Notice of Award

RESEARCH **Issue Date:** 05/03/2012

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

Grant Number: 1R01AI098775-01

Principal Investigator(s):
Maria Elena Bottazzi
PETER J HOTEZ (contact), PHD
SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Helen Shepherd
Business Manager
One Baylor Plaza, BCM320A
Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Budget Period: 05/04/2012 – 04/30/2013
Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $1,501,840 (see “Award Calculation” in Section I and “Terms and Conditions” in Section III) to BAYLOR COLLEGE OF MEDICINE 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 R01AI098775. 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 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. 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

NIH NGA R | Version: 409 - 03/06/2012 15:38:01 | Generated on: 05/03/2012 00:13:19

SECTION I – AWARD DATA – 1R01AI098775-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$190,031
Fringe Benefits	$52,093
Equipment	$298,503
Supplies	$65,231
Travel Costs	$20,000
Other Costs	$15,000
Consortium/Contractual Cost	$636,563

Federal Direct Costs	$1,277,421
Federal F&A Costs	$224,419
Approved Budget	$1,501,840
Federal Share	$1,501,840
TOTAL FEDERAL AWARD AMOUNT	$1,501,840

AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,501,840

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$1,501,840	$1,501,840
2	$1,154,596	$1,154,596
3	$1,134,359	$1,134,359
4	$1,165,726	$1,165,726
5	$1,165,855	$1,165,855

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:	1741613878A1
Document Number:	RAI098775A
Fiscal Year:	2012

IC	*CAN*	*2012*	*2013*	*2014*	*2015*	*2016*
AI	8472315	$1,501,840	$1,154,596	$1,134,359	$1,165,726	$1,165,855

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

NIH Administrative Data:
PCC: M51C B / **OC**: 414A / **Processed**: PII 04/27/2012

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI098775-01

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 – 1R01AI098775-01

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.)

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.

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/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI098775-01

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

Restriction: Under governing PHS Policy, Federal funds administered by the Public Health Service (PHS) shall not be expended for research involving live vertebrate animals without prior approval by the Office of Laboratory Animal Welfare (OLAW) of an Assurance to comply with the PHS Policy on Humane Care and Use of Laboratory Animals and the project has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The present award is being made without currently valid verification of IACUC approval for the portion of this project being completed in CHINA with the following restriction: No activities that involve live vertebrate animals may be conducted at Frontier Biosciences located in CHINA pending acceptance by the NIH awarding component of verification of IACUC approval. The Program Officer has approved the funding of this application without the portion of Frontier Biosciences located in CHINA in year 05 as the project is viable without it. No funds may be expended for the foreign site pending the resolution of internal administrative issues. Once these issues have been resolved, this award may be revised to include the study originally planned for the foreign site. Failure to comply with this special condition can result in suspension and/or termination of this award, withholding of support, audit disallowances, and/or other appropriate action.

The budget period anniversary start date for future year(s) will be May1.

None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-035.html Therefore, this award and/or future years are adjusted accordingly, if applicable.

This award includes funds awarded for consortium activity with NY Blood Center in the amount of $445,904 ($263,070 direct costs + $182,834 facilities and administrative costs);

This award includes funds awarded for consortium activity with the University of Texas Medical Branch in the amount of $190,659 ($124,614 direct costs + $66,045 facilities and administrative costs).

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2011/nihgps_ch15.htm .

The research proposed in this grant may involve Select Agents and/or Highly Pathogenic Agents. Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving approval from the NIAID,NIH Program Official. Awardee is responsible for having its subcomponent/subcontractor comply with the requirements pertaining to the use of Select Agents and/or Highly Pathogenic Agents. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that, under some circumstances, may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm), and your Institutional Biosafety Committee (IBC) or equivalent body, or appropriate designated institutional biosafety official. If there is ambiguity in the BMBL guidelines and/or there is disagreement among the BMBL, an institutional committee or institutional official, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

For work with Select Agents performed in the U.S. provide documentation of Registration status of all domestic organizations/entities where Select Agent(s) will be used. For work with Select Agents performed in a non-U.S. country prior NIAID approval is required.

Please be advised that changes in the use of a Select Agent will likely be considered a change in scope and, therefore, require NIH awarding office prior approval.

Please contact Donna Sullivan at 301-594-6361 or at dsullivan@niaid.nih.gov if there is a need to be provided additional assistance in a grant-related issue.

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: Mary Ann Anderson-garlic
Email: mgarlic@niaid.nih.gov **Phone**: 301-451-3680 **Fax**: 301-493-0597

Program Official: Rachelle Salomon
Email: salomonra@mail.nih.gov **Phone**: 301-402-2202 **Fax**: 301-496-8030

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AI098775-01

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

Budget	***Year 1***	***Year 2***	***Year 3***	***Year 4***	***Year 5***
Salaries and Wages	$190,031	$122,034	$174,866	$57,811	$54,221
Fringe Benefits	$52,093	$33,453	$47,936	$15,848	$14,864
Equipment	$298,503				
Supplies	$65,231	$60,485	$71,400	$10,000	$10,126
Travel Costs	$20,000	$10,000	$20,000	$5,000	$5,000
Other Costs	$15,000	$15,000	$95,000	$579,807	$609,000
Consortium/Contractual Cost	$636,563	$775,547	$490,684	$114,229	$75,434
TOTAL FEDERAL DC	$1,277,421	$1,016,519	$899,886	$782,695	$768,645
TOTAL FEDERAL F&A	$224,419	$138,077	$234,473	$383,031	$397,210
TOTAL COST	$1,501,840	$1,154,596	$1,134,359	$1,165,726	$1,165,855

Facilities and Administrative Costs	***Year 1***	***Year 2***	***Year 3***	***Year 4***	***Year 5***
F&A Cost Rate 1	57.3%	57.3%	57.3%	57.3%	57.3%
F&A Cost Base 1	$342,355	$240,972	$409,202	$668,466	$693,211
F&A Costs 1	$196,169	$138,077	$234,473	$383,031	$397,210
F&A Cost Rate 2	56.5%				
F&A Cost Base 2	$50,000				
F&A Costs 2	$28,250				

PI: **HOTEZ, PETER J**	Title: RBD recombinant protein-based SARS vaccine for biodefense	
Received: 05/26/2011	FOA: AI11-014	Council: 01/2012
Competition ID: ADOBE-FORMS-B1	FOA Title: PARTNERSHIPS FOR BIODEFENSE (R01)	
1 R01 AI098775-01	Dual:	Accession Number: 3392091
IPF: 481201	Organization: BAYLOR COLLEGE OF MEDICINE	
Former Number:	Department: Pediatrics	
IRG/SRG: ZAI1 RGK-M (J2)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 1,048,503 Year 2: 749,999 Year 3: 750,000 Year 4: 750,000 Year 5: 749,999	Animals: Y Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Peter Hotez M.D.	Baylor College of Medicine	PD/PI
Shibo Jiang M.D.	New York Blood Center	MPI
Maria Bottazzi Ph.D	Baylor College of Medicine	MPI
Sara Lustigman Ph.D	New York Blood Center	Co-Investigator
Chien-Te Tseng Ph.D	The University of Texas Medical Branch	Co-Investigator
Lanying Du Ph.D	New York Blood Center	Co-Investigator
Bin Zhan M.D.	Baylor College of Medicine	Co-Investigator
Tehsheng Chan PhD	University of Texas Medical Branch	Co-Investigator

Appendices

App1 a research paper related to rbd of sars-cov s protein,App2 review article vaccine development,App3 patents directly relevant to the project,Appendix summary sheet

OMB Number: 4040-0001
Expiration Date: 06/30/2011

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 GRANT10878569

b. Agency Routing Identifier

2. DATE SUBMITTED

Applicant Identifier 35116-I

5. APPLICANT INFORMATION

*** Organizational DUNS:** 051113330

* Legal Name: Baylor College of Medicine
Department: Office of Research Division: NA
* Street1: One Baylor Plaza
Street2:
* City: Houston County / Parish:
* State: TX: Texas Province:
* Country: USA: UNITED STATES * ZIP / Postal Code: 77030-3411

Person to be contacted on matters involving this application

Prefix: * First Name: Miki Middle Name:
* Last Name: Gillis Suffix:
* Phone Number: 832-824-2283 Fax Number: 713-790-1345
Email: myates@bcm.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN)*: 1741613878A1

7. * TYPE OF APPLICANT: O: Private 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:

RBD recombinant protein-based SARS vaccine for biodefense

12. PROPOSED PROJECT:

* Start Date: 05/01/2012
* Ending Date: 04/30/2017

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT**

TX-007

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: * First Name: Peter Middle Name: J
* Last Name: Hotez Suffix: M.D.
Position/Title: Professor
* Organization Name: Baylor College of Medicine
Department: Pediatrics Division: College of Medicine
* Street1: One Baylor Plaza, BCM320A
Street2:
* City: Houston County / Parish:
* State: TX: Texas Province:
* Country: USA: UNITED STATES * ZIP / Postal Code: 77030-3411
* Phone Number: 832-824-2283 Fax Number:
* Email: hotez@bcm.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	6,265,449.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	6,265,449.00
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 penalities. (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

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix:
* First Name: Helen
Middle Name:
* Last Name: Shepherd
Suffix:
* Position/Title: Executive Director, Office of Research
* Organization: Baylor College of Medicine
Department: Office of Research
Division: NA
* Street1: One Baylor Plaza, BCM310
Street2:
* City: Houston
County / Parish:
* State: TX: Texas
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 77030-3411
* Phone Number: 713-798-1297
Fax Number: 713-798-6990
* Email: spo@bcm.edu

* Signature of Authorized Representative	* Date Signed
Helen Shepherd	05/26/2011

20. Pre-application

Add Attachment | Delete Attachment | View Attachment

424 R&R and PHS-398 Specific Table Of Contents

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Appendix

Number of Attachments in Appendix: 4

OMB Number: 4040-0010
Expiration Date: 08/31/2011

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: BAYLOR COLLEGE OF MEDICINE
DUNS Number: 0511133300000
* Street1: ONE BAYLOR PLAZA
Street2:
* City: HOUSTON
County:
* State: TX: Texas
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 77030-3411
* Project/ Performance Site Congressional District: TX-007

Project/Performance Site Location 1

☐ 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: New York Blood Center
DUNS Number: 0732718270000
* Street1: 310 East 67 Street
Street2:
* City: New York
County:
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 10065-6275
* Project/ Performance Site Congressional District: NY-014

Project/Performance Site Location a

☐ 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 University of Texas Medical Branch
DUNS Number: 8007711490000
* Street1: 301 University Boulevard
Street2:
* City: Galveston
County: Galveston
* State: TX: Texas
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 77555-0156
* Project/ Performance Site Congressional District: TX-014

Tracking Number:GRANT10878584 Funding Opportunity Number:RFA-AI-11-014 Received Date:2011-05-26T09:28:29-04:00

Project/Performance Site Location(s)

Project/Performance Site Location 3

☐ 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: Texas Childrens Hospital

DUNS Number: 0746153940000

* Street1: 1102 Bates Street

Street2:

* City: Houston

County:

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 77030-2399

* Project/ Performance Site Congressional District: TX-007

Additional Location(s)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? [X] Yes [ ] No
   1.a If YES to Human Subjects
   Is the Project Exempt from Federal regulations? [ ] Yes [X] No
   If yes, check appropriate exemption number. [ ] 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] 6
   If no, is the IRB review Pending? [X] Yes [ ] No
   IRB Approval Date:
   Human Subject Assurance Number: 00000286
2. * Are Vertebrate Animals Used? [X] Yes [ ] No
   2.a. If YES to Vertebrate Animals
   Is the IACUC review Pending? [X] Yes [ ] No
   IACUC Approval Date:
   Animal Welfare Assurance Number A3823-01
3. * Is proprietary/privileged information included in the application? [ ] Yes [X] No

4.a. * Does this project have an actual or potential impact on the environment? [ ] Yes [X] 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 [X] No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? [ ] Yes [X] No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract 1234-Abstract.pdf — Add Attachment | Delete Attachment | View Attachment

8. * Project Narrative 1235-Project_Narrative_5-16-11.pdf — Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited 1236-Bibliography_5-15-11.pdf — Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources 1237-Resources_all1.pdf — Add Attachment | Delete Attachment | View Attachment

11. Equipment 1238-Equipment_all.pdf — Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments [X]

Abstract

The 2002-2003 pandemic of severe acute respiratory syndrome (SARS) posed an enormous threat to global public health and the social and economic stability. Its causative pathogen, the SARS-associated coronavirus (SARS-CoV), has been classified by NIAID as a Category C Priority Pathogen. SARS outbreaks remain a serious concern mainly due to possible zoonotic reintroduction of SARS-CoV into humans, accidental release from a laboratory or deliberate spreading of the virus by a bioterrorist attack. Therefore, an effective and safe vaccine is urgently needed for preventing future SARS outbreaks and for biodefense preparedness. We have identified a highly promising lead candidate vaccine antigen, the receptor binding domain (RBD) of the SARS-CoV spike (S) protein that contains the major neutralizing epitopes and can induce potent neutralizing antibody response and protection in animals against SARS-CoV infection. To rapidly translate our initial proof of concept findings into a solid platform of clinical trials, a consortium of experts was put together consisting of scientists from Baylor College Medicine, the new home of Sabin Vaccine Institute's product development partnership (BCM-Sabin), the New York Blood Center (NYBC) and the University of Texas Medical Branch (UTMB), and in partnership with industrial partners and non-profit organizations. The specific aims of this proposal are: **(1) Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate.** The rRBD protein will be expressed in bacteria and yeast expression systems and one of these expression systems will be selected for subsequent studies based on yields, purity, stability, antigenicity, functionality, immunogenicity, and efficacy of the rRBD protein. The immunization regimens will be optimized and the ability of rRBD protein to induce cross-neutralizing antibody response, cross-protection and long-term immune responses and protection will be assessed. **(2) Process development, characterization, formulation and stability profiling.** A scalable and reproducible fermentation process for rRBD (10 liter scale) and a purification process using chromatographic technologies will be developed. Reproducibility will be confirmed. The specific product quality assays and vaccine formulations with alum and/or glucopyrranosyl lipid A (GLA), an innate adjuvant, will be developed. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing. **(3) Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation.** The cell bank production, production processes and the formulation technology for the rRBD-based SARS vaccine will be transferred to Walter Reed Army Institute of Research (WRAIR) pilot facility for 60-L scale GMP manufacture, formulation and fill and finish. The clinical lots will be released by Sabin-Texas and following a pre-IND meeting with the U.S. FDA, GLP toxicology will be initiated at Frontier Biosciences, a Maryland-based contractor.

Project Narrative

SARS-CoV is the first new human infectious disease agent of this century classified by NIAID as a Category C Priority Pathogen, and SARS outbreaks still remain a serious global concern mainly due to possible zoonotic reintroduction of SARS-CoV into humans or accidental release from a laboratory or deliberate spreading of the virus by a bioterrorist. We have identified a highly promising lead candidate vaccine antigen, the receptor binding domain (RBD) of the SARS-CoV spike (S) protein that can induce potent neutralizing antibody response and protection against SARS-CoV infection. Our objective is to develop a highly effective and safe recombinant RBD-based SARS vaccine that can be used in humans for prevention of future SARS outbreak and for biodefense preparedness.

FACILITIES & RESOURCES

Baylor College of Medicine

Laboratory:

The Hotez lab occupies approximately 8500 square feet of well-equipped bench laboratory space in the recently renovated Feigin Research building at Texas Children's Hospital. A major expansion of which (an addition 8 floors has been added) was completed in 2009. The laboratory is equipped with –80° and –20° freezers as well as adequate bench space. Expression capabilities include *Pichia pastoris*, *Saccharomyces cerevisiae*, *Escherichia coli,* Baculovirus and mammalian cell culture expression systems. Generation and characterization of research cell banks. For Process Development and studies designed to optimize large-scale protein production include fermentation capabilities from 1-10L scales. Facility is capable of developing and optimizing protein purification processes. The optimal purification scheme is dependent upon the properties of the particular protein, which is being purified. Procedures may be included, but are not limited to ion exchange chromatography, (anion or cation), hydrophobic interaction chromatography, hydroxyapatite, reverse phase and size exclusion, IMAC and affinity chromatography. The laboratories are fully suited to perform biochemical characterization (testing for identity, purity, conformation, integrity, stability, consistency, antigenicity and residual host cell proteins) of in-process and final purified bulk protein samples. Formulation studies are also performed (testing for compatibility, stability, immunogenicity and potency) with multiple adjuvants. All process development and analytical work performed under a strictly-regulated environment with highly-trained individuals performing operations according to formal standard operating procedures, batch production records and protocols. In addition, the lab has access to all the Texas Children's core facilities and expertise as listed below.

Animal:

Baylor College of Medicine (BCM) maintains an animal program fully accredited by the American Association for the Accreditation of Laboratory Animal Care since October 26, 1995. The Center for Comparative Medicine (CCM) administers the program of veterinary care. All animals are maintained in accordance with the provisions of the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. Animals are housed in six central animal facilities. These animal facilities are located throughout the medical center and together comprise 56,000 square feet of conventional animal housing space. In addition CCM maintains a 7,400 square feet mouse barrier facility. Animals in all animal facilities are observed daily by members of CCM staff. The Center has a staff of 5 full time veterinarians. Three of the veterinarians are diplomats of the American College of Laboratory Animal Medicine (ACLAM) and have extensive experience with laboratory animal species. A comprehensive diagnostic laboratory is maintained by the veterinary staff and headed by a board certified veterinary pathologist.

In vivo imaging:

Animal imaging modalities including bioluminescence (IVIS Xenogen system), ultrasound, in vivo fluorescence, PET, CT/SPECT and MRI scanning are all immediately available in either the Feigin Center animal facility or in the imaging center at Baylor College of Medicine. All animals are manipulated under animal protocols reviewed and approved by the Baylor Animal Research Committee.

Computer:

Laboratories and offices have exclusive use Pentium-based personal computers, complete with word processing software, extensive scientific software, database programs, spreadsheet programs, electronic mail, scheduling, and Internet browsers. Computers are connected to a campus-wide network to provide access to central services such as file servers, data base servers, UNIX servers, printers, and film recorders, as well as to facilitate the sharing of information between colleagues both locally and long distance via the Internet. A DEC 8060 computer is available for statistical analysis plotting. Full time IT and data management support is provided by Baylor College of Medicine.

Office:

The principal investigators has adequate office space adjacent to his laboratory.

Other:

Texas Children's Hospital (TCH) is located in the Texas Medical Center (TMC) that comprises 675 acres and is

the world's largest medical center. The TMC encompasses 42 not-for profit institutions dedicated to the highest quality patient care, research, and education. These institutions include 13 hospitals, two medical schools, four schools of nursing, a school of public health, a college of pharmacy, two graduate schools of biomedical sciences, and a medical library. More than 5.4 million patient visits were recorded in 2000, and more than $573 million in funded research is conducted annually. Additionally, two other major universities with vigorous programs in biological sciences are within easy access; Rice University is adjacent to the TMC, and the University of Houston (UH) is 3 miles away. The UH College of Pharmacy is located in the TMC. Other resources available to the investigator include a Medical Illustration Department, which can meet all audio visual or printed material needs, and the Houston Academy of Medicine/TMC Library. TCH also has a 75 member Biomedical Engineering Department, and an excellent machine shop and electronic shop, which are available as-needed basis.

New York Blood Center

Laboratory: LABORATORY OF VIRAL IMMUNOLOGY

General laboratory area (2,946 feet^2) including tissue culture section; tissue culture laboratories (2 BSL-2 facilities, 760 feet^2); walk-in refrigerator (98 feet^2); area for centrifuges (120 feet^2); BSL-3 facility (400 feet^2) with air locks, limited access corridors and a ventilation system providing directional air flow incorporating high efficiency HEPA filters with water resistant interior surfaces which are sealed.

Clinical:
N/A

Animal:
N/A

Computer:
Computers: Two Silicon Graphics Octane, one HP xw 9300 Workstation, eight desktop computers, and twoIBM ThinkPad T30. Printers: HP Color LaserJet 4700dn, HP LaserJet 4200n, HP Officejet Pro 8500.

Office:
3 offices (420 feet^2).

Laboratory: LABORATORY OF MOLECULAR PARASITOLOGY
Molecular Parasitology at the Lindsley F. Kimball Research Institute of the New York Blood Center consists of three labs and occupies approximately 955 square feet, containing approximately 80 linear feet of active bench space (room 1, 655 square feet and room 2, 300 square feet). The largest laboratory is fully equipped for molecular biology research, whereas the second lab is mostly committed to research involved with tissue culture and RNA work.

Clinical:
N/A

Animal:
N/A

Computer:
There are presently 4 PC computers (Pentium) and 1 Macintosh computers (Power Macintosh G4) available in this department. They contain programs for DNA and protein analysis (Vector NTI Suite, DNASIS/PPROSIS, DNA Star), word-processing, data management, desktop publishing, bibliographic database, digital imaging software, web browsers and graphics presentations. All the computers have access to the Internet and Color Printer.

Office:
There are 2 offices (total of 225 square feet) for researchers. Photocopy, scanning and fax equipment is also available in this space.

Other:

The following Core facilities are available to us:

1. Flow Cytometry Laboratory (Fcl)
Staff: Wu He, Ph.D.

The Flow Cytometry Laboratory (FCL) provides investigators with equipment and support for cell sorting (separation) from single cell suspension using fluorescence and for acquisition and analysis of flow cytometric data. For analysis purposes, the FCL is equipped with a bench top alignment-free multipurpose analyzer from BD Immunocytometry Systems (FACSCanto). An additional FACSCalibur analyzer is also available. The laser configurations on these instruments allow for analysis of up to 6 colors on the FACS Canto and up to 4 colors on the FACSCalibur with 488 nm and 633 nm-excited dyes. A number of applications, including the multicolor analysis of cell phenotype, gene expression and cell cycle may be performed. For cell sorting, the FCL is equipped with a MoFlo high-speed cell sorter (DAKO Cytomation). This instrument provides high-speed, high purity (up to 99.0%) multilaser cell sorting and is capable of sterile sorts of single cells. The sorter is operated by FCL staff only.

2. Radioisotope suite:
Staff: Rawlinson Isaac

The radioisotope suite is a shared facility of the Lindsley F. Kimball Research Institute. The suite occupies 400 square feet and is equipped with seven scintillation counters, including the LKB Betaplate 1205 and a Tomtec 96 cell Harvester.

3. Laboratory of Electron Microscopy:
Staff: Yelena Oksov, M.S, Electron Microscopist

The facility provides transmission electron microscopy (TEM) services, including full specimen preparation through fixation to embedding, thin and ultrathin sectioning and staining and analyzing results of experiments. The electron microscopic methods include:

1. Conventional electron microscopy of the cells and tissues.
2. Negative staining to study particles and molecules in solution or in suspension.
3. Immunoelectron microscopy (IEM) to visualize the location of antigenic agents.

Equipment: Electron Microscope, EM 410, Philips Electronic Instruments/FEI equipped with a Digital Camera; Advantage HR Digital CCD Camera System; Ultramicrotome, MT-X, RMC; High Vac Evaporator, Denton Vacuum; Camera System, MP-4 Standard, Polaroid.

4. Laboratory of Confocalicroscopy:
Staff: Lyudmil Angelov, MS.

With the Zeiss LSM 510 META Confocal Microscope, a laser scanning fluorescent microscope, the laboratory provides three- and four-dimensional images of living cells and tissue. Researchers can study cellular structures and determine the location of multiple proteins simultaneously within the cell. Observed over time, the images make a film of the movement of proteins within the cell.

5. Shared Instrumentation Laboratory
Staff: Richard Sanford

Tri-Carb 2900RT (Packard BioScience); Minaxi Auto-Gamma 5530 (Packard BioScience); Beta plate Flatbed Scanner 1205-001 (PerkinElmer); Storm 860 (Molecular Dynamics/Amersham Bioscience); Konica SRX-101A Film Developer; Alpha Beta Sample Counter 3030 (Ludlum); Savant SpeedVac System; U-2000 UV/Vis Spectrophotometer (Hitachi); Vacamatic Stage 3 Autoclave 3023 (Steris); Better Built Glasswasher (LBR Scientific Inc); Irradiator (Isomedix); Phosphoimager (Molecular Dynamics); ABI PRISM 7300 sequence detection system (PE Applied Biosystems); Biacore 3000 (Biacore, Piscataway, NJ).

6. Laboratory for Animal Research Services (LARS)
Staff: Kathy Tang

The Animal Services facility of the New York Blood Center is a specifically designated unit to house small laboratory animals. These animal quarters are under the direction of a full-time supervisor who works closely with a consultant Veterinarian. The facility is registered with the U.S. Department of Agriculture, Animal Health Division #21-8, and is fully accredited by the American Association of Laboratory Animal Care.

Scientific environment: The project aims to be executed at the New York Blood Center are supported by the collegial environment at the Lindsley F. Kimball Research Institute (LFKRI) of the New York Blood Center and the close contact and collaboration between the groups of Dr. Shibo Jiang (PI#2 at NYBC), head of the Viral Immunology Laboratory and Dr. Sara Lustigman (Co-Investigator), head of Molecular Parasitology laboratory and. Both laboratories are located in the same building. Both PIs have common interest in developing vaccines against different pathogens using common and innovative adjuvants, one of which was discovered and is being developed for clinical use by both groups, thus having carried out several collaborative projects since 2005. Their past collaborative research resulted in 6 published manuscripts, and others are in preparations for publications. They have regular meetings at least once a week to discuss their collaborative projects. Their lab members have often shared the equipments, exchanged reagents and provided technical assistance for each other. They will report their progress of the project through the laboratory seminar on Wednesday as well as by having teleconference meetings and in person with the group at Sabin-Texas. Infrastructural support for research is provided at LFKRI in the form of several core facilities as indicated in the section of the Equipment located in the Core Science Laboratories.

<u>University of Texas Medical Branch</u>

Laboratory: Dr. Tseng has approximately 1,200 sq.ft. of laboratory space. Readily convenient to his laboratory are the vivarium, tissue culture room, and glassware and sterilizing services.

Office: Dr. Tseng has a up-to-date computer in his office and the laboratories are equipped with PC's for data retrieval and record-keeping

Other: On-campus support groups include a computation center in which hardware and software are available for data reduction and statistical analysis, a common darkroom facility, column chromatography, spectrophotometric analysis and routine biochemical and cellular assays. In addition, housed on the sixth floor of the Medical Research Building is the Sealy Center for Molecular Science. The Sealy Center for Molecular Science offers plasmid DNA preparation, plasmid DNA transformation and fragments and oligonucleotide spurification vector/plasmid DNA construction, DNA sequence analysis, genomic and cDNA library construction and screening, bacterial expression of recombinant genes, polymerase chain reaction technology and expertise in various types of mutagenesis. Dr. Tseng will conduct the proposed studies with SARS-associated coronavirus (SARS-CoV) in the campus' BSL-3 laboratory.

BIOHAZARDs AND BIOSAFETY:

The BSL-3 laboratory:

Most of the UTMB BSL-3 laboratories are located in the Keiller building as part of the Center for Biodefense and Center for Tropical Diseases. Laboratory space in Keiller Building includes ten standard BSL-2

laboratories (total space approximately 8,000 ft^2), plus three special BSL-3 laboratories (each 282 ft^2) for work with hazardous viral agents. The BSL-3 laboratories have been inspected by both CDC and USDA/APHIS and are approved for work with “select agents.” Core facilities include: a new, 6,550 ft^2 animal biosafety level 2 and 3 facility; a 750 ft^2 level 2 arthropod containment facility; a fully equipped Electron Microscopy Laboratory; and a darkroom equipped to develop X -Ray film. Additionally, UTMB has recently provided a new BSL-3 lab (235 ft^2) for SARS-related studies. This new lab, located at the fifth floor of Mary Moody Northen Pavilion, is equipped with two tissue culture hoods, two CO2 incubators, one refrigerated tabletop centrifuge, and a computer. The P.I. of this project will have access to this new lab.

All experiments that use infectious SARS-CoV in the preliminary study have been done in one BSL-3 lab in the Keiller building. This BSL-3 lab has one tissue culture hood and one CO 2 incubator. Dr. Tseng is highly trained and experienced in working with BSL-3 infectious agents.

Those who work on SARS-CoV will be under medical surveillance so that if any febrile or lower respiratory illness develops, they will be evaluated medically. The medical evaluation will be by the infectious disease fellow on call in a negative pressure room that contains specific protocols for suspected SARS patients.

Inactivation of SARS-CoV: We have access to a gammacell ^{60}Cosource (Model# 109A, JL Shepherd & Associates, San Fernando, CA) to provide an alternate mode of inactivation to the conventionally employed detergent or chaotropic ion extractions. Samples that require further experimental steps outside of a BSL-3 lab will be irradiated by ^{60}Co to complete inactive SARS-CoV infectivity. The kill curve of SARS-CoV b y gamma irradiation has been determined, in which a single dose of 10^6 rads is sufficient to completely inactivate 1 x 10^7 TCID50/ml.

UTMB’s Health and Safety Service s (HSS) office is responsible for ensuring the safety and security of all the high containment laboratories. The Keiller Building has electronic key card access. As of December, 2003, this building is guarded 24 hours a day, seven days a week, as required by the new federal regulations for buildings in which select agent research is conducted. Because of the high cost of security, UTMB has developed a plan to consolidate select agent research in only two buildings, Keiller and Pharmacology. A designated HSS staff member controls the issue of key cards and police undertake checks on select agent users as required by the USA Patriot Act.

We will update this policy in the future as required by law. Additions and deletions to the system are made through the University Police Department. Both systems track the employee through the UTMBI identification number, a unique number assigned to a new employee. The key card system identifies the ID number, date and time of entry into the building and biocontainment facilities. Removal of access can be accomplished within minutes should the need arise.

Encoded staff members have key card access at specific perimeter entry doors and to specific laboratories. An investigator and support staff are given access to only those laboratories approved for their work. All personnel have been finger printed and are registered with the CDC and the Department of Justice.

A senior Safety Coordinator-level HSS staff member is assigned to all the high containment laboratories and is on call at all times to respond to issues relating to safety and security. Both UTMB Facilities Operation & Management and HSS routinely conduct visual confirmation of performance several times per week. The frequency is increased according to project demands. Written authorization is required of any person entering the facilities that is not approved, and these individuals are kept at a minimum.

UTMB’s HHS is a division of Preventive, Occupational & Environmental Medicine designed to maximize workplace health, safety and well being through nine integrated prevention programs. One compo nent, the Biological and Chemical Safety Program has the mission of providing a comprehensive approach to health and safety for UTMB. The staff provides direction and consultation for the safe use, handling and storage of biological and chemical agents in the research, academic and healthcare arena. The program provides:

consultation and technical information for handling biological agents; reviews proposals and protocols for the use of hazardous biological agents, rDNA, and submits these to the Biological Safety Committee with recommendations; oversees the annual certification of biological safety cabinets by an outside contractor; presents biological safety seminars upon request; reviews and approves purchases of biological safety cabinets and other safety-related equipment; surveys laboratories for compliance with approved standards and policies of UTMB, CDC and NIH; provides assistance or advice in the disinfection of facilities and equipment; assists in the development of safety plans and training programs. Compliance The University of Texas Medical Branch has an existing safety program that is in accordance with appropriate Federal, State, and local regulations, and that hazards have been identified, eliminated, and/or controlled and that research can be performed safely under BSL1, BSL2, and BSL3 laboratory conditions. Similar safety programs are in development for the new BSL4 facilities that are under construction. All principal investigators are responsible for the implementation and adherence to University safety policies and procedures. Failure to do so could result in suspension or withdrawal of committee approvals for approved research projects.

Institutional BioSafety Committee UTMB researchers using BSL2 and BSL3 agents are required to have Institutional Biological Safety Committee (IBC) review and approval prior to use. The UTMB Biological Safety Committee requires the submittal of a Notification of Use for Biological Agents and rDNA (NOU) for biological agents meeting criteria for BSL2 and BSL3. Included in the NOU submittal are the written procedures for transport, surface decontamination, spill procedures, methods for disposal, methodology, transmission routes and a review of occupational health issues as well the description of Principal Investigators experience and scope of research. The IBC is directly linked into the University Employee Health Center and Health and Safety Services. The IBC meets once a month, allowing for timely review of the submitted NOU. Committee membership includes the County of Galveston Health Department Epidemiologist as a voting community member. CDC/HHS select biological agent users must have received Biological Safety Committee approval prior to CDC Select Agent Registration submission.

Training Additional training opportunities include Lab Safety Orientation, a 3-hour course covering laboratory safety issues in detail. Lab Safety Refresher is required every two years. EH&S staff are also available to develop and conduct topic specific training, as needed. Biosafety Level 3 users meet with Environmental Health and Safety prior to being allowed access to the BSL3 laboratory for initial BS L3 training. Users are referred to t he Director of the BSL 3 facility to complete initial check of f to prepare for entry procedures. First time users are mentored during an initial training period then allowed limited key card access to the facility. Once training has been completed 24 hour, 7-day-a-week access is granted. Blood-borne pathogen training is conducted during initial employee orientation by UTMB Healthcare Epidemiology personnel and updated periodically. Radiation Safety training is provided by the Radiation Safety Program. Basic Radiation Safety in the Laboratory and Authorized U ser training programs and refreshers are provided to radioactive material users.

Disposal of Hazardous Materials Hazardous chemicals and radioactive materials are disposed and/or recycled by the Chemical and Radioactive material Pickup Program administered through Environmental Health and Safety, Environmental Protection Program. Chemicals are evaluated for potential recycling, reuse or final disposal. The University contracts with outside contractors for ultimate disposal of chemical and Radioactive wastes. Biohazardous waste is placed into red bag lined single use biohazard boxes. The boxes are transported to the campus Services Building and are incinerated on site. The University owns and maintains a licensed medical waste incinerator, which is operated by EnviroClean Inc. All disposable material leaving the BSL3 facilities are autoclaved, then placed in a biohazard container for incineration or non-disposable items are either autoclaved or chemically disinfected then sent out for final processing. Sharps containers are used to dispose of needles, capillary tubes, glass Pasteur pipettes and glass slides as required by the State of Texas Municipal Solid Waste Regulations for Healthcare Facilities.

The Center for Biodefense and Emerging Infectious Diseases (Keiller Building) BioSafety Level 3 Manual:

The Keiller BSL3 Manual is focused toward safety procedures and protocols for the *in vitro* BSL3 laboratory and the BSL3 insectary. The manual provides information such as entry procedures, spills, personal protective equipment, transportation of material and disinfectants.

EQUIPMENT

Baylor College of Medicine

The BCM laboratories are equipped with 3 New Brunswick BioFlo 3000 Fermentors, which have the capacity to run up to 10L fermentations (testing parameters including temperature, pH, dissolved oxygen, agitation rate, induction time, wet cell weight, and yield). This information is then transferred to scale up protein production. In addition, it contains a UV/VIS Spectrophotometer Smartspec 3000 (Biorad) Percival L33NLC8 intellus environmental incubator Vortex (2), stirrer (2), shakers, dry bath (2).

Equipment available for purification processes include: FPLC (2 *ÄKTA Explorer and 1 AKTA Pilot*), HPLC (Waters Liquid Chromatography System) or peristaltic pumps for larger scale processes. Other equipment and resources include: Victor3 multilabel counter (OPA), Invitrogen Xcell protein gel electrophoresis apparatus; SDS-PAGE-related equipment for protein characterization; Agarose and polyacrylamide gel electrophoresis equipment for analysis of nucleic acids and proteins; Balances, pH meter, water baths, microwave oven, and Milli-Q water system.

The imaging facility will include the following instrumentation:

- Siemens Inveon PET/SPECT/CT
- Brucker 9.4T/20 MRI
- Xenogen IVIS 100 Bioluminescence
- VisualSonics Vevo Ultrasound
- Siemens Sequoia ECHO
- RadSource RS 2000 Biological Irradiator

New York Blood Center

Equipment in Viral Immunology Laboratory

Tecan Ultra 384 multi-function reader; Beckman-Coulter Analytic Ultracentrifuge; Beckman L7-65 Ultracentrifuge; Carver tablet press; Tablet four usage tester; Osmometer (Model 3320, Advanced); ÄKTA™ purifier (FPLC) from GE; Waters HPLC System; Cytofluor 2300 fluorescence measurement system (Millipore); IBI STS45 DNA sequencing unit; Dynatech ML2252 Microtiter Luminescence Detection System; Spex Fluorolog FL212 Spectrofluorometer. Perkin Elmer Cetus Gene Amp PCR system 9600; 3 biosafety hoods; Pro/Pette liquid handling system (Perkin Elmer Cetus); Nikon flourescence microscope; 3 microscopes, 2 inverted microscopes; DuPont Ommispin R; Sorvall RT 6000 and Sorvall RC-5C centrifuges; 5 freezers, 3 refrigerators, Isotec Fisher Laboratory Refrigerator; 3 CO_2 incubators; Coulter Electronics cell counter, 3 water baths; Oven; Labconco laboratory steam washer; Barnstead Nanopure system; Lab-line incubator-shaker; Savant Speed-Vac concentrator; 3 fraction collectors; Perkin Elmer spectrophotometer; Gilson Fluorometer; Biorad gel-dryer and slicer; Skatron 96-well cell harvester; Thermolyne Cryo Biological Storage System; Büchi Rotavapor R-124; The BSL3 facility has 2 biosafety hoods; an autoclave; Sorvall RC3, Beckman J-6B and Beckman L28-M. centrifuges; refrigerators and freezers.

Equipment in Laboratory of Molecular Parasitology

1 Leica 3MC dissecting scope, 2 Innova 4000 incubator shaker (New Brunswick Scientific Company), 1 Innova 44R incubator shaker (New Brunswick Scientific Company), 1 water bath; 1 Perkin-Elmer Cetus DNA thermal cycler, 1 Gene Machine II DNA thermal cycler, Equipment for gel electrophoresis and blotting, 1 dimensional and 2 dimential, Sub Cell GT nucleic Acid electrophoresis systems, Prep Cell model 491 (Biorad), Rotofor Cell (Bio-Rad), 2 Laboratory Refrigerators (Precision 812 and Sears Coldspot), 1 Microplate Autowasher Dynex Utrawash Plus, 2 Imperial III incubators, 1 bench top centrifuge (Fisher), 2 Eppendorf Centrifuge 5415C and 4 picofuges, Uvicord SII, pump, fraction collector and recorder (Parmacia Biotech), WL/Uv light box plus Polaroid Gel Cam camera, Microinjection appratus, including a micromanipulator, A Horizontal needle puller PN-30, Nikon Eclipse E600 microscope with attached digital camera and video monitor, Electrophoresis power supplies (5), DigiTerm incubator (Tritech Research), UV/VIS Spectrophotometer Smartspec 3000 (Biorad),

Percival L33NLC8 intellus environmental incubator Vortex (2), stirrer (2), shakers, dry bath (2), 1 biological safety cabinet, 1 CO2 water-jacketed incubator for tissue culture work (Forma Scientific), 1 Speed Vac Concentrator and a refrigerated vapor trap (Savant), 1 DrygelSr model SE 1160 (Hoefer Scientific), 1 Frost Free/Magic Chef Refrigerator, 2 -20°C freezers, So-Low Ultra Low and Cryo-Fridge, 2 vacuum pumps, 1 bench top Sorvall RT7 refrigerated centrifuge, 1 water bath, 1 Sorvall RC Plus centrifuge + SS-34 and SLA 3000 rotors, 1 Milli-Biocel and Milli-RiOs 8 water purification system (Millipore), Nikon Eclipse E400 microscope, Nikon TMS Tissue Culture Microscope, Eppendorf Centrifuge 5415C, Vortex, stirrer, pH meter, 2 balances, microwave, 1 Taylor Wharton K Series liquid nitrogen storage tank, 1 Revco ultra-low temperature freezer with 24-hour electronic temperature surveillance located in a central freezer facility, Zeiss Axioskop equipped with epifluoresece filter sets and digital camera.

Equipment located in the Core Facilities

Jasco circular dichroism (CD) spectropolarimeter (Model J-715, Jacobin, Japan); Biacore 3000 (Biacore, Piscataway, NJ); ; Electron Microscope, EM 410, Philips Electronic Instruments/FEI equipped with a Digital Camera; Zeiss LSM 510 META Confocal Microscope; Pi1er Peptide synthesizer (Applied Biosystems); Bench top alignment-free multipurpose analyzer from BD Immunocytometry Systems (FACSCanto); FACSCalibur analyzer; MoFlo high-speed cell sorter (DAKO Cytomation); Isothermal Titration Calorimetry (ITC); Differential scanning calorimetry (DSC); PEB Voyager DE Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometer; 492 Protein Sequencer; DNA Sequencer (3100 Genetic Analyzer); HPLC (HP 1100 & HP 1050); Sun Sparc Station I + computer workstation; 4 Packard Liquid Scintillation Counters: 3375, 3385, 2660, Minaxi 4430; 2 Nuclear Chicago Gamma Counters 1185, 1195; Viscometer (Model SVM 3000, Anton Paar); 3 Ludlum Survey Meters; Research Products International Series 900 Mini-Monitor; Packard Gamma Minaxi Counter; Shimadzu CS9000U Dual Wavelength Flying Spot Scanner; Tri-Carb 2900RT (Packard BioScience); Minaxi Auto-Gamma 5530 (Packard BioScience); Beta plate Flatbed Scanner 1205-001 (PerkinElmer); Storm 860 (Molecular Dynamics/Amersham Bioscience); Konica SRX-101A Film Developer; Alpha Beta Sample Counter

University of Texas Medical Branch

In Dr. Tseng's laboratory: 1 Microscope; Refrigerators; CO2 incubators; Laminar flow hoods; Deep freezers; Refrigerated Centrifuges; Spectrophotometer; Photometer; Thermal cycler; Gel electrophoresis equipment

Flow Cytometry Unit - In the nearby MRB is the Flow Cytometry Unit for the UTMB campus. The unit has a Coulter EPICS Profile flow fluorocytometer, a Becton-Dickinson FACScan and a Becton-Dickinson FACSVantage flow cytometer.

PRODUCT DEVELOPMENT STRATEGY

Milestones and Timeline

Overview. The development of a vaccine represents a major endeavor taking years to achieve. It requires a program overseen by effective management that is based on a strong scientific evidence base, product development and manufacturing under a quality umbrella, and requisite preclinical and clinical studies. A Product Development Strategy (PDS) is generally drafted to provide an overall guidance, a set of specific and achievable goals, the schedules required to reach the ultimate objective, a set of alternative approaches, and the regulations that must be addressed, such as those related to product quality and safety.

This section, Milestones and Timeline, will describe the major development milestones including a risk management strategy if impediments arise that could require a revision in the work plan.

The likelihood of success for this project to develop an rRBD-based vaccine to prevent SARS relies on:

1) An extensive body of preliminary proof of concept data,
2) New innovations for product development and vaccine formulation,
3) The strong expertise of the operational management group, the technical units and industry partners with a track record of transitioning antigens through process development, cGMP manufacture, and FDA regulatory filings, and
4) Risk management strategies that include interim objectives, alternatives and accordingly, the decision points during the 5 years timeline.

One of the strength of this proposal is the selection of a group of experienced scientists from different fields that bring into this project the expertise to support all the activities within the proposal. The vaccine will be developed by BCM-Sabin, the Sabin Vaccine Institute's product development partnership located at Texas Children's Hospital and Baylor College of Medicine, in collaboration with academic partners at New York Blood Center (NYBC) and University of Texas Medical Branch Galveston (UTMB) and two industrial partners, Immune Design Corporation and Frontier BioSciences. In addition, we will collaborate through subcontracts with Walter Reed Army Institute of Research (WRAIR) for cGMP manufacture. A strength of the proposal is BCM-Sabin's 11 year track record of tech transfer of recombinant vaccines through cGMP manufacture with WRAIR including a hookworm vaccine that completed phase 1 clinical testing (42, 74, 75). BCM-Sabin will also provide regulatory and quality assurance and link our project with the industrial partners. Thus, experts have been specifically chosen for their individual strengths and our history of collaborating in other areas of vaccine product and clinical development.

The multi PD/PIs will be responsible for managing, coordinating, monitoring and supervising the many facets of the program to mitigate risk and maximize the likelihood for achieving the goals of each project, as well as to ensure that the activities interrelate and follow a strategic plan in the proposed timelines. Each collaborator will manage their activities and tasks at their respective sites and will be independently responsible for the hiring of personnel, the day-to-day supervision of projects, the recording of data according as required by the project, the recording of financial expenditure and keeping of accounts, the preparation of reports and ensuring regular interaction with other members of the consortium., including weekly teleconferences and/or meetings, in addition to quarterly program reviews with an expert advisory committee comprised of senior vaccine development experts.

Developmental Milestones. This R01 proposal aims to maximize success in the development of an anti-SARS vaccine based on the receptor binding domain (RBD) of the SARS-CoV spike (S) protein, by establishing the feasibility of upscale expression of the recombinant RBD (rRBD) protein, selecting the most appropriate expression system, and scalable manufacturing process. These activities include the development of a series of critical biochemical and biophysical assays for in-process, release and stability testing, which will result in a high-yield reproducible production process and a high quality and stable product that is potent and stimulates the desired protective antibody and cellular responses with co-formulated adjuvants. These represent essential steps for downstream pilot cGMP manufacture, GLP toxicology testing, IND filing with the U.S. FDA and ultimately clinical testing using adjuvant/vaccine combinations of choice. Ultimately the proposed studies will accelerate the development of the rRBD-based vaccine for human use.

To rapidly translate our initial proof of concept findings on the rRBD-based vaccine into a solid platform that will yield new approaches and strategies for product development, vaccine formulations will be optimized and

tested in mice to establish dose, schedule, and routes as well as confirmatory efficacy of the optimally expressed rRBD proteins *in vitro* and *in vivo* using two animal models. Since vaccination with the rRBD protein alone may not induce strong immune responses, alum based adjuvants as well as a novel TLR4 immunostimulant, GLA, will be assessed alone or in combination.

The ***Ultimate Goal*** of the proposed studies is to develop a highly effective and safe RBD-based vaccine that can be used in humans. SARS outbreaks remain a serious global concern mainly due to possible zoonotic reintroduction of SARS-CoV into humans or accidental release from a laboratory or deliberate spreading of the virus by a bioterrorist.

The proposal has three specific aims that will be performed in part in parallel with one another, while some activities will depend on the completion of the product development aspects of the project: 1) Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate; 2) Process development, characterization, formulation and stability profiling 3) Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation. *Ultimately, these studies will provide the basis for accelerating the conduct of a "first-in-human" Phase 1 study of the rRBD-based vaccine for preventing future SARS outbreaks and for biodefense preparedness*.

The following milestones will be achieved through the performance and development of the specific aims detailed in this application:

Milestone 1. A suitable expression system is selected for expression of rRBD in small scale. Aim 1 will focus on the selection of the expression system expression based on yields, purity, antigenicity, functionality, immunogenicity, and efficacy (for inducing neutralizing antibody responses and protection against SARS-CoV challenge) of the rRBD protein. These attributes will be evaluated with qualified assays outlined in Table I. Based on the outcomes of assays each expression system will be scored and ranked as described in the proposal. An rRBD antigen preparation with a category score < 3 or final score < 15 will be removed from further consideration (see Table 1 in the proposal). A lead rRBD from one of the expression vectors with the highest final score will be selected for subsequent studies for optimization of immunization regimens (Aims 1E-G), and process development and pilot cGMP manufacture (Aims 2 and 3).

Table I. Assays for yield, purity, antigenicity, functionality, immunogenicity, and efficacy of rRBD protein

Application	Type of Assay
Yields	HPLC (RP-HPLC and/or SE-HPLC), protein concentration, SDS-PAGE stained with Coomassie Brilliant Blue or Silver Stain
Purity	HPLC and SDS-PAGE stained with Coomassie Brilliant Blue or Silver Stain, HPLC, Mass Spectrometry, Residual Host Proteins (by slot-blot)
Antigenicity	ELISA using RBD-specific and confirmation-dependent mAbs, including 33G4 (Conf V), 24H8 (Conf I), 31H12 (Conf II), and 35B5 (Conf IV), and human convalescent sera from at least three different SARS patients containing neutralizing antibodies.
Functionality	ELISA or ITC for measuring binding of rRBD protein to soluble ACE2 or flow cytometry for detecting the binding of rRBD protein to ACE2-expressing cells.
Immunogenicity	1) ELISA for determining the titers of the RBD-specific antibodies, including IgG1, IgG2a, IgG2b, IgG3 and IgA; 2) ELISPOT for MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte (CTL) responses, including the induction of IFN-γ, IL-2, IL-4 and IL-10.
Efficacy	1) Luciferase assays for measuring the titers of neutralizing antibodies against pseudoviruses expressing SARS-CoV S protein; 2) Cytopathic effect assay for determining the titers of neutralizing antibodies against live SARS-CoV infection; 3) use of the lethal MA15/BALB/c and/or SARS-CoV/ACE2 transgenic mouse models for evaluating the protection of rRBD-based vaccine against SARS-CoV challenge.

Milestone 2. Optimized immunization regimens that provide the best antigenicity, immunogenicity and efficacy for rRBD formulated with alum alone and/or in the presence of GLA, an innate immunostimulant. Once the most optimal expression system is selected, it will be used to generate research lots of rRBD protein for further characterization and confirmatory studies as well as for optimization of immunization regimens by testing different antigen doses and adjuvant formulations (Table II). Once the optimal dose and adjuvant formulation are identified, different routes of administration, number and interval of immunizations will be tested (Table III). The efficacy of the rRBD-based SARS vaccine to induce protection

and long-term immune responses will be evaluated in a SARS-CoV infection mouse model using an optimized immunization regimen (Table III) and challenge studies (Table IV). The goal is to find the best vaccine formulation and vaccination regimen that induces high titers of neutralizing antibodies and high levels of CTL responses as well as protection against homologous and heterologous virus challenge, while using the least amount of the rRBD antigen within the vaccine and number of immunizations.

Table II: Optimization of antigen doses and adjuvant formulation

Group	Treatment	Group	Treatment
1	rRBD (5 µg) + PBS, s.c., 3 immunizations	7	rRBD (10 µg) + GLA, s.c., 3 immunizations
2	rRBD (5 µg) + alum, s.c., 3 immunizations	8	rRBD (10 µg) + alum+GLA, s.c., 3 immunizations
3	rRBD (5 µg) + GLA, s.c., 3 immunizations	9	rRBD (20 µg) + PBS, s.c., 3 immunizations
4	rRBD (5 µg) + alum+GLA, s.c., 3 immunizations	10	rRBD (20 µg) + alum, s.c., 3 immunizations
5	rRBD (10 µg) + PBS, s.c., 3 immunizations	11	rRBD (20 µg) + GLA, s.c., 3 immunizations
6	rRBD (10 µg) + alum, s.c., 3 immunizations	12	rRBD (20 µg) + alum+GLA, s.c., 3 immunizations

Table III: Optimization of immunization route and dose frequency of immunizations

Group	Treatment	Group	Treatment
1	rRBD + adjuvant, s.c., 1 immunization	10	PBS + adjuvant, s.c., 2 immunizations, 4-week interval
2	PBS + adjuvant, s.c., 1 immunization	11	rRBD + adjuvant, i.m., 2 immunizations, 2-week interval
3	rRBD + adjuvant, i.m., 1 immunization	12	PBS + adjuvant, i.m., 2 immunizations, 2-week interval
4	PBS + adjuvant, i.m., 1 immunization	13	rRBD + adjuvant, i.m., 2 immunizations, 4-week interval
5	rRBD + adjuvant, i.d., 1 immunization	14	PBS + adjuvant, i.m., 2 immunizations, 4-week interval
6	PBS + adjuvant, i.d., 1 immunization	15	rRBD + adjuvant, i.d., 2 immunizations, 2-week interval
7	rRBD + adjuvant, s.c., 2 immunizations, 2-week interval	16	PBS + adjuvant, i.d., 2 immunizations, 2-week interval
8	PBS + adjuvant, s.c., 2 immunizations, 2-week interval	17	rRBD + adjuvant, i.d., 2 immunizations, 4-week interval
9	rRBD + adjuvant, s.c., 2 immunizations, 4-week interval	18	PBS + adjuvant, i.d., 2 immunizations, 4-week interval

Table IV: Efficacy of RBD-based vaccine to protect against SARS-CoV challenge in the lethal MA-15/Balb/c mouse model

Group	Treatment
1	Challenge mice with SARS-CoV 5 days post-last immunization, monitor the accumulated mortality, and sacrifice 3 survivals (if any) at day 3rd, 6th, and 9th post-infection
2	Challenge mice with SARS-CoV 10 days post-last immunization, monitor the accumulated mortality, and sacrifice 3 survivals (if any) at day 3rd, 6th, and 9th post-infection
3	Challenge mice with SARS-CoV 15 days post-last immunization, monitor the accumulated mortality, and sacrifice 3 survivals (if any) at day 3rd, 6th, and 9th post-infection

Milestone 3. Established analytical/biochemical and biophysical assays for rRBD. Process development and downstream manufacturing rely on developing assays for the product's characterization (appearance, identity, conformation, integrity and purity), identification of contaminants, and evaluation of stability. The optimization and development of antigen-specific assays such as those shown in Table I will lead to the qualifications for each assay (sensitivity, specificity, accuracy, and reproducibility) as well as product specifications, (qualitative and quantitative ranges of values) which are essential quality and consistency criteria during the production process. Therefore, it is planned to simultaneously use and evaluate these assays as the recombinant rRBD is made available. Biologic products may be especially susceptible to degradation and potential biochemical modifications. These assays will be able to monitor the proteins and provide meaningful information on the stability of the recombinant proteins. In addition, various denaturing/solubilizing agents will be evaluated using the biophysical techniques. The goal will be to complete a phase diagram. The information obtained from these assays will identify potential stabilizers for the recombinant protein with the goal to maximize multiple aspects of physical stability.

TABLE V contains a tool-box of qualified assays, combined with those described in Table I, which will be used to assess the identity, integrity, bioactivity and stability of recombinant proteins during in-process, release and stability testing for rRBD-based SARS vaccine; all of which will support the success of finding the optimized conditions for the production of a scalable manufacturing process development for potent rRBD to support downstream pilot cGMP manufacture, toxicology testing, IND filing with the FDA and clinical testing.

Table V. Qualified analytical/biochemical and biophysical assays for assess the identity, integrity, stability of rRBD proteins during in-process, release and stability testing

(b)(4); (b)(3):7 U.S.C. § 8401

Milestone 4. Established a reproducible 10L scale process for a stable rRBD-based vaccine in preparation for future technology transfer to a cGMP manufacturing facility. Biologics must be developed, produced and tested using a comprehensive framework that must consider a number of unique features related to yield and cost, purity, consistency, identity, antigenicity and immunogenicity, formulation, and thermostability. Under this aim, fermentation, purification and buffer formulation parameters will be further optimized and evaluated at the 10L scale. The goal will be to establish reproducible high-yield production processes. As these studies are completed, the stability of the rRBD will be assessed. Both accelerated and long term stability studies will be conducted on the final formulations by monitoring physical changes in the protein formulation over time as detected using biophysical assays. This activity is critical for the future success during technology transfer to a manufacturing facility. Based on the experiences of BCM-Sabin all the reagents, processes and equipment used at the 10L scale should be compatible to those to be used during the manufacturing process. Therefore, efforts will be made so that at the time of transfer, scale will be the only variable that could be modified, since changes in almost any other parameter could result in run failure.

Milestone 5. Successful pilot cGMP manufacture and completed GLP toxicology testing. Process development and formulation technology for the selected rRBD-based vaccine will be successfully transferred to the WRAIR pilot cGMP facility and both drug substance and drug product will be evaluated during the in process and final lot release. Certificate of Analyses (CoA) for the cell banks, drug substance and product will be generated and a formal stability program will be initiated. A pre-IND meeting will be requested with the U.S. FDA and GLP toxicology testing at Frontier Biosciences will be completed with an acceptable safety profile. The extensive array of assays developed and qualified throughout Aims 1 and 2 will be used to establish criteria for the evaluation of the expression during manufacturing and vaccine formulations. These same specifications will be used throughout the stability program.

Milestone 6. IND Regulatory Package Preparation and Submission. An IND package will be prepared containing all the information related to the chemical, manufacture and control (CMC) as well as results of all the preclinical animal studies, GLP toxicology results and a proposed clinical development plan. Similar to other vaccine development programs of BCM-Sabin, the ultimate intention is to have a completed IND package in order to advance into clinical testing and accelerate the *"first-in-human" Phase 1 safety study of the rRBD-based vaccine for* preventing future SARS outbreaks and for biodefense preparedness.

Risk management and alternative approaches that will support the above milestones are detailed within the proposal. As part of our risk management strategy, we build in alternative pathways to arrive at each milestone. For example in milestone 1, multiple expression systems are available to us both for *E. coli*

(including pET/BL21 systems, pTrcHis/BL21, and pBAD) and *P. pastoris* and *Saccharomyces*. For milestone 2 we have built in multiple immunization regimens, while for milestone 3 our system of assay development, qualification, and implementation has already led to two IND submissions with CMC section acceptable to the FDA. For milestone 4 through DOE and multiple process development runs we will maximize the likelihood of deriving a process to produce recombinant RBD at the highest yield and purity, while for milestone 5, we have an extensive track record of successful technology transfer to WRAIR, but we have also worked successfully with additional CMOs including Aeras Global TB Foundation, and our long-standing collaboration with Instituto Butantan, Brazil's major vaccine manufacturer. For milestone 6, we have a strong regulatory group, which has now successfully completed two INDs for neglected disease recombinant vaccines.

Invention report, patent status or other protection of project intellectual property (IP)

<u>We have had the following patent related to the proposed studies</u>: Jiang, S., and He, Y. Neutralizing monoclonal antibodies against severe acute respiratory syndrome-associated coronavirus. US Patent 7,629,443. Filed February 8, 2006. Issued December 8, 2009, assigned to the New York Blood Center. This invention shows that a recombinant protein containing the receptor-binding domain (RBD) in the SARS-CoV S protein (residues 318–510) could induce highly potent neutralizing antibody responses in the immunized mice, and thus a panel of 23 monoclonal antibodies (mAbs) with potent neutralizing activity and ability to recognize six different conformational epitopes in the RBD domain were discovered. Accordingly, the RBD of SARS S protein is an important target site for developing vaccines and immunotherapeutics. It is anticipated that BCM-Sabin will file an additional patent on the composition and use of the formulated rRBD SARS CoV vaccine.

TABLE VI. Proposed Project Timeline

	Year 1	Year 2	Year 3	Year 4	Year 5	Responsible Group
Specific Aim 1. Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate						
1.A. Feasibility of expression						BCM-Sabin
1.B. Antigenicity and functionality						NYBC
1.C. Immunogenicity						NYBC
1.D. Efficacy						NYBC/UTMB
1.E. Optimization of the immunization regimens						NYBC/UTMB
1.F. Efficacy of rRBD-based vaccine to induce cross-neutralizing antibody responses and cross-protection in mice						NYBC/UTMB
1G. Efficacy of the rRBD-based vaccine to induce long-term immune responses and protection.						NYBC/UTMB
Specific Aim 2. Process development, characterization, formulation and stability profiling						
2.A. Development and optimization of a 10L scale process (fermentation & purification)						BCM-Sabin
2.B. Assay development						BCM-Sabin
2.C. Execution of 3 successive process development runs at the 10L scale						BCM-Sabin/NYBC
2.D. Formulation and Stabilization						BCM-Sabin/NYBC
Specific Aim 3. Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation						
3.A. Strategy for Manufacture of Drug Substance and Drug Product						BCM-Sabin
3.B. Lot release and start of a stability program						BCM-Sabin
3.C. IND Regulatory Package Preparation and Submission						BCM-Sabin

Product Development Plan

Overview. The studies here will lead to the expression, process development, formulation, technology transfer, cGMP manufacture, GLP toxicology and preparation of a regulatory filing (IND submission) of a recombinant vaccine to prevent severe acute respiratory distress syndrome (SARS) caused by the SARS-associated coronavirus (SARS-CoV). The vaccine is comprised of the receptor binding domain (RBD) of the SARS-CoV S protein, expressed as a recombinant protein (rRBD), and formulated with alum and/or GLA, a innate immunostimulant. The studies here will accelerate the development and lead to the first Phase 1 clinical study of a recombinant anti-SARS-CoV vaccine. The vaccine will be developed by BCM-Sabin, the product development partnership of the Sabin Vaccine Institute located at the Feigin Center of Texas Children's Hospital Center and Baylor College of Medicine. BCM-Sabin has an eleven year track record of taking recombinant antigens for two neglected diseases, hookworm infection and schistosomiasis, from antigen discovery, through scale-up process development, formulation, cGMP, regulatory filing, and phase 1 clinical testing (2,20,42,44,67). To achieve the technological and product development objectives of this proposal, BCM-Sabin will collaborate with two academic partners at New York Blood Center (NYBC) and University of Texas Medical Branch Galveston (UTMB). NYBC has a seven year track record of discovering the rRBD antigen and accumulating preliminary data for its protection in experimental challenge models of SARS-CoV, while UTMB has developed an innovative transgenic mouse model that replicates human SARS. In addition two key industrial partners will be actively involved in the project: Immune Design Corporation and Frontier BioSciences as well as the Walter Reed Army Institute of Research (WRAIR) for cGMP manufacture.

Intended use / Indication of the product. The rRBD-based vaccine is a recombinant protein-based vaccine formulated with alum (either Alhydrogel® or aluminum phosphate) and/or GLA, a synthetic TLR4 (toll-like receptor 4) agonist known to induce potent antibody and cellular responses to by stander recombinant proteins (28). The initial indication for the vaccine is for adults and individuals over the age of 15 who are considered at greatest risk of mortality from SARS (68). The vaccine would be administered as an injectable product to protect against lethal SARS-CoV infection and as a single dose or two doses spaced closely together (within 2-4 weeks) so that vulnerable populations could be rapidly immunized in an outbreak setting (Table 1). Another aspect of the target product profile of the rRBD protein is that it would be highly stable (up to four years at 2-8°C) so that it can be stockpiled for emergency use. Finally, low-cost is desirable so that the vaccine could also be used in low- and middle-income country settings in Southeast Asia (where populations are at the greatest risk of acquiring SARS) as well as in the United States and Canada.

Table 1. Target Product Profile of the Recombinant RBD-based Vaccine.

Item	Desired Target
Indication	A preventative vaccine to protect against lethal infection caused by the SARS-CoV
Target Population	Adults and children $\geq$15 years
Route of Administration	Intramuscular injection
Product Presentation	Single-dose vials. 1.0 ml volume of delivery
Dosage Schedule	Maximum of 2 immunizations regardless of age, with the second injection given 2-4 weeks after the first immunization
Warnings and Precautions/Pregnancy and Lactation	Mild to moderate local injection site reactions such as erythema, edema and pain, the character, frequency, and severity of which is similar to licensed recombinant protein vaccines. Less than 0.01% risk of urticaria and other systemic allergic reactions. Incidence of SAEs no more than licensed comparator vaccines.
Expected Efficacy	80% efficacy at preventing SARS associated deaths
Co-administration	All doses may be co-administered with antiviral drugs and/or other vaccines used in public health emergencies
Shelf-Life	4 years
Storage	Refrigeration between 2 to 8 degrees Celsius. Cannot be frozen. Can be out of refrigeration (at temperatures up to 25 degrees) for up to 72 hours
Product Registration	Licensure by the U.S. Food and Drug Administration
Target Price	Less than $10 per dose for use in low- and middle-income country settings

Addressing the biodefense / public health gap. The SARS-CoV is classified by NIAID-NIH as a Category C pathogen. SARS is considered the first new infectious disease of this new century, having originated in Guangdong province of China in November 2002 and ultimately spreading along international air-travel routes to and across five continents and leading to over 8,000 infections and almost 800 deaths, before the outbreak was contained through an aggressive program of quarantine, patient isolation and travel restrictions (1). The majority of SARS patients to date have been adults with only a few cases in children less than 15 years of age (68). While the overall mortality rate from SARS during the 2002-03 pandemic was approximately 10%, the mortality among the elderly exceeded 50% (1). In April of 2003, a new coronavirus (CoV), SARS-CoV, was identified as the etiologic agent of this deadly human respiratory disease. Like other animal CoVs, SARS-CoV is an envelope virus with a single stranded, positive-sense RNA genome encoding a nonstructural replicase polyprotein and several structural proteins including a spike (S) and envelope (E) protein (1). SARS CoV is a zoonotic virus that resides in bats and other natural reservoirs, including palm civets as key intermediate hosts. The virus replicates primarily in airway epithelial cells of humans as well as many different zoonotic hosts. Since the original outbreak, subsequent outbreaks were reported in late 2003 and early 2004, with the latest one in Beijing and Anhui China that originated from accidental exposure to a laboratory virus isolate. SARS outbreaks remain a serious global concern because of possible zoonotic reintroduction of SARS CoV into humans, further escape of laboratory isolates or being used for bioterrorism. The movement of SARS-CoV into human populations suggests an urgent need to prepare vaccines for this emerging agent (68). There is a particular need for high-risk groups including the elderly, healthcare workers and laboratory personnel and for biodefense preparedness (68).

Scientific and technical value of the project. The proposed studies would lead to the development of the first recombinant vaccine for preventing human SARS-CoV infection. Key strengths the product development plan include a strong evidence base of preliminary data for the protective efficacy of the rRBD molecule accumulated by NYBC over the last seven years, and BCM-Sabin's eleven year track record of scale up process development, formulation and tech transfer for cGMP manufacture.

The key technology objectives are the development of a process for vaccine manufacture, utilizing an appropriate expression system selected based on small scale feasibility studies using primarily a bacterial expression system and with a parallel evaluation of the yeast system as back-up. Process development will emphasize inexpensive fermentation and purification methods to produce the rRBD protein antigen component of the vaccine with optimized yields and minimized costs. These activities would take place at BCM-Sabin with confirmatory preclinical testing at NYBC and UTMB. During scale up and while establishing reproducibility, Batch Production Records (BPRs) will be utilized to ensure that complete records are maintained, and to make certain that production conditions can be repeated with absolute accuracy. Before transferring technology to a cGMP facility, the final process will be repeated a minimum of three times at the 10 L scale, to assure that vaccine manufacture, from fermentation through formulation, reproducibly results in product of sufficient yield and quality. In addition, antigen/adjuvant formulation studies will be established both biochemically and by immunological assessment (potency). By performing a minimum 3 full runs with in process and final quality control, we will ensure the adequate planning of this transitional step and prevent a transfer of the technology that could otherwise lead to significant losses in yield or an improperly folded recombinant protein adjuvant. Preliminary accelerated and long term stability studies will be conducted on formulations by monitoring analytical/biochemical and biophysical changes in the protein and formulation over time. Studies of the chemical stability of the recombinant protein with an emphasis on oxidative and deamidation processes will also be conducted using high pH to induce deamidation and various oxidizing agents to produce oxidation. Isoelectric focusing studies will initially be used to detect deamidated as well as some oxidized species. In addition, accelerated stability will be assessed at different time points and after doing temperature incursion experiments (repeated freeze thaw cycles, and at different temperatures). Following formulation of the vaccine on alum and/or GLA in collaboration with the Immune Design Corporation, the technology would be transferred to Walter Reed Army Institute of Research (WRAIR) for cGMP manufacture. The final product would be subjected to a rigorous battery of assays for lot and product release at BCM-Sabin and WRAIR. An added strength of the proposal is BCM-Sabin's decade-long history of tech transfer to WRAIR for recombinant protein vaccines and its long standing collaboration with Dr. Steve Reed previously CEO of IDRI and now CEO of Immune Design Corporation. Following GLP toxicology testing at Frontier BioSciences, an IND regulatory filing

for clinical testing will be prepared and submitted to the U.S. FDA with BCM-Sabin as the sponsoring institution.

The major innovations for this product include:

- Selecting to use recombinant RBD rather than the full-length S-protein as the vaccine antigen in order to limit the possibilities of vaccine induced antibody enhancement (see below).
- Use of a novel combination of biochemical/analytical and biophysical tools to profile and maximize the long-term stability of the vaccine, which is an essential element for a vaccine that might require emergency stockpiling. Biophysical tools including circular dichroism, intrinsic and extrinsic fluorescence, light scattering, and differential scanning calorimetry have been used previously to evaluate vaccine stability (34,35). Collectively, the combination of assays during the development phases provides specific information concerning the physical state of the protein as a function of stresses (i.e temperature and pH fluctuations). Such data will be used to develop assays to screen and identify potential stabilizers such as sugars and other buffering agents, preventing degradation or aggregation of recombinant proteins as well as ensuring compatibility of buffering agents with the adjuvants to be used during vaccine formulations.
- Access to the TLR4 agonist and synthetic lipid A adjuvant known as GLA (glucopyrranosyl lipid A). This 1.7 kDa synthetic molecule with 6 acyl chains and a single phosphorylation site was developed by the Immune Design Corporation (Seattle, WA). In a study in mice with commercial Fluzone®, a killed influenza vaccine, chemically synthesized GLA was effective in enhancing IgG titers (both IgG 1 and IgG2a), both as an oil-water emulsion (squalene oil and surfactant), and as an aqueous formulation (GLA-AF) (3, 4). Key Th1 cytokines, IL-2 and gamma interferon, were also elevated in this study (13). Moreover, a oil-in-water emulsion of GLA with a malaria vaccine, GMZ2 adjuvanted by aluminum hydroxide, have been shown to enable the induction of the highest (a) vaccine-specific IgG2a and total IgG titers, (b) parasite-specific IFA titers, (c) levels of Type 1 cytokine responses (IFN-☐), and (d) number of long-lived-plasma cells secreting antibodies, which are thought to be essential for the development of long-term protective immunity against clinical malaria (28). Preclinical study of the efficacy of rRBD-based vaccine will be studied in two innovative and complementary lethal mouse models: 1) MA15/BALB/c mouse model using a mouse-adapted MA15 virus for homologous virus challenge since it originated from SARS-CoV Urbani strain and can cause SARS-like disease and mortality in BALB/c mice; 2) GD03/Tg AC70 mouse model: Transgenic mice, Tg AC70, express the human angiotensin-converting enzyme 2 (ACE-2) virus receptor (the human SARS-CoV receptor). They are susceptible to infection by both homologous and heterologous SARS-CoV of the clinical isolates, and thus they can be used for evaluation of cross protection. Following infection of such transgenic mice with SARS-CoV, they develop weight loss and other clinical manifestations before reaching 100% mortality within 8 days after intranasal infection (63). The severity of the disease developed in the Tg AC70 mice makes them valuable for preclinical testing of SARS vaccines (63).

Advantages compared to competing products. Currently there are no approved vaccine and antiviral drugs that can be used for prevention and treatment of SARS-CoV infection (68). Several different strategies have been proposed for developing SARS vaccines, including inactivated virus vaccines, and the live attenuated vaccines, adenovirus vectored vaccines, poxvirus vectors, and DNA vaccines encoding the full-length spike (S) of SARS-CoV (68). Safety is the major concern regarding the inactivated SARS vaccines since the production workers are at risk of infection during their handling of the highly concentrated live SARS-CoV and incomplete inactivation may cause SARS outbreaks among vaccinated populations. The live attenuated or genetically engineered vaccines expressing full-length S protein of the 2002-2003 SARS-CoV may induce antibodies that enhance infection by civet SARS-CoV(69), which has been noted with feline CoVs (68,70,71). The ferrets vaccinated with a recombinant poxvirus vector MVA expressing SARS S protein displayed increased liver pathology after challenge compared to other groups (8,68), while vaccination with an N protein expressed in Venezuelan equine encephalitis virus replicon particles was reported to increase eosiniophilic infiltration and damage in lungs of mice challenged with SARS-CoV (64). We are the first group that discovered that the RBD is the most important target for SARS vaccines since it contains the major neutralizing epitopes and some specific T cell epitopes, but no immunodominant non-neutralizing epitopes. Moreover, immunization of animals with the rRBD-based subunit vaccines could induce broad cross-neutralizing and protective activities against the predominant human SARS-CoV strains (9,10,29,40). Compared with the inactivated virus and live attenuated virus vaccines, the rRBD-based vaccine is safer since there is no risk to cause virus infection due to

the vaccination. It is also safer than the full-length S protein since the linear immunodominant domains in S protein could induce high titers of non-neutralizing antibodies (31), some of which could enhance infection by heterologous SARS-CoV, such as the civet SARS-CoV strains (32). Vaccination of ferrets with vaccinia virus-based SARS expressing full-length S protein also caused liver damage after challenge with SARS-CoV (7,33). So far, no one has shown that rRBD vaccines induce infection-enhancing antibodies or harmful immunity (1).

Project goals and key technological objectives. The major project goals to be completed during the 5 years award period include: 1) feasibility of expression evaluating both bacterial and yeast expression systems, and pre-clinical characterization of the differently expressed rRBD protein as a vaccine candidate; 2) rRBD and vaccine formulations will be compared for their ability to induce neutralizing antibodies and protection in laboratory animals against challenge infections with multiple strains of the SARS-CoV using an optimized vaccination regimen and adjuvant platforms; 3) development of a process for the high yield and low cost expression and purification of the RBD protein antigen, followed by formulation on alum (Alhydrogel® or aluminum phosphate) and/or an aqueous formulation with GLA, an TLR4 agonist; 4) development and qualification of product specific assays, followed by process and formulation optimization, which incorporates biophysical profiling; 5) Completing three process development pilot runs with in process and quality control testing; 6) technology transfer for the cGMP manufacture of master and production cell banks, drug substance and drug product; 7) formal release of the drug product based on qualified assays and initiation of a formal stability program; 8) a pre-IND meeting followed by completion of a GLP toxicology study, and 9) IND preparation and submission and approval for clinical testing. The major deliverable at the end of the project period is that the rRBD-based vaccine will be approved for Phase 1 clinical testing by the U.S. FDA. To date, BCM-Sabin, the product development partnership of the Sabin Vaccine Institute, has a track record of transitioning two recombinant parasite antigens for human hookworm infection all the way from discovery through process development, cGMP manufacture, and FDA approval for clinical testing (2,20,67). Another vaccine antigen for schistosomiasis is currently undergoing cGMP manufacture (2).

Performance specifications and features. In order to provide immunological benefit, the rRBD-based vaccine must elicit RBD-specific antibody and cytotoxic T lymphocyte (CTL) responses. In human infections, SARS CoV-specific IgG is detectable two weeks post-infection in humans reaching a peak approximately 60 days post-infection. High titers of neutralizing antibodies as well as specific CTL responses are detectable in patients who recover from SARS, with levels correlating with disease outcome. Neutralizing antibodies and/or CTLs from these individuals mainly target the S protein, specially the receptor-binding domain (RBD), which is comprised of 192 amino acids (amino acids 318-510) in the S1 subunit and is responsible for binding of the spike protein to the receptor ACE2. Indeed, we have found that more than 50% of the neutralizing activity in the sera of convalescent SARS patients is from anti-RBD specific antibody (10-12,30,39,40,72). Moreover, additional studies have shown that RBD can absorb and remove the majority of neutralizing antibodies in the antisera of rabbits, mice, and monkeys immunized with MVA expressing full length S protein (11). As noted below, RBD induces potent neutralizing antibody responses and long-term protective immunity in animal models, and RBD can induce potent cross-neutralizing antibodies against recombinant RBD from the SARS CoV of 2002-03 and 2003-04 human SARS-CoV isolates and the civet SARS CoV. The RBD of S protein contains multiple conformation-dependent epitopes and it is the main domain that induces neutralizing antibody and T-cell responses against SARS-CoV infection (1), making it an important vaccine target.

Description of the candidate product as currently configured. The rRBD-based vaccine is currently comprised of a purified recombinant RBD protein (expressed in *Escherichia coli*) and formulated on alum (either Alhydrogel® or aluminum phosphate) and/or an aqueous formulation of the synthetic TLR4 agonist glucopyrranosyl lipid A (GLA-AF). Shown in Fig. 1 is an rRBD protein expressed in pET-SUMO *E. coli* system containing and purified by HPLC. The purified rRBD (without a tag) exhibits a single band with a molecular weight of 24 kDa on SDS-PAGE and reacts strongly with the RBD-specific and conformation-dependent neutralizing mAbs: 24HB (Conf I), 31H12 (Conf II), 35B5 (conf IV), 33G4 (Conf V) and 12B2 (Conf VI), as well as the linear epitope-specific mAb 17H9 in Western blot.

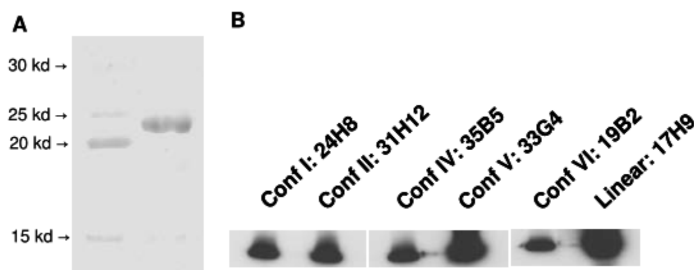

Fig. 1. Purification and identification of rRBD expressed in *E. coli.* (A) analysis of rRBD using SDS-PAGE and (B) identification of rRBD by Western blot using an panel of RBD-specific conformational and linear mAbs.

Assays for product release and characterization. Under a quality management system, BCM-Sabin has developed and implemented numerous assays for biochemical/immunological characterization. An existing panel of orthogonal techniques and assays are used in the earliest stages of process development, and then refined for the specific antigen concurrently with the program. These assays have provided important feedback to direct process development and formulation, regarding the state and stability of the molecule. Additionally, new assays and technology are typically identified and implemented where appropriate and to address specific characteristics of the molecule, such as multi-angle light scattering to detect protein aggregation. BCM-Sabin has performed similar assay qualifications to establish that the release and stability assays used for characterization are accurate, precise, sensitive, specific, reproducible, and robust. Several of these assays, currently used or being developed for RBD protein, have been qualified for other recombinant antigens by BCM-Sabin for Phase 1 clinical trials of hookworm vaccines. A description of the predominant assays proposed for characterization of the RBD protein antigen and formulation are presented below in Table 2. In addition, a variety of standard assays are developed and maintained to analyze pH, protein concentration determination by UV absorbance, and endotoxin content. These techniques will need be qualified and audited by BCM-Sabin's Quality Assurance unit prior to release testing of GMP lot.

Table 2. Qualified assays proposed for product release and characterization of the rRBD-based vaccine

Assay / (Application)	Description
SE-HPLC (bulk protein) In-process, Release, Stability	A size exclusion HPLC assay is used to analyze recombinant protein samples (crude fermentation supernatant to release of purified bulk). This assay has been developed for other antigens and adapted to RBD protein to quantitate target protein from other contaminants during in-process analysis. This assay also serves as a purity assay for RBD protein
RP-HPLC (Bulk protein) Release, Stability	Column and condition screening of a variety of RP-HPLC columns (including silica and polymer based) has been initiated to provide an assay that can better resolve full-length RBD protein from its product-derived cleaved forms (i.e. <1 kD difference).
Multi-angle light scattering (bulk protein) Information Only	A Wyatt Technologies multi-angle light scattering detector utilized both online (w/ SE-HPLC) and offline (Batch) has provided valuable data regarding the aggregation and native state of candidate antigens *IN SOLUTION*. This assay could be used to detect polydisperity of RBD protein and assist in stable formulation development
SDS-PAGE (Bulk protein & Formulation) In-Process, Release, Stability	SDS-PAGE assays under both non-reduced and reducing conditions allow determination of molecular weight, aggregation, and purity of samples. A calibrated scanning densitometer (Bio-Rad GS-800) allows a quantitative aspect to the assay, involving the determination of limit of detection and linear range of the assay to assess purity and relative quantity. This is assay, already adapted and implemented routinely for RBD protein analysis.
N-Terminal Sequencing (Bulk protein) Information Only	N-terminal sequencing has been completed on major, minor, and contaminant bands to identify whether contaminants are product derived and cleavage sites. SDS-PAGE is completed, transferred to a PVDF membrane and sent to a contracted service, audited by BCM-Sabin's Quality Assurance team and subject to a quality agreement.
Mass Spectrometry (Bulk protein) Information Only	Electrospray ionization mass spectrometry (ESMS) is carried out on a Micromass Q-Tof mass spectrometer on samples to monitor mass and any post-translational modifications. This assay will be used for RBD protein.
O-phtaladehyde (OPA) (Formulation) Release, Stability	The OPA assay measures protein content by fluorogenic detection using *o*-phthalaldehyde (OPA), an amine-reacting reagent, in microplates. This technique is especially applicable to vaccine formulations where measurement of pellet (Alhydrogel®-bound) and supernatant (free-unbound) protein content is desired. This assay will be adapted to RBD protein and its formulation buffers and is widely used to determine binding efficiency.
Host Cell Protein (HCP) Content (Bulk protein) In-Process, Release	Residual host cell protein (HCP) impurities are analyzed using a slot blot system with a scanning calibrated densitometer with a picogram level of sensitivity in process fermentation and purification products and final purified protein samples.
Potency, ELISA (Formulation) Release, Stability	Potency will be determined by the median Effective Dose 50 (ED50), i.e., the minimal dose that seroconverts 50% of the mice vaccinated. Measurement of anti-RBD protein IgG will be performed by the indirect ELISA. We have applied this method successfully for the release of several hookworm antigens: it includes a robust in-house animal model and qualified ELISA procedures. A dose ranging study and generation of standard reference sera will be completed prior to assessing the performance characteristics specific to this antigen and qualification of RBD protein ELISA.

The following *in vitro* and *in vivo* assays will be used for evaluating the efficacy of the rRBD-based vaccine to induce specific antibody and T cell responses that protect animals from infection by homologous and heterologous SARS-CoV strains:

1. RBD-specific antibody and T cell responses. We will collect the sera of the rRBD-immunized mice for testing the titers of anti-RBD antibodies, including IgG1, IgG2a, IgG2b, IgG3 and IgA, using a well-established sandwich ELISA as we previously described (12,29,30,41). We will also collect the spleen and lymph nodes for assessing MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte response by using an ELISPOT mouse kit following *in vitro* priming with peptides bearing specific CD8 or CD4 epitopes that we identified (55,56).

2. Neutralizing antibody responses against pseudotyped and live SARS-CoV homologous and heterologous strains. We will determine the titers of the neutralizing antibodies in the sera of mice immunized with rRBD proteins using the pseudotyped viruses expressing the S protein of homologous and heterologous SARS-CoV strains Tor2, GD03, and SZ3 isolated from SARS patients at 2002-2003 and 2002–2003 and 2003–2004 outbreaks, and palm civet, respectively (Table 3)(39), as we previously described (12,29,30,41). We will also test the neutralizing antibody titers using live SARS-CoV Urbani (homologous) and GD03 (heterologous) strains as described before (30,40,55,57,63,64).

3. Viral replication in lung tissues of mice challenged with live SARS-CoV viruses. Mouse lung tissues will be collected for determining the titer of SARS-CoV ($TCID_{50}$/g of lung tissue) with the standard infectivity assay using Vero E6 cells or viral load (RNA copies/μg of lung tissues) using Q-RT-PCR as previously described (30,57,63,64).

We will use the following two animal models for evaluating the *in vivo* efficacy of the rRBD-based SARS vaccine to protect animals from infection by homologous and heterologous SARS-CoV infection: **1.** The MA15/BALB/c mouse model. This mouse SARS-CoV infection model was established by Roberts et al (62), who have shown that the mouse-adapted MA15 virus originated from SARS-CoV Urbani strain could infect BALB/c mice, resulting in SARS-like disease and high mortality (62). We will use this mouse model for evaluating the efficacy of rRBD to induce protection in the immunized animals against challenge with homologous SARS-CoV Urbani strain as previously described (62). **2**. The icGD03-S/Tg AC70 mouse model. This is the first transgenic mouse model of SARS-CoV infection established by Dr. Tseng's group at UTMB. We will use this model to evaluate the efficacy of rRBD-based vaccine against challenge with the homologous (Urbani) and heterologous (icGD03-S) SARS-CoV strains as previously described (57,63,64).

***Rationale*:** An ideal vaccine for preventing SARS outbreaks must possess efficacy to induce high levels of neutralizing antibody response and protection in the vaccinated animals against a broad spectrum of SARS-CoV strains that may cause future outbreaks. We select both MA15/BALB/c and icGD03-S/Tg AC70 mouse models for evaluating the protective efficacy of RBD-based SARS vaccine because infection of SARS-CoV in these animal models can cause SARS-like disease and high mortality rate. Use of MA15/BALB/c mouse model is economic, but it cannot be used for evaluating the cross-protection against heterologous SARS-CoV challenge. To complement these studies, the icGD03-S/Tg AC70 mouse model can be used for evaluating the cross-protection against challenge with both homologous SARS-CoV strains, such as Urbani, and heterologous strains, e.g., icGDO3-S, which encodes the S glycoprotein gene of the most genetically divergent human strain, GDO3 (Table 3) (66) and could efficiently replicate in mouse lungs and in human airway epithelial cells (64). We will use the pseudotyped viruses expressing the S protein of the SARS-CoV strains Tor2, GD03, and SZ3, the representative strains of human 2002–2003 and 2003–2004 SARS-CoV and palm civet SARS-CoV, respectively (39), for evaluating the cross-neutralizing antibody responses in the rRBD-vaccinated mice because the future SARS outbreaks may be caused by a human SARS-CoV strain that may be accidentally released from a laboratory inventory or deliberately spread by a bioterrorist. The outbreak may also be caused by a new SARS-CoV isolate originated from the natural animal reservoir, like the palm civet SARS-CoV (73). We will use the intranasal route to challenge mice with SARS-CoV since this route was proven to be effective for SARS-CoV infection (55,64,74). The primary efficacy endpoint of rRBD-based SARS vaccine is the level of protection of the vaccinated animals against SARS-CoV challenge and the secondary endpoint is the ability of the rRBD protein to induce SARS-CoV neutralizing antibodies and specific T cell responses.

Table 3. Representative SARS-CoV strains that will be used for testing antibody neutralizing activities.

Virus	GenBank Accession#	Isolated from	Substitutes in S protein	Substitutes in RBD
Tor2	AY274119	SARS patients at 2002-2003 outbreak	0	0
Urbani	AY278741	SARS patients at 2002-2003 outbreak	0	0
MA15	DQ497008	Adapted from Urbani in BALB/c mice	1	1
GD03	AY525636	SARS patients at 2003-2004 epidemic	9	3
SZ3	AY304486	Palm civets	17	4

Regulatory experience and discussions with FDA. The vaccine will be developed by BCM-Sabin, Sabin's product development partnership (PDP) located at Baylor College of Medicine. BCM-Sabin is an established PDP for the development of vaccines to combat neglected diseases. BCM-Sabin serves as the regulatory sponsor for vaccines produced within the partnership, and has the lead role in filing INDs with the U.S. FDA and as well as other national regulatory authorities. BCM-Sabin submitted its first IND to the FDA in 2004, and conducted a Phase 1 clinical trial of the safety and immunogenicity of the *Na*-ASP-2 Hookworm Vaccine under BB-IND 12166 in the U.S (20,42,67). A second IND for a different hookworm vaccine has also been approved by the FDA and submitted to ANVISA for a phase 1 study in a hookworm-endemic area of Brazil in a field site established in collaboration with the Brazilian Ministry of Health's Fundação Oswaldo Cruz (2,44). In addition, a pre-IND meeting has been held for BCM-Sabin's new *Sm*-TSP-2 schistosomiasis vaccine. For this proposal, BCM-Sabin will review, synthesize, and assemble all preclinical data, CMC, toxicology data and a Phase 1 safety study protocol for the rRBD-based anti-SARS CoV vaccine and will submit an IND to the FDA.

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

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RESEARCH & RELATED Senior/Key Person Profile (Expanded)

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RESEARCH & RELATED Senior/Key Person Profile (Expanded)

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RESEARCH & RELATED Senior/Key Person Profile (Expanded)

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