/2018

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $1,273,089 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF CALIFORNIA SANTA CRUZ in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI113893. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Shellie M. Wilburn
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R01AI113893-01 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$348,715
Fringe Benefits	$114,614
Personnel Costs (Subtotal)	$463,329
Equipment	$698,266
Supplies	$111,500
Travel Costs	$3,000
Other Costs	$122,830
Consortium/Contractual Cost	$246,250
Federal Direct Costs	$1,645,175
Federal F&A Costs	$381,003
Approved Budget	$2,026,178
Federal Share	$2,026,178
TOTAL FEDERAL AWARD AMOUNT	$2,026,178
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,273,089

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$2,026,178	$2,026,178
2	$746,619	$746,619
3	$725,929	$725,929
4	$712,069	$712,069

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1941539563A1
Document Number:	RAI113893A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2014

IC	CAN	2014	2015	2016	2017
AI	8472297	$753,089	$746,619	$725,929	$712,069
AI	8480840	$1,273,089			

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: A14E / **OC:** 414A / **Released:** Pll 08/26/2014
Award Processed: 05/08/2014 01:52:21 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI113893-01 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01AI113893-01 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase V Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI113893. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award is funded by the following list of institutes. Any papers published under the auspices of this award must cite the funding support of all institutes.

National Institute Of Allergy And Infectious Diseases (NIAID)

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI113893-01 REVISED

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

REVISION #2

ADMINISTRATIVE SUPPLEMENT:
This revised Notice of Award (NoA) provides supplemental funds of $1,273,089 Total Costs ($1,145,413 Direct Costs and $127,676 F&A Costs) to purchase equipment and services to accelerate the production and immunogenicity testing of candidate HIV vaccine antigens designed to elicit protective antibodies to
glycandependent epitopes. These funds are restricted for stated purpose, in request dated July 21, 2014, from Lynn Crowder, University of California, Santa Cruz, and may not used for any other purpose, without NIAID awarding unit approval.

This supersedes NoA issued 07/24/2014.

REVISION #1

FUNDING ADJUSTMENT:
This Notice of Award (NoA) is revised to correct the project period end date to 06/30/2018 to allow 4 years of funding.

This supersedes NoA issued 07/18/2014.

ANNIVERSARY DATE:
The budget period anniversary start date for future year(s) will be **July 1**.

CONTACT INFORMATION:
NIAID Grants staff will be moving to a new building effective October 1, 2014. As a result of the move the telephone numbers listed on this NoA for your grants management specialist and program officers may no longer be active. Please check the website below to find their new telephone numbers https://ned.nih.gov/search/

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Leslie D. Boggs
Email: boggsl@mail.nih.gov **Phone**: 301-402-6450 **Fax**: 301-493-0597

Program Official: Michael N. Pensiero
Email: mpensiero@niaid.nih.gov **Phone:** 301-435-3749

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AI113893-01 REVISED

INSTITUTION: UNIVERSITY OF CALIFORNIA SANTA CRUZ

Budget	Year 1	Year 2	Year 3	Year 4
Salaries and Wages	$348,715	$241,005	$241,005	$241,005
Fringe Benefits	$114,614	$82,471	$82,471	$82,471
Personnel Costs (Subtotal)	$463,329	$323,476	$323,476	$323,476
Equipment	$698,266			
Supplies	$111,500	$115,500	$100,500	$91,500
Travel Costs	$3,000	$3,000	$3,000	$3,000
Other Costs	$122,830	$46,786	$46,786	$46,786
Consortium/Contractual Cost	$246,250			
TOTAL FEDERAL DC	$1,645,175	$488,762	$473,762	$464,762
TOTAL FEDERAL F&A	$381,003	$257,857	$252,167	$247,307
TOTAL COST	$2,026,178	$746,619	$725,929	$712,069

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4
F&A Cost Rate 1	53%	53.5%	54%	54%
F&A Cost Base 1	$718,873	$481,976	$466,976	$457,976
F&A Costs 1	$381,003	$257,857	$252,167	$247,307

PI: **Berman, Phillip Wayne**	Title: Re-engineering gp120 to include glycan-dependent epitopes	
Received: 12/02/2013	FOA: AI12056	Council: 05/2014
Competition ID: FORMS-C	FOA Title: FUNCTIONAL GLYCOMICS IN HIV VACCINE DESIGN (R01)	
1 R01 AI113893-01	Dual:	Accession Number: 3649288
IPF: 577510	Organization: UNIVERSITY OF CALIFORNIA SANTA CRUZ	
Former Number:	Department: Biomolecular Engineering	
IRG/SRG: ZAI1 KP-A (M1)	AIDS: Y	Expedited: Y
Subtotal Direct Costs (excludes consortium F&A) Year 1: 499,762 Year 2: 499,756 Year 3: 496,172 Year 4: 499,020 Year 5: 499,322	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Phillip Berman	The Regents of the University of California	PD/PI

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

○ Pre-application ○ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT11539547

5. APPLICANT INFORMATION **Organizational DUNS*:** 125084723

Legal Name*: The Regents of the University of California
Department:
Division:
Street1*: 1156 High Street
Street2:
City*: Santa Cruz
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 95064-1077

Person to be contacted on matters involving this application
Prefix: First Name*: Deirdre Middle Name: Last Name*: Beach Suffix:
Position/Title: contract & grant officer
Street1*: 1156 High Street
Street2:
City*: Santa Cruz
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 95064-1077
Phone Number*: 8314592778 Fax Number: Email: dbeach@ucsc.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 94-1539563

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ○ Resubmission
○ Renewal ○ Continuation ○ Revision

If Revision, mark appropriate box(es).
○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)* :

Is this application being submitted to other agencies?* ○Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Re-engineering gp120 to include glycan-dependent epitopes

12. PROPOSED PROJECT
Start Date* 07/01/2014
Ending Date* 06/30/2019

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-020

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Phillip Middle Name: Last Name*: Berman Suffix:
Position/Title: Professor
Organization Name*: The Regents of the University of California
Department: Biomolecular Engineering
Division: Engineering
Street1*: 1156 High Street
Street2:
City*: Santa Cruz
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 95064-1077
Phone Number*: 8314593529 Fax Number: Email*: pwb@soe.ucsc.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$3,807,141.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$3,807,141.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Lynn Middle Name: Last Name*: Crowder Suffix:
Position/Title*: Proposal Analyst
Organization Name*: The Regents of the University of California
Department:
Division:
Street1*: 1156 High Street
Street2:
City*: Santa Cruz
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 95064-1077
Phone Number*: 8314591378 Fax Number: Email*: lcrowder@usc.edu

Signature of Authorized Representative*
Lynn Crowder

Date Signed*
12/02/2013

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1235CoverLetter.pdf

Funding Opportunity Number: RFA-AI-12-056 . Received Date: 2013-12-02T19:07:07.000-05:00

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OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The Regents of the University of California Santa Cruz
Duns Number:	125084723000
Street1*:	1156 High Street
Street2:	
City*:	Santa Cruz
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	95064-1077
Project/Performance Site Congressional District*:	CA-020

File Name

Additional Location(s)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3859-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1236-SummaryAbstract.pdf
8. Project Narrative*	1237-ProjectNarrative.pdf
9. Bibliography & References Cited	1238-Bibliography.pdf
10.Facilities & Other Resources	1239-Facilities.pdf
11.Equipment	1240-Equipment.pdf

Summary Abstract

The goal of this work is the development of a safe, effective, and affordable vaccine to prevent HIV-1 infection resulting from injection drug use, unprotected sex, and exposure-contaminated blood products. We plan to accomplish this goal by improving a vaccine originally tested in injection drug users, AIDSVAX B/E. This proposal takes advantage of recent discoveries demonstrating that many of the most potent neutralizing antibodies in sera from HIV-1-infected people are directed to glycan-dependent epitopes. The recognition that broadly neutralizing antibodies recognize carbohydrate epitopes is revolutionary, and represents perhaps one of the greatest advances in the history of HIV-1 vaccine development. In this proposal, we plan to use this information to develop new vaccine immunogens consisting of recombinant gp120 and fragments of gp120 (scaffolds) produced with the glycosylation required for the binding of these antibodies. We plan to use these immunogens to improve the efficacy of the AIDSVAX B/E vaccine used in the RV144 and VAX003 clinical trials. Our recent studies showed that this vaccine lacked the carbohydrate structure required for the production of antibodies to this new and important class of epitopes. The key criteria of success in this proposal will be: 1) the identification of immunogens and an immunization regimen able to elicit broadly neutralizing antibodies in laboratory animals with activity similar to the prototypic PG9 monoclonal antibody, and 2) the identification of immunogens able to elicit antibodies to the V1/V2 domain of the type that correlated with protection in the RV144 trial. By improving an existing vaccine with an established record of safety and immunogenicity in more than 15,000 subjects, an existing GMP manufacturing process, and demonstrated efficacy, we believe that years of time and millions of dollars can be saved, compared with the cost of developing a new vaccine from scratch. Other useful information that will result from this proposal includes: 1) determining the best method to elicit antibodies to glycan-dependent epitopes in the V1/V2 domain of gp120; 2) the identification of new glycan-dependent and -independent epitopes in the V1 and V2 domains of gp120; 3) understanding the differences in the magnitude and specificity of antibody responses to V1/V2 domain that led to protection in the RV144 clinical trial and was unable to protect injection drug users in the VAX003 clinical trial, and 4) understanding the prevalence of antibodies to glycan dependent epitopes in people who make antibodies to gp120 as a consequence of immunization or virus infection.

Project Narrative

The goal of this work is the development of a safe, effective, and affordable vaccine to prevent HIV-1 infection. Our approach is to develop vaccine immunogens that target glycan-dependent epitopes recognized by broadly neutralizing antibodies. By improving an existing vaccine, extensively tested in human clinical trials, rather than developing a new vaccine from scratch, years of time and millions of dollars can be saved in delivering this vaccine to populations at risk of infection by HIV-1.

Facilities

Laboratories. The Berman research laboratories comprise ~2947 square feet of laboratory and support space. They are located in the Baskin Engineering (BE) building on the University of California, Santa Cruz campus. They consist of a completely refurbished Molecular Biology lab, a Biosafety Level 2 Cell Culture lab, and accessory instrument rooms as follows, and shown in gray on the diagram below:

Molecular Biology and Protein Chemistry Lab (BSL1)	BE292	910 sq ft
2 Cell Culture/ Fermentation Laboratory (BSL2)	BE288	910 sq ft
Centrifuge and Gel Imaging room	BE286	118 sq ft
Microscopy room: Immunofluorescence and DIC	BE288A	112 sq ft
Bacterial Shaker, gel dryer, SpeedVac room	BE285	197 sq ft
Freezer storage area	BE280	127 sq ft
Pseudotype Neut Assay - BSL2+	BE281	164 sq ft
Protein	BE299	409 sq ft

The Molecular Biology lab is currently set up with two 4-person laboratory benches for general biochemistry, molecular biology, and immunology, with separate work stations for gel electrophoresis, immunoblotting and instrumentation. The Tissue Culture lab also contains two 4-person laboratory benches, and is designed for small- and medium-scale cell biology and cell culture experiments as well as safe handling of potential pathogens. Communal facilities such as an autoclave, glassware washer, drying oven and cold room are housed in a nearby room, BE202.

A secured and dedicated BSL2+ laboratory space to safely conduct Pseudotype Neutralization Assays and radioactive metabolic labeling experiments. This lab contains a gowning atrium, biosafety cabinet and incubators for tissue culture, dedicated equipment for general bochemistry and refrigerators and freezers for storage of pseudovirus and ^{35}S-methionine/cysteine.

A dedicated laboratory for Analytical Chemistry and Protein Purification. This room contains a 4-person laboratory bench, and separate space for AKTApurifier FPLC instrument, Allega 6R benchtop centrifuge and peripheral equipment for protein purification. This laboratory can be adapted to bench top GMP production of protein antigens for vaccine trials.

Shared Research Core: Flow Cytometry Facility, Microsopy Center and Chemical Screening Center. Flow Cytometry facility has a BD Aria and LSRIII for multicolor cell sorting and quantitative analysis. The microscopy center has instruments for wide field live cell imaging, fluorescence deconvolution imaging and confocal microscopy as well as image analysis workstations.The Screening center has equipment to facility high throughput screening for antibody/antigen interaction: a PE Envision automated plate reader, BioTeck plate washer for high throughput walk away applications, and PE Janus robotic liquid handling system for 96 and 384 plate pipetting applications. All three facilities have support staff to support researchers and maintain the instruments.

Computers. The lab is currently equipped with four Windows-equipped PCs and five IMac computers for researchers, all with access to the internet and databases for proteomics and DNA manipulation tools, as well as a shared printer. One Mac is set up with crystallography software for modeling and viewing molecular structures. Additional computers are used to drive imagers, plate readers, etc.

Office space for the PI and staff (350 square feet) is provided in the nearby Physical Sciences Building. Each of the two offices is equipped with computer, printer and a shared scanner/fax machine. An additional 337 sq ft of cubicle space with seating for 8 researchers and students has recently been added to the lab, in close proximity to the research labs on the 2nd floor of the Baskin Engineering building.

Scientific Environment. The working environment at UCSC is highly interactive and encourages both formal and informal collaborations. Our department offers extensive administrative assistance to faculty, including access to grant processing assistance via the Office of Sponsored Projects and a professional grant writer. Computing help is provided by UCSC Information Technology Services (ITS). As PI, I have access to graduate students from both the School of Engineering and the Department of MCD Biology, and I am a full member of the Center for Biomolecular Science and Engineering (CBSE). In addition, CBSE and a group of BME faculty have hired additional computing staff to help maintain high performance computing cluster systems and some of their own desktop systems. The SoE Business Office has three research analysts who are responsible for the financial management and administration of extramural contracts, grants, and gifts awarded to SoE faculty.

The Biomolecular Engineering Department and the MCD Biology Department both benefit from NIH training grants designed to support graduate students involved in specified areas of biomedical research. In addition to directly supporting graduate students, the grants also provide flexible funding that departments can use to support graduate training programs. The Departments of MCD Biology, Chemistry and Biochemistry, Biomolecular Engineering, Environmental Toxicology, and Physics/SCIPP jointly recruit graduate students via the newly established graduate Program in Biomedical Science and Engineering (PBSE). This program offers a broad range of interdisciplinary research and training opportunities to incoming graduate students and allows access to a diverse range of students.

UCSC also takes advantage of its prime location in close proximity to several other academic institutions, including Stanford University, UCSF and UC Berkeley. This continues to lead to collaborative interactions between investigators at UCSC and other institutions. For example, the Berman Lab makes use of DNA sequencing facilities at the University of California, Berkeley, where gene sequences can be determined with a 1-2 day turnaround time. The Berman lab utilizes the Biacore facility at Stanford University for quantitative assessment of antibody-antigen affinity measurement. In addition, the Berman Lab makes use of the University of California, Davis protein sequencing facility to carry our sequencing by Edman degradation. Recently, the PI has been named a visiting investigator at the Gladstone Institute of Virology and Immunology at the University of California, San Francisco, and a member of the UCSF Center for AIDS Research. These appointments provide the PI with access to equipment and clinical services that are not available at UCSC. It also provides the Berman Lab with access to the rich intellectual environment provided by the many excellent AIDS researchers in the San Francisco Bay area.

UCSC has an excellent record of recruiting and supporting outstanding faculty. Because of this, biomedical researchers at UCSC have earned a reputation for excellence and accomplishment that far exceeds the campus's modest size and youth. In fact, research from our departments repeatedly ranks among the most influential in the nation. Our faculty receive important recognition for their work. They are fellows of the National Academy of Sciences, the American Academy of Arts and Sciences, and the American Association for the Advancement of Science. We have a Howard Hughes Medical Institute (HHMI) investigator

and an HHMI professor. We have faculty members working under a Searle Scholar award, Sloan foundation awards, NSF CAREER awards, the Pew Biomedical Scholar award, the Ellison Medical Foundation New Scholar Award, and Packard fellowships. Many other distinguished awards have accrued to our faculty, including the Dickson Prize, the Massry Prize, the Gairdner Award, and *R&D Magazine* Scientist of the Year.

Equipment

Molecular Biology and Immunology. Four molecular biology workstations in BE292. Each consists of an Eppendorf model 5415 microcentrifuge, a vortex mixer, a variable temperature heating block for enzymatic digestions, and multiple pipetting and micropipetting devices. The room contains a Beckman model GS6R table top refrigerated centrifuge and an Eppendorf model 5417R refrigerated centrifuge. Also present are a 37° C incubator of bacterial culture dishes, a microwave oven, two -20°C Sanyo lab freezers, a -4°C dairy case, household type refrigerator/freezer, a Beckman pH meter, analytical and preparative balances (Mettler) for preparing buffers and various solutions, and multiple table-top rocker and shaker platforms. The room is equipped with a chemical fume hood and cabinets for the safe storage of acids, bases and organic chemicals. Laboratory water is produced by a Millipore MilliQ Biocel water purification system to produce clean water by reverse osmosis and deionization. The lab also houses a new Eppendorf Vis/UV spectrophotometer, a new NanoVue spectrophotometer (GE Healthcare), and 3 thermocyclers (2 BioRad C-1000 tetras, and 1 ABI Veriti 96 well).

Protein Chemistry. The analytical chemistry laboratory (BE299) also contains a variety of equipment to support analytical and preparative scale protein purification activities. Particularly important are two AKTApurifier liquid chromatography system for purifying milligram to gram quantities of recombinant proteins. This system is equipped with a variety of columns used for affinity chromatography and ion exchange chromatography. Other protein chemistry equipment include power supplies and gel boxes for polyacrylamide gel electrophoresis. In addition we have recently acquired a PROTEAN isoelectric focusing apparatus (Bio-Rad Laboratories) that allows us to assess glycan heterogeneity of HIV envelope proteins. A number of other instruments used for protein chemistry are located across the street in shared use facilities maintained by the Department of Chemistry and Biochemistry. We routinely use reverse phase HPLC and LC-MS and LCMS/MS mass spectroscopy, and circular dichroism to analyze protein purity and for sequencing of protein fragments. If the mass spectra data obtained at UCSC is insufficient to identify protein fragments, we can send them to the UC Davis Molecular Structure Facility for sequencing by Edman degradation.

The Tissue Culture lab (BE288) has restricted access and contains two new Baker Sterilgard III Advance laminar flow biosafety cabinets, -20°C and -80°C freezers and a laboratory refrigerator (-4°C). Major equipment housed in the cell culture facility includes two Sanyo model MCO17A1 37°C CO2 cell culture incubators, and a large roller bottle incubator with revolving roller bottle racks. Of particular significance for this project are two newly purchased ISF-X1 CO2 controlled orbital shaking incubators (Kuhner AG, Birsfelden, Switzerland). These incubators are specifically designed to support the new large scale transient transfection technology (LSTT) and will be used to produce the immunogens described in this proposal. The lab is also equipped with a Molecular Devices Spectra 190 Vis/UV microtiter plate reader for ELISA assays, a Tecan microtiter plate washer for ELISA assays, and a Beckman table top model GS6 preparative centrifuge. The lab is equipped with a Nikon inverted microscope (Eclipse TS100) for routine cell culture, and an upright Nikon model Eclipse 50i microscope for cell counting and general microscopy. Within BE288, a dedicated microscopy room (BE288A) for fluorescence imaging is equipped with an AxioskopII fluroescense microscope with filter systems and lenses appropriate for immunofluorescent staining of cells with UV, FITC/GFP, and PE fluorophores. This in-lab imaging capability is augmented with deconvolution and confocal imaging capacity at the UCSC Microscopy Center. The lab also contains multiple shaker and rocker platforms suitable for microtiter plates. New equipment which has been added includes a 5L Wavebag fermentation system (GE Healthcare) and an Alpha Innotech high resolution imaging system with fluorescent detectors suitable for immunofluorescent labeling of proteins on western blots. Finally we have recently added a 3-D computer workstation for visualizing 3-D structures of the HIV envelope protein alone and in complexes with CD4 and monoclonal antibodies using PyMol 3-D imaging software.

Pseudotype Neutralization Assay Lab (BE281). We have constructed a new lab in BE281 in order to carry out pseudotype virus neutralization assays. Entry to this room is secured, and an atrium allows for a gowning area to facilitate PPE for conducting BSL2+ research. This room contains a 4' Baker SterilGard III laminar flow biosafety cabinet, a pair of Sanyo stacking CO2 incubators, and a Perkin Elmer VictorX3 multilabel plate reader luminometer. In addition there is an inverted cell culture microscope (Nikon Diaphot) and 10 linear ft of lab bench for miscellaneous equipment such as a waterbath and microfuge This room also has a dedicated Sanyo -80 freezer and commercial refrigerator for storage of pseudovirus. This room is also certified for the

use of 35^S for metabolic labeling experiment. A Mettler Model3 survey meter with pancake detector is present for procedural monitoring and a Beckman 3801 Scintillation counter along with a GE Lifescience Typhoon imaging system in an adjacent building belonging to the Department of Molecular, Cell and Developmental Biology.

Instrument room (BE286). Adjacent to the main lab is an instrument room containing a new Beckman Avanti J.E. centrifuge, a transilluminator, UltraCam Gel Imaging System, and a rack for six CO2 cylinders with automatic changeover valves to supply the incubators in rooms 288 and 281. Along the walls are storage shelves for lab consumables.

The Instrument Room (BE285) has been reconfigured to provide space for two 37° bacterial shaking cabinets, two gel dryers with vacuum pumps and a SpeedVac with a vacuum pump. Along the walls are storage shelves for lab consumables.

The laboratory is conveniently located near a Biomolecular Engineering (BME) labs common area (BE202) that includes an autoclave (Consolidated Model SR-24A-PB), a glasswasher (Lanser Inc Model Laberxia 1400 XP) equipped with an RO-DI Rinse Water Filter and storage tank (Siemens ELGA Labwater Purelab), an icemaker (Scotsman Model AFE 325) and a glassware drying oven (Thermoelectron Oven MDL160). This room allows access to a walk-in cold room, which is also shared with other labs in the vicinity (BE202A). A new Nanodrop mass spectrophotometer purchased for use by the BME Labs is kept in a neighboring lab, BE201. In addition, we have access to a BD Biosciences Aria FACS flow cytometer and LSRIII analyzer through the IBSC Flow Cytometry Center located in the adjacent Biomedical Science Building.

Other BME department labs sharing the floor include the UCSC Genome Sequencing Center run by Dr. Nader Pourmand of the Department of Biomolecular Engineering. This center is available to all UCSC faculty and is designed to support genome and transcriptome sequencing. It is equipped with state-of-the-art "next generation" sequencing instruments. These include an Applied Biosystems SOLiD genetic analysis system and a 454 Life Sciences Genome sequencer. Other nearby labs focus on the analysis of small regulatory RNAs and nanodevices designed for the next generation of high throughput DNA sequencing ($1000 genome project). These instrument-intensive laboratories include equipment for DNA sequencing, quantitative PCR, microarray printing and analysis, and computational biology.

Additional facilities off campus: We contract with the Berkeley DNA sequencing facility, UC Berkeley, DNA Sequencing Facility 303 Barker Hall #3202, Berkeley CA 94720-3202 for standard gene sequencing. We utilize the PAN Biacore facility, Stanford University Medical Center, B017 Beckman Center, 291 Campus Dr, Stanford CA 94305-5301 for quantitative assessment of antibody-antigen affinity measurement.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Phillip Middle Name Last Name*: Berman Suffix:

Position/Title*:	Professor
Organization Name*:	The Regents of the University of California
Department:	Biomolecular Engineering
Division:	Engineering
Street1*:	1156 High Street
Street2:	
City*:	Santa Cruz
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip/ Postal Code*:	95064-1077

Phone Number*: 8314593529 Fax Number: E-Mail*: pwb@soe.ucsc.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1977

	File Name
Attach Biographical Sketch*:	1243-BermanBiosketch.pdf
Attach Current & Pending Support:	

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Berman, Phillip W.	Baskin Distinguished Professor Biomolecular Engineering Department Jack Baskin School of Engineering University of California, Santa Cruz
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of California, Berkeley, CA	B.A.	12/1971	Biology
Dartmouth Medical School, Hanover, NH	Ph.D.	6/1977	Biochemistry
Salk Institute, Neurobiology Lab, La Jolla, CA	Post doctoral	1977-1981	Autoimmunity
UCSF, Dept. of Biochemistry/Biophysics, San Francisco, CA	Post doctoral	1981-1982	Molecular biology export and secretion in E. coli

A. Personal Statement

The goal of my research is to develop a safe and effective HIV vaccine based on recombinant HIV envelope glycoproteins. This approach has been successful with other vaccines, such as Hepatitis B and human papilloma virus, and we think it will ultimately be successful for HIV. Recently the results of a Phase 3 clinical trial (RV144) were reported which showed, for the first time in more than 25 years of research, that a vaccine could prevent HIV infection. The RV144 trial involved a prime boost regimen; subjects were given priming immunizations with a recombinant pox-virus vaccine (vCP1521) and booster immunizations with a bivalent gp120 vaccine created in my lab (AIDSVAX B/E). Although the overall efficacy was modest, this was an important result that has rekindled interest in subunit vaccines and given an indication that we are on the right track. I believe that I have the commitment, experience, and leadership skills required to be successful in this endeavor. I began working on HIV vaccines in 1984 at Genentech, Inc. and have been involved in many of the key advances in the field, evidenced by my listing as inventor on multiple patents. For example, my lab was the first to show that antibodies raised against gp120 could neutralize HIV *in vitro*, and that immunization with recombinant gp120 could protect chimpanzees from experimental HIV infection. My colleagues and I were also the first to develop stable mammalian cell lines to express fully glycosylated, properly folded recombinant envelope proteins, which are notoriously difficult to express. In this regard we were first to develop a commercial scale GMP manufacturing process carried out at 10,000 L scale. In 1997, I co-founded a company, VaxGen, to carry out clinical development of candidate HIV vaccines developed at Genentech, and we carried out the first two large scale Phase 3 clinical trials of any HIV vaccine. Although these trials failed to show efficacy, they did show that Phase 3 HIV vaccines were possible, and proved that many concerns regarding the technical and ethical conduct of such trials were unfounded. Following the disappointing results in the VaxGen Phase 3 trials, I began a new "back to the drawing board" AIDS vaccine research program as a professor at University of California, Santa Cruz. I have set up a new lab focused on genetic analysis of viruses from the VaxGen Phase 3 trials, and improving the immunogenicity of HIV vaccine antigens.

My current research is focused on improving the protective efficacy of the gp120 vaccines. The RV144 trial showed that the AIDSVAX B/E vaccine, developed in our laboratory to elicit antibody responses, could provide protection from HIV infection when given in conjunction with vCP1520, designed to elicit cell mediated immunity. Although the level of protection was significant, it was too low for registration and clinical deployment. The goal of our current research is to improve the efficacy of the AIDSVAX B/E vaccine from the 31.2 % level seen in RV144 to the level of 60% or more thought to be the threshold for product approval. By improving the efficacy of an existing vaccine with safety data from more than 12,000 subjects rather than developing a new vaccine from scratch, years of time, millions of dollars, and many lives can be saved. Our lab is currently following three approaches to improve gp120 vaccines: 1) re-engineering gp120s to possess the glycan-dependent epitopes recognized by broadly neutralizing antibodies such as PG9 and PGT128; 2) improving the immunogenicity of gp120 vaccines by deletion of conserved protease cleavage sites that appear to degrade key neu-

tralizing epitopes before they can be recognized by the immune system; and 3) the production of new gp120 immunogens from a rare group of individuals, termed elite neutralizers, documented to have elicited broadly neutralizing antibodies. If we are successful in developing vaccine immunogens that elicit broadly neutralizing antibodies, we hope to advance these into human clinical trials such as the VAX003, VAX004, and RV144 trial that made use of the AIDSVAX B/B and AIDSVAX B/E vaccines developed in our lab.

B. Positions and Honors

Positions and Employment

1982-1991	Scientist/Senior Scientist, Department of Molecular Biology, Genentech, South San Francisco, CA
1991-1997	Staff Scientist, Process Sciences and Immunology Departments, Genentech, South San Francisco, CA
1997-2004	Co-founder, Senior VP, Research & Development, VaxGen, Inc., Brisbane, CA
2004-2006	Co-founder, Chief Scientific Officer, Global Solutions for Infectious Diseases, South San Francisco, CA
2006-2010	Chair, Department of Biomolecular Engineering, Baskin School of Engineering, UCSC
2006-present	Professor, Department of Biomolecular Engineering, Baskin School of Engineering, UCSC

Other Experience and Professional Memberships

2013	NIH U19 IPCAVD External Scientific Advisory Committee, U. Mass Medical School
2011-present	Visiting Investigator, UCSF Gladstone Institute of Virology & Immunology
2011-present	Permanent member, UCSF Center for AIDS Research Scientific Council (CFAR, CSC)
2005-present	Scientific Advisory Board, Global Solutions for Infectious Diseases
2006-2009	Scientific Advisory Board, Celltrion Inc., Inchon, Korea
2005-2009	Scientific Advisory Committee, European Union AIDS Vaccine Integrated Program (AVIP)

Honors

July 2013	Jack Baskin Distinguished Professor Chair, Biomolecular Engineering Department, UCSC
July 2012	Distinguished Professor of Biomolecular Engineering, UCSC
Sep 2009	Invited speaker at symposium "The Discovery of AIDS and HIV: Contributions of California Community to the Early Years of AIDS Research" at UC Davis
2008-present	Chair of UCSC Center of Excellence, "Information Technology & Infectious Diseases", for UC School of Global Health
2007-present	UCSC Executive Committee, California Institute for Quantitative Biomedical Research (QB3)
2007-2013	Jack Baskin Endowed Chair, Biomolecular Engineering Department, UCSC
2006-2008	Executive Committee, U.C. Biotechnology Research & Education Program (BREP)

C. Selected Peer-reviewed Publications in chronological order (selected from **111** peer-reviewed publications and **20** patents)

1. Weiss, R.A., Clapham, P.R., Weber, J.N., Dalgleish, A.G., Lasky, L.A., and Berman, P.W. (1986). Variable and Conserved Neutralization Antigens of Human Immunodeficiency Virus. Nature 324:572-575. PMID2431324.

2. Lasky, L.A., Nakamura, G., Smith, D.H., Fennie, C., Shimasaki, C., Patzer, E., Berman, P.W., Gregory, T., and Capon, D.J. (1987). Delineation of a Region of the Human Immunodeficiency Virus gp120 Glycoprotein Critical for Interaction with the CD4 Receptor. Cell 50:975-985. PMID2441877.

3. Berman, P.W., Gregory, T.J., Riddle, L., Nakamura, G.R., Champe, M.A., Porter, J.P., Wurm, F., Hershberg, R.D., Cobb, E.K., and Eichberg, J.W. (1990). Protection of Chimpanzees from Infection by HIV-1 after Vaccination with Recombinant Glycoprotein gp120 But Not gp160. Nature 345: 622-625. PMID2190095.

4. Berman, P.W., Huang W., Riddle, L., Gray, A.M., Wrin, T., Vennari, J., Johnson, A., Klaussen, M., Prashad, H., Koehne, C., deWit, and Gregory, TJ. (1999) Development of Bivalent (B/E) Vaccines Able to Neutralize CCR5-Dependent Viruses from the United States and Thailand. Virology 265: 1-9. PMID10603312.

5. Berman, P.W. (1998) Development of Bivalent rgp120 Vaccines to Prevent HIV type 1 Infection. AIDS Res Hum Retroviruses 14 Suppl 3:S277-89. PMID: 981495

6. Flynn, N.M, Forthal, D.N. Harro, C., Judson, F.N., Mayer, K.H., Para, M. F., Gilbert, P.B., Hudgens, M.G., Metch, B.J., Self, S.G., Berman, P.W., Francis, D. P., Gurwith, M., Heyward, W.L., Jobes, D.V., Peterson, M.L., Papovic, V., and Sinangil, F.M. (2005). Placebo controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. JID 191:654-665. PMID15688278.

7. Gilbert, P.B., Peterson, M.P., Follman, D., Hudgens, M.G., Francis, D.P., Gurwith, M., Heyward, W.L., Jobes, D.V., Popovic, V., Self, S.G., Sinangil, F., Burke, D., and Berman, P.W. (2005). Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventative vaccine trial. JID 191:666-675. PMID15688279.

8. O'Rourke, S., Schweighardt, B., Scott, W.G., Wrin, T., Fonseca, D., Sinangil, F., and Berman, P.W. (2009). Novel ring structure in the gp41 trimer of human immunodeficiency virus type 1 that modulates sensitivity and resistance to broadly neutralizing antibodies. J. Virol. 83:7728-38. PMCID2708639.

9. Yu, B., Fonseca, D., O'Rourke, S., and Berman, P.W. (2010). Protease cleavage sites in HIV-1 recognized by antigen processing enzymes are conserved and located at receptor binding sites. J. Virol. 84: 1513-26. PMCID2812349.

10. Smith, D.H., Winters-DiGiacinto, P., Mitiku, M., O'Rourke, S.M., Siangil, F., Wrin, T., Montefiori, D.C. and Berman, P.W. (2010). Comparative Immunogenicity of HIV-1 Clade C Envelope Proteins for Prime/Boost Studies. PLoS ONE 5(8): e12076. doi:10.1371/journal.pone.0012076. PMCID2920315.

11. O'Rourke, S.M., Schweighardt, B., Phung, P., Fonseca, D., Terry, K., Wrin, T., Sinangil, F., and Berman, P.W. (2010). Mutation at a single position in the V2 domain of the HIV-1 envelope protein confers neutralization sensitivity to a highly neutralization resistant virus. J. Virol 84:11200-9. PMCID2953176

12. Haynes B.F., Gilbert, P.B., McElrath, M.J., Zolla-Pazner, S., Tomaras, G.D., Alam, S.M., Evans, D.T., Montefiori, D.C., Karnasuta, C., Sutthent, R., Liao, H.X., DeVico, A.L., Lewis, G.K., Williams, C., Pinter, A., Fong, Y., Janes, H., DeCamp, A., Huang, Y., Rao, M., Billings, E., Karasavvas, N., Robb, M.L., Ngauy, V., de Souza, M.S., Paris, R., Ferrari, G., Bailer, R.T., Soderberg, K.A., Andrews, C., Berman, P.W., Frahm, N., De Rosa, S.C., Alpert, M.D., Yates, N.L., Shen, X., Koup, R.A., Pitisuttithum, P., Kaewkungwal, J., Nitayaphan, S., Rerks-Ngarm, S., Michael, N.L., Kim, J.H. (2012) Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med. 2012 Apr 5;366(14):1275-86.

13. Nakamura, G. R., D. P. Fonseca, S. M. O'Rourke, A. L. Vollrath, and P. W. Berman. (2012) Monoclonal Antibodies to the V2 Domain of MN-rgp120: Fine Mapping of Epitopes and Inhibition of alpha4beta7 Binding. PLoS One 7:e39045.

14. Yu, B., J. F. Morales, S. M. O'Rourke, G. P. Tatsuno, and P. W. Berman (2012) Glycoform and Net Charge Heterogeneity in gp120 Immunogens Used in HIV Vaccine Trials. PLoS One 7:e43903.

15. O'Rourke, S., B. Schweighardt, P. Phung, K. A. Mesa, A. L. Vollrath, G. P. Tatsuno, B. To, F. Sinangil, K. Limoli, T. Wrin, and P. W. Berman (2012) Sequences in gp41, the CD4 Binding Site, and the V2 Domain Regulate Sensitivity and Resistance to Broadly Neutralizing Antibodies J Virol 86:12105-14.

D. Research Support

Ongoing Research Support

R01 1R01DA026801-01A1 08/01/09 – 06/30/14
HIV Variation in Injection Drug Users: Mapping Broadly Neutralizing Antibodies
NIH/NIDA
The goal of this grant is to map mutations that affect sensitivity and resistance to broadly neutralizing antibodies using viruses from an injection drug user cohort from Thailand. The cohort is unique in because of many transmission linkages where people became infected with similar viruses through needle sharing.
Role: PI

R01 1R01AI089378-01 02/15/10 – 01/31/14
Refocusing the Immune Response to the HIV Envelope Glycoprotein
NIH/NIAID
The goal of this grant is to refocus the antibody response to the HIV envelope protein in a way that promotes the formation of broadly neutralizing antibodies. To accomplish this we plan to inactivate conserved cleavage sites recognized by proteases responsible for antigen processing and presentation. The fact that HIV protease cleavage sites occur at neutralizing epitopes may explain the difficulties in eliciting neutralizing antibodies to this protein.
Role: PI

R56 1R56 AI 106556-01A1 07/04/2013 – 06/30/2014
HIV Vaccines Targeting Glycan Epitopes: Improvement of a Vaccine Tested In IDUs
NIH/NIAID
The major goal of this project is to develop vaccine antigens that elicit antibodies to glycan-dependent epitopes recognized by broadly neutralizing monoclonal antibodies.
Role: PI

R01 DA 036335-01 08/01/2013 – 07/31/2018
Enhanced Anti-HIV-1 Antibody Responses in African American Women at Risk for HIV Infection Through Injection Drug Abuse
NIH/NIDA
The major goal of this project is to characterize the viruses and broadly neutralizing antibody responses in African American females that possess the dual elite neutralizer-elite controller phenotype.
Role: PI

Completed Research Support

Development of Strategies to Inhibit the Function of RANK, the Major Receptor Required for the Growth and Differential of Osteoclasts 04/01/13 – 08/31/2013
NASA/Ames/University Affiliated Research Center
The goal of this project is to characterize splice variants of RANK occurring as a consequence of space habitation, cancer, or aging. ROLE: Sub investigator

ARRA Administrative Supplement 1R01AI089378-01S1 10/01/10 – 08/31/12
Refocusing the Immune Response to the HIV Envelope Glycoprotein
NIH/NIAID ROLE: PI

Specificity of the gp120 Antibody Response in the RV144 Trial 05/01/10 – 04/30/13
Private Source
The goal of this project is to determine whether the three HIV envelope protein antigens contained in the AIDSVAX B/E and vCP1521 vaccines used in the RV144 clinical trial were similarly immunogenic and correlated with protection from HIV infection. This project is part of a larger consortium, carrying out a retrospective analysis of the clinical trial. ROLE: Sub investigator

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: The Regents of the University of California

Start Date*: 07-01-2014 **End Date*:** 06-30-2015 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Phillip		Berman		PD/PI	Institutional Base Salary		EFFORT		26,955.00	7,817.00	34,772.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **34,772.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			45,670.00	12,331.00	58,001.00
1	Graduate Students		EFFORT		22,109.00	553.00	22,662.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Analyst	EFFORT			16,041.00	9,785.00	25,826.00
1	Lab Manager				25,641.00	12,564.00	38,205.00
1	Research Specialist				33,829.00	11,840.00	45,669.00
1	Protein chemist Researher				29,972.00	17,384.00	47,356.00
1	Project Scientist				40,788.00	10,197.00	50,985.00
7	**Total Number Other Personnel**					**Total Other Personnel**	**288,704.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**323,476.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: RFA-AI-12-056 , Received Date: 2013-12-02T19:07:07.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2014 **End Date*:** 06-30-2015 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
1 . Microscope upgrade light source	7,100.00
2 . 2x CO2 incubator shaker platforms and clamps	7,900.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**15,000.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2014 **End Date*:** 06-30-2015 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	110,000.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . other - immunization (pocono)	40,000.00
9 . other - graduate student fes	6,786.00
Total Other Direct Costs	**158,286.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**499,762.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53.00	477,976.00	253,327.00
		Total Indirect Costs	**253,327.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**753,089.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: 1234-BudgetJustification2.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: The Regents of the University of California

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period:** 2

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	Phillip		Berman		PD/PI	Institutional Base Salary		EFFORT		26,955.00	7,817.00	34,772.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **34,772.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			47,040.00	12,701.00	59,741.00
1	Graduate Students		EFFORT		22,109.00	553.00	22,662.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Analyst	EFFORT			16,522.00	10,409.00	26,931.00
1	Lab Manager				26,410.00	13,469.00	39,879.00
1	Research Specialist				34,844.00	12,892.00	47,736.00
1	Protein Chemist Researcher				30,871.00	18,523.00	49,394.00
1	Project Scientist				42,012.00	11,343.00	53,355.00
7	**Total Number Other Personnel**					**Total Other Personnel**	**299,698.00**

Total Salary, Wages and Fringe Benefits (A+B) **334,470.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: RFA-AI-12-056 , Received Date: 2013-12-02T19:07:07.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2015 **End Date*:** 06-30-2016 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	114,000.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . other - Immunazation Pocono	40,000.00
9 . other - graduate student fees	6,786.00
Total Other Direct Costs	**162,286.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**499,756.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	53.50	492,970.00	263,739.00
		Total Indirect Costs	**263,739.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**763,495.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: 1234-BudgetJustification2.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: The Regents of the University of California

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period:** 3

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	Phillip		Berman		PD/PI	Institutional Base Salary		EFFORT		26,955.00	7,817.00	34,772.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **34,772.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			48,452.00	13,082.00	61,534.00
1	Graduate Students		EFFORT		22,109.00	553.00	22,662.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Analyst	EFFORT			17,018.00	11,062.00	28,080.00
1	Lab Manager				27,202.00	14,417.00	41,619.00
1	Protein Chemist Researcher				31,797.00	19,714.00	51,511.00
1	Research Specialist				35,890.00	13,997.00	49,887.00
1	Project Scientist				43,272.00	12,549.00	55,821.00
7	**Total Number Other Personnel**					**Total Other Personnel**	**311,114.00**

Total Salary, Wages and Fringe Benefits (A+B) **345,886.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: RFA-AI-12-056 , Received Date: 2013-12-02T19:07:07.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period:** 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2016 **End Date*:** 06-30-2017 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	99,000.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . other - Immunization Pocono	40,000.00
9 . other - gradute stuent fees	6,786.00
Total Other Direct Costs	**147,286.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**496,172.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	54.00	489,386.00	264,268.00
		Total Indirect Costs	**264,268.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**760,440.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: 1234-BudgetJustification2.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: The Regents of the University of California

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period:** 4

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	Phillip		Berman		PD/PI	Institutional Base Salary		EFFORT		26,955.00	7,817.00	34,772.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **34,772.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			49,905.00	13,474.00	63,379.00
1	Graduate Students		EFFORT		22,109.00	553.00	22,662.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Analyst	EFFORT			17,528.00	11,744.00	29,272.00
1	Lab Manager				28,018.00	15,410.00	43,428.00
1	Protein Chemist Researcer				32,751.00	20,961.00	53,712.00
1	Research Specialist				36,966.00	15,156.00	52,122.00
1	Project Scientist				44,570.00	13,817.00	58,387.00
7	**Total Number Other Personnel**					**Total Other Personnel**	**322,962.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**357,734.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number: GRANT11539590
Funding Opportunity Number: RFA-AI-12-056 . Received Date: 2013-12-02T19:07:07.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	90,000.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . other - Immunization Pocono	40,000.00
9 . other - graduate student fees	6,786.00
Total Other Direct Costs	**138,286.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**499,020.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	54.00	492,234.00	265,806.00
		Total Indirect Costs	**265,806.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**764,826.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: 1234-BudgetJustification2.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: The Regents of the University of California

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Phillip		Berman		PD/PI	Institutional Base Salary	EFFORT			26,955.00	7,817.00	34,772.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **34,772.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.00			51,402.00	13,879.00	65,281.00
1	Graduate Students		EFFORT		22,109.00	553.00	22,662.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Analyst	EFFORT			18,054.00	12,457.00	30,511.00
1	Lab manager				28,859.00	16,450.00	45,309.00
1	Protein Chemist Researcher				33,734.00	22,264.00	55,998.00
1	Research Specialist				38,075.00	16,372.00	54,447.00
1	Project Scientist				45,907.00	15,149.00	61,056.00
7	**Total Number Other Personnel**					**Total Other Personnel**	**335,264.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**370,036.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: RFA-AI-12-056 . Received Date: 2013-12-02T18:07:07.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 1250847230000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	78,000.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . other - Immunization Pocono	40,000.00
9 . other - graduate stuent fees	6,786.00
Total Other Direct Costs	**126,286.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**499,322.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	54.00	492,536.00	265,969.00
		Total Indirect Costs	**265,969.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**765,291.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: 1234-BudgetJustification2.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

PERSONNEL/CONSULTANTS:

Personnel justification: The proposal describes a labor-intensive Thomas Edison type empiric approach that requires molecular biology, cell culture, and protein chemistry and glycobiology support throughout the project, as well as a high level of immunoassay and neutralization assay support. To meet this need, all of our lab members have been cross-trained to support immunoassays, cell culture, and molecular biology. To access analytical methods and expertise not available in our lab or at UCSC we have established collaborations in glycobiology (Drs Raju, Fisher, Witkowska), structural biology (Drs Chen, Dubois, and Karplus), immunology (Drs Coffman and Berzofsky), and virology (Drs Montefiori , Duke University; and Marielle Cavrois, Gladstone Institute of Immunology and Virology, UCSF). We also make extensive use of UC wide core facilities at other campuses for activities such as: DNA sequencing (U.C. Berkeley core sequencing facility); N-terminal sequence analysis (U.C. Davis core protein sequencing facility), and Biacore analysis (Stanford University PAN Facility). Over the 5 year course of this proposal, we plan to produce and carry out immunization studies with 12 different proteins and scaffolds. While this is a large number of molecules, it represents less than three novel proteins per year. However, the production of new proteins and scaffolds immunologic evaluation is not simply a matter of constructing plasmids with the relevant sequence, transfecting cells, purifying the proteins by a generic immunoaffinity purification process, and then immunizing the animals. The preparation of each antigen requires a customized fermentation process and purification process, and analytical assays to insure high quality, reproducible glycoprotein preparations. Proteins derived from gp120 are difficult to purify to homogeneity due to proteolysis, aggregation, disulfide exchange reactions and glycan variation. Therefore, to obtain reproducible, well-defined immunogens, multiple experiments are required to work out the fermentation and purification conditions required to produce unclipped monomers with uniform glycosylation. Often this requires changes to the fermentation process (media composition, cell densities, viability limits, and time of harvest) or the purification conditions (exposure to low pH, salt concentration, methods of desalting, buffer exchange, and storage conditions) to optimize the recovery of the species for immunogenicity studies. Although it is often possible to produce preparations with 80-90% or more of the protein intact (unclipped), our previous studies suggested that clipped proteins are much more immunogenic than unclipped proteins and even this small amount of clipping can have a major deleterious effect on the immune response. Indeed we previously reported that inhibition of proteolytic clipping appeared to be the major factor that accounted for protection of chimpanzees from HIV infection following immunization with gp120 (Berman et al., *PNAS* 1988 85:5200-4; Berman et al., *Nature* 345:622-5). Moreover, we have also presented data suggesting that several neutralizing epitopes on gp120 (~~including the~~ V2 domain, the V3 domain, and the CD4 binding site) possess highly conserved protease cleavage sites that appear to have evolved as a way of preventing the formation of bNAbs (Yu et al., *PLoS ONE* 7:e43903). Therefore, controlling proteolysis and glycosylation heterogeneity are key elements in developing effective HIV-1 vaccine immunogens. Therefore the production of each immunogen requires much more than a single transfection and single generic purification process. The work described in this proposal will easily occupy the headcount requested for all 5 years of the grant.

Proposed Project Dedicated Personnel (Salaries funded through grant)

Phillip Berman (PI). The PI is requesting EFFORT salary support in years 1-5 of this grant, up to the NIH limit. The remainder of his salary beyond this limit ~~will be provided~~ by endowed chair funds.

Molecular Virology and Immunology Project Scientist: David Alexander, Ph.D. Dr. Alexander completed his Ph.D. in Biochemistry and Toxicology at the University of Wisconsin, Madison in 1999, then carried out postdoctoral training at Stanford University with John Boothroyd examining the secretion and trafficking of glycoproteins in model pathogenic eukaryotic organisms. Applying this special expertise in cell biology and protein trafficking, Dr. Alexander will have overall responsibility for the design and implementation of expression strategies to control glycosylation heterogeneity. His training in protein in protein biochemistry and immunoaffinity methodologies will be employed in the implementation of analytical methods to characterize heterogeneity, proteolysis, and aggregation of the novel antigen described here. This includes application of surface plasmon resonance (Biacore) and the development of immunodepletion protocols for GDE identification and quantitation. His responsibilities will also include neutralization assays and the coordinating subcontracted assays. Dr. Alexander is a senior scientist with broad experience in biochemical and cell biological assay design and implementation, and equally important is his expertise implementing safe

laboratory conduct and the mentoring of students.

Protein Chemistry Research Specialist: Bin Yu, Ph.D. Dr. Yu completed his Ph.D. in Chemistry at the University of California, Santa Cruz in October 2007. Dr. Yu's research has focused on defining the disulfide structure of agouti related protein in the laboratory of Dr. Glenn Millhauser. Dr. Yu has expertise in protein purification and defining the disulfide structure in soluble proteins. In particular he has considerable experience in the use of HPLC and mass spectrometry to determine the primary sequence and disulfide structure of complex proteins and to purify fragments resulting from protease digestion of purified proteins. Dr. Yu has cross-trained in protein chemistry, analytical biochemistry, and immunoassay development. Dr. Yu will be responsible for purification of the recombinant proteins described in this proposal. He will also be responsible for biochemical characterization and stability studies of the proteins and scaffolds that are produced. This includes PAGE gels, isolelectric focusing, characterization of disulfide structures, N-terminal sequence analysis, circular dichroism studies, and mass spectroscopy studies. Using immunoaffinity chromatography, he has purified more than 20 envelope proteins, monoclonal antibodies and fragments of gp120 in the 10-20 mg of scale. Dr. Yu completed his postdoctoral training in our lab and has now accepted an academic appointment as a Research Specialist in our lab.

Cell Biology/ Molecular Biologist: Gwen Tatsuno B.A., M.S. Ms. Tatsuno is an experienced, versatile Research Specialist with a solid background in molecular biology, cell culture, protein expression, immuno-assays, virology, and antibody production. Over the last two decades she has worked at a variety of San Francisco Bay area biotechnology companies including Synergenics, Avigen, and Elan Pharmaceuticals. She is a graduate of the University of California, Berkeley and earned a Masters degree in Genetic Counseling at Rutgers University. Ms. Tatsuno produced most of the gp120 fragments described in this proposal and has produced most of the glycosylation mutants. Besides the molecular biology, she expressed most of the proteins by large scale transient transfection and evaluated them for binding to broadly neutralizing monoclonal and polyclonal sera. We will rely on Ms. Tatsuno for molecular biology, cell culture, and immunoassay support during the course of this proposal.

Graduate Student Researcher: Rachel Doran. Ms. Doran is a second year student at the University of California, Santa Cruz Graduate Program for Biomedical Sciences and Engineering. She is doing her thesis on glycan-dependent epitopes (GDEs) in gp120. Recently she isolated a mouse monoclonal antibody to a GDE in the V1 domain and discovered that antibodies to GDEs can be elicited with normal 3-dose immunization regimens. Ms. Doran graduated with honors from University of California at Berkeley with a degree in Molecular Biology, emphasis on Microbiology. At Berkeley, she worked in a plant genetics lab at the Richmond branch of the USDA Plant Gene Expression Center, and a soil microbiology lab, where she cultured and characterized novel ammonium-oxidixing archaea. She also enjoyed working on an HHMI-funded revamping of the Berkeley introductory molecular biology program under Jasper Rine. Upon graduation, she traveled to China for half a year to continue her undergraduate research on the OXS3 protein in relation to heavy metal stress tolerance in rice plants. Ms. Doran joined the Berman lab in early 2012, and was the recipient of the QB3 (California Institute for Quantitative Bioscience) scholarship for the 2011-2012 academic year. Ms. Doran has a particular interest in immunology and the molecular basis of eliciting B-cell memory responses to T cell independent antigens.

Lab Manager/Molecular Biologist: Kathryn Mesa, B.A., M.S. Ms. Mesa is an experienced lab manager and molecular biologist with 16 years experience at UCSC. Ms. Mesa is responsible for maintaining all of the lab equipment, ordering lab supplies and consumables, and assigning tasks to student workers. Besides managing lab operations for staff of 13 researchers, she is a gifted molecular biologist and has contributed significantly to the work described in this proposal. Going forward, Ms. Mesa will continue to contribute to the project by providing molecular biology support. In this regard she will carry out *in vitro* mutagenesis, make and purify plasmid constructs, and organize the storage plasmids and sera. She also serves as the lab safety officer and serves as a liaison between the EH&S Dept and the UCSC Facilities Dept.

Data Analyst: Ann Durbin, B.A. Ms. Durbin has an undergraduate degree in molecular biology, with a professional certificate of Clinical Trials Design & Management from UCSD Extension and special training in the protection of human subjects. She has extensive experience in the management of therapeutic clinical trials. In a prior Clinical & Regulatory position at Quidel, Inc., Ms. Durbin was responsible for the compilation

and analysis of preclinical data for *in vitro* diagnostic products. In this proposal, Ms. Durbin will be responsible for the computational infrastructure and relational databases required to track all of the plasmids, proteins, sera and all of the assay results generated in the course of this proposal. She will be responsible for all of the graphic representations of the data for progress reports, final reports, and publications from these studies including charts, graphs, molecular images, and spreadsheets. She will serve as the liaison with the UCSC IT group and the computational resource for database and statistical analysis software.

Postdoctoral Fellow: TBD. We are requesting funding for a Postdoctoral Fellow with expertise in protein chemistry and mass spectrometry to help define the glycan structure of our envelope proteins and scaffolds that bind glycan-dependent epitopes recognized by the broadly neutralizing PG9 and PGT128 monoclonal antibodies. The work will be carried out at the Mass Spectrometry facilities at UCSC and at UCSF.

Graduate and Undergraduate Rotation Students. We are fortunate that many talented undergraduate and graduate students seek rotations or individual research projects in our laboratory. This experience enriches their academic experience and in many cases stimulates the commitment to a career in biomedical research. We have trained multiple students in basic plasmid constructions, the isolation and screening of monoclonal antibodies, and epitope mapping.

Ad Hoc Advisors/Consultants/Collaborators (Unpaid, no salary or fees requested)

David Montefiori, Ph.D: David Montefiori is Professor and Director of the Laboratory for AIDS Vaccine Research and Development in the Department of Surgery at Duke University Medical Center. His major research interests are viral immunology and AIDS vaccine development, with a special emphasis on neutralizing antibodies. One of his highest priorities is to identify immunogens that generate broadly cross-reactive neutralizing antibodies against HIV-1 for inclusion in vaccines. His work with HIV-1 began in 1985 while in the Department of Pathology at Vanderbilt University Medical Center, where he developed one of the earliest high throughput assays to measure HIV-1 neutralization. He moved to Duke in 1993, where he continued his studies of the antibody responses in HIV-1 infected people and in nonhuman primate models of AIDS virus infection and vaccination. For the past two decades, his laboratory has served as a national and international resource for standardized assessments of neutralizing antibody responses in preclinical and clinical trials of candidate AIDS vaccines. His laboratory is funded by the National Institutes of Health as part of the HIV Vaccine Trials Network (HVTN), the Center for HIV/AIDS Vaccine Immunology (CHAVI) and the Primate Core Immunology/Virology Laboratories. His laboratory also receives funding from the Bill and Melinda Gates Foundation as part of the Collaboration for AIDS Vaccine Discovery (CAVD). He has published over 300 original research papers that have helped shape the scientific rationale for antibody-based HIV-1 vaccines.

Dr. Montefiori will assay all the sera produced in this proposal for neutralizing antibodies against standardized panels of tier 1, tier 2, and tier 3 viruses. Dr. Montefiori has not requested funding for the neutralization assays themselves, but has requested funding for serum shipping costs. These costs have been built in to our budget.

Grant Pogson, Ph.D: Dr. Grant Pogson, Ph.D is a Professor in the department of Ecology & Evolutionary Biology at UC Santa Cruz. He earned his Ph.D. at the University of British Columbia in Vancouver and went on to do postdoctoral work with Dr. Eleftherious Zouros at Dalhousie University and Dr. Robert Boutilier at the University of Cambridge, working on the population genetics of marine bivalves and fishes. Dr. Pogson has over 25 years of experience investigating the signals of natural selection at protein and DNA sequence levels. He is an expert on the application of statistical tests for natural selection, using both DNA polymorphism data within species and DNA sequence divergence data between species. In the proposed grant, Dr. Pogson will assist in performing statistical tests for selection in the V1-V2 domain of the HIV1 envelope protein and interpreting the assay and neutralization data.

T. Shantha Raju, Ph.D. Dr. Raju earned his doctorate from the University of Mysore (India), and completed postdoctoral work in the Stanley lab at Albert Einstein College of Yeshiva University. He has specialized in bioanalytic chemistry and pursued a career in the biotech industry, including Genentech, Centocor, and Johnson and Johnson. Dr. Raju now holds the position of Scientific Director for Biologics Research at Centocor

Research & Development (Radnor, PA). Dr. Raju is considered to be one of the world's exerts in the glycosylation of therapeutic antibodies. While at Genentech, Dr. Raju characterized the carbohydrate composition of MN-rgp120 used in the AIDSVAX B/B and B/E vaccines. This work was never published but was submitted to the FDA as part of the IND package. Dr. Raju has maintained an interest in HIV vaccines and gp120 in particular. In this proposal, he will determine the carbohydrate composition of Env proteins and scaffolds designed to elicit antibodies to glycan-dependent epitopes.

Susan Fisher, Ph.D. Dr. Fisher is a Professor in the Department of Obstetrics, Gynecology & Reproductive Sciences, UCSF School of Medicine, as well as Faculty Director of the Sandler-Moore Mass Spectrometry Core Facility that serves as a resource for the UCSF proteomics research community. She received her Ph.D. in Anatomy awarded by the University of Kentucky, Lexington. She carried out postdoctoral work in Mass Spectrometry at UK Lexington. In their work on cytotrophoblast invasion during normal pregnancy and preeclampsia, her lab applies mass spectrometry-based approaches for proteome analyses and protein structure determination. In her own research and as director of SMMS facility, she develops mass spectrometry workflows for mapping the microheterogeneity of protein populations. This expertise in applying label free and tagless strategies in proteome mapping has direct extension to our efforts to define the glycan epitope structures on vaccine antigens. *Dr. Fisher's biosketch is attached.*

Ewa Witkowska, Ph.D. Dr. Witkowska is a Professor in the Department of Obstetrics, Gynecology & Reproductive Sciences at the UCSF School of Medicine. She has an outstanding publication record for mass spectrometry based protein structure analysis with specific applications in proteomic biomarker discovery, and the application of native MS techniques for discovery of protein-protein interactions. Her work at the Sandler-Moore Mass Spectrometry Core Facility at UCSF demonstrates expertise in developing robust MS workflows for discovery of protein architecture and modification in protein structure resulting from proteolytic processing. Dr. Witkowska will contribute to the development of analytical methods to define the structure of new glyco-immunogens. *Dr. Witkowska's biosketch is attached.*

Denon Wang, Ph.D.: Denong Wang, Ph.D., is a Distinguished Scientist and Senior Program Director of the Tumor Glycome Laboratory in the Center for Cancer and Metabolism of SRI Biosciences (Menlo Park, CA). Wang's long-term research interest is in the carbohydrate moieties that are critical for self/non-self recognition and induction of antibody responses. His team has established multiple platforms of carbohydrate microarrays and introduced these glycomics tools to explore the structural and antigenic diversities of the glycome. The main research focus of his lab is in the immunogenic sugar moieties. In the past few years, his group has contributed to the identification of immunologically potent glycan markers of SARS-CoV, *Bacillus anthracis exosporium*, and a number of human cancers. Dr. Wang received his Ph.D. in immunology and glycobiology with the late Professor Elvin A. Kabat at Columbia University in 1993. After that, he entered the developing field of post-genomics research. Before joining SRI in 2010, he served as head of the Functional Genomics Division at Columbia University's Genome Center from 1998 to 2003 and was director of Stanford University's Tumor Glycome Laboratory from 2007 to 2010.

Robert Coffman, Ph.D. Dr. Coffman is one of the most highly respected immunologists in the world. He is widely credited with the discovery of the TH1/TH2 control system of the immune response, whereby helper T lymphocytes can elicit either a TH1, cell-mediated immune response, or a TH2, antibody-mediated immune response. This basic distinction in the type of the immune response has been fundamental to our current understanding of the regulation of the immune response, and provided the basis for understanding the pathobiology of bacterial and viral infections, the pathogenesis of autoimmune diseases, the regulation of allergic reactions, and elements of the inflammatory response. The insights imparted by Dr. Coffman have provided the rationale for a number of vaccines and therapeutic products designed to prevent and treat allergic disease, inflammatory diseases, and autoimmune diseases. Dr. Coffman has made significant contributions to our understanding of the innate immune system which provides the "early warning system" that triggers TH1 or TH2 responses. He has provided basic insights into the specificity of Toll-like receptors (pattern recognition receptors) that are triggered by bacterial and viral products, and has created oligonucleotide Toll receptor agonists and antagonists. In his current position as Vice President and Chief Scientific Officer at the Bay Area biotechnology company, Dynavax, he has had the opportunity to apply insights from basic research to product development. In this regard, Dr. Coffman has had responsibility for the development of new vaccine adjuvants used to augment the immune response of vaccines such as Hepatitis B and influenza A virus in the

elderly and in immune-compromised patients, such as those undergoing kidney dialysis. Recently Dr. Coffman has become an adjunct professor in the Department of Biomolecular Engineering at UCSC.

Rebecca Dubois, Ph.D. Dr. Dubois is an Assistant Professor in the Department of Biomolecular Engineering at UCSC. She received her Ph.D. at UC San Diego and pursued postdoctoral training both at the Pasteur Institute with Dr. Felix Ray and at St. Judes Children's Research Hospital in Memphis TN with Dr. Charles Russell. Her research applies structural and biochemical methods to identify mechanisms of antibody neutralization and to discover novel antiviral inhibitors. She developed a novel methodology for the production of a structurally validated H1N1 virus vaccine. This expertise in methods to structurally validate vaccine immunogens will advance our efforts to establish standardized protocols for immunogen production.

Bing Chen, Ph.D. Dr. Chen is Assistant Professor of Pediatrics at Harvard Medical School. Dr. Chen received his PhD from Ohio State University and received his postgraduate training at Children's Hospital Boston and Harvard Medical School. His research centers on elucidating molecular mechanisms of HIV viral entry, with particular focus on structural studies of HIV envelope glycoprotein in distinct conformational states. His lab uses biochemical and structural approaches to elucidate the molecular details of the interaction between HIV envelope glycoprotein and the host co-receptor CCR5, recapitulating the membrane fusion *in vitro*, using purified viral and host components. Another research focus is on antibody neutralization mechanism and immunogen design, studying the molecular mechanisms of neutralization by broadly neutralizing antibodies.

Statistical Center for HIV/AIDS Research and Prevention (SCHARP), Seattle, WA. Dr. Berman has collaborated with Dr. Gilbert, Ph.D., at SCHARP for many years on the analysis of results from the VAX003 and VAX004 Phase 3 trials. He will interact with Dr. Gilbert and other members of SCHARP on an informal basis to make sure that data from this new project is analyzed in such a way that can be compared to previous clinical data and new data that is acquired during the course of this proposal.

MATERIALS AND SUPPLIES:

General lab supplies: Our cost for laboratory supplies and consumables is currently about $2500-3500 per FTE per month. Some of the expenses we incur are somewhat unusual for academic labs because much of our work requires the production of 20-50 mg batches HIV envelope proteins. The serum free culture media and reagents required to produce DNA for large scale (2-10 L) transient transfection experiments are expensive. For example, kifunensine, a glycan inhibitor used to produce proteins with mannose 9 glycans in cell culture, costs $2800 for 250 mg and is used at a concentration of 25 mg/liter of media. Each large scale transient transfection experiment used to produce 10-20 mg of recombinant gp120 or scaffold requires approximately $3000 for Qiagen Maxiprep plasmid DNA kits and lipofectamine and "plus" reagent (Invitrogen, Inc.). These costs could easily double for hard-to-express isolates. Additional expenses are incurred for disposable 2-3 L shake flasks with integral sterility filters for large scale transient transfection. To the extent possible, we try to obtain our immunoassay reagents from the NIH AIDS Regent and Reference Reagent Program; however they are only able to supply limited quantities of many of the antibodies critical for our research. Therefore we have to source many of the monoclonal antibodies (PG9, PG16, 4E10, 2F5, 2G12) used for AIDS research from Polymun Scientific (Vienna, Austria) where our cost for antibodies exceeds $12,000 per year. Additional expenses are incurred in the synthesis of synthetic codon optimized envelope genes ($3,000/gene), primers for mutagenesis and sequencing, and peptides for immunoassays. Any modest shortfalls in funding for supplies that we experience will be made up by funding to the PI from other sources (e.g. endowed chair funds).

Animal Studies: We plan to test 2-3 new candidate vaccine antigens or formulations per year during each of the 5 years of this grant in rabbits and mice. Each experiment will involve 5-6 groups of 6 animals per group for a total of 36 animals of each species per year. Because the antibody responses we seek to elicit take more than a year to develop in HIV-1 infected humans, we plan to continue these studies for at least 12 months. Based on bids from the vendors, the estimated cost for these studies is approximately $1000 per rabbit for the immunization and bleeding schedule specified in the proposal. Therefore we expect a base rate of $36,000 per year plus the cost of shipping sera and immunogens by overnight mail. We have used Pocono labs for the last three years and have found them to be responsive, conscientious, and reliable. Mouse studies will be

carried out in the new UCSC Biomedical Research Building Vivarium. The cost for these mouse studies is included in our materials and supplies budget.

Equipment: We are requesting funding for a microscope upgrade and for two shaker platforms to increase our capacity for mammalian cell shake flask cultures. The microscope upgrades include a replacement light source (Leica SFL4000 LED illumination source, $5800) and a new manipulator stage ($1300) for an old Zeiss Axioskop II used for immunoflourescence microscopy. This light source allows us to use new dyes useful to assess protein expression without having to go to another building as we do now. The new LED source is stable for 10,000 hrs whereas our current burner is stable for 200-400 hrs and costs >$1800. We are expecting the current lamp to fail shortly. We are also requesting two CO2 incubator shaking platforms. These in-incubator shaking platforms are an economic way to provide surge capacity for cell culture production using our current CO2 staking incubators. Each platform (Benchmark Orbishaker BT4000) costs $3000 and the appropriate platform clamps cost $950 each. It is likely that existing equipment used on this project may require servicing or repair during the course of this proposal. If so, the repair/replacement cost will be split among the different grants that make use of the equipment and taken out of materials and supply funds. Although not broken out as a separate budget item, we anticipate approximately $10,000 per year in recharge fees and consumables for use of Biacore instruments at the Stanford PAN facility (http://pan.stanford.edu). Recharge fees for utilizing these instruments are $350/day. The antibody affinity data generated from these experiments is a key element to characterizing our novel antigens and analytical profiling of our immunogens as we refine production methodologies.

Travel: The PI is requesting funding ($4,500/year) in each year of the grant for the PI and lab members (graduate students, postdocs, research specialists) to attend scientific conferences. Members of the Berman lab attend one of two AIDS vaccine meetings every other year: typically the Keystone symposium on AIDS vaccine and/or AIDS pathogenesis, and the International AIDS Vaccine meeting sponsored by the Global HIV Vaccine Enterprise which alternates between venues in the US and outside of the US (Europe or Asia).

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Fisher, Susan J.	Professor
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Hope College, Holland, MI	A.B.	1972	Biology, Chemistry
University of Michigan, Ann Arbor, MI		1973	Anatomy
University of Kentucky, Lexington, KY	Ph.D.	1977	Anatomy
University of Kentucky, Lexington, KY	Postdoc	1982	Mass Spectrometry

A. Personal Statement

I will oversee all aspects of the mass spectrometry-based carbohydrate structural work. I have 34 years of experience in biological mass spectrometry (MS). A major focus has been glycan analyses, specifically the structural characterization of human complex oligosaccharides. My group is also experienced in the MS-based methods of protein analysis that are proposed in this application. Therefore, I have the background and expertise that are required to direct this work.

B. Positions and Honors

Positions and Employment

1982-1988 Assistant Professor, Dept. of Stomatology, University of California, San Francisco (UCSF), CA
1988-1994 Associate Professor, Dept. of Stomatology, UCSF, CA
1994-2008 Professor, Dept. of Cell and Tissue Biology, UCSF, CA
2003- Faculty Director, UCSF Sandler-Moore Mass Spectrometry Core Facility, UCSF, CA
2004- Director, Human Embryonic Stem Cell Program, UCSF, CA
2008- Professor, Dept. of Obstetrics, Gynecology and Reproductive Sciences, UCSF, CA

Honors (Since 2000)

2000, Co-Chair, Gordon Conference (Reproductive Tract Biology); 1997-2000, Member, Reproductive Biology Study Section (NIH); 2000-2002, Chair, Reproductive Biology Study Section (NIH); 2000, Sadler Award from NICHD; 2000, NIH MERIT Award (DE07244); 2001, Faculty Director, UCSF Biomolecular Resource Center; 2001, Scientific Advisory Board, Society for Women's Health; 2002, UCSF Graduate Association Outstanding Mentor Award; 2003, Organizer, NIH Workshop on the Immunology of Implantation; 2003, Anita Payne Lectureship (University of Michigan); 2004, UCSF School of Dentistry Faculty Research Award; 2005, Organizer of the first Keystone Symposium on Reproductive Biology (with Drs. S.K. Dey and R. Michael Roberts); 2006, Dean's Distinguished Lecturer (University of Colorado); 2006, Jerry Elliott Memorial Lecture (New England Perinatal Research Society); 2007, 18th Eliot L. Silbar, MD, Memorial Lecturer and Visiting Professor, Northwestern University, Chicago, IL; 2010, Organizer of The National Academies workshop on Stem Cell Models for Environmental Health, Washington, DC; 2011, Fellow of the American Association for the Advancement of Science; 2012, Nina B. Schwarz Lectureship, Northwestern University; 2012, Honorary Bingzhi Forum Professorship, Institute of Zoology, Chinese Academy of Sciences; 2013, Frontiers in Reproduction Research Program Pioneer Award (NIH, MBL and Burroughs Welcome); 2013, Society for Reproductive Biology (Australia) President's Lecturer; 2013, NICHD MERIT Award (HD076253); 2013, Advisory Committee, Burroughs Wellcome Preterm Birth Committee; 2014, NIH State of the Art Lecture, International Federation of Placenta Associations

C. Selected peer-reviewed publications (Selected from 187 peer-reviewed publications; 208 total)

1. Genbacev OD, Prakobphol A, Foulk RA, Krtolica AR, Ilic D, Singer MS, Yang ZQ, Kiessling LL, Rosen SD,

Fisher SJ. (2003) Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. Science. 299(5605):405-8.

2. Hardt M, Witkowska HE, Webb S, Thomas LR, Dixon SE, Hall SC, Fisher SJ. (2005) Assessing the effects of diurnal variation on the composition of human parotid saliva: quantitative analysis of native peptides using iTRAQ reagents. Anal Chem. 77(15):4947-54.
3. Hardt M, Thomas LR, Dixon SE, Newport G, Agabian N, Prakobphol A, Hall SC, Witkowska HE, Fisher SJ. (2005) Toward defining the human parotid gland salivary proteome and peptidome: identification and characterization using 2D SDS-PAGE, ultrafiltration, HPLC, and mass spectrometry. Biochemistry. 44(8):2885-99.
4. Prakobphol A, Genbacev O, Gormley M, Kapidzic M, Fisher SJ. (2006) A role for the L-selectin adhesion system in mediating cytotrophoblast emigration from the placenta. Dev Biol. 298(1):107-17.
5. Denny P, Hagen FK, Hardt M, Liao L, Yan W, Arellanno M, Bassilian S, Bedi GS, Boontheung P, Cociorva D, Delahunty CM, Denny T, Dunsmore J, Faull KF, Gilligan J, Gonzalez-Begne M, Halgand F, Hall SC, Han X, Henson B, Hewel J, Hu S, Jeffrey S, Jiang J, Loo JA, Ogorzalek Loo RR, Malamud D, Melvin JE, Miroshnychenko O, Navazesh M, Niles R, Park SK, Prakobphol A, Ramachandran P, Richert M, Robinson S, Sondej M, Souda P, Sullivan MA, Takashima J, Than S, Wang J, Whitelegge JP, Witkowska HE, Wolinsky L, Xie Y, Xu T, Yu W, Ytterberg J, Wong DT, Yates JR, 3rd, Fisher SJ. (2008) The proteomes of human parotid and submandibular/sublingual gland salivas collected as the ductal secretions. J Proteome Res. 7(5):1994-2006. PMCID: PMC2839126.
6. Addona TA, Abbatiello SE, Schilling B, Skates SJ, Mani DR, Bunk DM, Spiegelman CH, Zimmerman LJ, Ham AJ, Keshishian H, Hall SC, Allen S, Blackman RK, Borchers CH, Buck C, Cardasis HL, Cusack MP, Dodder NG, Gibson BW, Held JM, Hiltke T, Jackson A, Johansen EB, Kinsinger CR, Li J, Mesri M, Neubert TA, Niles RK, Pulsipher TC, Ransohoff D, Rodriguez H, Rudnick PA, Smith D, Tabb DL, Tegeler TJ, Variyath AM, Vega-Montoto LJ, Wahlander A, Waldemarson S, Wang M, Whiteaker JR, Zhao L, Anderson NL, Fisher SJ, Liebler DC, Paulovich AG, Regnier FE, Tempst P, Carr SA. (2009) Multi-site assessment of the precision and reproducibility of multiple reaction monitoring-based measurements of proteins in plasma. Nat Biotechnol. 27(7):633-41. PMCID: PMC2855883.
7. Drake PM, Cho W, Li B, Prakobphol A, Johansen E, Anderson NL, Regnier FE, Gibson BW, Fisher SJ. (2010) Sweetening the pot: adding glycosylation to the biomarker discovery equation. Clin Chem. 56(2):223-36. PMCID: PMC2849286.
8. Drake PM, Schilling B, Niles RK, Braten M, Johansen E, Liu H, Lerch M, Sorensen DJ, Li B, Allen S, Hall SC, Witkowska HE, Regnier FE, Gibson BW, Fisher SJ. (2011) A lectin affinity workflow targeting glycosite-specific, cancer-related carbohydrate structures in trypsin-digested human plasma. Anal Biochem. 408(1):71-85.
9. Hunkapiller NM, Gasperowicz M, Kapidzic M, Plaks V, Maltepe E, Kitajewski J, Cross JC, Fisher SJ. (2011) A role for notch signaling in trophoblast endovascular invasion and in the pathogenesis of preeclampsia. Development.138: 2987-98.
10. Genbacev O, Donne M, Kapidzic M, Gormley M, Lamb J, Gilmore J, Larocque N, Goldfien G, Zdravkovic T, McMaster MT, Fisher SJ. (2011) Establishment of human trophoblast progenitor cell lines from the chorion. Stem Cells. 29(9):1427-36.
11. Drake et al. A lectin chromatography/mass spectrometry discovery workflow identifies putative biomarkers of aggressive breast cancers. J Proteome Res (2012) pp.
12. Hromatka BA, Drake PM, Kapidzic, M, Stolp H, Goldfien GA, Shih leM, Fisher SJ (2012) Polysialic Acid Enhances the Migration and Invasion of Human Cytotrophoblasts. Glycobiology. Epub ahead of print.
13. Drake PM, Schilling B, Niles RK, Prakobphol A, Li B, Jung K, Cho W, Braten M, Inerowicz HD, William K, Albertolle M, Held JM, Iacovides D, Sorensen DJ, Griffith OL, Johansen E, Zawadzka AM, Cusack MP, Allen S, Gormley M, Hall SC, Witkowska HE, Gray JW, Regnier F, Gibson BW, Fisher SJ. (2012) Lectin chromatography/mass spectrometry discovery workflow identifies putative biomarkers of aggressive breast cancers. J Proteome Res. 11(4):2508-20
14. Drake PM, Schilling B, Gibson B, Fisher, SJ (2013) Elucidation of N-glycosites within human plasma glycoproteins for cancer biomarker discovery. Methods Mol Biol. vol. 951 pp. 307-22
15. Bartlett JA, Albertolle ME, Wohlford-Lenane C, Pezzulo AA, Zabner J, Niles RK, Fisher SJ, McCray PB Jr, Williams KE (2013) Protein composition of bronchoalveolar lavage fluid and airway surface liquid from newborn pigs. Am J Physiol Lung Cell Mol Physiol. vol. 305 (3) pp. L256-66

D. Research Support

1 R01 HD076253-01 (Fisher; Merit Award) 09/25/13 – 06/30/23 EFFORT
NIH $207,500
Dissecting Gene Dysregulation at the Maternal-Fetal Interface in Preeclampsia
The goal of this project is to use laser capture microdissection and global transcriptomics approaches to profile gene expression of specific placental and maternal cell types across gestation (in normal pregnancies) and in pregnancy complications (preterm labor and preeclampsia).

1 P01 ES022841-01 (Woodruff) 06/15/13 – 05/31/18 EFFORT
NIH/NIEHS
The UCSF Pregnancy Exposures to Environmental Chemicals (PEEC) Children's Center

The University of California, San Francisco (UCSF) Pregnancy Exposures to Environmental Chemicals (PEEC) Children's Center will uncover how environmental chemicals affect early development by linking basic biology research on new pathways by which environmental chemicals adversely affect development, with unprecedented data about maternal and fetal chemical exposures during mid-gestation, and new insights on the combined impacts of environmental chemicals and chronic psychosocial stress on pregnancy outcomes. We will also improve methods and approaches to translate the science into action in the clinical and policy communities.

Project 1 $125,000 EFFORT
Modeling the Effects of EDCs on the Early Stages of Human Placental Development
The goal of this project is to study the effects of EDCs in terms of the human trophoblast epigenome.

1 P01 ES022841-01 (Woodruff) 04/01/13 – 03/31/18
EFFORT
EPA

The UCSF Pregnancy Exposures to Environmental Chemicals (PEEC) Children's Center

The University of California, San Francisco (UCSF) Pregnancy Exposures to Environmental Chemicals (PEEC) Children's Center will uncover how environmental chemicals affect early development by linking basic biology research on new pathways by which environmental chemicals adversely affect development, with unprecedented data about maternal and fetal chemical exposures during mid-gestation, and new insights on the combined impacts of environmental chemicals and chronic psychosocial stress on pregnancy outcomes. We will also improve methods and approaches to translate the science into action in the clinical and policy communities.

Project 1 $120,000 EFFORT
Modeling the Effects of EDCs on the Early Stages of Human Placental Development
The goal of this project is to study the effects of EDCs in terms of the human trophoblast epigenome.

2 R01 DE022031-02 (Hall) 05/01/12 – 04/30/17 EFFORT
NIH $240,000
Mass Spectrometry Tools in Pursuit of Salivary Biomarkers of Sjogren's syndrome

The goal of this project is to use state-of-the-art mass spectrometry approaches to identify salivary biomarkers that enable a definite diagnosis of Sjogren's syndrome.

5 U54 HD055764-07 (Giudice) 04/01/12 – 03/31/17
NIH
Origins and Biological Consequences of Human Infertility

The objectives of the NIH U54 SCCPIR at the University of California, San Francisco Center are: (a) to understand mechanisms underlying normal and abnormal early development fundamentally and for developing novel strategies to diagnose, prevent and treat infertility; (b) optimize fertility and pregnancy

outcomes and minimize adverse outcomes; (c) educate aspiring students, the public, health care professionals and patients about human development and disorders associated with abnormal development; and (d) inspire career choice and nurture career development in this important area of reproductive health and research.

Project 2 $195,000 EFFORT
Molecular Analysis of the Early Stages of Human Trophoblast Differentiation
The goal of this project is to understand the major regulators that govern the early steps in human trophoblast differentiation and to use this information to understand preeclampsia-associated defects in this process.

Placenta Bank $96,486 EFFORT
The goal of this project is to establish a Human Placenta Bank to acquire human placental tissue from well-characterized clinical specimens. The bank will be unique with regard to its composition, consisting of samples from normal singleton pregnancies and form women who are diagnosed with the major forms of preeclampsia (PE) or preterm birth (PTB).

5 R01 DE021041-03 (Fisher) 04/01/11 – 03/31/16 EFFORT
NIH $240,000
Functional Glycomics of Human Saliva

The goal of this project is to use mass spectrometry-based methods to characterize the oligosaccharide structures that comprise the human salivary glycome and to assess their functions in terms of mediating bacterial and/or immune cell adhesion.

TG2-01153 (Fisher) 09/01/09 – 08/31/15 EFFORT
Private Source $1,219,000
Training Program in Stem Cell Research at UCSF

The goal of this project is to provide training opportunities for Graduate Students, Postdoctoral Scholars and Clinical Fellows at UCSF who are interested in pursuing research in the realm of stem cells.

CL1-00523-1 (Giudice) 06/01/09 – 05/31/15 EFFORT
Private Source $400,000
The University of California San Francisco Shared Research and Teaching Laboratory: a Non-Federal Human Embryonic Stem Cell Resource for the Bay Area Community

The major goal of this project is to establish a human embryonic stem cell teaching and resource center.

1 R21 ES019944-01 (Fisher) 09/20/12 – 08/31/14 EFFORT
NIH $150,000
A Novel Model for Assessing the Effects of BPA Exposures on Human Placentation

The goal of this project is to use a new cell culture model, continuously self-renewing human trophoblast progenitor cells, to study the effects of bisphenol A (BPA) exposures during pregnancy in terms of placental development.

17UB-8705 (Werb) 07/01/11 – 06/30/14 EFFORT
Private Source $289,875
Biomarkers for Environmental Exposures in Breast Cancer

The goal of this project is to use a mouse model and mass spectrometry approaches to identify biomarkers of environmental chemical exposures as related to the mammary gland.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Witkowska, Halina Ewa (aka H. Ewa Witkowska)	Adjunct Professor
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Technical University of Gdansk, Gdansk, Poland	M.S.	1973	Organic Chemistry
Technical University of Gdansk, Gdansk, Poland	Ph.D.	1988	Organic Chemistry
Children's Hospital Oakland Research Institute, Oakland, CA	Postdoc	1986-1990	Enzymology, fetal-to-adult globin switch, hemoglobinopathy diagnosis, protein mass spectrometry

A. Personal Statement

I will provide oversight for all experiments aimed at profiling *N*-glycosylation and disulfide bridge assembly in gp120. To this end, I will work closely with Dr. Susan Fisher on development of analytical strategies, optimizing protocols and interpreting the data. I note that mass spectrometry analysis of oligosaccharide structures remains highly manual thus requiring significant expertise, in addition to time and effort. I will also maintain close communications with Dr. Phillip Berman, the grant PI to ensure timely interpretation of the MS findings in the context of the biology of viral infection that she will study. I have utilized MS to address complex questions posed by biological systems for more than 20 years, specializing in the areas of protein structure analysis and proteomics, including glycosite mapping, cross-linking, glycation, phosphorylation, as well as saponin oligosaccharide structures, with over 70 publications in MS field. As a Co-Director of Operations of the UCSF Sandler-Moore Mass Spectrometry (SMMS) Core Facility, I manage diverse collaborative research projects that involve proteomics and protein structure characterization in various body fluids and biological specimens and I understand well the challenges presented by structural complexity that is inherent to biological systems. I have been involved in efforts that develop and optimize proteomics technologies, including instituting quality control measures, by serving as a member of the Association of Biomolecular Facilities Proteomics Research Group and participating in the NCI-funded consortium Clinical Proteomics Technologies Assessment for Cancer. For seven years (2005-2012), I served as one of PIs of the DOE-funded Scientific Focus Area consortium ENIGMA leading the mass spectrometry group responsible for identification of protein-protein interactions in *Desulfovibrio vulgaris*, a sulfate reducing bacterium of environmental interest due to its potential utilization as a reducing agent in bioremediation of heavy metal-contaminated soils. I am a recipient of the ARRA NIH High End Shared Instrumentation Grant to a purchase state-of-the-art mass spectrometer (Waters Synapt G2) equipped with a T-Wave® ion mobility analyzer to offer MALDI imaging and native MS capabilities to the UCSF research community. I am currently involved in a project aimed at characterization of salivary glycome. As an experienced PI, mass spectrometrist and protein chemist, I am well prepared to face the challenges of this project of great potential for HIV vaccine development.

B. Positions and Honors

Positions and Employment

1990-2000 Assistant Research Biochemist, Deputy Director, Mass Spectrometry Facility, Children's Hospital Oakland, Oakland, CA
2000-2003 Application Specialist, Mass Spectrometry, Applied Biosystems, Foster City, CA
2003-Present Adjunct Professor, Co-Director of Operations, UCSF Sandler-Moore Mass Spectrometry Core Facility, Dept. of Obstetrics, Gynecology & Reproductive Sciences, UCSF, San Francisco, CA

Professional Memberships

1991-Present American Society for Mass Spectrometry and Allied Techniques
1993-Present Bay Area Mass Spectrometry
2005-Present Association of Biomolecular Resource Facilities
2007-2009 American Chemical Society

Other Professional Activities/Honors

2002	*Ad hoc* reviewer for one grant application: NHLBI
2005-2006	Member, Special Study Section: NIDDK
2007	Member, Special Study Section: DOE Genomics:GTL Program review of solicitation for "Analytical and Imaging Technologies for Lignocellulosic Material Degradation"
2009, 2010	Member, Special Study Section: NCRR, Shared Instrumentation Grant Program
2010, 2012-3	*Ad hoc* reviewer, Training Grants: NIDDK
1995-	Reviewer for Analytical Chemistry, Journal of Mass Spectrometry, Analytical Biochemistry, Biochemistry, Journal of Proteome Research, Nucleic Acid Research, Journal of American Society of Mass Spectrometry, Molecular & Cellular Proteomics.
1997	Reviewer and session chair (DNA adducts and Interactions) at the 45th Conference on Mass Spectrometry and Allied Topics, Palm Springs CA
2005-2007	Member: Proteomics Research Group, Association of Biomolecular Resource Facilities

C. Selected peer-reviewed publications (in chronological order)

Most relevant to the current application

1. Chiu YL, Witkowska HE, Hall SC, Santiago M, Soros VB, Esnault C, Heidmann T, and Greene WC. High-molecular-mass APOBEC3G complexes restrict Alu retrotransposition. *Proc Natl Acad Sci U S A.* 2006;103(42):15588-15593. PMCID: 1592537.
2. Dong M, Yang LL, Williams K, Fisher SJ, Hall SC, Biggin MD, Jin J, and Witkowska HE. A "tagless" strategy for identification of stable protein complexes genome-wide by multidimensional orthogonal chromatographic separation and iTRAQ reagent tracking. *J Proteome Res.* 2008;7(5):1836-1849. PMID: 18336004.
3. Witkowska HE, Bialy Z, Jurzysta M, and Waller GR. Analysis of Saponin Mixtures from Alfalfa (Medicago sativa L.) Roots using Mass Spectrometry with MALDI Techniques. *Natural Product Communications.* 2008;3(9):1395-1410.
4. Roan NR, Muller JA, Liu H, Chu S, Arnold F, Sturzel CM, Walther P, Dong M, Witkowska HE, Kirchhoff F, Münch J, and Greene WC. Peptides released by physiological cleavage of semen coagulum proteins form amyloids that enhance HIV infection. *Cell Host Microbe.* 2011;10(6):541-550. PMCID: 3257029.
5. Drake PM, Schilling B, Niles RK, Prakobphol A, Li B, Jung K, Cho W, Braten M, Inerowicz HD, Williams K, Albertolle M, Held JM, Iacovides D, Sorensen DJ, Griffith OL, Johansen E, Zawadzka AM, Cusack MP, Allen S, Gormley M, Hall SC, Witkowska HE, Gray JW, Regnier F, Gibson BW, and Fisher SJ. Lectin chromatography/mass spectrometry discovery workflow identifies putative biomarkers of aggressive breast cancers. *J Proteome Res.* 2012;11(4):2508-2520. PMCID: 3383053.

Additional recent publications of importance to the field

1. Robinson S, Niles RK, Witkowska HE, Rittenbach KJ, Nichols RJ, Sargent JA, Dixon SE, Prakobphol A, Hall SC, Fisher SJ, and Hardt M. A mass spectrometry-based strategy for detecting and characterizing endogenous proteinase activities in complex biological samples. *Proteomics.* 2008;8(3):435-445. PMID: 18186022.
2. Niles R, Witkowska HE, Allen S, Hall SC, Fisher SJ, and Hardt M. Acid-catalyzed oxygen-18 labeling of peptides. *Anal Chem.* 2009;81(7):2804-2809.
3. Friedman DB, Andacht TM, Bunger MK, Chien AS, Hawke DH, Krijgsveld J, Lane WS, Lilley KS, MacCoss MJ, Moritz RL, Settlage RE, Sherman NE, Weintraub ST, Witkowska HE, Yates NA, and Turck CW. The ABRF Proteomics Research Group studies: educational exercises for qualitative and quantitative proteomic analyses. *Proteomics.* 2011;11(8):1371-1381. PMID: 21394914.
4. Liu H, Yang L, Khainovski N, Dong M, Hall SC, Fisher SJ, Biggin MD, Jin J, and Witkowska HE. Automated Iterative MS/MS Acquisition: A Tool for Improving Efficiency of Protein Identification Using a LC-MALDI MS Workflow. *Anal Chem.* 2011;83(16):6286-6293.

5. Price MN, Deutschbauer AM, Kuehl JV, Liu H, Witkowska HE, Arkin AP. Evidence-based annotation of transcripts and proteins in the sulfate-reducing bacterium Desulfovibrio vulgaris Hildenborough. J Bacteriol 2011;193:5716-27. PMCID: 3187205.
6. Walian PJ, Allen S, Shatsky M, Zeng L, Szakal ED, Liu H, Hall SC, Fisher SJ, Lam BR, Singer ME, Geller JT, Brenner SE, Chandonia JM, Hazen TC, Witkowska HE, Biggin MD, and Jap BK. High-throughput Isolation and Characterization of Untagged Membrane Protein Complexes: Outer Membrane Complexes of Desulfovibrio vulgaris. *J Proteome Res.* 2012;11(12):5720-5735. PMCID: 3516867.
7. Witkowska HE, Hall SC, and Fisher SJ. Breaking the bottleneck in the protein biomarker pipeline. *Clin Chem.* 2012;58(2):321-323. PMID: 22140214.
8. Ounjai P, Kim KD, Liu H, Dong M, Tauscher AN, Witkowska HE, and Downing KH. Architectural insights into a ciliary partition. *Curr Biol.* 2013;23(4):339-344. PMCID: 3580003,
9. Zhu L, Hwang P, Witkowska HE, Liu H, and Li W. Quantitatively and kinetically identifying binding motifs of amelogenin proteins to mineral crystals through biochemical and spectroscopic assays. Methods Enzymol. 2013;532:327-341. PMID: 24188774.
10. Remis JP, Wei D, Gorur A, Zemla M, Haraga J, Allen S, Witkowska HE, Costerton JW, Berleman JE, and Auer M. Bacterial social networks: structure and composition of *Myxococcus xanthus* outer membrane vesicle chains. Environ Microbiol. 2013. Epub ahead of print. PMID: 23848955

D. Research Support

Ongoing Research Support

• R01 DE021041 Fisher (PI) 04/01/11 – 03/31/16
Functional Glycomics of Human Saliva
The goal of this project is to use mass spectrometry-based methods to characterize the oligosaccharide structures that comprise the human salivary glycome and to assess their functions in terms of mediating bacterial and/or immune cell adhesion.
Role: Co-investigator

• R01 DE015821 Li, Wu (PI) 07/05/11 – 06/30/15
Amelogenin Degradation by MMP20 and KLK4 in Enamel Biomineralization Amelogenin Degradation by MMP20 and KLK4 in Enamel Biomineralization
This grant will focus on the enamel mineralization modulated by interactions between apatite crystals, amelogenin and proteinases during different development stages and the defective growth of crystals in amelogenesis imperfecta.
Role: Co-investigator

• R56 DE013508 DenBe.ten (PI) 09/01/11 – 03/31/14
Enamel Fluorosis: Mechanism of Action
The major goals of this project are to test two possible mechanisms for the formation of dental fluorosis, including a differential binding of enamel proteins to fluoride containing apatite, and a direct effect of fluoride on ameloblast function.
Role: Co-investigator

Past Research Support

• 5 U24 CA126477-07 Fisher (PI) 09/28/06 – 08/31/13
Targeted and Global Proteomic Strategies for Early Breast Cancer Detection
The goal of this project is to identify biomarkers to be used in blood-based tests that will enable the early detection of several tumor types with our group focusing on breast cancer.
Role: Co-investigator

• DE-AC02-05CH11231 Adams (PI) 10/01/05 – 09/30/12
US Dept of Energy
Project Title: Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA).
Subproject Title: Characterization of Proteomes and Interactomes of Key ENIGMA Organisms Using Mass Spectrometry (Witkowska, PI)
The goal of this project are to extensively characterize the biology of bacterial communities at the systems level using tools of functional genomics, proteomics and metabolomics. The UCSF subcontract focuses on

characterization of soluble and membrane interactomes of *Desulfovibrio vulgaris*, a bacterium of potential use in bioremediation of heavy metal contamination in soil.
Role: Co-Principal Investigator

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**173,860.00**
Section B, Other Personnel		**1,557,742.00**
Total Number Other Personnel	35	
Total Salary, Wages and Fringe Benefits (A+B)		**1,731,602.00**
Section C, Equipment		**15,000.00**
Section D, Travel		**15,000.00**
1. Domestic	15,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**732,430.00**
1. Materials and Supplies	491,000.00	
2. Publication Costs	7,500.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	200,000.00	
9. Other 2	33,930.00	
10. Other 3		
Section G, Direct Costs (A thru F)		**2,494,032.00**
Section H, Indirect Costs		**1,313,109.00**
Section I, Total Direct and Indirect Costs (G + H)		**3,807,141.00**
Section J, Fee		

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
First Name*: Phillip
Middle Name:
Last Name*: Berman
Suffix:

2. Human Subjects

Clinical Trial? ○ No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	1241-SpecificAims.pdf
3. Research Strategy*	1242-ResearchStrategy3.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	1244-VertebrateAnimals.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	1245-AllLettersSupportAndContractual2.pdf
13. Resource Sharing Plan(s)	1246-ResourceSharing.pdf
Appendix (if applicable)	
14. Appendix	

Goals of the Research:

A major goal of HIV vaccine research is to develop immunogens able to elicit broadly neutralizing antibodies (bNAbs) such as those seen in rare HIV infected individuals, termed elite neutralizers (ENs). However, after more than 30 years of HIV vaccine research, none of the vaccine antigens developed to date are able to elicit antibodies of this type. Recent studies of monoclonal antibodies (mAbs) recovered from ENs have shown that several of the most potent bNAbs ever described (e.g. PG9 and PGT128) are directed to glycan-dependent epitopes (GDEs) in the V1/V2 and V3 domains of gp120 [1, 2]. This totally unanticipated discovery led to the hypothesis that previous HIV vaccines were ineffective in eliciting bNAbs because they lacked the glycan structures required for the binding of these antibodies.

In this proposal we will test this hypothesis using novel vaccine antigens (monomeric gp120s and scaffolds from the V1/V2 and V3 domain) possessing the glycans required for the binding of the PG9 and PGT 128 mAbs. A unique aspect of this approach is that we plan to re-engineer the two envelope proteins (MN and A244-rgp120) that appeared to provide protection in the RV144 trial when given in the context of a prime/boost immunization protocol. Therefore our goal is to increase the level of protection (31.2%) achieved in the RV144 trial to one of 60% or more required for product registration and clinical deployment.

An additional goal of this proposal is to develop to produce immunogens, with the glycans required for PG9 and PGT128 binding, in cell lines (such as CHO DG44) that can be used for the GMP production of proteins for clinical trials. While we and others have produced Env proteins and scaffolds able to bind PG9 and PGT 128 in cell culture systems appropriate for basic research (e.g. GnTI- 293 cells, 293 and CHO cells treated with kifunensine), these are difficult and expensive to scale up to prepare even the modest amounts of GMP protein required for clinical trials. The perspective we have gained from pursuing the development of gp120 subunit vaccines (e.g. AIDSVAX B/B and AIDSVAX B/E), from benchtop through to Phase 3 clinical trials, provides evidence that our lab extremely is well qualified to undertake the activities described in this proposal. These activities extend beyond antigen discovery and immunogenicity testing and include biochemical characterization, stability testing, and purification process optimization. Attention to these details will avoid the lengthy and expensive delays, such as those recently encountered by the NIAID-supported P5 Partnership for the production of envelope proteins for RV144 follow up studies in Africa and Thailand. They will also insure that our preclinical results are reproducible and will facilitate the transition of successful vaccine concepts into non-human primate studies and clinical development. Our lab at UCSC and our network of collaborators and advisors possess all the skills, experience, and equipment required to ensure the timely and efficient development of new vaccine immunogens with the potential to save millions of lives worldwide.

A. Specific Objectives:

1. To engineer novel monomeric gp120s and fragments (scaffolds) from the V1/V2 and V3 domains that will elicit PG9- and PGT128-like bNAbs. We plan to follow up on preliminary data suggesting that monomeric gp120s and scaffolds from the V1/V2 domain are able to bind bNAbs such as PG9 and PGT128 provided the proper glycans are incorporated. Besides engineering these for high affinity antibody binding of bNAbs, these proteins will also be engineered to minimize proteolysis and to allow expression in normal cell lines (e.g. 293 and CHO-DG44 cells) suitable for the production of clinical materials.

2. To implement new analytical methods for characterization of the novel immunogens. Because gp120s and scaffolds are complex molecules that are particularly prone to glycosylation heterogeneity, proteolysis, and aggregation, robust analytical methods are required to define the structure of each new immunogen prior to production for immunogenicity studies. For this purpose, we will augment the analytical assays previously used in the production of the gp120s used in the RV144 trial with additional assays such as mass spectrometry, isoelectric focusing, and circular dichroism to characterize glycan structure and proteolysis.

3. To identify immunization regimens effective in eliciting bNAbs to GDEs, and antibodies that correlated with protection in the RV144 trial. Mice and rabbits will be immunized with single immunogens, cocktails of immunogens, and using prime-boost regimens to identify the most effective strategy to elicit PG9- and PGT128-like bNAbs and antibodies to the V2 domain that correlated with protection in the RV144 trial. Neutralization studies using pairs of PG9- and PGT128-sensitive and -resistant viruses, immunodepletion studies using V1/V2 and V3 domain scaffolds, and binding to synthetic peptides and glycan microarrays will be used to assess the magnitude of antibody responses to specific epitopes.

B. Significance.

The development of immunogens able to elicit broadly neutralizing antibodies (bNAbs) is a major goal of HIV vaccine research. The experiments described in this proposal will examine the possibility that previous HIV vaccines failed to elicit bNAbs because they lacked the specific glycan structures required for their binding. The PI proposes to improve the gp120s contained in the AIDSVAX B/E vaccine used in the RV144 trial [3] by altering their carbohydrate structures at key epitopes recognized by the broadly neutralizing (bN) monoclonal antibodies (mAbs) PG9 and PGT128 [1, 2]. He also plans to produce small glycopeptide fragments (scaffolds) to target antibody responses to the glycan-dependent epitopes (GDEs) in the V1/V2 and the V3 domains recognize by these mAbs. Preliminary data suggest that these scaffolds are able to enhance the antibody response to the region of the V2 domain (termed V2cop) that correlated with protection in the RV144 trial. Because PG9 and PGT128 are able to neutralize viruses from all of the major clades of HIV-1, an immunogen that elicits antibodies to the epitopes defined by these mAbs may provide the basis for the long-sought universal HIV vaccine. Re-engineering the AIDSVAX B/E vaccine to include these GDEs may raise its efficacy from the 31% observed in the RV144 trial to the level of 60%, or higher, required for registration and clinical deployment. ***By improving an existing vaccine with an established record of safety and immunogenicity in more than 13,000 subjects [3-5], and demonstrated efficacy in the RV144 trial, years of time and millions of dollars in manufacturing costs can be saved, compared with the cost of developing a new vaccine from scratch.***

C. Innovation.

C1. We are attempting to improve the immunogenicity of the gp120s (MN and A244) contained in the AIDSVAX B/E vaccine, that provided protection in RV144 trial, by re-engineering them to incorporate the GDEs required for the binding of bN-mAbs. Because these novel gp120 monomers now bind both the PG9 and PGT128 bN-mAbs with high affinity, we question whether trimeric Env proteins are required to elicit antibodies to the PG9 and PGT128 epitopes.

C2. We have developed monomeric V1/V2 scaffolds from diverse strains able to bind the PG9 bN-mAb with high affinity. Preliminary data suggests that these target the PG9 epitope and enhance the antibody responses that correlated with protection in the RV144 trial.

C3. We have developed monomeric gp120s and V3 scaffolds able to bind the PGT128 bN-mAb, one of the most potent bN-mAbs ever described. Immunization with these molecules may provide a practical approach to elicit PGT128-like antibodies.

C4. We are attempting to improve the stability of gp120s and scaffolds in vitro *and* in vivo *by deletion of conserved protease cleavage sites that affect epitopes recognized by bNAbs [6].* Initially we will focus on the cathepsin D site located between positions 179 and 180 in the V2 domain. If promising results are obtained, we will attempt to delete conserved cathepsin cleavage sites in the V3 domain.

C5. We are attempting to engineer our gp120s and scaffolds for production in normal cell lines (e.g., 293 and CHO DG44) suitable for GMP production. This will provide a practical approach to produce proteins for clinical trials.

C6. We have developed gp120s and V1/V2 scaffolds from one of the few strains (A244) able to bind to the inferred ancestral IgVH germ line gene of the PG9 mAb [7]. These molecules should facilitate studies to determine if immunogens able to bind germline B-cell receptors can enhance the formation of PG9-like bNAbs.

C7. The V1/V2 and V3 scaffolds can be used in combination with other vaccine concepts (DNA immunization, recombinant viruses, mosaic Env proteins, trimeric Env proteins).

D. Preliminary Results.

D1. Understanding the antigenic structure of the V1/V2 and V3 domains of gp120. Recently, the location of the epitopes recognized by the prototypic, broadly neutralizing monoclonal antibodies (bN-mAbs) PG9 and PGT128 were determined [8, 9]. Surprisingly, both bN-mAbs recognized glycan-dependent epitopes (GDEs) in the V1/V2 and V3 domains of HIV gp120. PG9 binding requires mannose-5 glycans at positions 156 and 160, whereas PGT128 binding requires terminal mannose-9 at positions 332 and 301. The recently published gp140 trimer structure [10] provides new information that places the PG9 epitope in the V1/V2 domain in close proximity to the PGT128 epitope in the V3 domain (Fig 1A). Thus, while once thought of as independent sites of vulnerability, the four strands of the V1/V2 β-sheet structure occur adjacent to and below the two β-sheet strands of the V3 domain creating a 6-stranded β-sheet structure (Fig 1B). To further define the antigenic structure of the V1/V2 domain, we recently mapped the epitopes recognized by 9 conformation-dependent mAbs to the V1/V2 domain of MN-rgp120 [11]. These studies (Fig 2) showed that there are two clusters of highly immunogenic epitopes within the V1/V2 domain. These epitopes occur at two of the three turns at the

junctions between adjacent strands of the 4-stranded, anti-parallel β-sheet structure. One cluster of epitopes occurs at the junction of the B and C strands (B-C peptide) and the other cluster is located in the junctional sequence connecting the C and D strands (C-D peptide). Although some of the mAbs to these sites possessed neutralizing activity, they differ from PG9 in that their activity was effective only against lab-adapted and tier 1 isolates [12].

The epitope landscape of the V3 domain is in part similar to that of the V1/V2 domain. Both have a highly immunogenic epitope, termed the principal neutralizing determinant (PND) [13], located in the connecting region between two β-sheet strands (1B). Most mAbs to this region are effective in neutralizing tier 1 and lab adapted viruses, but not the tier 2 and 3 viruses characteristic of clinical isolates [14]. However, the stem of the V3 domain also possesses a GDE recognized by the bN-mAb, PGT128. Interestingly, the PGT128 epitope occurs in close proximity to the peptide connecting the A and B strands of the V1/V2 domain, whereas the PND of the V3 domain occurs in close proximity to the V1/V2 B-C connecting peptide. The close proximity of the V1/V2 and V3 β-sheet structures provides a structural explanation for the observation that antibodies to the V1/V2 or V3 domain sometimes compete with each other in certain assays [1] and ultimately suggests that both regions should be considered a unified antigenic structure.

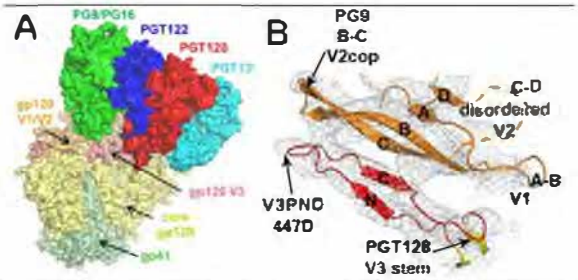


Figure 1. Location of epitopes recognized by PG9 and PGT128 mAbs. (**A**) Superimposition of the co-crystal structures of PG16 (green), PGT128 (red), and PGT135 (cyan) on the PGT122 (blue) Env trimer crystal structure reveals that these glycan-dependent epitopes overlap. (**B**) Structural arrangement of 4-stranded gp120 V1/V2 β-sheet structure (orange) and the 2-stranded V3 β-sheet structure (red) in the context of the gp140 trimer. Locations of A-B, B-C (V2cop), and C-D connecting peptides linking β-sheet strands are indicated. Approximate locations of epitopes of the PG9, PGT128, and 447D mAbs are indicated. Data from Julian et al. *Science* 2013 10.1126/science. 1245625

D2. Overlap between bNAbs to the V1/V2 domain and antibodies that correlated with protection in the RV144 vaccine trial. When the binding site for the PG9 mAb was defined by structural and mutational analyses [9, 10, 15], it was found to require basic amino acid side chains at the B-C junction (e.g., positions 167-171) as well as glycans at positions N156 and N160, located in the middle of the B strand. Remarkably, this exact region of the B-C junction of the V1/V2 domain was independently highlighted in the RV144 "Correlates of Protection" study [16]. Using synthetic peptides, and MLV gp70-V1/V2 fusion proteins, a sequence in the V2 domain (spanning positions 165-178) was identified that correlated with protection from HIV-1 infection. ***We term this sequence the V2 correlates of protection (V2cop) epitope***. Interestingly, the antibodies to the V2cop epitope do not appear to possess virus neutralizing activity [17]. They are thought to have provided protection in RV144 by neutralization-independent mechanisms, such as ADCC [18], or ADCVI. ***Thus, the B-C junction of the V1/V2 domain appears to possess two functionally significant overlapping epitopes: the PG9 epitope that includes glycans at positions 156 and 160, and the V2cop epitope.***

The recognition that many of the bNAbs found in HIV-infected humans are directed to GDEs [19] is a major discovery as it may account for the inability of virtually all HIV vaccines developed to date to elicit bNAbs. Thus while gp120 vaccines have been able to elicit antibodies to sites in the V1/V2 and V3 domains that overlap those recognized by bNAbs [20-22], they were produced without regard to the specific glycan structures in the V1/V2 and V3 domains necessary for the binding of antibodies such as PG9 and PGT128. ***These observations provide an alternate explanation to the Env trimer theory to account for the inability of previous gp120 subunit vaccines to elicit bNAbs; namely, previous vaccines were unable to elicit bNAbs because they lacked the glycan structure required to elicit PG9 and PGT128-like antibodies.***

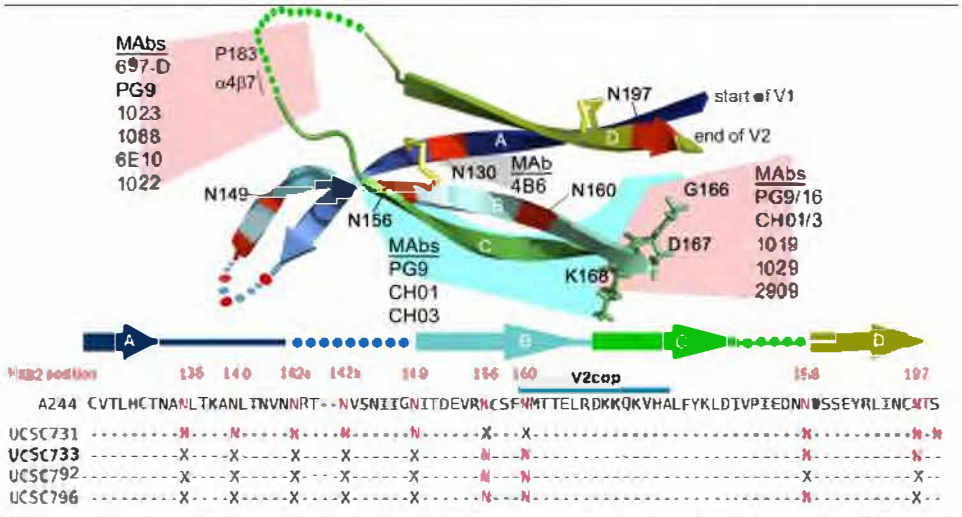


Figure 2. Location of glycosylation sites and epitopes in the V1/V2 domain. The structure of the V1/V2 domain determined by McLellan et al. (*Nature* 2011. 480:336) has been modified to show the approximate positions of glycosylation sites (red) and epitopes recognized by MAbs (shaded), and disulfide bonds (yellow). Each strand in the 4-stranded β-sheet structure is indicated as A-D. The sequence of the V1/V2 domain of A244 is shown below, as is the location of mutations (X) that destroy specific glycosylation sites (UCSC731,733, 792, and 796) in scaffolds used for immunization studies and/or immunoassays. Glycosylation sites (N) are shown with reference to the HXB2 numbering system adjusted for insertions by the method of Rolland et al. (*Nature* 2012, 490:417).

D3. Glycan composition and bN-mAb binding of MN and A244-rgp120s incorporated in the AIDSVAX B/E vaccine. To determine whether the inability of the AIDSVAX B/E vaccine to elicit PG9-like antibodies might have been the result of inappropriate glycosylation, we characterized the glycan heterogeneity of the two gp120s contained in this vaccine (MN- and A244-rgp120) by isoelectric focusing and endoglycosidase digestion [23]. Our results showed that both immunogens consisted of a highly heterogeneous mixture of proteins that varied in net charge and sialic acid content. Thus it is possible that the AIDSVAX B/E vaccine failed to elicit PG9-like antibodies because it lacked the requisite high mannose glycans. Another possibility is that these proteins failed to elicit PG9-like antibodies because they lacked the trimeric structure reported to be required for the binding of these antibodies [2, 24]. To further explore this possibility, we measured the binding of PG9 to the CHO cell-produced MN- and A244-gp120s. We found that of the CHO-produced vaccine immunogens, PG9 exhibited little, if any, binding to MN-rgp120, whereas A244 exhibited weak binding [23]. To determine if the binding of PG9 could be improved by altering the glycan structure, both proteins were expressed in GnTI- 293 cells, deficient in N-acetylglucosaminyltransferase I. A mutation in this enzyme limits N-linked glycans to the mannose-5 structure required for PG9 binding. We found that production of both proteins in GnTI- cells greatly enhanced binding of PG9 (Fig 3A-B). Thus the 50% effective concentrations (EC_{50}) for PG9 binding to MN and A244-rgp120s were 0.05 and 0.02 ug/mL, respectively. ***This experiment demonstrated that monomeric Env proteins were able to bind PG9-like antibodies, provided that the proper glycosylation was present. These studies also demonstrated that trimeric forms of the Env protein are not required for the binding of PG9.***

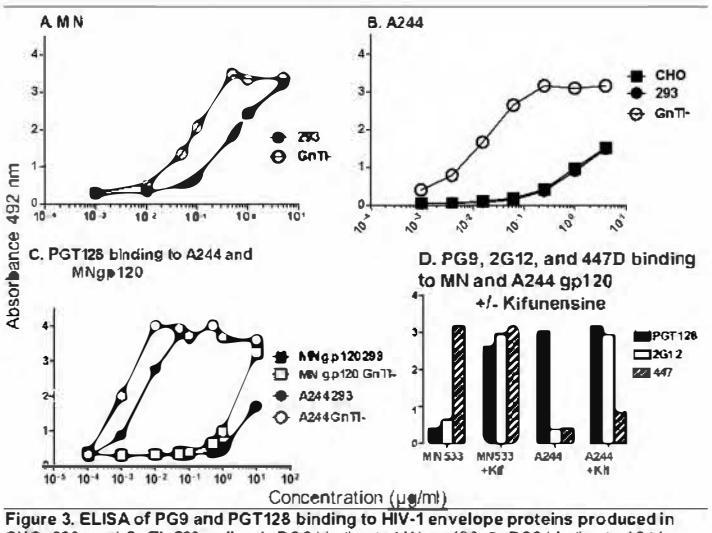


Figure 3. ELISA of PG9 and PGT128 binding to HIV-1 envelope proteins produced in CHO, 293, and GnTI- 293 cells. A, PG9 binding to MN-rgp120; B, PG9 binding to A244-rgp120. C, PGT128 binding to A244 and MN–rgp120 MN; D. PGT128 , 2G12, and 447D MAb binding to MN and A244 gp120 produced in normal and kifunensine treated(+Kif) 293 cells.

To explore the binding of PGT128, we compared its binding to MN- and A244-rgp120s in normal 293 cells and GnTI- 293 cells (Fig 3C). We found little or no binding of PGT128 to MN-rgp120 produced in normal 293 cells or GnTI- 293 cells. Surprisingly, A244-rgp120 produced in both cell lines exhibited high affinity binding to PGT128 with EC_{50}s of 0.005 and 0.001 ug/mL, respectively. In pilot studies, we then expressed both proteins in 293 cells treated with kifunensine, a glycosylation inhibitor that limits N-linked glycans to mannose-9 structures. Although we observed little improvement in the binding of kifunensine-produced A244-rgp120, kifunensine treatment improved the binding to MN-rgp120. We found that kifunensine treatment enhanced the binding of another glycan-dependent mAb (2G12), but had no effect on the binding of the 447D mAb that recognizes the PND of the clade B, MN-rgp120 but not the PND of the A244-rgp120 (Fig 3D). ***This experiment demonstrated that: 1) PGT128 could bind to monomeric gp120s provided that the proper sequence and glycosylation were present; 2) trimeric forms of the Env protein were not required for binding; and 3) the ability to elicit bNAbs to both MN-rgp120 and A244-rgp120 could be improved by incorporating the glycans required for binding of bNAbs.***

D4. Development of V1/V2 scaffolds that bind glycan-dependent bN-mAbs. Although these studies showed that we could improve the PG9 and PGT128 binding activity of MN and A244 gp120, there is a major difference between immunogenicity and antigenicity. ***Thus, while incorporation of the glycans required for the binding of PG9 and PGT128 improves the antigenic structure of gp120, there is no guarantee that this will improve the immunogenicity of these epitopes.*** This would explain why A244-rgp120 did not elicit bNAbs in the RV144 trial, despite the fact that it possessed the PGT128 epitope. The antibody response to gp120 is complex and only a small percentage of the overall antibody response to any Env protein is directed to epitopes that mediate virus neutralization [25]. Moreover, the epitopes recognized by bNAbs appear to be immunorecessive, and the virus may have evolved other immunodominant "decoy" epitopes to divert the immune response from epitopes targeted by neutralizing antibodies. Therefore we reasoned that additional strategies would likely be required to target antibody responses to the PG9 and PGT128 GDEs in the V1/V2 and V3 domains. One way this might be accomplished would be through the use of gp120 fragments (scaffolds), possessing the proper conformation and glycan structures required for the PG9 and PGT128 binding. We could then combine these with the gp120s to create a multivalent vaccine cocktail.

To determine if we could produce scaffolds able to bind PG9-like antibodies, we expressed a series of fragments containing the V1/V2 domain from a diverse collection of viruses. These fragments were expressed as fusion proteins with an amino-terminal flag epitope of HSV glycoprotein D (gD) as described previously [11]. When we tested the fragments produced in normal 293 cells, most (e.g., MN, 108060, TRO.11, REJO, SC422, WITO and JRFL E168K) exhibited little or no binding to PG9 (data not shown). However the scaffolds from several viruses such as A244, Bal.01, ZM197, and clinical isolate UCSC1015 bound PG9 with high affinity (Fig 4A-D). The first V1/V2 scaffold that we studied in depth was that of A244 gp120 (Fig 4A). We found that a 97 amino acid fragment of A244-rgp120, that encompassed the entire V1/V2 domain and possessed two disulfide bonds, exhibited weak binding (0.2 mg/mL) to PG9 when expressed in normal 293 cells and high affinity binding (0.01 ug/mL)when expressed in GnTI- 293 cells (Fig 4B). To further investigate the properties of this fragment, which contained 9 potential N-linked glycosylation sites (PNGS), we measured binding to three additional PG9-like antibodies: CH01, CH03, and PG16 [1, 7, 9]. We found that CH01 and CH03 also bound well to the A244-V1/V2 scaffold when expressed in GnTI- cells but not 293 cells. The PG16 also bound weakly to the scaffold produced in GnTI- cells (data not shown). ***The discovery that a V1/V2 scaffold from A244 could bind PG9-like antibodies is significant for three reasons: 1) it provides an approach to enhance the magnitude of the antibody response that correlated with protection in the RV144 trial, 2) it shows that A244 has the potential to elicit PG9-like antibodies, and 3) it indicates that A244 should be especially immunogenic in humans, since it is one of the few envelope proteins known to bind directly to the inferred germline precursor of the PG9 antibody [7].***

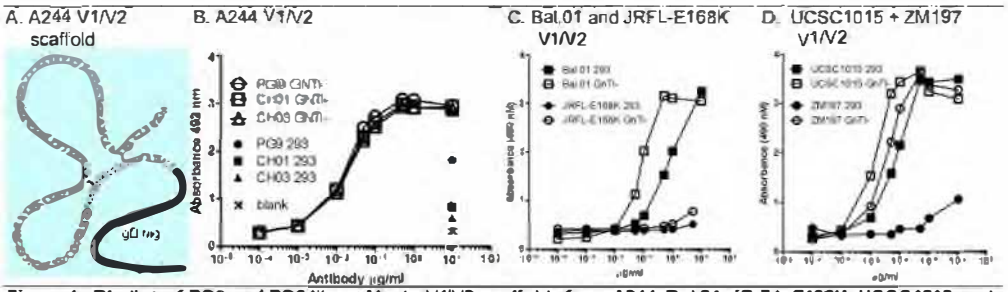
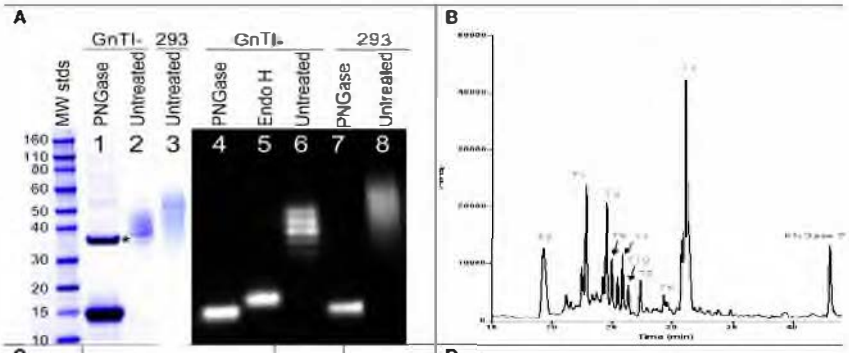


Figure 4. Binding of PG9 and PG9-like mAbs to V1/V2 scaffolds from A244, Bal.01, JR-FL E168K, UCSC1015, and ZM197 rgp120 expressed in normal and GnTI- 293 cells. A. diagram of A244-V1/V2 scaffold expressed with a gD flag epitope and signal sequence. B, Binding of PG9-like MAbs (PG9, PG16, CH01, CH03, and PGT145) binding to the A244 V1/V2 scaffold. C. PG9 binding to clade B Bal.01 and JRFL-E168K V1/V2 scaffolds; D. PG9 binding to clade C ZM109 and UCSC1015 V1/V2 scaffolds.

D5. Characterization of the structure and biochemical properties of the A244 V1/V2 scaffolds. We first examined the size of the scaffolds produced in normal and GnTI- 293 cells by PAGE (Fig 5A). We found that the V1/V2 scaffold produced in 293 cells ran as a diffuse smear of 38-55 kDa. However, the A244-V1/V2 scaffold produced in GnTI- cells ran as 3 discrete bands of approximately 38-42kDa. One explanation was that these bands represented proteolyzed forms of the scaffold. Another possibility was they represented differentially glycosylated forms of the scaffold. To distinguish between these possibilities, we treated the A244-V1/V2 scaffold with either PNGase to remove all of the N-linked carbohydrate, or with endoglycosidase H (Endo-H) to remove the high mannose carbohydrate. Treatment with PNGase resulted in a single band of 14 kDa, whereas treatment with Endo-H resulted in a single band of approximately 17 kDa. ***These results demonstrated that the scaffold was highly glycosylated and that the three bands were due to differences in glycosylation.*** The large difference in size of the glycoprotein scaffold before and after treatment with



V1/V2 peptide	Glycopeptides	PNGS	PNGS Utilized
T4	LTPPCVTLHCTNANLTK	1	1
T5	ANLTNVNNR	2	0, 1, and 2
T6	TNVSNIIGNITDEVR	2	1 and 2
T7	NCSFNMTTELR	2	2
T9	LDIVPIEDNDSSEYR	1	0 and 1
T10	LINCNTSVIK	1	1

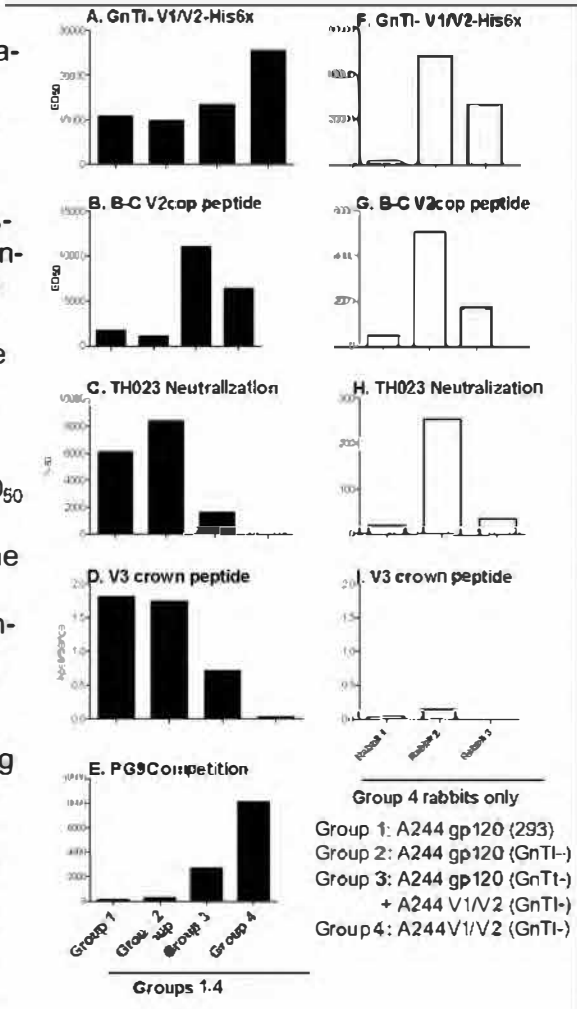
Figure 5. Characterization of the A244 V1/V2 scaffold produced in GnTI- cells. (A) Purified A244-V1/V2 scaffold analyzed using 4-12% reducing SDS-PAGE gels. Lanes 1 and 2, purified scaffolds before and after treatment with PNGase F and visualized with Coomassie staining. The location of the PNGase enzyme is indicated by an asterisk. Lanes 3 and 8, scaffolds produced in normal 293F cells. Lanes 4-8, immunoblots where scaffolds were detected with a mAb to the gD flag epitope. Lanes 4-5, A244-V1/V2 fragment expressed in GnTI- cells after treatment with PNGase and Endo H. Lane 6, untreated A244 V1/V2 scaffold produced in GnTI- cells; Lane 7, V1/V2 scaffold expressed in 293F cells treated with PNGase F. (B) Mass spectroscopy analysis of V1/V2 peptides resulting from the treatment of the V1/V2 scaffold with trypsin and PNGase F. Site occupancy (PNGS utilized) was determined by the change in mass of asparagine to aspartate. (C), table of the glycopeptides generated by trypsin digestion of the V1/V2 scaffold and the location of potential glycosylation sites (red N). Peptides T5, T6, and T9 showed variable incorporation of glycans whereas T7, possessing the glycans at positions 156 and 160 required for PG9 binding, were fully occupied. The linear sequence of the peptides included in this panel is provided in Figure 2. (D) Circular dichroism of purified A244-V1/V2 scaffold before (blue solid line) and after (red dotted line) denaturation by reduction and carboxylmethylation.

PNGase and Endo H is explained by the fact that 9 PNGS occur within the 97 amino acid A244 V1/V2 fragment. To determine if the difference in glycosylation might affect the binding of PG9, we carried out mass spectroscopy studies (Fig 5B and 5C). Our results demonstrated that the T5, T6, and T9 peptides containing PNGS at 140 and 140e; 142h and 149; and 188, respectively (Fig 2) were subject to differential glycosylation with 0, 1, or 2 glycans being incorporated. However, the T7 peptide possessed both the PNGS required for PG9 binding, with both glycans occupied. Because the V1/V2 domain is a 4-stranded anti-parallel β-sheet, we reasoned that we should be able to monitor its conformation spectroscopically. For this purpose we carried out circular dichroism studies (Fig 5D). ***We observed an absorbance pattern at 218 nm clearly indicating that the A244 V1/V2 scaffold produced in GnTI- cells possessed the β-sheet structure reported as necessary for PG9 binding to other scaffolds [9] and for the gp140 trimer [10].*** When we examined the A244 V1/V2 scaffold that had been reduced and carboxymethylated to destroy the tertiary structure, the absorbance pattern changed to that characteristic of a random coil [26]. Thus circular dichroism provides a convenient method to verify that the β-sheet structure is preserved in V1/V2 scaffolds and in variants of the V1/V2 domain possessing altered glycan structures.

D6. Preliminary immunogenicity studies. A pilot immunogenicity study was carried out in rabbits to investigate the immunogenicity of A244 gp120 and the A244 V1/V2 scaffold produced in GnTI- 293 cells. Because of funding constraints, these studies were of smaller size (3 rabbits/group) and shorter duration (5 months) than we would have liked. However, they allowed us to answer several important questions with respect to the immunogenicity of gp120 and V1/V2 domain scaffolds produced in GnTI- cells. The overall study consisted of 4 groups treated with different immunogens: Group 1, A244-rgp120 produced in 293 cells; Group 2, A244-rgp120 produced in GnTI- cells; Group 3, a priming immunization with A244-rgp120 produced in GnTI- cells and booster immunizations with the A244 V1/V2 scaffold produced in GnTI-293 cells; and Group 4, the A244 V1/V2 scaffold produced in GnTI- cells. The animals received 5 immunizations given at months 0, 1, 2, 3, and 4, with blood taken 2 weeks after each immunization as well as 4 weeks after the last immunization (month 5). Sera from each group were then pooled and analyzed by ELISA and TZM-bl neutralization assays to profile the antibody responses. Results obtained with sera collected after the 5th immunization are shown in Fig 6.

We first examined antibody titers to the V1/V2 scaffold elicited by either gp120s alone, the scaffold alone, or by the prime/boost protocol. To avoid measuring antibodies to the highly immunogenic gD flag epitope, our assays made use of an A244 V1/V2 scaffold in which the gD flag was replaced by a hexa-histidine tag. Pre-immune rabbit sera served as the negative control. It can be seen that the animals in all 4 groups developed high titers to the V1/V2 scaffold, with ED_{50} titers of 1:10,000 for the gp120s and 1:30,000 for the animals that received the V1/V2 scaffold (Fig 6A). These studies suggested that the V1/V2 domain is highly immunogenic either within the context of gp120 or as an isolated fragment.

We then examined the antibody response to a synthetic peptide possessing the V2cop epitope in the B-C junction. This region is significant for several reasons. First, it possesses the amino acid side chains, including lysine (K) at position 168, 169, and 171, required for the binding of PG9 and PG9-like antibodies [15]. Second, antibodies to this region correlated with protection in the RV144



Group 1: A244 gp120 (293)
Group 2: A244 gp120 (GnTI-)
Group 3: A244 gp120 (GnTI-) + A244 V1/V2 (GnTI-)
Group 4: A244V1/V2 (GnTI-)

Figure 6. Immunogenicity of gp120s and V1/V2 scaffolds. Rabbits were immunized with A244-rgp120 produced in 293 cells (Group 1), A244-rgp120 produced in GnTI- cells (Group 2), or the A244-V1/V2 scaffold produced in GnTI- cells (Group 4). A prime/boost protocol was used in Group 3, where rabbits received one injection of A244-rgp120 and 4 injections of the V1/V2 scaffold, both produced in GnTI- cells. Data obtained with pooled sera from each group is show in A-E. Panels F-I represent sera from the three individual rabbits in Group 4. 50% Endpoint dilution titers (ED_{50}) were determined by standard ELISA for antibody binding to the A244V1/V2 scaffold (panel A and F); the B-C (V2cop) peptide (NMTTELRDKKQKVHA) from the V1/V2 domain (panels B and G), and the V3 crown peptide (SITIGPGQVFYR) from A244 gp120 (panels D and I). TZM-bl neutralization titers were carried out using pseudoviruses from the TH023 strain of HIV-1 (panels C and H). A competitive binding ELISA was used to measure the dilution of rabbit sera required to prevent the binding of a fixed amount of PG9 mAb to the A244-V1/V2 scaffold produced in GnTI- 293 cells. Pre-immune rabbit sera served as a negative control in all experiments.

trial [16, 27]. Finally, it contains K169 that has been shown by sieve analysis to be a key determinant of protection [28]. The sequence of this V2cop peptide, NMTTELRDKKQKVHA (positions 160-174), was designed to replicate the hairpin structure at the B-C junction. Thus this peptide included sequences on both sides of the hairpin turn flanking the B-C junction. When we measured the antibody titers to the V2cop peptide in rabbits immunized with full length gp120 (Fig 6B), we found that they were at least 3-fold higher (~1:1000) than those reported for the participants in HIV vaccine trials, where immunization with recombinant gp120 or HIV infection rarely elicited titers above 1:300 [5, 21, 29]. However, when we measured the titers obtained by immunization with the V1/V2 scaffold (groups 3 and 4), we found that the antibody titers to the V2cop peptide were approximately 30-fold higher (exceeding 1:10,000 in Group 3) than those elicited by immunization with gp120 alone. ***Thus, immunization with the A244 V1/V2 scaffold significantly improved antibody response to the region of gp120 reported to correlate with protection from HIV-1 infection. Additionally, we learned that a prime/boost immunization regimen was the most effective way to improve the antibody response to the V2cop epitope.***

We next measured the neutralizing antibodies in the TZM-bl assay. We screened pooled sera from groups of immunized rabbits against representative tier 1 and tier 2 viruses. These included TH023, MN533, JR-FL, and SF62. We observed robust neutralizing activity against the CRF01_AE clade, TH023 strain of HIV-1 (Fig 6C). We observed high titers of neutralizing antibodies (1:6000 to 1:8000) in groups 1 and 2 that received the full length gp120 immunogens, modest titers of 1:1000 in sera from Group 3 that received the prime/boost regimen, and low titers (1:96) in Group 4 that received the V/V2 scaffold alone. The higher titers in the animals that received gp120 were not unexpected, since full length gp120 possesses epitopes in the V3 domain and the CD4 binding site known to be recognized by neutralizing antibodies, not present in the V1/V2 scaffold. Because tier 1 viruses are particularly sensitive to antibodies directed to the V3 domain [30-33], we next examined the antibody titers to a synthetic peptide corresponding to the principal neutralizing determinant at the crown of the V3 domain (Fig 6D). The sequence of this peptide is identical in both the A244 and TH023 strains of HIV-1. We found that the antibody titers to the V3 peptide closely paralleled the neutralizing antibody response in the gp120 immunized animals, suggesting that most of the neutralizing activity was directed to the V3 domain. The presence of V3 antibodies in Group 3 was somewhat surprising, since these animals only received full length gp120 on day 0 in the primary immunization and received the V1/V2 scaffold alone for the four additional immunizations. Subsequent time course studies (data not shown) demonstrated that the V3 response in rabbits is far more sustained than in humans [22]. As expected, there was no significant antibody response to the V3 domain in Group 4 since these animals only received the V1/V2 immunogen. While the majority of the neutralizing response in Groups 1-3 was likely to be due to V3 antibodies, the low (e.g. 1:96) but significant level of neutralizing antibodies in group 4 could only be due to antibodies to the V1/V2 domain.

We next wanted to determine whether antibodies to the V1/V2 scaffold were directed to the epitopes in close proximity to the PG9 epitope. To answer this question, competitive binding studies were carried out using an ELISA format. In these studies we measured the ability of rabbit antibodies to inhibit the binding of PG9 to the A244 V1/V2 scaffold (produced in GnTI- cells) and captured onto wells of microtiter plates. The results of this study are shown in Fig 6E. ***We found that all four groups of animals produced antibodies able to prevent the binding of PG9 to the A244 V1/V2 scaffold; however, the group that received the scaffold alone had PG9-blocking antibody titers at least 20-fold higher than animals that were immunized with gp120 alone.*** In this case immunization with the V1/V2 scaffold alone, rather than a prime/boost regimen, appeared to be the most effective way of eliciting antibodies with this specificity. Thus it appears that immunization with the V1/V2 scaffolds elicited antibodies to the PG9 epitope, but these antibodies lacked the broadly neutralizing activity characteristic of PG9.

To better understand the neutralizing antibody response in Group 4, we measured the antibody response in each animal individually. When we examined the antibody responses to the V1/V2 scaffold (Fig 6F), we found that only rabbits 2 and 3 developed robust titers. Thus rabbit 1 appeared to be a non-responder, and the scaffold was immunogenic in only two of the three animals. When we examined antibody titers to the V2cop peptide, we found considerable differences among all three animals (Fig 6G). We found that the response in rabbit 1 was barely detectable, whereas rabbit 2 developed titers greater than 1:5000. Rabbit 3 had a modest antibody response of approximately 1:2000. When we examined the titers of neutralizing antibodies in these 3 rabbits (Fig 6H), we found that rabbit 2 had the highest titers (1:304), rabbit 3 had intermediate titers (1:60), and rabbit 1 had no detectable neutralizing response. ***Thus, there was an excellent correlation (R^2=0.9922) between neutralizing antibodies and antibodies to the V2cop epitope.***

Another finding from this study was that neutralizing antibodies to the V1/V2 scaffold only occurred after a 5-month immunization regime, but these did not appear to neutralize tier 2 viruses. ***However, the titers in the***

rabbits had not plateaued by the time the study terminated and further boosting might result in higher levels of neutralizing antibodies. The immune responses that we have observed in rabbits may be similar to those reported for humans, where bNAbs to the V1/V2 domain are not observed until at least 1 year post infection [34, 35]. Clearly, longer studies with higher doses of scaffold, and larger numbers of animals per group will be required to define the potential of these scaffolds to elicit bNAbs.

D7. Production of gp120s and scaffolds in normal cells. Because scale-up production of immunogens for clinical trials in either GnTI- 293 cells, or cells treated with glycosylation inhibitors (e.g. kifunensine) is impractical, we wanted to determine whether gp120s and scaffolds could be produced in cell lines (e.g. 293 or CHO DG44) acceptable for biopharmaceutical production. The observation that A244 gp120 exhibited strong PGT128 and modest PG9 binding activity when expressed in normal 293 cells was intriguing (Fig 3C). We wondered whether MN-rgp120 could be mutagenized to improve both PG9 and PGT128 binding when expressed in normal cells. To explore this possibility, we compared the location of PNGS in MN- and A244-rgp120 (Fig 7A). Notably, MN-rgp120 lacked PNGS at positions 301 and 332 in the V3 stem known to be important for PGT128 binding. Therefore we mutagenized the MNgp120 gene to replicate the pattern of glycosylation found in A244-rgp120 (Fig 7A). We initially screened the 5 constructs (UCSC467-471) for PG9 binding (Fig 7B). Remarkably, we found that addition of PNGS sites at positions 289, 301, and either 332 or 334 (UCSC467, 468, and 469) all resulted in proteins able to bind PG9 when expressed in normal 293 cells (Fig 7B). Because UCSC468 consistently exhibited high binding affinity, it was selected for further studies. We next compared PG9 binding to MN and UCSC468 gp120 produced in 293, GnTI- 293, and CHO cells (Fig 7C). We found that UCSC468 produced in normal 293 cells exhibited binding to PG9 that was approximately 5-fold better (EC_{50} =0.08 ug/mL) than binding to MN-rgp120 produced in GnTI- 293 cells. We also found that the UCSC468 produced in CHO cells bound PGT9, albeit with significantly lower affinity (EC_{50}=0.4 µg/mL) compared to MN-rgp120 produced in GnTI- 293 cells. When we examined PGT128 binding to UCSC468 and MN-rgp120 (Fig 7D), we found that UCSC468 expressed in normal 293 cells bound PGT128 with high affinity (EC_{50}=0.001 µg/mL). When we examined PGT128 binding to UCSC468 produced in CHO DG44 cells, we also observed high affinity binding with an EC_{50} of 0.005 ug/mL. ***Thus, UCSC468 has a remarkable ability to bind PGT128 with high affinity.***

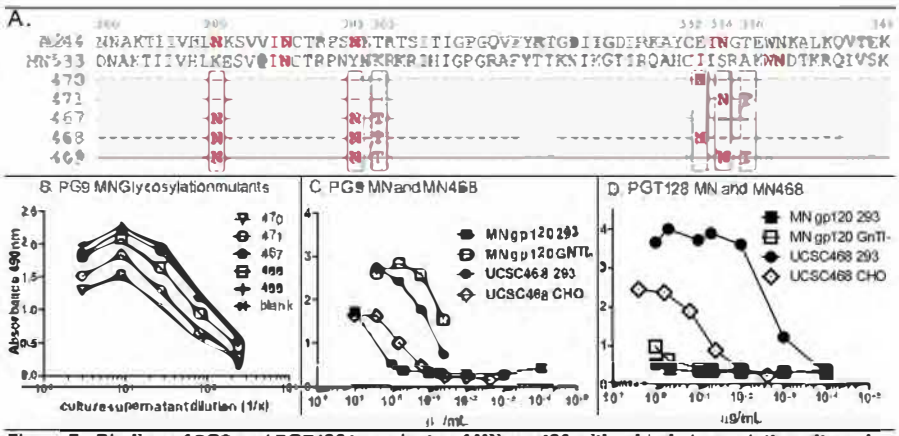


Figure 7. Binding of PG9 and PGT128 to variants of MN-rgp120 with added glycosylation sites. A, comparison of PNGS locations in the V3 domain of MN and A244 gp120. B, binding of PG9 to PNGS variants of MN-rgp120. C, binding of PG9 to MN-rgp120 and UCSC468 gp120 expressed in 293, GnTI- 293 and CHO (DG44) cells. D, Binding of PGT128 to MN and UCSC468 gp120 expressed in the same cells as panel C. Data for panel B was acquired using cell culture supernatants where the gp120 concentration was normalized after quantitation using a mAb to the gD flag epitope. Data in panels C and D was obtained by ELISA using purified proteins.

There appeared to be a consistent difference in UCSC468 binding by PG9 between human 293 and CHO DG44 cells that appears to reflect differences in the glycan structures incorporated in transient transfection experiments. However, these results were encouraging and suggest that we might be able to improve the binding of PG9 to UCSC468 produced in CHO cells. Previously we produced stable CHO cell lines expressing multiple gp120s for clinical trials (IIIB, MN, GNE8, and A244) [4, 5, 36-38]. In these studies, CHO cell lines possessing complex, sialic acid-containing glycans were the exception rather than the rule. Thus we discarded many clones with high mannose glycans because immunogens with complex carbohydrate were thought to maximize the *in vivo* half-life. It now appears that the selection for immunogens with complex glycans inadvertently selected for gp120s unable to bind PG9 and PGT128. Once we identify an immunogen that we want to advance into clinical trials, we believe we can screen for CHO clones that bind PG9 and PGT128 with the glycans required to bind these antibodies with high affinity.

Encouraged by these results, we explored the possibility that we might be able to produce V1/V2 scaffolds able to bind PG9 with high affinity when expressed in normal cells. For this purpose, we examined a number of the scaffolds that exhibited good PG9 binding when expressed in GnTI- cells. ***We found that the Bal.01 and UCSC1015 scaffolds were able to bind PG9 with high affinity when expressed in normal 293 cells (Fig 4C and 4D).*** Thus, our results suggest that both monomeric gp120s and V1/V2 scaffolds can be engineered to bind PG9 with high affinity when expressed in normal cell lines commonly used for the production of human biopharmaceutical products.

D8. Production of V3 scaffolds able to bind PGT128 antibodies. Based on the success in developing V1/V2 scaffolds able to bind to glycan-dependent PG9-like antibodies, we have begun efforts to produce scaffolds of the gp120 V3 domain able to bind PGT128 when produced with the appropriate glycosylation. For this purpose we screened V3 fragments from several isolates expressed in normal 293 cells and kifunensine-treated 293 cells. Although these studies are still at an early stage, we have identified two fragments of the 108060 clinical isolate [39] (UCSC959 and UCSC322) that were able to bind PGT128 when expressed in kifunensine-treated 293 cells (Fig 8A-C). The EC_{50} for PGT128 binding to the UCSC959 scaffold was 0.03 µg/mL. We are in the process of preparing V3 scaffolds from other strains of HIV that also exhibit high affinity binding to PGT128 when expressed in normal cells. We are also working with a crystallographer (Dr. Rebecca Dubois) and a protein structure prediction expert (Dr. Kevin Karplus), both in our department at UCSC, to better understand the structural features that define and stabilize the V1/V2 and V3 scaffolds in relation to neutralizing epitopes. Using these insights, we aim to develop a scaffold that redirects the immune response to the 6-stranded β-sheet structure that binds both PG9 and PGT128 within the gp140 trimer (Fig 1B).

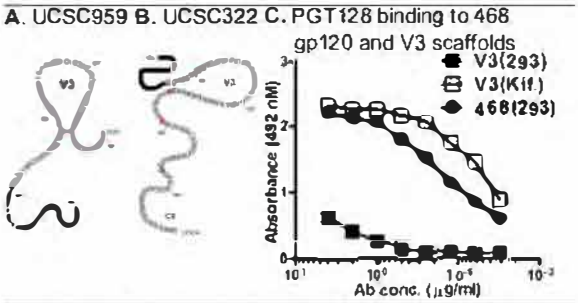


Figure 8. The structure and antibody binding V3 scaffolds from the 108060 isolate of HIV-1. Two V3 fragments were identified that bound PGT128 when expressed in kifunensine-treated 293 cells. (A) UCSC959 scaffold included a long sequence from the C2 domain and a short sequence from the C3 domain. (B) UCSC322 V3 scaffold included a short sequence from the C2 domain and a long sequence from the C3 domain. (C) PGT 128 binding to 468 gp120, expressed in normal 293 cells, and the UCSC959 scaffold expressed in normal and kifunensine (Kif) treated 293 cells.

E. Approach: Experimental Strategy/ Methodology.

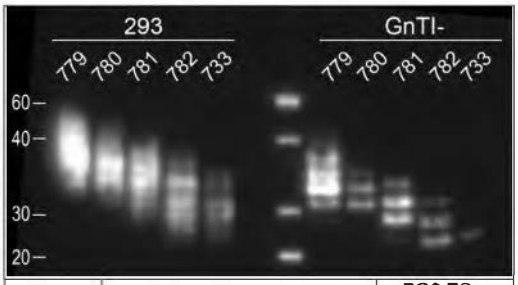
E1. Schedule of the specific tasks and timelines. A schedule of the major tasks and timelines required for this project is provided in Table 1. A schedule of the 12 novel immunogens that will be prepared over the 5 year course of this proposal is provided in Table 2. We plan to produce 10-20 mg of each protein and scaffold to support the long-term immunogenicity studies and immunoassays (Tables 3 and 4).

Table 1. Summary of Major Tasks	Year:	1	2	3	4	5
1	Cloning, expression and purification (10-20 mg) of gp120s and scaffolds able to bind PG9 and PGT128	X	X	X	X	
2	Repeat immunization with PG9-binding immunogens (MN and A244-rgp120s and A244 V1/V2 scaffolds) in more animals per group, higher dose, and longer immunization schedule	X	X			
3	Pilot immunization study with PGT128 binding gp120 (UCSC468), and 108060 V3 scaffold produced in kifunensine treated 293 cells	X	X			
4	Comparative immunization with gp120s from different clades and protease resistance mutations	X	X	X		
5	Comparative immunization with V1/V2 scaffolds from different clades, with protease resistance mutations, and deletions of glycosylation sites		X	X	X	
6	Comoarative immunization with V3 scaffolds from different clades		X	X		
7	Prime/boost immunizations with the best gp120s and scaffolds from comparative immunization studies			X	X	X
8	Biochemical characterization of scaffolds and gp120s used for immunization	X	X	X	X	X
9	ELISA assays to measure magnitude/specificity of antibody response to GDEs in rabbit, mouse, and human sera	X	X	X	X	X
10	Neutralization assays in rabbit and mouse sera	X	X	X	X	X

E2. Production of gp120s able to bind PG9 and PGT128. Now that we have identified monomeric gp120s and scaffolds that possess the epitopes recognized by PG9 and PGT128, we can for the first time determine how best to elicit antibodies to these GDEs. Because protein antigenicity doesn't predict immunogenicity, an empirical "Thomas Edison" approach involving comparative immunogenicity studies of gp120s with different sequences should increase our chances of identifying immunogens able to elicit PG9- and PGT128-like antibodies. Our baseline control for these studies will be the 50:50 mixture of MN- and A244-gp120 (AIDSVAX B/E), produced in CHO cells and used in the RV144 trial. This will allow us to determine whether our immunogens result in improved antibody responses relative to those that provided protection in the RV144 trial. The novel monomeric gp120s that will be used for immunization experiments will include A244-gp120 (CRF01_AE), and UCSC1015-gp120 (clade C), expressed in GnTI- cells. We will also evaluate the glycan-engineered variant of the clade B, MN-rgp120 (UCSC468) able to bind PG9 and PGT128 when expressed in normal 293 cells (described above). In addition we will examine the immunogenicity of a variant of UCSC468 that has been mutagenized to delete a highly conserved cathepsin D cleavage site in the V2 domain [6]. We have found that cleavage at this site destroys PG9 binding activity (data not shown). We plan to first examine the immunogenicity of these proteins alone to determine which has the strongest responses in the binding assays that measure antibodies likely to correlate with protection from HIV infection. These include virus neutralization assays, antibodies to the V2cop epitope, and antibodies to the PG9 and PGT128 epitopes measured by competition assays. The gp120 with the best profile of functionally significant epitopes will then be used in prime/boost regimens with the most promising V1/V2 scaffolds. If two or more proteins appear equally effective in eliciting antibodies to functionally significant antibodies, we will select the one that either expresses best or has fewest problems (e.g. proteolysis, aggregation) in purification. The proteins will all be produced by large-scale transient transfection and purified as described previously [6, 23]. The proteins used for immunization studies will all be monomers, free of higher order aggregates, and with no more than 5% proteolysis as determined by N-

terminal sequence analysis.

E3. Production of three types of V1/V2 scaffolds. In this proposal, we plan to analyze three different types of V1/V2 scaffolds. The first type will be scaffolds with wild-type sequences (A244, Bal.01, and UCSC1015) that bind PG9 with high affinity, but are derived from viruses that differ in sequence, number, and location of PNGS, and lengths of the V1 and/or V2 domains. As described above, the scaffold from A244-rgp120 required production in GnTI- cells in order to bind PG9, whereas the scaffolds from Bal.01 and UCSC1015 bind PG9 when expressed in normal 293 cells. The second type of scaffold will be engineered to enhance the stability of the V1/V2 domain to proteolysis. This scaffold is based on A244 gp120 and possesses a mutation in a highly conserved cathepsin D cleavage site (residues L179 to 180). Deletion of this highly conserved cathepsin D cleavage site, adjacent to the $\alpha 4\beta 7$ binding site in the V2 domain [23], has two distinct benefits. First is its enhanced protein stability throughout fermentation and purification. We have found that some V1/V2 scaffolds are particularly sensitive to proteolysis at this site; this results in conformational changes that prevent PG9 binding. Second, cathepsin D is a key enzyme responsible for antigen processing by the immune system. We have postulated that this site has been conserved in gp120 by positive selection as part of an immune escape mechanism that serves to minimize the humoral immune responses to this critical region of the HIV-1 envelope protein. Indeed, we recently observed that deletion of this site in gp120 by mutagenesis enhances both antibody and cell mediated immune responses (Morin, Berman et al., 2013 (in preparation); Frey, Berzofsky et al., 2013 (in preparation).



UCSC #	PNGS deletions (HXB2 numbering)	PG9 EC_{50} (μg/ml) 293	PG9 EC_{50} (μg/ml) GnTI-
779	N14 2e	0.13	0.0079
780	N14 N142e	0.13	0.0074
781	N140, N142e, N142h	0.13	0.008
782	N135, N140, N142e, N142h	0.13	0.008
733	N140, N142e, N142h, N135, N149	0.133	0.0079
792	N135, N140, N142, N142h, N149, N188,N197	0.14	0.147

Figure 9. Expression of V1/V2 glycosylation mutants in normal and GnTI- 293 cells. Upperpanel shows the migration of V1/V2 domain scaffolds with glycosylation site deletions on 4-12% PAGE gels following immunoblot detection. The mobility of molecular weight markers is shown on the left. The location of PNGSs is indicated with reference to HXB2 numbering (Rolland et al., 2012). The location of individual mutations within the entire A244 V1/V2 domain sequence is provided in Figure 2. The binding of the scaffolds produced in 293 or GnTI- to PG9 was determined measured by ELISA using cell culture supernatants where the scaffold concentration was normalized by quantitation using a rabbit polyclonal sera raised against the V1/V2 scaffold.

The third type of scaffold will be engineered to enhance antibody responses to the PG9 epitope as well as glycan independent epitopes in other regions of the V1/V2 domain. Because our primary goal is to target the immune response to the two glycans in the V1/V2 domain required for PG9 binding (N156 and N160), we plan to delete as many of the other PNGS as possible without affecting PG9 binding. In preliminary studies (Fig 9), we found that we could delete at least 5 glycosylation sites from the A244 V1/V2 scaffold (UCSC733) without impairing the binding of PG9. We are in the process of evaluating constructs that delete N197 (e.g., UCSC796). Besides the PG9 epitope, the V1/V2 domain appears to possess two glycan-independent epitopes recognized by neutralizing antibodies. One is located in the connecting peptide between the A and B strands (residues 135 to 142) of the V1 domain (O'Rourke and Berman, 2013, submitted) and the other is located in the C-terminus of the V2 domain (position 197) [40, 41]. These are epitopes that are destroyed, rather than created, by N-linked glycosylation. Thus scaffolds with deletions in glycosylation sites should enhance PG9 binding as well as the binding of antibodies to these two glycan-independent epitopes.

E4. Biochemical characterization of gp120s and scaffolds. Because gp120 possesses a large number of glycosylation sites and is subject to proteolysis, aggregation, and disulfide exchange reactions, the development of reproducible fermentation and purification processes is a formidable challenge. To obtain reproducible preparations of immunogens, many aspects of the fermentation and purification process need to be controlled. It is therefore essential to have biochemical and biophysical assays to verify the structure and stability of proteins used as immunogens. As described above, we have identified a variety of biochemical and biophysical assays to monitor protein structure and integrity. These include assays to measure glycan heterogeneity, proteolysis, aggregation, proteolysis, β-sheet structure, and mAb binding activity. We plan to use mass spectrometry to monitor both carbohydrate composition and disulfide structure of the V1/V2 scaffolds. For this purpose, we will rely on our collaborators Drs. Ewa Witkowski and Susan Fisher (UCSF) and Dr. Shantha Raju (Janssen Research & Development Center, Radnor, PA) to carry out these disulfide and glycan characterization studies.

E5. Immunogenicity studies to enhance neutralizing antibody responses to epitopes in the V1/V2 domain recognized by bNAbs. Based on our preliminary data described above, two different strategies appear to hold the greatest promise for eliciting protective antibodies to the V1/V2 and V3 domains. The first is the prime/boost strategy, which appears to enhance antibody binding to the V2cop epitope that correlated with protection in the RV144 trial (Fig 7B). The second approach is to immunize exclusively with the V1/V2 scaffolds

that appeared to enhance the immune response to the PG9 epitope (Fig 7E). We will compare the immunogenicity of gp120s and scaffolds that possess the PG9 and PGT128 epitopes to identify the scaffold that that gives the best antibody response. The criteria for the "best response" includes: 1) broad neutralization of tier 2 and 3 viruses, 2) the highest titers to the V2cop epitope, 3) competitive binding by the PG9 and PGT128 antibodies (e.g. Fig 7E), and 4) the magnitude of the antibody response to V1/V2 and V3 scaffolds.

We will then independently compare the immunogenicity of the V1/V2 scaffolds (identified in Table 2) as well as at least two new V3 scaffolds developed during the course of this project. In the final two years of the grant, we will evaluate the immunogens most effective in eliciting PG9- and PGT128-like antibodies in sequential immunization (prime/boost) studies and in multivalent vaccine cocktails to discover the most advantageous combination of components and the best immunization protocol to elicit PG9- and PGT128-like antibodies (Table 2). Because bNAbs in humans take a year or more to develop, we plan to use a longer than normal immunization regimen for antibody production. Rabbits and mice will receive at least 6 immunizations over a 1-year period at 0, 1, 2, 6, 9, and 12 months, with blood collected 2 weeks after each immunization. The protocol may be extended longer in rabbits if bNAb titers are still rising. Each immunogen will be tested in 6 rabbits and 6 mice. We will use a dose of 200 µg of each immunogen (gp120) for each rabbit and a 20 µg dose for each mouse injection. For rabbit studies we plan to formulate the immunogens in a lipid A adjuvant similar to that described by Alving et al. [42, 43] that has been shown to give comparable results in rabbits and humans. For mouse immunization, we plan to use a TLR9 directed CpG oligodeoxynucleotide adjuvant provided by Dr. Robert Coffman (DynaVax, Emeryville, CA) that is particularly effective in eliciting follicular T helper cells [44] and, in humans, has been shown to be particularly effective in sustaining the duration of antibody responses [45, 46]. This is a well known problem with HIV vaccines [22, 47, 48] that may be solved by this adjuvant. Unfortunately this adjuvant is not effective in rabbits or guinea pigs. The results from these animal studies will be evaluated using statistical methods selected by our project statistician, Dr. Grant Pogson (UCSC). To the extent possible, we will use assays recommended by Dr. Peter Gilbert at the Statistical Center for HIV/AIDS Research & Prevention (Seattle, WA). A detailed description of each experiment is provided in Table 2.

Table 2. Schedule of Immunogens and planned immunization experiments

Immunogens	Experiment	Immunogens (AM) for each experiment*					
gp120s		***group 1***	***group 2***	***group 3***	***group 4***	***group 5***	***group 6***
A AIDSVAX B/E	1	A/A	B/B	B/F	F/F	–	–
B A244 †	2	A/A	C/C	C/K	K/K	C / (F+K)	–
C UCSC468	3	A/A	B/B	C/C	D/D	E/E	–
D UCSC468ΔCatD	4	A/A	F/F	G/G	H/H	I/I	J/J
E UCSC1015	5	A/A	K/K	L/L	M/M	C/L	C/M
V1/V2 Scaffolds	6 §	gp120 / V1V2 scaffold	gp120 / V3 scaffold	gp120 + V1V2 scaffold	gp120 + V3 scaffold	gp120 / (V1V2+V3 scaffold)	(gp120+ V1V2+V3) / (gp120+ V1V2+V3)
F A244 †							
G A244ΔCalD †							
H UCSC1015							
I Bal							
J UCSC792 †							
V3 Scaffolds							
K 108060 V3‡							
L V3 TBD ‡							
M V1/V2/V3 ‡							

*Groups of 6 animals will be immunized by either a prime/boost protocol (/) or by repeated doses of single antigens or protein mixtures (+). †, indicates proteins produced in GnTI- cells. ‡, indicates proteins produced in kifunensine-treated cells. §, For experiment 6, the best gp120s, V1/V2 scaffold, and V3 scaffold selected from experiments 3, 4, and 5 will be used for immunization.

Experiment 1: Repeat the pilot A244 V1/V2 scaffold immunogenicity studies to see if higher doses of immunogen, given over a longer time, can elicit PG9-like antibodies. As described above, our original pilot study was terminated at a time when neutralization titers were still rising and had not plateaued. In addition, the rabbits were boosted with small doses of scaffold (10-33 µg). We think we might achieve better titers using larger doses of immunogens that were not available at the time. This study will also determine whether a prolonged immunization schedule is required to elicit PG9-like antibodies, as is the case in HIV-infected humans.

Experiment 2. A pilot immunogenicity study to determine if bNAbs can be elicited by immunization with gp120s and scaffolds possessing epitopes recognized by both the PG9 and PGT128 mAbs. We plan to carry out new pilot immunogenicity studies using the UCSC468 gp120 produced in 293 cells and the 108060 V3 scaffold produced in kifunensine-treated 293 cells (Table 2). Although we expect other V3 scaffolds to become available in later years of the grant, there is a possibility that the scaffold we have now might be effective in eliciting PGT128-like antibodies. An additional group will be added to the prime/boost studies such that booster injections will be given with a mixture of V3 and V1/V2 scaffolds as well as V3 scaffold alone.

Experiment 3. Comparative immunogenicity studies to identify the gp120s best able to elicit both PG9- and PGT128-like mAbs. We will collect data on the antibody responses to 4 different gp120 proteins that bind PG9 and PGT128 antibodies. These include A244-rgp120 produced in GnTI- cells, UCSC468, UCSC468ΔCatD, and UCSC1015 produced in 293 cells (Table 2). The UCSC468ΔCatD envelope protein possesses a mutation in the V2 domain that deletes the cathepsin D cleavage site that facilitates inactivation of the PG9 binding site *in vitro* and *in vivo* (described above). A fifth group will be immunized with the AIDS B/E vaccine containing a mixture of MN and A244-rgp120 formulated in alum adjuvant. This will serve as our baseline control group.

Experiment 4: Comparative immunogenicity studies to identify the V1/V2 scaffolds best able to elicit PG9-like antibodies. These will compare three variants of the A244 V1/V2 scaffold produced in GnTI- 293 cells as

well as scaffolds from the Bal.01 and UCSC1015 isolates produced in normal 293 cells for the ability to elicit PG9-like antibodies. The A244-derived scaffolds include the scaffold with wild-type sequence and 9 PNGS (UCSC588), the UCSC733 scaffold where 5 of 9 glycosylation sites in A244 V1/V2 domain have been deleted; and a scaffold (A244 V1/V2ΔcatD) where the conserved cathepsin D site in the V2 domain has been deleted.

Experiment 5. Comparative immunogenicity studies to identify the V3 scaffolds best able to elicit PGT128-like antibodies. We will collect data on the antibody responses to 3-4 different V3 scaffolds able to bind PGT128-like antibodies. Currently we have identified only one V3 scaffold, 108060, with the ability to bind PGT128 when expressed in kifunensine-treated 293 cells. However, we have identified a number of other envelope proteins from clade C viruses able to bind PGT128 when expressed in normal 293 cells without kifunensine, and we think that by screening V3 scaffolds from these envelopes we will be able to find ones that bind PGT128 with high affinity. Thus we will follow an approach that was successful in identifying V1/V2 scaffolds able to bind PG9. We also plan to test a scaffold engineered to possess the sequences from both the V1/V2 and V3 domains. We anticipate creating this by fusion of the V1/V2 scaffold best able to bind PG9 with the scaffold best able to bind PGT128 with connecting sequences selected by our structural consultants.

Experiment 6. Optimization of the immunization regimen to elicit PG9- and PGT128-like antibodies. In these studies we will evaluate the antibody responses of the best gp120s and scaffolds identified in Experiments 3, 4, and 5 above. We will compare a prime/boost strategy whereby animals are primed with the best gp120s and then boosted with a mixture of the best V1/V2 and V3 scaffolds given alone or in combination. We will also carry out experiments to see if a cocktail consisting one gp120, one V1/V2 scaffold, and one V3 scaffold is more effective than the prime/boost approach or the immunization with individual proteins or scaffolds described in Experiments 3, 4, and 5 above.

E6. Measurement of antibodies to gp120 and the V1/V2 domain. We will use an ELISA format to measure the antibody responses elicited by our vaccine immunogens. These will include assays described previously [21-23, 49], as well as a number of new assays specific for the glycan-dependent immune responses in the V1/V2 domain (Table 3). These include assays to directly measure antibody binding to gp120s, V1/V2 scaffolds, and peptides from the V2 and V3 domains (Figs 3, 4, 6, 7, and 8). To dissect the antibody response to GDEs, we will compare antibody binding to fully glycosylated V1/V2 scaffolds with scaffolds where glycosylation sites have been selectively deleted (Figs 2, 9).

Table 3. ELISA assays to measure antibodies to gp120 and epitopes in the V1/V2 domain in rabbit and mouse sera

gp120 (A244, MN468, UCSC1015)
V1/V2 scaffolds (A244, Bal.01, UCSC1015)
V1/V2 scaffolds with protease cleavage site and glycosylation site mutations
PNGase treated V1/V2 scaffolds
V3 scaffolds
V2 peptides (V2cop, A-B and C-D junctions)
V3 peptide (V3 crown)
Competitive binding with PG9 and PGT128
Examples of these assays are provided in Figs 3, 4, 6, 7, and 8.

To measure antibodies to the PG9 epitope, we will carry out antibody depletion studies. These studies will make use of the A244 V1/V2 scaffolds described in Figs 2 and 9. Scaffolds of this type will then be used in one of two ways. First, they will be biotinylated, and then bound to streptavidin-magnetic beads (Dynal-M280 Streptavidin, Invitrogen) and used to deplete sera of antibodies to all epitopes in the V1/V2 domain except those dependent on glycans at positions 156 and 160. The binding of the adsorbed sera to native A244 V1/V2 scaffold or gp120 will then provide a measure of the antibodies specific for positions 156 and 160. An alternative strategy to measure antibodies to GDEs, dependent on glycans at positions 156 and 160, would be to measure the binding of sera to normal V1/V2 scaffolds after preincubation of sera with a large molar excess of the UC731 mutant scaffolds that lacks glycosylation at positions 156 and 160. Methods similar to this have been described previously [19, 50]. An analogous strategy will be used to measure glycan-dependent antibody responses to the V3 scaffolds. In these studies we will make use of scaffolds where the glycosylation sites at positions 332 and 301 have been deleted.

We also plan to use this serum depletion strategy to measure antibody responses to other GDEs in the V1/V2 domain. To measure antibody responses to GDEs in the V1 domain, we plan to use the UC733 scaffold that lacks 5 PNGS between positions 131 and 150 (Fig 2). To measure the antibody response to conformation-independent epitopes, we will use a reduced and carboxymethylated V1/V2 scaffold as described in Fig 5D. Finally, the availability of V1/V2 and V3 scaffolds from other strains and clades that differ in primary sequence provide additional reagents that can be used to detect the broadly cross-reactive antibody responses to GDEs in the V1/V2 domain that we hope to elicit.

E7. Virus neutralization assays. Our ability to elicit bNAbs is perhaps the most important criteria of success in this proposal. For this purpose, the antisera produced in this proposal will be tested in a two step approach. First, we will screen the sera in our laboratory in the standard TZM-bl assay [51]. Inclusion of tier 1 viruses neutralized by the existing AIDSVAX B/E vaccine such as MN, TH023, and DU965.26 will allow us to

determine if the immunogens or immunization regimens used in these studies are an improvement over the previous vaccines. All of the viruses have been grown and tested in our lab, and should prove useful for initial screening. If we detect antibodies that neutralize the tier 2 or 3 viruses in this panel (Table 4), we will then collaborate with David Montefiori (Duke University) to test them against a more extensive virus panel such as those described previously [17, 19].

To measure antibodies to the PG9 and PGT128 epitopes, we will make use of pairs of viruses that differ by the presence or absence of glycans required for the binding of these antibodies. For example the JRCSF is resistant to neutralization by PG9, whereas JRCSF_N160K is sensitive to neutralization by this antibody. Similarly, JRCSF is sensitive to PGT128, whereas JRCSF_N332A is resistant to neutralization by this antibody. Because there are few viruses that are resistant to neutralization by PGT128, we may need to construct envelope proteins for pseudovirus production where the glycosylation sites at 332 and 301 are deleted by *in vitro* mutagenesis. However, the identification of any immunogen that consistently elicits bNAbs against tier 2 and 3 viruses would represent a major advance in HIV vaccine research.

Table 4. Properties of viruses used at UCSC to detect PG9- and PGT128-like bNAbs in rabbit and mouse serum

Virus	Clade	Tier	PG9	PGT128
TH023	AE	1	+	ND
MN-3	B	1	-	ND
MW965.26	C	1	+	ND
JRCSF	B	2	+	+
JRCSF_N160K	B	2	-	+
JRCSF_N332A	B	2	+	-
113039	AE	2	+	ND
113039_K168E	AE	2	-	ND
Du422.1	C	2	+	ND
Du422.1_N160K	C	2	-	ND
PV04	B	3	+	+

(+) indicates neutralization sensitive, (-) indicates neutralization resistant, ND indicates not done.

E8. Things that could go wrong and what we will learn in the event we are unable to achieve our primary objective. PG9 and PGT128 possess some of the longest CDRH3 domains (28 AA) ever observed in humans [52, 53]. Long CDRH3 domains such as those in PG9 and PGT128 are rare in all species, found only at the extreme trailing end of the CDRH3 length distribution curves www.bioinf.org.uk/abysis/sequences). Thus the problem of eliciting antibodies with long CDRH3 domain represents a conundrum for the entire HIV vaccine field and may ultimately require immunization studies in humans to determine the ability to elicit such responses by vaccination. However, we plan to collaborate with investigators (e.g., L. Stamatatos, Seattle Biomedical Research Institute) who are in the process of constructing B-cell lines expressing germline genes encoding the PG9 and PGT128 mAbs [54].

In the event none of the immunogens tested elicit PG9-like antibodies, these studies will advance HIV vaccine research and our understanding of the immune system in a variety of ways. First, we will gain considerable knowledge regarding the capacity to elicit antibodies to GDEs. While vaccinologists have considerable experience in eliciting antibodies to bacterial polysaccharides, antibody responses to hybrid epitopes consisting of amino acids and glycans common to every cell in the body (e.g., PG9 and PGT128 epitopes) are unusual. Through the use of comparative ELISA assays with glycosylated and deglycosylated scaffolds, we will expand our knowledge of the magnitude, frequency, kinetics, and specificity of antibodies to GDEs. Second, since many virus and parasite proteins are highly glycosylated, bNAbs against GDEs represent a new and potentially important type of antibody response. Understanding the factors that favor the formation of these antibodies is a significant contribution to basic immunology that might find utility in the development of vaccines to other pathogens. Finally, the proteins and scaffolds developed in this proposal can be combined with other vaccine concepts such as DNA immunization, viral vectors, mosaic Env genes or gp140 trimers in order to develop vaccines able to stimulate both antibody and cell mediated immune responses.

E9. Previous studies with V1/V2 scaffolds. Fragments of the gp120 V1/V2 domain have previously been considered as potential vaccine immunogens [55-57]; however their activity was considered too strain specific. Recently, V1/V2 scaffolds able to bind PG9 have been produced for several purposes (e.g. structural studies [9] and epitope mapping [58, 59]); however, their activity as vaccine immunogens, particularly when expressed with the glycans required for PG9 binding has not been described. There are several reasons why the A244 scaffolds developed in our laboratory may be better immunogens than those described previously. First, the scaffolds previously produced for structural studies were heterogeneous, and an imaginative purification scheme involving purification of Fab-scaffold complexes was required to produce sufficient scaffold for crystallography studies [9, 60]. Second, A244-rgp120 was a key component of the AIDSVAX B/E vaccine in the RV144 trial [3] where protection correlated with non-neutralizing antibodies binding to the V2cop peptide [27]. Envs from other viruses may not elicit this binding activity. Third, the A244 protein appears to be unique in its ability to bind directly to inferred germline V_H region precursors of PG9-like antibodies [7]. Scaffolds from other viruses may not have this property. Fourth, some of the gp120s and scaffolds we have developed are able to bind PG9 with high affinity when expressed in cell lines (e.g. CHO DG44) suitable for vaccine production and manufacturing. In principle, these gp120s and scaffolds can be advanced into human clinical trials much faster than other scaffolds that have been described.

Vertebrate animals

The research in this proposal involves immunization studies in small animals (rabbits and mice). Immunization of rabbits will be carried out by a commercial vendor, while mouse immunizations will be performed at the UC Santa Cruz vivarium. Data for each institution is provided below.

A. Rabbits. Immunizations of rabbits will be carried out by a commercial vendor specializing in immunization services (Pocono Rabbit Farm & Laboratories, Canadensis, PA) as detailed below. PRF&L has a current USDA research license and a current Animal Welfare Assurance with NIH's Office of Laboratory as follows:

- AAALAC Accreditation number: 926
- USDA Research license 23-R-0141
- OLAW / NIH Assurance number A3886-01 (expiration date: 1/31/2017)

A1. Rabbit immunization studies: A maximum of 200 rabbits total will be required for the R01 proposal. Six rabbits will be immunized for each of six experimental groups in each of five years. These will be New Zealand white rabbits (NZW), approximately six months old.

A2. The use of mammals is essential in order to evaluate *in vivo* the novel immunogens created by modifications to gp120. Since we are interested in evaluating the humoral immune response and neutralizing antibodies in sera, rabbits are a good choice, since each will produce a sufficient volume of serum. This requirement rules out the option of small rodent species. NZW rabbits are known to respond to a wide range of antigens. The use of six rabbits per antigen is justified to compensate for variation in individual animal responses and to obtain the required amount of antisera necessary for this project.

A3. PRF&L is provided veterinary care by a consulting veterinarian who lives approximately one and half hours away from PRF&L. He reports to the General Manager of PRF&L. The payment for services rendered is based on a formalized arrangement of an annual contract. The contract specifies that the Attending and Consulting Veterinarian be on call every day 24 hours per day and visit PRF&L regularly at least one day per month for 8 to 10 hours. During the Veterinarian's visit the following business may be discussed or conducted: employee training, treatment and prescribed treatment for any and all animals, attendance at PRF&L Animal Care and Use Committee meetings, attendance at PRF&L Safety Committee meetings, facility design, regulatory concerns, necropsy, disease diagnosis, facility tours, and telephone/fax/written communication with research investigators. Dr. April endorses and follows the recommendations made in the most recent Report of the American College of Laboratory Animal Medicine (ACLAM) on Adequate Veterinary Care in Research, Testing and Teaching and the American Veterinary Medical Association (AVMA) Principles of Veterinary Ethics. The Institutional Official has given the Veterinarian the authority to execute the duties inherent in assuring the adequacy of veterinary care and overseeing other aspects of animal care and use to ensure that the program meets applicable standards by using sound professional veterinary judgment.

All animals at PRF&L are observed for signs of illness, injury, or abnormal behavior at least once daily by experienced, trained, qualified, full time, regular animal care personnel. All animal care personnel are extensively trained by PRF&L management staff and/or the Veterinarian to recognize animal illnesses, injuries, or abnormal behavior. When an animal is found ill, injured, or exhibiting abnormal behavior, the employee promptly places an identifying tag on the outside of the cage or pen that identifies the problem observed. The employee then promptly obtains the animal's Permanent Record/Health Status Card and records and initials the observations made of the animal. The card is then given to the Laboratory Manager for further instructions. A treatment is determined by the Laboratory Manager or he may consult with the General Manager. The General Manager may determine a treatment or a decision will be made to euthanize the animal or contact the Veterinarian for further instructions. All PRF&L personnel are required to make written observations of animals. A communication with the Veterinarian regarding ill, injured, or abnormal behavior of animals is written down on the animal's card. The Record of Contact sheet or a written letter or fax is also filled out when any PRF&L staff member has contact with the Veterinarian concerning an animal health problem. An extensive file of all communication between PRF&L and the Veterinarian is located in the Office and is made available to all PRF&L personnel.

For all non-routine cases, the Veterinarian is contacted and consulted. The animal care personnel upon finding an animal requiring veterinary medical care will promptly contact the Laboratory Manager, who in turn will promptly contact the General Manger. The General Manager will then promptly contact the Veterinarian by phone, pager, or fax, who is on call 24 hours a day. In the absence of the Laboratory Manager or General Manager, such as on weekends or holidays, personnel may contact the Veterinarian directly. In the case that the Veterinarian is not on call, he is responsible for appointing a suitable substitute. All communication with the Veterinarian regarding veterinary medical care prescribed for an animal is documented on the animal's Permanent Record/Health Status Card. A complete Record of Contact sheet or written letter or fax is also obtained from the Veterinarian regarding instructions for an animal's veterinary medical care. An extensive file of all communication with the Veterinarian is located in the Office and is made available to all PRF&L personnel.

In the case of a veterinary medical emergency or complication, PRF&L has a special arrangement with two local, on call 24 hours a day Veterinarians who can respond directly to PRF&L (Canadensis Veterinary Clinic, Canadensis, PA). All veterinary medical care treatments performed on an animal are recorded and initialed on the animal's Permanent Record/Health Status Card.

A4. Antigen injections are generally considered to be pain free, particularly with the use of adjuvants (such as alum) that have been approved for use in human vaccine products and clinical trials. However, if animals exhibit signs of pain or discomfort in response to the use of an adjuvant, the PRF&L veterinarian shall be consulted and will provide appropriate relief. The potential for pain or distress will be attenuated by using procedures known to minimize the adverse affects associated with FCA. By following IACUC policy and approved procedures, we expect no signs of pain or distress. The animals' pain or distress, if any, will be relieved according to the recommendation of the PRF&L veterinarian.

The PRF&LACUC (PRF&L Animal Care and Use Committee) has established guidelines for euthanizing animals in apparent pain and distress Guidelines for Euthanasia of Animals in Pain or Distress). The PRF&LACUC has also established Guidelines for Blood Collection, Guidelines for the Use of Freund's Adjuvant in Laboratory Animals, Injection Volume Guidelines by Species and Route, and pre-calculated Bleeding, Analgesia, Anesthesia, and Euthanasia Charts for all species. All of these guidelines are designed to assist personnel in avoiding unnecessary pain or distress in animals. PRF&L follows the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, which specifically addresses that it is imperative when using animals properly that the avoidance or minimization of discomfort, distress, and pain must be considered. It is PRF&L policy to prevent or alleviate pain associated with procedural protocols.

A5. All euthanasia methods at PRF&L used follow the guidelines established in 2007 by the American Veterinary Medical Association Panel on Euthanasia and have been prescribed by the Veterinarian. Euthanasia methods for Rabbits: When the Principal Investigator requests that a final terminal bleed be performed, the rabbit is first anesthetized with Ketamine and Xylazine and then exsanguination is performed by a cardiac puncture. When no serum is requested, the rabbit is euthanized by an intravenous injection of a commercial euthanasia solution (Euthasol ™) containing sodium pentobarbital and phenytoin sodium.

B. Mice: Housing and immunization of mice will be carried out at the UCSC campus vivarium, as detailed below.

B1. Proposed use of animals: We plan to utilize the immunization of mice to generate polyclonal and monoclonal antibodies against HIV virus envelope proteins, and to assess the immune response as potential immunogens for a vaccine that might one day be used in humans. Mice will be handled under standard protocols, subject to approval by the Chancellor's Animal Research Committee (CARC), which is UCSC's Institutional Animal Care and Use Committee (IACUC) that oversees and approves studies on mice and sets guidelines for sacrifice, anesthesia and other manipulations. The mice will be housed under the care of the UCSC Vivarium, an AAALAC-certified facility that is responsible for the management of laboratory animal resources at UCSC. This includes the daily care and maintenance of the animals and communication with the researchers to ensure that campus policies regarding animal use are followed. Standard mouse strains to be

used in this proposal include Balb/c or C57BL. All proposed experiments will be initiated with young female animals approximately 6 weeks of age (approximately 20 +/- 5 g). We estimate that a maximum of 200 mice will be used in this proposal with an average of 40 mice per year.

B2. Justification: Studies in mice have proved highly relevant in the development of vaccines and therapeutics to prevent and treat human diseases. By using mice for immunization studies, we will be able to compare the results obtained with new vaccine antigens to a tremendous amount of archival data and the immune response to candidate vaccines developed in the past. Because well established protocols exist for mice, but not other species (e.g. rabbits) the use of mice provides the opportunity to make monoclonal antibodies from animals that make neutralizing antibodies. These antibodies will allow us to determine the specific epitope on the HIV envelope proteins that are the targets of neutralizing antibodies. A great deal is known about the cellular, molecular and genetic aspects of mouse immunology. Studies in mice can be done efficiently at relatively low cost, given the potential benefits. We will make every effort to minimize the number of animals necessary for these studies.

B3. Veterinary care: Animals are cared for by the full-time veterinary staff of the UCSC vivarium. The colonies are monitored daily by a caretaker and experimental mice are checked regularly by laboratory staff.

B4. Procedures to alleviate pain or discomfort: Every effort will be made to ensure that the level of pain and discomfort in our mice is minimal. Mice will be anesthetized with inhaled isofluorane or with intraperitoneally injected avertin prior to invasive procedures. Ill appearing mice will be sacrificed. Sentinel cages are used routinely in the animal facility.

B5. Methods for euthanasia: Animals will be euthanized by inhalation of CO2 in accordance with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association.

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**Obstetrics, Gynecology &
Reproductive Sciences**

November 22, 2013

Phillip W. Berman, Ph.D.
Professor, Biomolecular Engineering, UCSC
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Dear Phil,

I am writing to express our enthusiastic support for your research proposal entitled: "Re-engineering gp120 to include glycan dependent epitopes". I enjoyed meeting with you and the members of your lab at UCSF last month and look forward to a productive collaboration.

As you know I serve as the Academic Director of the Sandler-Moore Mass Spectrometry (SMMS) core facility at UCSF that makes its services available to all campus investigators as well as to members of the Gladstone Institute of Virology and Immunology and QB3, where you have appointments. The core has 5 mass spectrometers. They include a Thermo-Fisher LTQ Velos Orbitrap equipped with ETD, a Waters Synapt G2 HDMS mass spectrometer (with a TriWave® ion mobility analyzer) that can be operated in electrospray or MALDI ionization modes, and an AB Sciex 5600 TripleTOF capable of MS/MS SWATH acquisition. We also have other ancillary equipment (e.g., nano and analytical scale HPLC systems and protein digesters), all dedicated to proteomics and well suited to the needs of your project. Our lab has particular expertise in determining the glycan and disulfide structures of complex glycoproteins. Characterization of the glycans on the gp120 proteins and scaffolds that you describe in the proposal is well within our capabilities. The analyses we provide should also help solve the gp120 aggregation and clipping problems that you described.

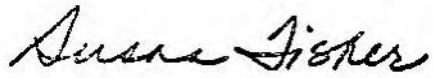
It is certainly surprising that neutralizing antibodies to HIV-1 recognize glycan-dependent epitopes. Previously, I thought that native glycans were poor immunogens. I am intrigued by the underlying biology and the opportunity to explain these findings in structural terms. I understand how the results of this project have added importance because they might be applicable to other viruses and pathogens with highly glycosylated envelope proteins.

As discussed during the meeting with our facility, we are happy to host a

graduate student or postdoctoral fellow who will carry out the proposed experiments and are looking forward to getting started. I understand that your graduate student, Javier Morales, and your Postdoctoral Fellow, Bin Yu, will be providing samples shortly.

In summary, we are here to help you accomplish the important goals set forth in your application. We look forward to a very productive and interesting collaboration.

Sincerely,



Susan J. Fisher, Ph.D.
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Department of Obstetrics, Gynecology and Reproductive Sciences
Division of Maternal Fetal Medicine
Department of Anatomy
The Eli & Edythe Broad Center for Regeneration Medicine and Stem Cell Research at UCSF
Director, Human Embryonic Stem Cell Program
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http://cancer.ucsf.edu/cores/mass-spectrometry

Phillip W. Berman, Ph.D.
Professor, Biomolecular Engineering, UCSC
1156 High Street
Santa Cruz, CA 95064
(831) 459-3529
pwb@soe.ucsc.edu

November 29, 2013

Dear Phil,

This isto reiterate my enthusiasm for our planned collaboration on characterizing glycosylation and disulfide bond arrangement of the re-engineered gp120. Your proposal on "Re-engineering gp120 to include glycan dependent epitopes" introduces very convincing approaches to delineate potential target epitopes for HIV vaccine development, and I am looking forward to participating in your studies.

As you know, I will provide day-to-day supervision for all mass spectrometry assays that will be performed at the UCSF Sandler-Moore Mass Spectrometry Core Facility where I serve as a Co-Director of Operations. I have over 20 years of experience in protein structure analysis and mass spectrometry. Our laboratory is very well equipped with modern high resolution mass spectrometers that are capable of novel technologies, e.g., ion mobility separation (IMS), electron transfer dissociation (ETD) and higher-energy collision dissociation (HTD), all enabling state-of-the-art experimental workflows for glycosite analysis and elucidation of oligosaccharide structures.

I will mentor your graduate student or post-doctoral fellow and will provide him or her with all support needed for developing professional expertise in mass spectrometry.

With my best wishes, sincerely,

H. Ewa Witkowska, Ph.D.
Adjunct Professor
UCSF School of Medicine
Dept. of Obstetrics, Gynecology & Reproductive Sciences

GLADSTONE INSTITUTES

SCIENCE OVERCOMING DISEASE

November 22, 2013

Phillip Berman, PhD
Visiting Investigator
Gladstone Institute of Virology and Immunology
Jack Baskin Endowed Professor
Biomolecular Engineering
University of California, Santa Cruz
1156 High Street, SOE2
Santa Cruz, CA 95064

Dear Phil,

I am writing in support of your grant application: "Re-engineering gp120 to include glycan dependent epitopes." I've enjoyed your presentations at GIVI and am pleased that we have found a project on which to collaborate. I share your interest in the structure of the gp120 V1/V2 domain and am intrigued with the idea that virus transmission involves a selection for viruses with compact V1/V2 domains with few potential N-linked glycosylation sites. As you know, we have assembled a set of 37 wild-type Envs and 19 isogenic clones with variations in the length of V1 or V2. We have found that species with compact V1/V2 domains incorporate more copies of Env proteins than viruses with longer V1/V2 domains and were also more fusogenic.

We would be happy to share our clones from pairs of viruses with short and long V1/V2 domains for use in your neutralization assays. I understand that you believe that your new vaccines will produce antibodies to epitopes in both the V1 and V2 domains and that you have a particular interest in viruses from new infections. The viruses we are studying are clade C viruses that came from four transmission pairs of the Lusaka cohort of discordant couples. These Envs, which have been subcloned in expression vectors and in provirus, have short V1/V2 domains with few glycosylation sites. These seem ideally suited for the studies you describe. I am also looking forward to testing the Env species from the CRF01_AE viruses identified in Thailand that similarly have short V1/V2 domains and few glycosylation sites. It will be interesting to see if they have the same phenotype as the viruses we have been studying.

I am looking forward to working with you.

Best regards,

MCAVROIS

Marielle Cavrois, PhD
Assistant Adjunct Professor, Department of Experimental Medicine
University of California, San Francisco
Staff Research Scientist
Director, Flow Cytometry Core
Gladstone Institute of Virology and Immunology

MC/sc

1650 Owens Street
San Francisco, CA 94158
Phone 415.734.2000
www.gladstoneinstitutes.org

centocor
research&development Inc

T. Shantha Raju
Scientific Director
Biologics Research

November 22, 2013

To:
Phillip W. Berman, Ph.D
Jack Baskin Professor
Department of Biomolecular Engineering
University of California Santa Cruz

Dear Phil,

I was happy to hear from you again and am pleased that you are still trying to make an AIDS vaccine. It is an important project and the world needs it. The recent work showing that neutralizing antibodies target glycan epitopes on gp120 is remarkable. It's too bad we never got around to publishing the results on the carbohydrate structure of MN-rgp120 that I determined while we were at Genentech. But I'm glad that we could include the data in the package for the FDA. Those were busy times and there was no shortage of exciting projects. Our work on the carbohydrate structure of therapeutic antibodies to treat cancer has been interesting and rewarding.
I'm pleased to collaborate with you on your new grant titled: "Re-engineering gp120 to include glycan dependent epitopes". We have a well equipped facility that contains all of the instrumentations required to characterize the glycan structure of your new gp120 based vaccine antigens. This includes: a MALDI-TOF-MS instrument, a Q-trap MS instrument and several HPLC instruments. As you know the main focus of my lab is structure and functions of glycans on therapeutically important glycoproteins and I am now considered as an expert on glycosylation of therapeutic proteins including antibodies? In this regard I serve as a reviewer on mAbs and BioProcess International Journals.
I am pleased to provide any analytical support on this important project and wish you every success with your project.

Sincerely,

T. Shantha Raju
Scientific Director
Biologics Research

145 King of Prussia Road
Radnor, PA 19087
phone: 610.651.6000
fax: 610.651.6100

November 26, 2013

Phillip Berman, Ph.D.
Distinguished Professor
Department of Biomolecular Engineering
University of California Santa Cruz
1156 High Street
Santa Cruz, CA 95064

RE: R01 Application: Re-engineering gp120 to include glycan dependent epitopes

Dear Phil:

I would like to confirm that I am more than happy to support your R01 application, entitled "Re-engineering gp120 to include glycan dependent epitopes " that will be submitted to NIH/NIAID in response to RFA-AI-12-056, Functional Glycomics in HIV Vaccine Design (R01).

We were pleased to learn of your interest in carbohydrate cluster microarrays. These allow for the highly sensitive detection of anti-carbohydrate antibodies and anti-oligommanosyl antibodies of both IgM and IgG isotypes. As you know, my major research interests are in Glycobiology , Immunology, and Vaccine Development. It will be very interesting to see if we can detect antibodies to glycan dependent epitopes in the rabbits and mice that you have immunized with proteins and scaffolds designed to elicit broadly neutralizing antibodies to glycan dependent epitopes. The results you recently presented in your recent seminar at SRI appear very promising, especially for antibodies to the PG9 epitope. I agree with you that it is very important to extend this investigation to specimens from HIV-1 vaccine trials in order to better understand of human immune responses to glycan dependent epitopes elicited by the gp120 glycoprotein and by natural HIV infection. In this regard it will be interesting to examine the sera from your VaxGen Phase 3 clinical trials and from the elite neutralizers that you have identified in San Francisco and Thailand.

I am very pleased to be able to help you characterize the glycans present on our vaccine antigens and scaffolds using the GlycoStation-based lectin array technology that has been fully established in our glycomics facility.

Sincerely yours,

D. N. Wang

Denong Wang
SRI Distinguished Scientist and Senior Program Director
Tumor Glycomics Laboratory
SRI International (formerly Stanford Research Institute)
Menlo Park, California, USA

Duke University Medical Center
David C. Montefiori, Ph.D.
Professor

November 21, 2013

Phillip W. Berman, Ph.D
Department of Biomolecular Engineering
Baskin School of Engineering, MS-SOE2
University of California Santa Cruz
Santa Cruz, CA 95064

Dear Phil,

I would be delighted to serve as a collaborator on your NIH grant application entitled "Re-engineering gp120 to include glycan dependent epitopes." I understand that you plan to immunize rabbits and macaques. My Laboratory will perform neutralizing antibody assays with sera from both animal species to determine the magnitude and breadth of the neutralizing antibody responses elicited by your immunogens.The assays will be performed with the standard panels of HIV-1 Env-pseudotyped reference strains in a validated luciferase reporter gene assay. We have standard panels of reference strains for all major genetic subtypes of HIV-1. We will be happy to assess the neutralizing antibody responses in your immunized animals using a multi-subtype panel of reference strains that exhibit Tier 1 and Tier 2 neutralization phenotypes. We may also assay a subset of interesting samples in the more sensitive A3R5 assay. As you know, my laboratory has served as a national and international resource for assessments of neutralizing antibody responses in preclinical and clinical HIV-1 vaccine trials for more than 20 years. We have performed the majority of neutralizing antibody assays for human clinical trials Conducted by the HIV Vaccine Trials Network (HVTN) since1988, including our collaborations on major human efficacy trials (Vax003, Vax004, RV144). In addition, my laboratory functions as a NIH-funded immune monitoring laboratory for animal models of HIV and as a central reference laboratory for the Bill & Melinda Gates Foundation's "Collaboration for AIDS Vaccine Discovery." We are well-poised to assist you in your studies of novel HIV-I Env immunogens.

I wish you all the best with your application.

Sincerely,

David C. Montefiori, Ph.D.
Professor and Director
Laboratory for AIDS Vaccine Research & Development

Children's Hospital
3 Blackfan Circle
Boston, MA 02115
617-355-7372

Division of Molecular Medicine

November 21, 2013

Children's Hospital

Phillip W. Berman, Ph.D
Jack Baskin Professor
Department of Biomolecular Engineering
University of California Santa Cruz

Dear Phil,

I would be delighted to assist your studies proposed in your NIH R01 grant application entitled "Re-engineering gp120 to include glycan dependent epitopes". As you know, the glycan shield of HIV-1 envelope protein has been increasingly recognized to play critical roles in the viral transmission, antigenicity and immunogenicity. For that reason, we have recently crystallized a fully glycosylated HIV-1 gp120 derived from a primary isolate. The structure is expected to provide important information about all the glycans on the surface of gp120 at the atomic level, and may greatly facilitate your vaccine efforts to target these glycans.

We have already made progress on crystallizing the A244 gp120 produced in GnTI- 293 cells that you have provided and we are now in the process of further optimizing these crystals for structure determination. We will continue to work on any additional gp120s from your laboratory including variants of monomeric gp120 that you have developed able to bind monoclonal antibodies to glycan dependent epitopes with high affinity such as your MN-rgp120 mutants. Once the structure is completed, I will be happy to provide structural interpretation of your epitope mapping data.

I look forward to this collaboration and I wish you the best in securing funding for this interesting and important project.

Best regards,

Bing Chen, Ph.D.
Associate Professor of Pediatrics
Harvard Medical School/Boston Children's Hospital

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2929 SEVENTH STREET, SUITE 100
BERKELEY, CALIFORNIA 94710

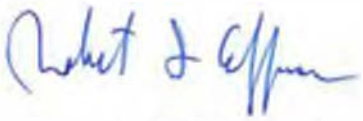
November 27, 2013

Phillip W. Berman, Ph.D
Department of Biomolecular Engineering
Baskin School of Engineering, MS-SOE2
University of California, Santa Cruz
Santa Cruz, CA 95064

Dear Phil,

As we have discussed, I would be pleased to provide several of our CpG oligonucleotide based adjuvants for you to test with the HIV vaccine candidates you are developing as part of your grant application **"Re-engineering gp120 to include glycan dependent epitopes"**. We have both soluble and nanoparticle formulations that are very promising and one (1018 ISS) which has been studied as a vaccine adjuvant in over 5000 human subjects. When used as an adjuvant for Hepatitis B surface antigen in humans, 1018 ISS produces considerably higher antibody titers with a two dose regimen than alum. Importantly for your proposal, the response using the 1018 ISS adjuvant exhibits a much slower rate of decline than the same vaccine antigen with alum when followed for a year after vaccination. We have not yet formally demonstrated a mechanism for this long-lived response in man, but studies in animals, both our unpublished work and published studies from others, demonstrates that CpG oligonucleotide adjuvants are particularly efficient in stimulating T follicular helper development and germinal center formation, conditions well known to favor the generation of long-lived plasma cells.

Very sincerely,



Robert L. Coffman, Ph.D.
Vice-President and Chief Scientific Officer

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SANTA BARBARA • SANTACRUZ

November 21, 2013

Dr. Phillip Berman
Department of Biomolecular Engineering
School of Engineering
University of California, Santa Cruz
Santa Cruz, CA 95064

Dear Phil,

I would be happy to serve as the statistical consultant on your grant application, "Re-engineering gp120 to include glycan-dependent epitopes". As you know I am a Population Geneticist in the Department of Ecology & Evolutionary Biology at UC Santa Cruz. I have an extensive background in statistical methodology, particularly the detection at natural selection at the molecular level, and teach several statistics-oriented courses including Evolution (Bioe 109) and Population Genetics (Bioe 172). I also serve as a reviewer for many journals that publish papers in molecular evolutionary genetics such as *Genetics*, *Molecular Biology and Evolution*, and *Molecular Ecology*. I often provide advice and input to colleagues regarding experimental design and interpretation of assay results. I have enjoyed working with you and your group on the evolution of viral DNA sequences from elite neutralizers and have been particularly interested in the evolution we've observed at glycosylation sites in the V1-V2 domain and other areas of the HIV envelope protein. I look forward to continuing this collaboration and am confident that I can provide whatever advice you need regarding the proper way to interpret your antibody binding assays and virus neutralization data.

Sincerely,

Grant Pogson

Grant Pogson
Professor of Ecology & Evolutionary Biology

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JACK BASKIN SCHOOL OF ENGINEERING
BIOMOLECULAR ENGINEERING DEPARTMENT
MAILSTOP SOE2

SANTA CRUZ, CALIFORNIA 95064
Tel (831) 459-1833

Dear Phil,

I am pleased to provide a letter of support for your NIAID grant application "Re-engineering gp120 to include glycan dependent epitopes." As you know, I recently joined your department as an Assistant Professor and my research interests are focused on the structures of virus glycoproteins. My training is in X-ray crystallography and I have solved a number of structures including those of Rubella virus envelope glycoprotein E1, H5N1 Influenza virus hemagglutinin, and Astrovirus capsid. Particularly relevant to your work is our success in engineering monomeric fragments of the influenza virus hemagglutinin that are able to elicit protective neutralizing antibody responses.

I have enjoyed attending your lab meetings and am please that I can help you and your students in rational structure-based design of new scaffolds to elicit neutralizing antibodies, a strategy known as structural vaccinology. The new structure of the gp140 trimer published in Science (Oct. 31, 2013) provides a tremendous amount of new information that can help guide the design of your new vaccine antigens. It should also help to explain the shortcomings of previous HIV vaccine efforts. I have enjoyed seeing the progress made by your students in improving the stability and glycan structure of their V1/V2 scaffolds. I have every confidence that they will be similarly successful in stabilizing the V3 domain. Once that is accomplished, I am particularly excited about your idea to do engineer a scaffold consisting of the V1/V2 and V3 domains, which you anticipate will lead to a more effective antigen able to elicit broadly neutralizing antibodies.

I am please that our labs are located in close proximity and that we are able to share the department's new BSL2+ facility and the array of protein fermentation and purification equipment that you have assembled.

Sincerely,

Rebecca M. DuBois

Rebecca Dubois, Ph.D.
Assistant Professor of Biomolecular Engineering
Department of Biomolecular Engineering

PRF&L
Quality Antibody Production Since 1960

Pocono Rabbit Farm & Laboratory Inc.
Dutch Hill Road, P.O. Box 240, Canadensis, PA 18325
Phone: (800) 622-6381 • Fax: (570) 595-9277
E-mail: antibody@prfal.com

November 21, 2013

Dr. Phillip Berman
Department of Biomolecular Engineering
1156 High Street, M/S SOE2
University of California
Santa Cruz, CA 95064

Dear Dr. Berman,

I am please to offer you our price for custom polyclonal antibody production in Rabbits. You will provide the antigens at suitable concentrations and buffers for the immunizations.

Each time there will be six rabbits per antigen. There will be between 180 to 200 rabbits during the five years.

The price quote assumes that you will provide us with antigen and all adjuvants. The antigen can be lyophilized, in PBS, or another suitable buffer.

$499 per pair of Rabbits - First three months (91 days)
Month 0 - Setup / Small Prebleed / SC Immunization
Month 1 - SC Immunization
Month 1.5 – Large Bleed
Month 2 - SC Immunization
Month 2.5 – Large Bleed

Project extension after 3 months. Pricing is per rabbit - PRF&L Pricing is based on large bleed at $25, boosts at $15 and daily maintenance fee at $2.50. All protocols will run for 12.5 months.
Month 6 - IM Immunization
Month 6.5 – Large Bleed
Month 9 - IM Immunization
Month 9.5 – Large Bleed
Month 12 - IM Immunization
Month 12.5 – Exsanguination
Month 3 to Month 12.5 or 289 Days Maintenance Fee at $2.50 per day.

Total per two rabbits for 12.5 month protocol - $2184
Total per four rabbits for 12.5 month protocol - $4368
Total per six rabbits for 12.5 month protocol - $6552

Shipping to CA - per package $77. Shipping charge is the $77, regardless of the number of bleeds in the package. If all six groups are started at the same time per year, there will be five shipping charges.

Terms – Payment for the first 91 days must be received prior to shipment of any bleeds. Project extensions is charged a-la-carte and is net 30 days. I am able to hold these prices until December 31, 2014.

Quote 13K211

To setup a project, please visit our website – www.prfal.com, complete the:
1. Client Information Form
2. Antibody Production Order Form

I look forward to working with you on these projects.

Best regards,

Scott Conklin

This price quote is prepared to give an estimate of expenses for custom antibody production for your convenience. Because result of custom antibody production is dependent on many factors such as antigen preparation and success with an animal with a variable immune system, you may wish to change the protocol. This price quote is intended to give an estimate of services to be provided and is not a contract of services to be provided. Actual charges for animal work will be calculated using the Custom Antibody Production Price Schedule in effect at the time the services are rendered.

Resource Sharing Plan

We expect that the materials and data produced in this proposal will be of considerable interest to other investigators in the field. The envelope genes and antisera produced in this proposal represent valuable reagents that can be used for follow-on studies beyond the scope of this project. In this respect, both the cloned genes and purified proteins could be studied in a variety of B-cell and immunization modalities including DNA vaccines, recombinant virus vaccines, and prime/boost approaches.

We expect that any interesting results will be published and presented as posters or oral presentations at scientific meetings in a timely fashion. We are under no obligations to 3rd parties to withhold publication or distribution of materials or data. The results, mutant genes, recombinant proteins and antisera produced in this grant proposal will be made available to qualified researchers subject to the Material Transfer policy of the University of California.