PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

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

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

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

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1241-Specific_Aims.pdf	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1242-Research_Strategy_PJH.p	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects	1257-Human_Subjects.pdf	Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities	1258-Inclusion_wom_Min_PJH.p	Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table	1259-Targeted_enrollment.pd	Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children	1260-INCLUSION_OF_CHILDREN.p	Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1261-Vertebrate_animals.pdf	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan	1262-Multiple_PI_Plan_PJH.pd	Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements	1263-Contractual agreement_1	Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1264-Support_Letters.pdf	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	1265-Resource_Sharing_Plan.p	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

List of Research Plan Attachments

Specific Aims

Severe acute respiratory syndrome (SARS), the first emerging infectious disease of the 21st century, can inflict devastating disease outcomes and disrupt the social, economic and political stability on an international scale as it did during the 2002-03 pandemic. Its causative pathogen, SARS coronavirus (SARS-CoV), has been classified by NIAID as a Category C Priority Pathogen. Therefore, development of an effective and safe vaccine for preventing future SARS outbreaks and for biodefense preparedness is urgently needed. In response to RFA-AI-11-014, Baylor College Medicine, the new home of Sabin Vaccine Institute's product development partnership (BCM-Sabin), will link with SARS research groups located at the New York Blood Center (NYBC) and University of Texas Medical Branch Galveston (UTMB) to develop, test and manufacture a novel recombinant SARS vaccine. **The strengths of the proposal include BCM-Sabin's eleven-year track record of advancing recombinant vaccines for neglected tropical diseases through product development, manufacture, regulatory filing, and clinical testing, together with NYBC's extensive body of preliminary data to justify the selection of the proposed candidate vaccine antigen, and UTMB's first transgenic mouse model for SARS-CoV infection.** The NYBC's group is the first to show that the receptor-binding domain (RBD) of SARS-CoV spike (S) protein contains the major neutralizing epitopes and RBD-based vaccine can induce potent neutralizing antibody responses and protection in animals against SARS-CoV infection. Another strength of the proposal is a high level of **innovation**, which include biophysical techniques to optimize product stability (for emergency stockpiling), selection of the RBD portion of the S protein to reduce the risk of immune enhancement, adjuvant access, and testing in a transgenic model of SARS in mice that resembles human disease. For this proposal NYBC will evaluate the antigenicity, functionality, and immunogenicity of the RBD-based SARS vaccine; UTMB will evaluate the *in vivo* efficacy of the RBD-based vaccine; and BCM-Sabin will express the recombinant vaccine and perform process development and technology transfer activities for manufacture, in addition to providing regulatory affairs and quality assurance. As an additional strength BCM-Sabin has enlisted key industrial partners for: 1) cGMP manufacture; 2) access to glucopyrranosyl lipid A (GLA), a novel TLR4 adjuvant; and 3) GLP toxicology testing. Ultimately, at the end of the project, all infor-mation and processes will be in place for compilation/submission of an IND and entry into clinical development.

Specific Aim 1*: *Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate. We will evaluate the expression of the recombinant RBD (rRBD) in bacteria and yeast expression systems and select one of them for expression of rRBD protein for subsequent studies based on yields, purity, stability, antigenicity, functionality, immunogenicity, and efficacy (for inducing neutralizing antibody responses and protection against SARS-CoV challenge) of the rRBD protein when formulated in alum. We will use the rRBD protein from the selected expression system for optimization of immunization regimens, and assess the ability of rRBD protein formulated with alum based adjuvants and/or GLA, a TLR4 antagonist adjuvant, to induce cross-neutralizing antibody response, cross-protection and long-term immune responses and protection in mouse models using the optimized immunization regimen. (Timeline Year 1-3)

Specific Aim 2: Process development, characterization, formulation and stability profiling. In parallel to Aim 1, 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 and specific product quality assays will be developed and used to characterize the recombinant vaccine protein. Vaccine buffer formulations will be developed and characterized using an innovative approach, combining analytical/biochemical tools with biophysical assays to test different excipients and stabilizers and establish an optimal stability profile. The stabilized protein will be formulated with alum and/or GLA. The binding and effect on the structure stability will be examined. Immunogenicity and efficacy of the vaccine formulations will be evaluated in parallel as described in Aim 1. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing. (Timeline Year 2-4)

Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation. The cell bank production, production processes and formulation technology for the selected rRBD-based 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 BCM-Sabin and following a pre-IND meeting with the U.S. FDA, GLP toxicology will be initiated at Frontier Biosciences, a Maryland-based contractor. BCM-Sabin will prepare and submit an IND in preparation for the initiation of the clinical development plan. (Timeline Year 4-5)

Research Strategy:

(a) Significance

SARS. SARS is considered the first new infectious disease identified in the 21st century and classified by NIAID as a Category C biodefense agent with an intense capacity to inflict devastating disease outcomes and disrupt local, national, and global economies as it did during the 2002-03 pandemic. It originated in Guangdong province of China in November 2002 and ultimately spread along international air-travel routes to and across five continents. This spread lead 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. 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, SARS-CoV, was identified as the etiologic agent, and subsequently shown to be 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) proteins (1). SARS-CoV is a zoonotic virus with the Chinese horseshoe bat and other exotic species (e.g., palm civets, raccoon dogs, etc.) as its putative natural reservoirs. Although SARS-CoV was believed to be eliminated from human circulation in July 2003, its brief recurrences in laboratory workers in China between late 2003 and early 2004, and its continued existence in nature pose a threat for its reemergence in human populations through either zoonotic reintroduction, laboratory escape or when used as a bioterrorism agent. Thus, there is an urgent need for effective strategies against this devastating viral agent especially in high-risk groups including the elderly, healthcare workers and laboratory personnel and for biodefense preparedness (2).

The RBD protein as the vaccine antigen. An important strength of this proposal is our seven years of preliminary data justifying RBD as a key vaccine target. 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 cytotoxic T lymphocyte (CTL) responses are detectable in patients who recover from SARS, with levels correlating with disease outcome (3-6). In laboratory animal models, neutralizing anti-RBD IgG is linked to protective efficacy following virus challenge (1). Neutralizing antibodies and/or CTLs can be raised against several SARS-CoV structural proteins, especially the spike (S) protein, which is responsible for receptor recognition, virus attachment, and entry, thereby suggesting that S protein is a promising target for the development of anti-SARS vaccines (1). The spikes of SARS-CoV are comprised of trimers of the S protein, which belong to a group of class 1 viral fusion glycoproteins, including HIV glycoprotein 160, influenza hemagglutinin, paramyxovirus F, and Ebola glycoprotein. The SARS-CoV S protein encodes a glycoprotein precursor of 1,256 amino acids (aa), with the amino terminus outside of the cell surface or virus particle. The precursor consists of a signal peptide (aa 1-12), an extracellular domain (aa 13-195), a transmembrane domain (aa 1,196-1,215), and an intracellular domain (aa 1,216-1,215) (Fig 1a). Similar to other coronaviruses, the SARS-CoV S protein can be cleaved into S1 and S2 subunits by proteases, and a fragment located in the S1 subunit spanning aa 318-510 (192 aa) is the minimal receptor binding domain (RBD) required for binding to the functional viral receptor, human angiotensin-converting enzyme 2 (ACE2). Crystallographic studies reveal that RBD binds to ACE2 through a tyrosine-rich 14 aa receptor binding motif (Figs 1b and 1c). The RBD also contains multiple cysteine residues linked by disulphide bonds, while two residues at positions 479 and 487 of the RBD are associated with enhanced animal to human disease transmission and human to human transmission. SARS-CoV S protein also mediates class 1 viral fusion (1).

The essential role of S protein in initiating receptor binding and the subsequent fusion between the viral envelop and membrane of susceptible cells makes it not only a major player for determining host specificity, tissue tropism, and viral pathogenicity, but also an attractive target for vaccine and antiviral development. Indeed, S protein-based vaccines developed by different approaches have been proven effective in inducing specific antibodies in many laboratory animal species (e.g., mice, nonhuman primates, etc.) capable of neutralizing homologous SARS-CoV infection in Vero E6 cells and in vaccinated animals. While these full-length S protein-based vaccines are promising candidates, they may induce antibody-dependent enhancement (ADE) of disease in vaccinated animals upon challenge with homologous SARS-CoV (7), thus raising concerns about the safety and ultimate protective efficacy of these vaccines (8).

We have shown that the recombinant RBD (rRBD) antigen of SARS-CoV could be recognized by the neutralizing antibodies in the antisera of mice and rabbits immunized with inactivated SARS-CoV (9). The rRBD also strongly reacts with the convalescent sera obtained from SARS patients, and depletion of the RBD-

specific antibodies from SARS patients results in significant elimination of the neutralizing activity (10). Chen *et al.* (11) have also shown that most neutralizing antibodies of antisera of mice, rabbits and monkeys induced by a live-attenuated MVA virus that expressed the full-length S protein could be absorbed and removed by rRBD. Furthermore, using an RBD fusion protein linked to human IgG1 Fc fragment (designated RBD-Fc) as an immunogen, we have successfully raised high titers (> 1:10,000) of neutralizing antibodies in rabbits. Importantly, these antibodies could also effectively cross-neutralize the infection by SARS pseudoviruses bearing S proteins of both homologous and heterologous SARS-CoV isolates, including the representative strains of human 2002–2003 and 2003–2004 SARS-CoV (Tor2 and GD03, respectively) and palm civet SARS-CoV (SZ3). Immunization of mice with RBD-Fc also induced long-term protective immunity against challenge with the homologous SARS-CoV BJ01 strain (12). Administration of an adeno-associated virus (AAV)-based vaccine that contains RBD (RBD-rAAV) by intramuscular and mucosal pathways elicits sufficient neutralizing antibodies to inhibit homologous SARS CoV (GZ50) challenge in the established mouse model, and the immune responses can be enhanced by priming with RBD-rAAV and boosting with RBD-specific peptides. In addition to the B cell-epitopes, SARS-CoV S protein contains T cell-epitopes capable of eliciting CD8+ T-cell responses. One H-2(b)- and one H-2(d)-restricted T-cell epitope were mapped to RBD (S436–S443 and S366–S374, respectively). Immunization of mice with a rRBD-based subunit vaccine (S318–S510) elicits both antibody and cellular immune responses against SARS-CoV (13). Notably, the RBD of S protein contains multiple conformation-dependent epitopes and is the main domain that induces neutralizing antibody and T-cell immune responses against SARS-CoV infection without revealing exacerbated pathogenesis in vaccinated animals in response to viral challenge, making it a desirable target for vaccine development.

To alleviate safety and efficacy concerns inherited by the full-length S protein based vaccines, we will continue and extend our previous efforts and in this proposal fully evaluate the potential of RBD as a subunit of S-protein-based, to be developed as a vaccine. Importantly, the approaches adopted in this proposal for developing an rRBD-based vaccine against SARS-CoV will provide a useful roadmap for designing vaccines against other viruses carrying class I fusion proteins, as these proteins also contain RBDs in their S proteins.

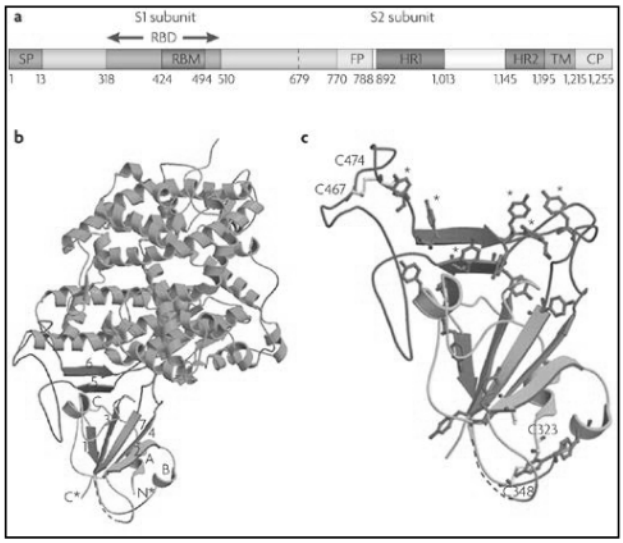


Fig. 1. Schematic representation of the S protein and its RBD (1). (a) Residue numbers of each region represent their positions in the S protein of SARS-CoV. CP, cytoplasm domain; FP, fusion peptide; HR, heptad repeat; RBD, receptor-binding domain; RBM, receptor-binding motif; SP, signal peptide; TM, transmembrane domain. (b) Crystal structures of the RBD complexed with ACE-2. (c) the RBD tyrosine (magenta) and cysteine (yellow) distribution.

Novel Adjuvants. Currently, alum salts are the only adjuvants licensed in the United States (14). To date, two recombinant protein vaccines formulated on alum – hepatitis B virus (HBV) and human papilloma virus (HPV) vaccines – have undergone licensure (14). Their formulation on alum was approved in part on their safety record of alum, together with evidence that alum can stabilize antigens and augment antibody responses to the target antigen (14). However, for both HBV and HPV vaccines the major antigen components also exhibit the ability to self-assemble into virus-like particles, which enhance immunogenicity and elicit high levels of antibody even when alum is used as the solitary adjuvant (15). In contrast, many of the vaccine antigens under development, including RBD, are soluble recombinant proteins. Notably, soluble proteins, such as the malaria antigens AMA-1, MSP-1, and Pfs25, when formulated on alum although had adequate safety profiles in both Phase 1 and 2 clinical trials; they were only modestly immunogenic in terms of the magnitude of antibody titers and the length of time when the antibody titers are detectable (16-19). Similarly, we found that a recombinant hookworm larval antigen when formulated on alum also showed modest antibody titers, although the titers persisted for up to a year after immunization (20). Although in our previous studies, rRBD when formulated in alum induced neutralizing antibodies and protection, it might be necessary to increase the vaccine's efficacy, in particular the induction of cellular immunity, by replacing alum with an innate adjuvant or by adding to the alum formulation a second immunostimulant. One approach is to add a mimetic of a pathogen-associated molecular

pattern (PAMP) that can bind to one or more TLR (21). For instance, TLR9 agonist CpG 7909 oligodeoxynucleotide has been formulated with AMA-1 and other recombinant malaria antigens and shown to augment IgG antibody titers (19,22,23). However, CpG 7909 was also associated with an increase in adverse events including neutropenia, possibly due to immune stimulation and white blood cell sequestration (22-24). In addition, one case of Wegeners granulomatosis was noted to occur in a volunteer who received hepatitis B formulated with an oligodeoxynucleotide similar to CpG 7909 (24,25). An alternative strategy is to use lipid A, the active principle of lipopolysaccharide (LPS), which is a potent TLR4 stimulator (21). To reduce the pyrogenicity and other toxicities, a lipid A from *Salmonella minnesota* has been modified by selective hydrolysis of two moieties (21). The resulting monophosphoryl lipid A (MPL®) is licensed for use in Europe as Fendrix®, a human hepatitis B vaccine (13,26,27). Recently, the biotechnology company, Immune Design Corp. (IDC, http://immunedesign.com, Seattle, WA), developed a novel 1.7 kDa synthetic toll-like receptor 4 (TLR4) agonist known as GLA (glycopyranosyl lipid A), which is equivalent of MPL® and is a clinical stage adjuvant being utilized in multiple internal and partnered vaccine programs by our industrial partner, Immune Design Corp. 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) (13,26). IL-2 and IFN-γ, Key Th1 cytokines, were also elevated (13). A oil-in-water emulsion of GLA with a malaria vaccine, GMZ2 adjuvanted by aluminum hydroxide, have been also shown to enable the induction of the highest (a) vaccine-specific IgG2a and total IgG titers, (b) parasite-specific IFA titers, (c) levels of IFN-γ, 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). In support of the use of GLA in human trials, in an IND filing this year the FDA has allowed BCM-Sabin to include the GLA as part of the clinical plan of one of our hookworm vaccines, which will initiate phase 1 clinical testing in June, 2011.

(b) Innovation

The use of rRBD, rather than full-length S protein or inactivated or attenuated viruses, for developing SARS vaccines. Our preliminary studies have shown that rRBD is the best candidate for a SARS vaccine since it is highly effective in eliciting high titers of neutralizing antibodies against SARS-CoV and the pseudovirus expressing S protein (29), and it induces protective immunity in animal models against SARS-CoV homologous and heterologous challenge (30). Compared with the inactivated virus and live attenuated virus-based vaccines, the rRBD-based vaccine is much 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 vaccine expressing full-length S protein also caused liver damage after animals were challenged with SARS-CoV (7,33). So far, no one has shown that rRBD-based vaccines may induce infection-enhancing antibodies or harmful immune responses (1).

Combining analytical/biochemical tools with biophysical assays to test different excipients and stabilizers and establish an optimal stability profile for the rRBD-based vaccine. In our experience, long-term stability 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 these assays during the product development phases will provide specific information concerning the physical state of the protein as a function of stresses (i.e temperature and pH fluctuations). Such data allows the development of specific assays to screen and identify potential stabilizers such as sugars and other buffering agents to prevent degradation. The type of assays (including light scattering for aggregation of recombinant proteins as well as ensuring compatibility of buffering agents with the adjuvants) are determined by the nature of the structural changes of the protein. Once the assays are established, a supplemented GRAS (“generally regarded as safe”) library is screened and potential stabilizers identified. The ability of these excipients to prevent aggregation or conformational changes of the protein is a key goal during vaccine formulations.

Preclinical testing of efficacy in two innovative animal models: we will use two stringent and complementary lethal mouse models: 1) MA15/BALB/c mice using a mouse-adapted MA15 virus for homologous virus challenge. It originated from SARS-CoV Urbani strain, which can cause SARS-like disease and mortality in BALB/c mice; 2) GD03/Tg AC70 mice developed by Dr. Tseng at UTMB. Transgenic Tg AC70

mice, express the human angiotensin-converting enzyme 2 (ACE-2), the virus receptor for human SARS-CoV. These mice can be also used for heterologous infections and thus evaluation of cross protection. Following infection of such transgenic mice with SARS-CoV they develop weight loss and other manifestations before reaching 100% mortality within 8 days post-intranasal infection with clinical isolates of SARS-CoV (36). The severity of the disease in Tg AC70 mice makes them valuable for preclinical testing of SARS vaccines (36).

A Product Development Partnership (PDP) to accelerate vaccine product development. An ideal vaccine to prevent SARS would be one that protects against devastating outcomes of the infection after a single dose or two doses spaced closely together so that vulnerable populations could be rapidly immunized in an outbreak setting (see Product Development Strategy Section). In addition, the vaccine should be highly stable so that it can be stockpiled for emergency use. Finally, the vaccine should be high yield and low cost so that it could 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. *In addition to the innovations described above an added strength of the proposal is using the PDP approach as an innovative first-in-human model for accelerating vaccine product development and entry into clinical trials of the rRBD-based vaccine.* BCM-Sabin integrates and coordinates industry and academic/public partners and contractors and permits the implementation of innovative business-like practices with traditional basic science approaches and applied product and clinical development technology exchanges. BCM-Sabin has a track record of producing safe and immunogenic recombinant protein vaccines (2). As an added strength, BCM-Sabin is uniquely positioned to develop vaccines at the lowest possible costs so that it can be used in resource-poor settings. This goal has been achieved based on product requirements for its anti-parasitic vaccines intended for use in the poorest countries of Africa, Asia, and the Americas (37). Such economic requirements likely obviate expensive vaccine biotechnologies including mammalian cell culture, insect expression vectors, and prime-boost strategies using adenovirus vectors or DNA vaccines. Therefore, BCM-Sabin focuses its vaccine development strategies on extremely low-cost bacteria and yeast expression vectors (2). BCM-Sabin and its collaborators will employ the PDP approach for innovation which will also include the use of several novel and innovative discovery biotechnologies for adjuvant formulations with IDC's TLR4 GLA adjuvant (13,26,38). In addition, through the application of parallel analytical, biophysical and immunological profiling success will be ensured during process development. A key element of BCM-Sabin's success is regular program/risk management reviews, organized to evaluate each step in the development cycle, by inviting a team of external technical advisors that will include established vaccine development scientists. *Such activities will provide the basis for accelerating cGMP manufacture and ultimately a "first-in-human" Phase 1 study of the rRBD-based SARS vaccine.*

Product Attributes of the rRBD-based Vaccine
1. Effective in a one or two doses 2. Stable for emergency stock-piling 3. Low cost for use in both Southeast Asia and the U.S.

(c) Approach. The strengths of our approach rely on an extensive evidence base of preliminary data collected over the last seven years by NYBC, which point to the RBD protein as a lead candidate vaccine antigen, together with an eleven-year track record of product development and testing for recombinant vaccines by BCM-Sabin. Additionally, BCM-Sabin has had previous success in tech transferring processes for recombinant protein vaccines to WRAIR our cGMP contractor (CMO) of choice. Further, our group has demonstrated the feasibility of developing rRBD-based subunit vaccines. We have shown that mammalian cell-expressed rRBD fused induces high titer of RBD-specific neutralizing antibodies in vaccinated animals and long-term (over a year) immune responses and protection against subsequent SARS-CoV challenge (12), while using the pseudotyped viruses expressing S protein originated from Tor2, GD03, or SZ3, the representative strains of human 2002–2003 & 2003–2004 SARS-CoV and palm civet SARS-CoV, respectively, we also found that mouse and rabbit antibodies raised against rRBD derived from either one of the aforementioned strains of SARS-CoV and the palm civet SARS-CoV cross-neutralize one another, suggesting their protective efficacy against challenge with heterogeneous viruses (39). We have demonstrated that immunization of mice with rRBD derived from various expressing systems, including mammalian cells (293T and CHO), insect sf9 cells, and *E. coli* was capable of producing high-levels of RBD-specific neutralizing antibody and potent T cell responses against both pseudotyped and live SARS-CoV. Importantly, these rRBD proteins appear to maintain intact conformation and authentic antigenicity, reacting with the RBD-specific and conformation-dependent mAbs with neutralizing activity (30,40,41) . These rRBD proteins elicited immunity that protected all vaccinated mice from SARS-CoV challenge (30). These preliminary data and expertise by our collaborators at NYBC, will now be paired with the track record at BCM-Sabin/WRAIR for transitioning recombinant protein-based

vaccines through process development and formulation, technology transfer for cGMP manufacture, lot release and stability testing, and regulatory filing with the FDA.

Overview: In response to this RFA, BCM-Sabin will apply its proven product development partnership approach and strong peer-reviewed publication track record in research and development of parasitic recombinant protein vaccines to accelerate the development of a recombinant RBD-based vaccine to prevent SARS-CoV infection. To ensure success, BCM-Sabin will provide program management throughout the project timeline and leverage the collaborations with the research and industry partners to augment the expertise for anti-SARS-CoV vaccine testing. A strength of the proposal are based on the supporting proof of concept data that identifies the RBD protein as a lead candidate vaccine antigen selected for further process development, characterization and preclinical evaluation. Following feasibility of expression in both bacterial and yeast expression systems, the recombinant proteins will be assessed at BCM-Sabin for scalability, yield, quality and stability. Antigenicity, functionality, immunogenicity and potency of the vaccine formulations will also be developed and evaluated at NYBC and UTMB. Furthermore, rRBD protein from the selected expression system will be compared at NYBC and UTMB for its ability to induce neutralizing antibodies and protection in laboratory animals against challenge infections with multiple strains of the SARS-CoV using different adjuvant platforms; alum (either Alhydrogel® or aluminum phosphate) and/or GLA, a TLR4 antagonist adjuvant. Once the most efficacious expression system is selected, BCM-Sabin will perform scale-up process development (PD) at the 10 liter fermentation scale followed by protein purification under detailed documentation. Reproducibility will be confirmed and specific product quality control assays will be developed in collaboration with NYBC and used to characterize rRBD. In addition, vaccine buffer formulations will be developed and characterized, and excipients and stabilizers will be evaluated. The stabilized molecule will then be formulated with alum and/or GLA and their effect on the structure stability will also be examined. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing.

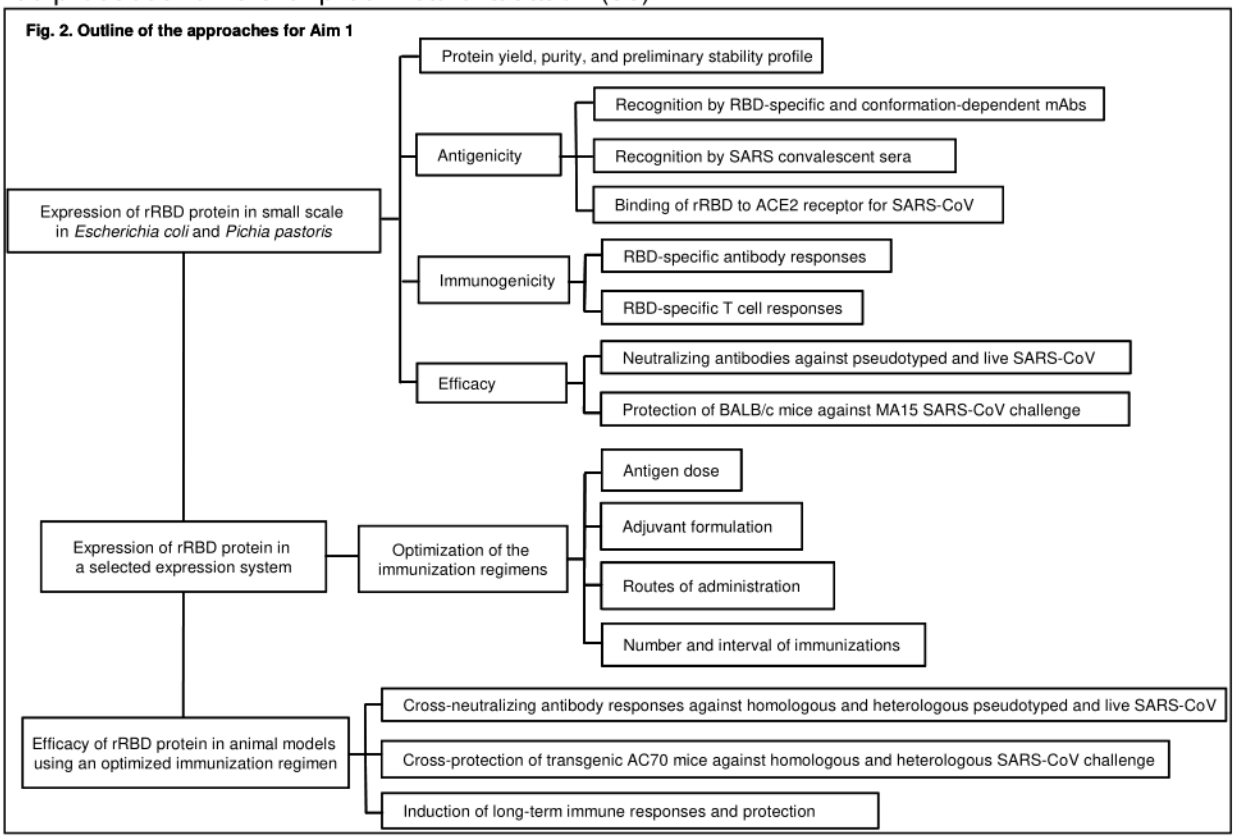
The Major Deliverables
1. Development of a scalable, reproducible process for expression of the rRBD protein and formulation of the RBD-based vaccine with high quality and stability. 2. Establishment of an immunization regimen, including the optimized antigen dose, vaccination schedule and route and adjuvant formulation, for evaluation of the immunogenicity and efficacy of the vaccine. 3. Successful pilot cGMP manufacture of a subunit SARS vaccine comprised of rRBD protein and an adjuvant formulation. 4. Completion of the GLP toxicology testing with acceptable safety profile. 5. IND preparation and submission with U.S. FDA.

The developed process will then be transferred to WRAIR that successfully manufactured two hookworm vaccines with BCM-Sabin. After pilot lot material is produced and released and following a pre-IND meeting with the U.S. FDA, toxicology testing will commence and an IND application will be prepared. Thus a key strength is clear-cut project deliverables (see adjoining box).

Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate. Two protein expression systems, *E. coli* and *Pichia pastoris*, will be evaluated in parallel for the feasibility of scalable expression of the rRBD protein. Preliminary studies have shown that *E. coli*-expressed rRBD protein was able to elicit high levels of neutralizing antibodies and protection in an animal model (30). However, the bacterially expressed protein was not evaluated and formally characterized for suitable scalable vaccine processes hence a more formal evaluation is proposed. While we have no knowledge whether the yeast expression system will be better than a bacterial system, BCM-Sabin has strong capabilities for both systems. To date at least two hookworm vaccine antigens, *Na*-ASP-2 and *Na*-GST-1, expressed by BCM-Sabin were shown to be suitable for producing high resolution crystals for X-ray diffraction (42,43), and were highly protective in animal models - these vaccines have already advanced through product and clinical development pathways (2,20). An expression systems will be selected for the subsequent studies based on the following criteria: 1) the yield, scalability, purity and preliminary stability profile of the rRBD protein expressed; 2) the antigenicity - recognition of rRBD by conformation-dependent monoclonal antibodies (mAbs) and by human convalescent sera, and the functionality – ability of rRBD to bind to ACE2 receptor for SARS-CoV; 3) the immunogenicity - the ability of the rRBD to elicit RBD-specific humoral and cellular immune responses; and 4) the efficacy – the ability of the rRBD to induce neutralizing antibodies and protection in vaccinated mice against subsequent lethal challenge with SARS-CoV Urbani strain (MA15/BALB/c mouse model). 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 (i) antigen doses and adjuvant formulations, and (ii) routes of administration, number and interval of immunizations. The efficacy of the rRBD-based SARS vaccine to induce neutralizing antibodies, protection and long-term immune responses will be evaluated in a SARS-CoV infection mouse model (GDO3/Tg AC70) using the optimized immunization regimen (Fig. 2). **The strength of this aim is two-fold**: 1) BCM-Sabin already has ample expertise in the evaluation of expression systems to determine the suitability for a molecule entering into a vaccine development pathway; and 2) preliminary data with rRBD expressed using a bacterial expression system at the shake-flask has been shown to elicit high levels of neutralizing antibodies and protection in an animal model, which provides proof of principle that this molecule could be produced for further preclinical evaluation (30).

Fig. 2. Outline of the approaches for Aim 1



1.A. Feasibility of scalable expression. BCM-Sabin has extensive experience with bacterial and yeast expression systems and in efforts at both the shaker-flask and up to 10 L fermentation level to optimize scalable expression. As evidence of our track-record in protein expression, four recombinant antigens derived from either hookworm or schistosomes have been successfully expressed at a level of purity and yield ideal for the creation of crystals and solving crystal structures as well as for use in vaccine trials in humans (42-45).

1.A.1. *E. coli* and yeast expression systems. We will use a parallel approach to evaluate both expression systems. Briefly, for bacterial expression, the NYBC investigators previously evaluated the pET-SUMO *E. coli* system (Invitrogen) and pET14b for the expression of rRBD protein, thus providing proof of principle that a bacterial system may be suitable for this protein. The highly-purified rRBD expressed from these systems reacted with the conformational epitope-specific mAbs targeting S protein, suggesting that this rRBD protein maintain intact conformation and authentic antigenicity. These proteins were able to induce strong neutralizing antibody responses and full protection in the vaccinated mice (30). Moreover, the pET-SUMO expressed rRBD was able to induce memory when formulated with alum. A strength of this sub-aim is BCM-Sabin's history and expertise in scaling up recombinant protein antigens both in *E. coli* and in yeast. Because we have found that some proteins expressed with SUMO tags may not be suitable for scale-up and downstream manufacture, we propose to evaluate additional and possibly more suitable *E. coli* systems. Some of these systems include the use of traditional pET/BL21 systems, as well pTrcHis/BL21, and pBAD (araBAD promoter), and the cloning of either harmonized or optimized RBD sequences. Since yeast expression systems have proven to overcome production hurdles of the *E. coli* system, the rRBD DNA sequence will also be optimized and engineered into several yeast expression vectors such as pPICZαA and pPink and evaluated for antigenicity and

immunogenicity. Initial cell growth and protein expression will be conducted in shake flasks and protein will be purified by column chromatography.

1.A.2. Protein yield, purity, and preliminary stability profile. The yield and purity of rRBD will be determined by assays such as HPLC (RP-HPLC and/or SE-HPLC), protein concentration, SDS-PAGE stained with Coomassie Brilliant Blue or Silver Stain, Mass Spectrometry and Residual Host Proteins (by slot-blot) as previously described (30,46). The preliminary stability profile of rRBD protein will also be determined using the assays above and by others such as circular dichroism, intrinsic and extrinsic fluorescence and light scattering (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).

1.B. Antigenicity and functionality. For this sub-Aim we will build on a 7 yr track record of evaluating SARS vaccine candidates in preclinical testing leading to the identification of rRBD as a key protective antigen.

1.B.1. Antigenicity of rRBD protein. The antigenicity of the rRBD protein expressed in the different expression systems will be evaluated by a sandwich ELISA using RBD-specific and conformation-dependent mAbs, including 33G4 (Conf V), 24H8 (Conf I), 31H12 (Conf II), and 35B5 (Conf IV) (40), and human convalescent sera from at least three different SARS patients containing neutralizing antibodies (47). Briefly, rRBD protein at 1 µg/ml will be coated onto the plate, followed by addition of the mAb at 10 µg/ml or the convalescent sera at 1:100 dilution. After addition of HRP-labeled anti-mouse or anti-human IgG, respectively, the binding of antibodies to the coated rRBD protein will be measured. Recognition of the rRBD by these mAbs will also be assessed by Western blot using native and SDS-PAGE gels (30).

1.B.2. Functionality of rRBD protein. The RBD in the SARS-CoV S protein is responsible for virus binding to host cell receptor – ACE2 and mediates the viral entry into the host cell (1,48,49). Our previous studies have shown that the recombinant RBD and S1 proteins could bind to the soluble and cell-associated ACE2, and that anti-RBD antibodies could significantly inhibit the binding of rRBD and rS1 to ACE2 molecule (29,50,51)**.** We will evaluate the binding activity of the differently expressed rRBD proteins to ACE2 by ELISA. Briefly, recombinant soluble ACE2 (R&D Systems, Minneapolis, MN) at 2 µg/ml will be coated onto a plate, followed by addition of rRBD, HRP-conjugated anti-RBD antibody, and the substrate, sequentially. The OD value will be measured (29,50,51). The binding of rRBD to sACE2 can also be detected by isothermal titration calorimetry (ITC) (52,53). The binding of rRBD to the cell-associated ACE2 will be measured by flow cytometry (29,50,51).

1.C. Immunogenicity. The immunogenicity of the rRBD protein will be evaluated by assessing RBD-specific antibody and T cell immune responses in mice immunized with rRBD plus alum as the adjuvant using our previously optimized vaccination regimen (priming subcutaneously with 20 µg and boosting twice with 10 µg rRBD three-weeks apart)(30).

1.C.1. Anti-RBD-specific antibody responses. The sera of the rRBD-immunized mice will be collected 10 days after each boost and the anti-RBD antibody titers will be determined using ELISA as described above (1.B) (30). To measure the isotypes of the RBD-specific antibodies in mouse sera, the HRP-conjugated goat anti-mouse IgG will be replaced by HRP-conjugated goat anti-mouse IgG1, IgG2a, IgG2b, IgG3 and IgA (54).

1.C.2. RBD-specific T cell responses. The RBD-specific T cell responses will be assessed using spleen and lymph node cells collected at day 10^{th} after the last immunization by analyzing MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte (CTL) responses using ELISPOT (Mabtech, Mariemont, OH) following *in vitro* priming with peptides encoding specific CD8 or CD4 epitopes previously identified by us (55,56). Briefly, 96-well ELISPOT plates will be coated with anti-IFN-γ, -IL-2, -IL-4 and -IL-10 mAbs. Single-cell suspensions prepared from the spleens and lymph nodes of the vaccinated mice will be added to the coated plate ($2x10^5$ cells/well), followed by an incubation at 37 °C for 24 h in the presence of MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte (CTL) peptide (N50) or Th peptide (N60), and then with biotinylated labeled anti-mouse IFN-γ, IL-2, IL-4 and IL-10 mAbs. Spots of cytokine-producing T cells will be counted and expressed as the number of spot-forming cells (SFC) per 10^6 input cells. If necessary, a more complete cytokine profile will be obtained by evaluating culture supernatants three days after *ex-vivo* stimulation using Bioplex/Milliplex methodology (57).

1.D. Efficacy. We previously demonstrated that rRBD-immunized aged mice with serum neutralizing antibody titers ranging from 1:189 to 1:505 were fully protected from SARS-CoV challenge (12). Other groups reported that mice immunized with attenuated virus or DNA vaccines encoding the SARS-CoV S protein, or inactivated virus had serum neutralizing antibody titers ranging from 1:50 to 1:640 and most of these mice were protected from SARS-CoV challenge (32,58,59), suggesting that the level of neutralizing antibody response is correlated

with the efficacy of protection and that a vaccine that induces in the immunized mice neutralizing serum antibody titers of 1: >500 would be considered as highly effective. Therefore, the efficacy of the rRBD expressed in the different expression systems will be judged based on their abilities to induce both neutralizing and protective responses when formulated in alum (30,40,55,57).

1.D.1. Neutralizing antibody responses. The titers of the neutralizing antibodies in the sera of mice immunized with the rRBD proteins will be determined using the pseudotyped and live SARS-CoV by Dr. Jiang's group at NYBC and Dr. Tseng's team at UTMB, respectively. The titer of serum neutralizing antibodies against SARS pseudovirus will be determined as previously described (29,30). Briefly, the pseudotyped viruses expressing SARS-CoV Tor2 S protein will be preincubated with serially diluted mouse sera at 37 °C for 1 h before adding to the ACE-2 expressing cells. Fresh medium will be added 24 h later, followed by lysis and the addition of the luciferase substrate (Promega). Relative luciferase activity will be determined in Ultra 384 luminometer. The 50% neutralization titer (NT_{50}) will be calculated using CalcuSyn program (60). Positive control will be neutralizing Mab's and negative control will be pre-immune mice sera. The neutralization assay against infection by live SARS-CoV Urbani strain will be performed as previously described (30,40,55). In brief, serial 2-fold dilutions of sera will be mixed with 100 $TCID_{50}$ of SARS-CoV, incubated at 37 °C and added to the monolayer of Vero E6 cells in triplicate. Cytopathic effect (CPE) in each well will be recorded on day 3 post-infection. The neutralizing titers that completely prevent CPE in 50% of the wells (NT_{50}) will be calculated (61).

1.D.2. Protection against SARS-CoV challenge. The mouse models have been widely used for evaluation of the *in vivo* efficacy of SARS vaccines and passive transfer of protective antibodies (32,58) because mice are much less expensive and more convenient for handling than ferrets and monkeys. It was reported that a mouse-adapted MA15 virus originated from SARS-CoV Urbani strain could cause SARS-like disease and mortality in BALB/c mice (62). Thus, we will use this well-established mouse model for evaluating the efficacy of rRBD to induce protection in the immunized animals. Ten days after the last boost, mice will be lightly anesthetized and bled for determining the titers of neutralizing antibodies prior to intranasal (i.n.) inoculation with 10^5 $TCID_{50}$ of MA15 virus in 50 μl. For each vaccinated and non-vaccinated group (n=15 per group), five mice will be scarified 2 days post-challenge for collection of lung tissues for detecting viral titers by using Vero E6 cells as described above (1.D.1) and expressed as $TCID_{50}$/g of lung tissue, and for examination of histopathogenic changes. In addition, Bioplex analyses will be performed for profiling cytokine and chemokine responses of the infected lungs. The remaining 10 mice in each group will be monitored for their weight loses, clinical symptoms and mortality rate for at least 14 days, as previously described (63). Because antibody-dependent enhancement (ADE) is a genuine concern of the overall vaccination strategy against SARS-CoV (64,65), to ascertain that rRBD protein is an effective and safe vaccine candidate, we will also investigate whether the rRBD could induce eosinophilia and other harmful inflammatory responses in the infected lungs.

Anticipated outcomes. Based on the assays described above, each expression system will be scored and ranked on the basis of a number of parameters as outlined in Table 1. This selection system is a key strength and we have determined previously that ranking by this mechanism helps to identify the most promising expression systems for vaccine candidates for subsequent process development and manufacture. An rRBD antigen preparation with a category score < 3 or final score < 15 will be removed from further consideration. 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).

1.E. Optimization of the immunization regimens. Induction of high levels of neutralizing antibody and protection responses is dependent on not only the antigenicity and immunogenicity of the antigen, but also the optimal antigen dose, adjuvant formulation, administration route, and number and interval of immunizations. Through our partnership with IDC, we have access to a novel adjuvant, GLA that can be formulated on its own or in the presence of alum. Thus, we will also optimize vaccine formulations using the rRBD protein from the selected expression system. The goal is to find the best vaccine formulation and regimen that induces highly potent neutralizing antibody and CTL responses as well as protection against homologous and heterologous virus challenge, while using the least amount of rRBD antigen within the vaccine and number of immunizations.

1.E.1. ***Optimization of antigen dose and adjuvant formulation.*** In our preliminary studies, s.c. vaccination of 20 μg followed by two 10 μg boosts of *E. coli* expressed rRBD plus Sigma Adjuvant System (SAS) or Alum was able to induce effectively strong neutralizing antibody and protective immunity (30). For optimizing both dosing and adjuvant formulation, we will immunize (s.c.) BALB/c mice with three different concentrations of

rRBD (e.g., 5, 10, or 20 μg) in the presence of alum (Alhydrog el® or aluminum phosphate) and/or GLA and boost twice at 3-week intervals. The immunogenicity of the differentially formulated rRBD vaccines will be assessed and compared, according to established protocols as described above, in order to select the most effective dose and formulation of the rRBD-based vaccines for the subsequent studies.

1.E.2. ***Optimization of route of administration, number and interval of immunizations.*** An appropriate route, and number and interval of immunizations are important for determining the nature (e.g., quantitative and quality) of effective immune responses. To optimize these, BALB/c mice will be immunized subcutaneously (s.c.), intramuscularly (i.m.) or intradermally (i.d.) with rRBD protein with the optimized dose-and-adjuvanted rRBD, as selected above in 1E1. The animals will be either boosted once at 2- or 4-week intervals or not boosted. Mouse sera, spleens and lymph nodes will be collected 10 days after last boost for detection of the RBD-specific antibody and T cell immune responses as described above (1.C).

Table 1. Proposed antigen ranking system

1. Expression Feasibility (yield & purity)	2. Antigenicity and functionality	3. Immunogenicity	4. Induction of neutralizing antibody responses	5. Protection against SARS-CoV challenge	Final Score (tally of scores from each category)
0-5	0-5	0-5	0-5	0-5	0-25

rRBD expression feasibility: A major impediment to the success of efficacious vaccine antigens is the ease and cost-effectiveness with which their production can be scaled-up to cGMP manufacture. 0 = unfeasible; 1 = low yields but potentially feasible; 2 = modest yields but unstable protein 3 = modest yields and relatively stable protein but significant process development required; 4 = modest yields and relatively stable protein but feasible through process development approaches; 5 = high yield of soluble, stable protein with straightforward scale-up for manufacture.

Antigenicity and functionality of rRBD: (1) Recognition by conformation-specific mAbs targeting RBD will be determined by an ELISA using rRBD protein (1 μg/ml) as the coating antigen and the mAb 38D4 at 10 μg/ml (40). The score will be recorded based on the OD value: 0 = <0.1; 1 = 0.1 – 0.25; 2 = 0.25 - 0.49; 3 = 0.5 – 0.74; 4 = 0.75 – 1.0; and 5 = >1.0; **(2) Recognition by convalescent sera** will be measured by an ELISA with rRBD protein (1 μg/ml) as the coating antigen and the SARS convalescent sera at 1:100 dilution (47) and scored based on the OD value: 0 = <0.1; 1 = 0.1 – 0.25; 2 = 0.25 - 0.49; 3 = 0.5 – 0.74; 4 = 0.75 – 1.0; and 5 = >1.0; **(3) functionality of rRBD protein.** The ability of rRBD to bind ACE2 receptor for SARS-CoV will be measured by ELISA using rACE2 as the coating antigen (2 μg/ml) and rRBD protein at 1 μg/ml (51), and scored based on the OD value: 0 = <0.1; 1 = 0.1 – 0.25; 2 = 0.25 - 0.49; 3 = 0.5 – 0.74; 4 = 0.75 – 1.0; and 5 = >1.0. The scores of (1), (2) and (3) will be averaged and entered into category 2.

Immunogenicity of rRBD: (1) RBD-specific antibody responses will be assessed with an ELISA with rRBD protein as the coating antigen (40) and scored based on the serum titers anti-RBD antibodies (reciprocal dilution)**:** 0 = < 10^2; 1 = ≥ 10^2 ~ < 10^3; 3 = ≥ 10^3 ~ < 10^4; 4 = ≥ 10^4 ~ < 10^5; and 5 = ≥ 10^5. **(2) RBD-specific CTL responses** will be tested with an ELISPOT (55) and scored based on the number of IFN-γ+ CD8+ cells in 10^6 splenocytes: 0 < 5; 1 = ≥ 5 ~ < 15; 2 = ≥ 15 ~ < 25; 3 = ≥ 25 ~ < 35; 4 = ≥ 35 ~ < 45; and 5 = ≥ 45. The scores of (1) and (2) will be averaged and entered into the category 3.

Induction of neutralizing antibody responses: Neutralization of pseudotyped virus bearing SARS-CoV S protein (1) and live SARS-CoV (2) will be determined as we previously described (40,55) and scored based on the serum titer of 50% neutralization (NT_{50}): 0 = < 20; 1 = ≥ 20 ~ < 100; 2 = ≥ 100 ~ < 250; 3 = ≥ 250 ~ < 500; 4 = ≥ 500 ~ < 1,000; and 5 = ≥ 1,000. The scores of (1) and (2) will be averaged and entered into category 3.

Protection against SARS-CoV challenge: Protection of BALB/c mice against MA15 SARS-CoV challenge will be determined as described (19, 24). The score will be recorded based on: **(1)** viral burden within the lungs ($TCID_{50}$/g of lung tissue): 0 = 5 out of 5 (5/5) or 100% detectable; 1 = 4/5 or 80% detectable; 2 = 3/5 or 60% detectable; 3 = 2/5 or 40% detectable; 4 = 1/5 or 20% detectable; and 5 = 0/5 or 0% detectable. **(2)** Body weight lose: 0 = > 20%; 1 = 15-20%; 2 = 10-15%; 3 = 5-10%; 4 = 0-5%; and 5 = ≤ 0%, **(3)** Accumulated mortality rate (%): 0 = 80-100%; 1 = 60-80%; 2 = 40-60%; 3 = 20-40%; 4 = 10-20%; and 5 = 0%, and **(4)** Protective efficacy against histopathological changes: 0 = severe, > 50% area; 1 = severe, < 50% area; 2 = moderate; 3 = mild; 4 = minimal/nonspecific; and 5 = none or negligible. The scores of (1), (2), (3), and (4) will be averaged and entered into category 5.

1.F. Efficacy of rRBD-based vaccine to induce cross-neutralizing antibody responses and cross-protection in mice vaccinated with rRBD using the optimized immunization regimen. Based on the above studies, we will select an optimized immunization regimen for vaccination of mice with the most effective adjuvanted rRBD vaccine and evaluate the cross-neutralizing antibody responses and cross-protection against infection by homologous and heterologous SARS-CoV strains

1.F.1. Cross-neutralizing antibody responses. We will immunize BALB/*c* mice with the most effective adjuvanted rRBD vaccine using an optimized immunization regimen. The titers of neutralizing antibodies (NT_{50}) will be determined in sera collected 10 days after the last boost as described above (1.D.1) (29,30) against the pseudotyped viruses expressing the S protein of homologous and heterologous 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). The antisera will also be tested against the live homologous SARS-CoV Urbani strain (its S protein sequence is 100% identical to Tor2) and heterologous SARS-CoV strain GD03.

1.F.2. Cross-protection. While infection of BALB/*c* mice with MA15 virus is a useful lethal model for assessing conveniently the vaccine efficacy, this animal model cannot be used for evaluating the efficacy of anti-SARS vaccines to induce cross-protection against heterologous SARS-CoV strains. To evaluate such efficacy the transgenic mouse model developed by Dr. Tseng's group at UTMB will be used. Briefly, four groups of transgenic AC70 mice (N=15 each group) will be immunized with the adjuvanted rRBD protein using the optimized vaccination regimen or remain non-vaccinated, as control. Ten days after the last immunization, mice will be bled for assessing $PRNT_{50}$ prior to challenging with 10^3 $TCID_{50}$ (~ 10X LD_{50}) live homologous SARS-CoV strain Urbani and heterologous SARS-CoV strain icGD03-S, which bears the S protein gene of the most genetically divergent human strain, GDO3 (66), and which could efficiently replicate in mouse lungs and in human airway epithelial cells (64). Ten of the challenged mice in each group will be monitored for their weight loses, clinical symptoms and mortality rate for at least 14 days, and the remaining five of challenged mice in each group will be sacrified 2 days post-infection for collection of lung and brain tissues for detecting viral titer and for examining histopathogenic changes (63) as described above (1.D.2).

1G. Efficacy of the rRBD-based vaccine to induce long-term immune responses and protection. Our preliminary studies show that immunization of rRBD with Freund's adjuvant induced long-term (as up to one year) and potent SARS-CoV S-specific antibodies with strong neutralizing activity, and protected mice against subsequent SARS-CoV challenge (12). We will fully assess the potential of the rRBD-based vaccine in providing long-term immune responses and protection against MA15 SARS-CoV infection in BALB/c mice. Briefly, we will immunize a total of six groups of BALB/c mice (N=20 per group) using the optimized vaccination regimen; three with adjuvant only as control groups, the other three groups with adjuvanted rRBD as test groups, thereby resulting in three pairs of "control and test" groups of animals. Five mice in each paired group will be scarified 10 days, 6 months and 12 months, respectively, after the last boost for collection of sera, spleen and lymph nodes for evaluating RBD-specific antibody responses, including neutralizing antibodies, and T cell responses as described above (1.C. and 1.D.1) (29,30). The remaining 15 mice in each paired group will be challenged with 10^3 $TCID_{50}$ SARS-CoV Urbani strain 10 days, 6 months and 12 months, respectively, after the last boost. Five challenged mice in each paired group will be sacrificed at day 2^{nd} after infection for assessing viral yields and histopathological changes in the lungs, whereas the remaining 10 mice will be monitored daily for the onset of clinical manifestations (e.g., weight loses) and accumulated mortality, as described above (1.D.2) (63).

Additional anticipated outcomes. In summary, we will use a series of well-optimized methodologies and two novel and complementary mouse models that have been developed in our laboratories to fully evaluate the vaccine potential of rRBD protein against SARS-CoV-induced illness and mortality. It will be based on five highly stringent criteria, including (i) expression feasibility, (ii) antigenicity and functionality, (iii) immunogenicity, iv) ability to elicit neutralizing antibodies, and (v) capacity to induce protection against SARS-CoV challenge. We anticipate establishing a reproducible expression system suitable for the scale-up production of the proven effective candidate vaccine. Although all the five criteria will be closely followed for the selection (each of the category score must be ≥ 3), we will put more weight on the protective efficacy in vaccinated animals since the major outcome endpoints are titers of the neutralizing antibodies and levels protection of vaccinated animals against SARS-CoV infection. Therefore, we will select the antigen with the scores of categories (iv) and (v) at 5. To avoid an undesirable bias, each experiment will be repeated twice and the positive and negative controls will always be included.

Specific Aim 2: Process development, characterization, formulation and stability profiling. We will extend the initial work at the shake flask level described in Aim 1 to generate a research cell bank and optimize expression of rRBD in the selected expression system. To improve yield, purity, stability and activity of the soluble protein, the optimal growth and induction conditions for the fermentation processes will be determined. Temperature, pH, dissolved oxygen, and agitation will be evaluated for their effect on cell growth, protein expression and activity. Once the best parameters are selected, these will be evaluated up to the 10L scale, with the aim of reproducible high-yield production (minimum of 100 mg/L target). The major sub-aims include: (A) production and purification of the rRBD protein at the 10 liter fermentation scale and using ion-exchange, hydrophobic interaction, and other column chromatographies. (B) Development of product specific assays for use to follow the expression and purification in process and for purposes of downstream lot release of clinical lots. (C) Documentation of three successive process development runs comprised of both fermentation and protein purification. (D) Product stability studies to establish a profile of the clinical grade lots.

2.A. Development and optimization of a 10L scale process (fermentation & purification). For fermentation protein expression bioreactors (BioFlo systems from New Brunswick Scientific) will be used. Scale up experiments will be performed with the selected expression system to determine the optimal induction conditions for the fermentation processes. This will be assessed by first optimizing a series of small scale fermentations. Once the best parameters are selected these fermentation parameters will be evaluated up to the 10 L scale, with the aim of reproducible high-yield production. Briefly, the fermentation will be optimized by evaluating six basic areas: growth rate, temperature, pH, dissolved oxygen, shear sensitivity, and foam. As for fermentation, purification processes will be started at the small scale, and these also are developed with the aim of keeping costs as low as possible. The AKTA Explorer and Pilot purifier systems and software will be used. Each isolation procedure will be carefully analyzed and assessed for its ability to improve both protein yield and purity. Both cation and anionic ion-exchange resins will be used as well as hydrophobic affinity resins, and gel filtration resins when necessary. For the overall process development strategy, purification is typically conducted at the 1/6 scale, meaning that if the desired cGMP manufacturing run will be conducted using a 60 L fermentation, purification would be developed using 10 L fermentations that represent a one-sixth scale purification. Alternative strategies used before (35) will be tested to increase yield, purity, and stability.

2.B. Assay development. Protein-specific assays (described further in the product development strategy) will be developed to characterize the rRBD during development (in-process), post-production and post-formulation. The same assays will establish the chemical stability. This aim will ultimately allow for future qualification of the assays to confirm accuracy, precision, sensitivity, specificity, reproducibility, and robustness. Based on these qualification procedures, a set of release specifications are established to support the GMP manufacturing and downstream regulatory package of the recombinant antigen vaccine. The majority of these assays are antigen-specific, and will be developed through application and/or modification of methods currently existing in our laboratories, including HPLC, SDS-PAGE (under reduced and non-reduced conditions), N-Terminal sequencing, Mass Spectrometry and ELISA, among others. These assays establish identity, yields, purity, conformation and integrity after expression. All assays will be complemented with biophysical methods such as light-scattering. Careful attention will be placed on the evaluation of endotoxin levels and the analysis of residual host cell protein (HCP) impurities. The HCP method, includes the use of a slot blot system with scanning laser densitometry to allow picogram level sensitivity in the detection of residual HCP in the in process fermentation products and final purified protein samples. Briefly, proteins will be analyzed using a generic rat polyclonal pool of sera with antibodies that are generated against soluble and insoluble proteins. Furthermore, analysis of contamination by exogenous endotoxin with Limulus amebocyte lysate will be done.

2.C. Execution of 3 successive process development runs at the 10L scale. Upon the completion of the optimization of process development and assay development, we will demonstrate reproducibility with three consecutive production and purification runs at the 10L scale. Such reproducibility is a key strength of our approach to process development. The consecutive batches will be characterized by using the in-process and release quality control testing. This activity is critical for the future success during technology transfer to a manufacturing facility. Based on the experiences of BCM-Sabin all reagents, processes and equipment used at the 10L scale should be compatible to those to be used during manufacture. At the time of transfer, scale will be the only variable that will be modified to avoid run failure.

2.D. Formulation and Stabilization. An important strength is the pairing of GRAS-designated excipients with biophysical techniques in order to produce a formulation of a recombinant vaccine of maximal stability potentially suitable for emergency stockpiling. Pre-formulation studies of rRBD in solution will provide key information for selecting appropriate buffer systems, optimal pH and stabilizers for a potent adjuvanted vaccine. Briefly, characterization with biophysical techniques including light scattering, circular dichroism, and intrinsic and extrinsic fluorescence will provide information concerning the physical state of the vaccine protein as a function of temperature and pH (34,35). GRAS-designated excipients and stabilizers such as sugars and other buffering agents will be evaluated to maximize the stability of the rRBD in solution. Temperature, pH and oxidizing agents will be used to induce deamidation and oxidative stress, which will be assessed with isoelectric focusing studies. In addition, accelerated stability protocols will be developed and assessed at different time points and after doing temperature incursion experiments (repeated freeze thaw cycles, and different temperatures).The stabilized molecule will then be formulated with alum (Alhydrogel® or aluminum phosphate), GLA or in combination, based on the results contained in Aim 1E. There is no way to predict whether Alhydrogel® vs. aluminum phosphate is the preferred form of alum. Assays to establish the percentage of protein bound with different combinations of the aluminum salts will be developed to ensure that

buffers and excipients present in the formulation do not interfere with binding capacity. Titrations of both antigen and adjuvant will be conducted. After the generation of binding isotherms, desorption studies will be performed using increasing amounts of sodium chloride, citrate buffer and a mild chaotrope. Desorption of antigens with increasing salt concentration is highly suggestive of electrostatic binding of the protein to adjuvant. Alternatively, citrate can sometimes be used to dissolve aluminum salts. In addition, formulations will be established both biochemically and by immunological potency. The interaction between rRBD with alum and/or GLA and its effect on stability of the protein will be examined based on IDC recommendations.

Anticipated outcomes/Alternative approaches*:* It is anticipated that by following well documented production processes, these experiments will result in a very consistent, high yield fermentation and purification process at the 10L scale. Deficits in any given process development run would be approached by investigating and establishing corrective actions and embarking on additional runs. If necessary, temperature, pH, dissolved oxygen, agitation and other parameters will be further evaluated performing Design of Experiments (DoE) to establish robustness of the different steps of fermentation and purification (35). DOE is an important strength for advancing candidate antigens through process development. However, based on the prior success of the small scale expression (1L) from Aim 1, we envision that the process will be easily scaled up directly from 1L to 10L scale of production. We expect a low probability that the 10L process scale up may lead to difficulties since the proposed activities will be maximized from the information obtained from Aim 1 that will provide feedback for how to improve the in-process procedures. We try to avoid the use of polyhistidine and other peptide tags as well as nickel affinity resins. However, in the event of low yield or inefficient purification processes using ion-exchange, hydrophobic interaction, and gel filtration, this approach remains a possibility. The buffer formulation and storage conditions of the purified protein will be optimized to support stability with and without adjuvant.

Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation. The process development and formulation technology for the selected rRBD-based vaccine will be transferred to the WRAIR pilot cGMP facility, an experienced vaccine manufacturer with a track record of working with BCM-Sabin for successful tech transfer (20,67). Assays developed and qualified in Aims 1 and 2 will then be used for lot release and to initiate stability testing. The protein-specific assays will be conducted at BCM-Sabin, the antigenicity/immunogenicity and efficacy will be conducted at the NYBC, while other regulated assays will be contracted to industrial partners. BCM-Sabin will be providing regulatory, quality assurance and link our project with the industrial partners for: 1) cGMP manufacture; 2) access to GLA, a TLR4 adjuvant from Immune Design Corp. and 3) GLP toxicology testing. Following a pre-IND meeting with the FDA GLP toxicology testing at Frontier Biosciences will be performed and an IND prepared.

3.A. Strategy for Manufacture of Drug Substance and Drug Product. Based on our previous success with technology transfer CMOs (contract manufacturing organizations) in the U.S. and Brazil, we expect that the production processes will yield rRBD-based vaccine of sufficient yield, purity, and stability for use in Phase 1 clinical testing. An investigational biologic must be manufactured in a prescribed manner, within a controlled environment, and by experienced staff. Initially the technology transfer staff will provide the research clone for the generation of Master and Production Cell Banks. Following fermentation and purification, antigens then undergo final formulation, which will involve the addition of stabilizers or diluents. Steps are taken to ensure final purity, sterility, and potency. The active ingredient (rRBD) is the “drug substance” formulated with buffers, excipients and adjuvants. Final fill is the formulated and vialed vaccine, the “drug product.”

3.B. Lot release and start of a stability program. An extensive array of assays developed and qualified in Aims 1 and 2 will establish criteria for the expression during formulation and manufacture and during stability (long-term and accelerated at different temperatures and during freeze/thaw). Quality control responsibilities will be performed by BCM-Sabin, the contract manufacturer and testing labs. BCM-Sabin will compile and generate the Certificate of Analyses (CoA) for the cell banks, drug substance and product.

3.C. IND Regulatory Package Preparation and Submission. A pre-IND briefing package including preclinical data, human immunoepidemiological data, and plans for GLP toxicology study. A concept clinical protocol will be submitted to the U.S. FDA after the release of the cGMP material. After the Pre-IND meeting and concurrence with our program plan and toxicology study design, a GLP study for rRBD and rRBD-based vaccine will be initiated with the selected AAALAC-accredited contract testing laboratory. Similar to other programs of BCM-Sabin, the ultimate intention is to have a completed IND package in order to advance into phase 1 clinical testing.

Human Subjects:

Human Subjects Involvement and Characteristics: Healthy human adult volunteer subjects are donors of serum specimen and convalescent serum specimens for recovered SARS patients with conformed specific antibody activity against SARS-CoV, a generous gift from our oversea collaborator, will be used for the *in vitro* studies to confirm the antigenicity of rRBD protein. The only use of human subjects for these studies is for the donated serum samples. Any healthy individual who, by virtue of anemia, might have additional risk (to those described below) from blood donation will be excluded. The healthy donors are commonly employees or students of the University of Texas Medical Branch at Galveston. (No student donors are required to participate, nor does student participation affect in any way evaluation or education.) Our volunteer donor pool will typically include Hispanic, African-American, and Asian (approximate male:female ratio of 1:1) individuals reflecting the population of Southeast Texas. Their age range will 18-55 years. Convalescent serum from three individuals recovered from SARS will be used.

Sources of Materials: Peripheral blood will be the only research material obtained from individually identifiable living human subjects. The peripheral blood will be obtained specifically for research purposes.

Potential Risks: Risks involved are those related to donation of 500 ml or less of blood and, therefore, are minimal. Criteria for advising of the risk of venipuncture have been formulated by a Human Use Committee 109 as follows: The risks of simple venipuncture include: Commonly, the occurrence of discomfort and/or bruise at the site of puncture; and less commonly, the formation of a small blood clot or swelling of the vein and surrounding issue, and bleeding from the puncture site.

Recruitment and Informed Consent: Subjects will be asked to donate blood. Risks for healthy subjects, as outlined above, are delineated by an investigator or by one of the trained designated research personnel, such that consent is informed. Such consent is documented by written confirmation, with the approval of the consent procedures and the written consent form by the Institutional Review Board of the University of Texas Medical Branch.

Protection Against Risk: Venipuncture will be performed by trained personnel in order to minimize the above risks. All blood samples are coded in the laboratory to establish donor confidentiality and records are kept in a locked cabinet in the Principal Investigator's locked laboratory.

Potential Benefits Of The Proposed Research To The Subjects And Others: There are no immediate or direct benefits to the volunteer subject. Financial reimbursement for time and effort is warranted and standard practice of our laboratory and will be given to the volunteers. Benefits to the volunteer and to society in general are those that come from extending medical knowledge of viral pathogenesis, with potential application to better management (prevention or therapy) of disease. The risks are limited in relation to the anticipated benefits to the subjects and to society.

INCLUSION OF WOMEN AND MINORITIES: Normal volunteers, including women and minorities, between the age range of 18 – 65 will be recruited to participate in this study (with the exception of pregnant females). Patients will be interviewed for any pre-existing conditions that may disqualify them as a blood donor. If the person(s) qualifies, a trained phlebotomist will conduct blood collection from the volunteers.

Program Director/Principal Investigator (Last, First, Middle): Tseng, Chien-Te K ent PhD.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants.

Study Title: RBD recombinant protein-based SARS vaccine for biodefense (IRB-pending)

Total Planned Enrollment: 3

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino			
Not Hispanic or Latino	1	2	3
Ethnic Category: Total of All Subjects *	1	2	3
Racial Categories			
American Indian/Alaska Native			
Asian	1	2	3
Native Hawaiian or Other Pacific Islander			
Black or African American			
White			
Racial Categories: Total of All Subjects *	1	2	3

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”

***INCLUSION OF CHILDREN**:* Children will NOT be included in these studies. As stated previously, we will **not** include any volunteer under the age of 18.

Vertebrate animal research:

1. Description of animal use. Both wild-type (*wt*) Balb/c mice and mice transgenically expressing hACE2, designated Tg^+ mice, will be used for experiments to test the efficacy of the recombinant receptor binding domain (rRBD) subunit of SARS-CoV spike (S) protein to protect against lethal infection by mouse adapted SARS-CoV (MA-15) and homologous and heterologous strains of clinical isolate/s of SARS-CoV, respectively. Mice will be immunized according to the optimized vaccination regimens with regard to the dosing/frequencies, route/s of vaccination, intervals between vaccination, and the formulation of adjuvant/s, etc, prior to lethal challenge through intranasal (i.n.) infection with either MA-15 or clinical isolate/s of SARS-CoV in Balb/c and Tg^+ mice, respectively. Some vaccinated and non-vaccinated mice will be sacrificed at various time points after viral infection for harvesting lungs and brain (for Tg^+ mice only), for assessing viral yields, pathology, cytokine profiling, and IHC for assessing the protective efficacy. Other infected mice will be monitored daily for the clinical manifestations (e.g., weight loss, etc), and mortality (if any). Mice will be bled from the saphenous vein or via cardiac puncture (terminal bleeds only, under isoflurane anesthesia). Weights will be measured on a laboratory balance. This animal study will be conducted under approved ABSL-3 facilities at UTMB.

Species: *Mus musculus*

Strains: *Balb/c* mice will be obtained from the commercial sources, whereas Tg^+ mice will be generated in house at UTMB by Dr. Chan, Tehsheng, Department of Microbiology and Immunology, UTMB.

Ages: 8-12 weeks

Sex: male and female

Numbers: Grand total of mice: N=420 (i.e., *Balb/c* mice: 300, Transgenic AC70 mice: 120)

Aim 1.D.2. Protection against SARS-CoV challenge.
■ Two treatments (i.e., vaccinated and non-vaccinated) X 15 *Balb/c* mice/cohort X 2 (repeat once) = 60 *Balb/c* mice.

Aim 1.F.2: Cross-protection.
■ Two different treatment (vaccinated and non-vaccinated) X 2 different viruses (i.e., homologous and heterologous) X 15 Tg^+ mice/cohort X 2 (repeat once) = 120 Tg^+ mice

Aim 1.G: Efficacy of the rRBD-based vaccine to induce long-term immune response and protection.
■ Two different treatment X three different time points X 20 Balb/c mice X 2 (repeat once) = 240 *Balb/c* mice

2. Justification: Both *Balb/c* mice and transgenic AC70 mice HACE2 transgenic mice are well-characterized models for mouse-adapted MA-15 SARS-CoV and clinical isolate/s of SARS-CoV infection, respectively, resulting in robust replication of SARS-CoV and onsets of diseases (e.g., weight loss), and mortality. In the absence of suitable *in vitro* or computer-based method that can be used to predict the protective efficacy of any given candidate vaccine against SARS-CoV infection, these mouse models are of valuable for reaching the goal of the proposed studies. The numbers to be used are the minimum required, bases on preliminary power analyses, to detect protective efficacy against illness and fatal disease of SARS-CoV infection.

3. Veterinary care: All animals will be housed in a veterinarian supervised, approved animal facilities that have been awarded Accreditation by the American Association for Accreditation of Laboratory Animal Care Council. The following statement explains UTMB's animal welfare compliance: "The University of Texas Medical Branch (UTMB) operates to comply with the USDA Animal Welfare Act (Public Law 89-544) as amended by PL91-579 (1970) PL94-279 (1976) and 45 CFR37618 (6-3-80); Health Research Extension Act of 1985 (Public Law 99-158); follows the Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised September 1986); and the Guide for the Care and Use of Laboratory Animals DHEW (NIH) 85-23 revised 1985. UTMB is a registered Research Facility under the Animal Welfare Act. It has a current Letter of Assurance on file with the Office of Protection from Research Risks, in compliance with NIH policy. The Animal Care Center is under the direction of a Doctor of Veterinary Medicine and staffed by veterinarians with training and experience in laboratory animal medicine and surgery, clinical care and diagnostic pathology. Routine veterinary care is provided for the animals in compliance with federal and state regulations. The Animal Care staff monitors the animals and the veterinarians personally make rounds each day, calling the investigator to ensure humanitarian care of all laboratory animals.

The animals are maintained in the UTMB Animal Care Facility at an ambient temperature of 30±1°C on a 12:12 hour light:dark cycle. Food is provided once daily in an amount sufficient for the cage containing no more than 5 mice, and water is provided *ad libitum*. Cages are cleaned on alternating days.

Housing and Care for infected mice: Infected animals will be housed in microisolator cages within the ABSL-2 or ABSL-3 and monitored daily for signs of disease associated with SARS-CoV infection. Blood samples will be taken via the saphenous vein, by or cardiac puncture (for terminal bleeds, under isoflurane anesthesia) to determine seroconversion. Analgesics cannot be used because they might have unintended physiological effects that would influence pathogenesis and the ability to detect protective effects of the vaccines.

4. Procedures for ensuring that discomfort, distress, pain, and injury. The goal of this project is to evaluate rRBD-based lead candidate vaccine against SARS-CoV infection and associated diseases and mortality. Unfortunately, it is not possible to use moribund endpoints in this study because we need to determine if the vaccine will protect against a lethal outcome, in addition to disease and viremia. It would be impossible to interpret our results if additional anti-inflammatory or any other analgesic drugs were used. Also, all analgesics interact in some way with the inflammatory response, which is tied to pathogenesis, and some can affect the blood brain barrier, which could also affect pathogenesis.

5. Euthanasia: Mice will be euthanized with an intravenous overdose of pentobarbital, or by expos

Multiple PD/PI Leadership Plan

Administration: This proposal involves the collaboration of Dr. Peter Hotez and Dr. Maria Elena Bottazzi at the Department of Pediatrics, Baylor College of Medicine (the new home of Sabin Vaccine Institute's product development partnership (BCM-Sabin), and Dr. Shibo Jiang and Dr. Sara Lustigman at the Lindsley F. Kimball Research Institute of the New York Blood Center (NYBC). Due to the complexities and broad scope of the proposed research, successful completion will require diverse areas of expertise and interests as represented by the Principal Investigators (PIs) and their laboratories. Since the success of this application depends on critical contributions from each of the named PIs, a multi-PI model is the best fit for this project. The responsibility for the named PIs is described below and each PI will be responsible for her/his own fiscal and research management.

PI#1 Peter Hotez, MD, Ph.D. Professor, Department of Pediatrics, Baylor College of Medicine; President, Sabin Vaccine Institute. He is a laboratory and clinician investigator with expertise in recombinant protein vaccines for neglected tropical diseases (NTDs). He is an elected member of the Institute of Medicine and founding director of one of the first non-profit public private partnerships (PDP) for developing vaccines to combat NTDs. He will be the PI#1 and the point of contact for the project. He works closely with PI# 3 to lead all the product development activities and will interact with PI#2 and Dr Sara Lustigman and Chien-Te K. Tseng at the Department of Microbiology and Immunology, University of Texas Medical Branch (UTMB) for the progress of the Aims.

PI#2 Shibo Jiang, M.D., Ph.D., Member and Head of the Viral Immunology Laboratory, Lindsley F. Kimball Research Institute of New York Blood Center. He is a well-established virologist and immunologist with medical background. He has extensive experience in the development of vaccines against SARS, influenza and HIV/AIDS. **PI#2** will plan, direct and execute the majority of the studies in Aim 1. He will supervise Dr. Lanying Du (Co-investigator) and Dr. Peng Zou (Postdoctoral Research Fellow), and participate in the related experiments, evaluate the experimental results, and the writing of progress reports and papers. He will interact with **PI#1**, **PI#3** and Dr. Sara Lustigman (NYBC), and be the liaison for the efficacy studies in collaboration with Dr. Chien-Te K. Tseng (UTMB).

PI#3 Maria Elena Bottazzi, Ph.D. Professor, Department of Pediatrics, Baylor College of Medicine; Director for Product Development, Sabin Vaccine Development. Her major expertise is in translational research and vaccine development for neglected tropical diseases and the role of vaccines as control tools in international public health programs and initiatives. Dr. Bottazzi will supervise all the expression process/analytical development and technology transfer activities. She will interact with Dr Sara Lustigman, who will be the NYBC liaison for all aspects involving the product development of recombinant RBD and adjuvant formulation.

Communication: The PIs, key personnel, and the subcontractors will have bi-monthly phone or web-conferences through Skype (http://www.skype.com/intl/en-us/features/allfeatures/conference-calls) or Arkadin Global Conferencing service (https://www.anywhereconference.com) to discuss experimental design, data analysis, and updates regarding administrative and regulatory responsibilities. PIs, key personnel, and the subcontractors will meet annually for a one day meeting to formally present data, and discuss scientific and administrative issues. PIs will share their respective research results with each other, and with the key personnel, subcontractors and consultants. They will work together to discuss any changes in the direction of the research projects and the reallocation of funds, if necessary. Dr. Hotez (**PI#1**) will serve as contact PI and be responsible for the submission of the annual progress report to NIH and all communications with the sponsoring organization.

Publication and Intellectual Property: Publication authorship and patent inventorship will be determined based on the relative scientific contributions of the PIs and key personnel.

Conflict Resolution: If a potential conflict develops in connection with this grant, the PIs shall first meet for attempted resolution in good faith within thirty (30) days of the notice of such dispute. If they fail to resolve the dispute, the disagreement may be referred to any dispute resolution mechanism that the institution chooses, including an arbitration committee consisting of senior executives mutually agreed upon by all PIs. No members of the arbitration committee will be directly involved in the research grant.

Change in PI Location: If a PI moves to a new institution, attempts will be made to transfer the relevant portion of the grant to the new institution.

Bibliography & References Cited

1. **Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. (2009). The spike protein of SARS-CoV: a target for vaccine and therapeutic development. *Nat. Rev. Microbiol.* 7:226-236. http://dx.doi.org/10.1038/nrmicro2090**

2. **Hotez PJ, Bethony JM, Diemert DJ, Pearson M, Loukas A. (2010). Developing vaccines to combat hookworm infection and intestinal schistosomiasis. *Nat Rev Microbiol* 8:814-826. PM:20948553**

3. Nie Y, Wang G, Shi X, Zhang H, Qiu Y, He Z, Wang W, Lian G, Yin X, Du L, Ren L, Wang J, He X, Li T, Deng H, Ding M. (2004). Neutralizing antibodies in patients with severe acute respiratory syndrome-associated coronavirus infection. *J Infect. Dis.* 190:1119-1126. PM:15319862

4. Temperton NJ, Chan PK, Simmons G, Zambon MC, Tedder RS, Takeuchi Y, Weiss RA. (2005). Longitudinally profiling neutralizing antibody response to SARS coronavirus with pseudotypes. *Emerg Infect Dis* 11:411-416. PM:15757556

5. Zhang L, Zhang F, Yu W, He T, Yu J, Yi CE, Ba L, Li W, Farzan M, Chen Z, Yuen KY, Ho D. (2006). Antibody responses against SARS coronavirus are correlated with disease outcome of infected individuals. *J Med. Virol* 78:1-8. PM:16299724

6. Li T, Xie J, He Y, Fan H, Baril L, Qiu Z, Han Y, Xu W, Zhang W, You H, Zuo Y, Fang Q, Yu J, Chen Z, Zhang L. (2006). Long-term persistence of robust antibody and cytotoxic T cell responses in recovered patients infected with SARS coronavirus. *PLoS ONE* 1:e24. PM:17183651

7. Weingartl H, Czub M, Czub S, Neufeld J, Marszal P, Gren J, Smith G, Jones S, Proulx R, Deschambault Y, Grudeski E, Andonov A, He R, Li Y, Copps J, Grolla A, Dick D, Berry J, Ganske S, Manning L, Cao J. (2004). Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. *J. Virol.* 78:12672-12676. PM:15507655

8. Czub M, Weingartl H, Czub S, He R, Cao J. (2005). Evaluation of modified vaccinia virus Ankara based recombinant SARS vaccine in ferrets. *Vaccine* 23:2273-2279. PM:15755610

9. **He Y, Zhou Y, Siddiqui P, Jiang S. (2004). Inactivated SARS-CoV vaccine elicits high titers of spike protein-specific antibodies that block receptor binding and virus entry. *Biochem. Biophys. Res. Commun.* 325:445-452**

10. **He Y, Zhu Q, Liu S, Zhou Y, Yang B, Li J, Jiang S. (2005). Identification of a critical neutralization determinant of severe acute respiratory syndrome (SARS)-associated coronavirus: importance for designing SARS vaccines. *Virology* 334:74-82**

11. Chen Z, Zhang L, Qin C, Ba L, Yi CE, Zhang F, Wei Q, He T, Yu W, Yu J, Gao H, Tu X, Gettie A, Farzan M, Yuen KY, Ho DD. (2005). Recombinant modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. *J. Virol.* 79:2678-2688. PM:15708987

12. **Du L, Zhao G, He Y, Guo Y, Zheng BJ, Jiang S, Zhou Y. (2007). Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. *Vaccine* 25:2832-2838**

13. **Baldwin SL, Shaverdian N, Goto Y, Duthie MS, Raman VS, Evers T, Mompoint F, Vedvick TS, Bertholet S, Coler RN, Reed SG. (2009). Enhanced humoral and Type 1 cellular immune responses with Fluzone adjuvanted with a synthetic TLR4 agonist formulated in an emulsion. *Vaccine* 27:5956-5963. PM:19679214**

14. Li F, Li W, Farzan M, Harrison SC. (2005). Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 309:1864-1868. PM:16166518

15. Nassal M, Skamel C, Vogel M, Kratz PA, Stehle T, Wallich R, Simon MM. (2008). Development of hepatitis B virus capsids into a whole-chain protein antigen display platform: new particulate Lyme disease vaccines. *Int. J Med Microbiol* 298:135-142. PM:17888729

16. Malkin EM, Diemert DJ, McArthur JH, Perreault JR, Miles AP, Giersing BK, Mullen GE, Orcutt A, Muratova O, Awkal M, Zhou H, Wang J, Stowers A, Long CA, Mahanty S, Miller LH, Saul A, Durbin AP. (2005). Phase 1 clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for Plasmodium falciparum malaria. *Infect Immun.* 73:3677-3685. PM:15908397

17. Dicko A, Sagara I, Ellis RD, Miura K, Guindo O, Kamate B, Sogoba M, Niambele MB, Sissoko M, Baby M, Dolo A, Mullen GE, Fay MP, Pierce M, Diallo DA, Saul A, Miller LH, Doumbo OK. (2008). Phase 1 study of a combination AMA1 blood stage malaria vaccine in Malian children. *PLoS ONE* 3:e1563. PM:18270560

18. Sagara I, Dicko A, Ellis RD, Fay MP, Diawara SI, Assadou MH, Sissoko MS, Kone M, Diallo AI, Saye R, Guindo MA, Kante O, Niambele MB, Miura K, Mullen GE, Pierce M, Martin LB, Dolo A, Diallo DA, Doumbo OK, Miller LH, Saul A. (2009). A randomized controlled phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. *Vaccine* 27:3090-3098. PM:19428923

19. Qian F, Rausch KM, Muratova O, Zhou H, Song G, Diouf A, Lambert L, Narum DL, Wu Y, Saul A, Miller LH, Long CA, Mullen GE. (2008). Addition of CpG ODN to recombinant Pseudomonas aeruginosa ExoProtein A conjugates of AMA1 and Pfs25 greatly increases the number of responders. *Vaccine* 26:2521-2527. PM:18423804

20. Bethony JM, Simon G, Diemert DJ, Parenti D, Desrosiers A, Schuck S, Fujiwara R, Santiago H, Hotez PJ. (2008). Randomized, placebo-controlled, double-blind trial of the Na-ASP-2 hookworm vaccine in unexposed adults. *Vaccine* 26:2408-2417. PM:18396361

21. Johnson DA. (2008). Synthetic TLR4-active glycolipids as vaccine adjuvants and stand-alone immunotherapeutics. *Curr. Top. Med Chem* 8:64-79. PM:18289078

22. Ellis RD, Mullen GE, Pierce M, Martin LB, Miura K, Fay MP, Long CA, Shaffer D, Saul A, Miller LH, Durbin AP. (2009). A Phase 1 study of the blood-stage malaria vaccine candidate AMA1-C1/Alhydrogel with CPG 7909, using two different formulations and dosing intervals. *Vaccine* 27:4104-4109. PM:19410624

23. Ellis RD, Martin LB, Shaffer D, Long CA, Miura K, Fay MP, Narum DL, Zhu D, Mullen GE, Mahanty S, Miller LH, Durbin AP. (2010). Phase 1 trial of the Plasmodium falciparum blood stage vaccine MSP1(42)-C1/Alhydrogel with and without CPG 7909 in malaria naive adults. *PLoS ONE* 5:e8787. PM:20107498

24. Krieg AM, Efler SM, Wittpoth M, Al Adhami MJ, Davis HL. (2004). Induction of systemic TH1-like innate immunity in normal volunteers following subcutaneous but not intravenous administration of CPG 7909, a synthetic B-class CpG oligodeoxynucleotide TLR9 agonist. *J Immunother.* 27:460-471. PM:15534490

25. DeFrancesco L. (2008). Dynavax trial halted. *Nat Biotechnol.* 26:484. PM:18464766

26. Fox CB, Friede M, Reed SG, Ireton GC. (2010). Synthetic and natural TLR4 agonists as safe and effective vaccine adjuvants. *Subcell. Biochem* 53:303-321. PM:20593273

27. Ulrich JT, Myers KR. (1995). Monophosphoryl Lipid A as an adjuvant. Past experiences and New Directions. In Vaccine Design: The Subunit and Adjuvant Approach. Plenum Press: New York,

28. Lousada-Dietrich S, Jogdand PS, Jepsen S, Pinto VV, Ditlev SB, Christiansen M, Larsen SO, Fox CB, Raman VS, Howard RF, Vedvick TS, Ireton G, Carter D, Reed SG, Theisen M. (2011). A synthetic TLR4 agonist formulated in an emulsion enhances humoral and Type 1 cellular immune responses against GMZ2 - A GLURP-MSP3 fusion protein malaria vaccine candidate. *Vaccine* 29:3284-3292. PM:21349366

29. **He Y, Zhou Y, Liu S, Kou Z, Li W, Farzan M, Jiang S. (2004). Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. *Biochem. Biophys. Res. Commun.* 324:773-781**

30. Du L, Zhao G, Chan CC, Sun S, Chen M, Liu Z, Guo H, He Y, Zhou Y, Zheng BJ, Jiang S. (2009). Recombinant receptor-binding domain of SARS-CoV spike protein expressed in mammalian, insect and E. coli cells elicits potent neutralizing antibody and protective immunity. *Virology* 393:144-150. PM:19683779

31. He Y, Zhou Y, Wu H, Luo B, Chen J, Li W, Jiang S. (2004). Identification of immunodominant sites on the spike protein of severe acute respiratory syndrome (SARS) coronavirus: implication for developing SARS diagnostics and vaccines. *J. Immunol.* 173:4050-4057. PM:15356154

32. Yang ZY, Kong WP, Huang Y, Roberts A, Murphy BR, Subbarao K, Nabel GJ. (2004). A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature* 428:561-564. PM:15024391

33. Enserink M. (2004). One year after outbreak, SARS virus yields some secrets. *Science* 304:1097

34. Kueltzo LA, Ersoy B, Ralston JP, Middaugh CR. (2003). Derivative absorbance spectroscopy and protein phase diagrams as tools for comprehensive protein characterization: a bGCSF case study. *J Pharm. Sci* 92:1805-1820. PM:12949999

35. Peek LJ, Brandau DT, Jones LS, Joshi SB, Middaugh CR. (2006). A systematic approach to stabilizing EBA-175 RII-NG for use as a malaria vaccine. *Vaccine* 24:5839-5851. PM:16735084

36. Brooker S, Bethony JM, Rodrigues LC, Alexander N, Geiger S, Hotez PJ. (2005). Epidemiologic, immunologic and practical considerations in developing and evaluating a human hookworm vaccine. *Expert Rev. Vaccines* 5:35-50

37. Bottazzi ME, Miles AP, Diemert D, Hotez PJ. (2006). An ounce of prevention on a budget: a nonprofit approach to developing vaccines against neglected diseases. *Expert Rev Vaccines* 5:189-198. PM:16608419

38. Reed SG, Bertholet S, Coler RN, Friede M. (2009). New horizons in adjuvants for vaccine development. *Trends Immunol* 30:23-32. PM:19059004

39. **He Y, Li J, Li W, Lustigman S, Farzan M, Jiang S. (2006). Cross-neutralization of human and palm civet severe acute respiratory syndrome coronaviruses by antibodies targeting the receptor-binding domain of spike protein. *J. Immunol.* 176:6085-6092. PM:16670317**

40. He Y, Lu H, Siddiqui P, Zhou Y, Jiang S. (2005). Receptor-binding domain of SARS coronavirus spike protein contains multiple conformation-dependant epitopes that induce highly potent neutralizing antibodies. *J. Immunol.* 174:4908-4915

41. He Y, Li J, Du L, Yan X, Hu G, Zhou Y, Jiang S. (2006). Identification and characterization of novel neutralizing epitopes in the receptor-binding domain of SARS-CoV spike protein: Revealing the critical antigenic determinants in inactivated SARS-CoV vaccine. *Vaccine* 24:5498-5508. PM:16725238

42. **Asojo OA, Goud G, Dhar K, Loukas A, Zhan B, Deumic V, Liu S, Borgstahl GE, Hotez PJ. (2005). X-ray structure of Na-ASP-2, a pathogenesis-related-1 protein from the nematode parasite, Necator americanus, and a vaccine antigen for human hookworm infection. *J Mol Biol* 346:801-814. PM:15713464**

43. Asojo OA, Loukas A, Inan M, Barent R, Huang J, Plantz B, Swanson A, Gouthro M, Meagher MM, Hotez PJ. (2005). Crystallization and preliminary X-ray analysis of Na-ASP-1, a multi-domain pathogenesis-related-1 protein from the human hookworm parasite Necator americanus. *Acta Crystallogr. Sect. F. Struct Biol Cryst. Commun.* 61:391-394. PM:16511050

44. Asojo OA, Homma K, Sedlacek M, Ngamelue M, Goud GN, Zhan B, Deumic V, Asojo O, Hotez PJ. (2007). X-ray structures of Na-GST-1 and Na-GST-2 two glutathione S-transferase from the human hookworm Necator americanus. *BMC Struct Biol* 7:42. PM:17594497

45. Asojo OA, Goud GN, Zhan B, Ordonez K, Sedlacek M, Homma K, Deumic V, Gupta R, Brelsford J, Price MK, Ngamelue MN, Hotez PJ. (2010). Crystallization and preliminary X-ray analysis of Na-SAA-2 from the human hookworm parasite Necator americanus. *Acta Crystallogr. Sect. F. Struct Biol Cryst. Commun.* 66:172-176. PM:20124715

46. Du L, Zhao G, Chan CC, Li L, He Y, Zhou Y, Zheng BJ, Jiang S. (2010). A 219-mer CHO-expressing receptor-binding domain of SARS-CoV S protein induces potent immune responses and protective immunity. *Viral Immunol* 23:211-219. PM:20374001

47. Cao Z, Liu L, Du L, Zhang C, Jiang S, Li T, He Y. (2010). Potent and persistent antibody responses against the receptor-binding domain of SARS-CoV spike protein in recovered patients. *Virol. J.* 7:299. PM:21047436

48. Wong SK, Li W, Moore MJ, Choe H, Farzan M. (2004). A 193-amino-acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. *J. Biol. Chem.* 279:3197-3201. PM:14670965

49. Xiao X, Chakraborti S, Dimitrov AS, Gramatikoff K, Dimitrov DS. (2003). The SARS-CoV S glycoprotein: expression and functional characterization. *Biochem. Biophys. Res. Commun.* 312:1159-1164. PM:14651994

50. He Y, Li J, Heck S, Lustigman S, Jiang S. (2006). Antigenic and immunogenic characterization of recombinant baculovirus-expressed severe acute respiratory syndrome coronavirus spike protein: implication for vaccine design. *J. Virol.* 80:5757-5767. PM:16731915

51. He Y, Li J, Jiang S. (2006). A single amino acid substitution (R441A) in the receptor-binding domain of SARS coronavirus spike protein disrupts the antigenic structure and binding activity. *Biochem. Biophys. Res. Commun.* 344:106-113. PM:16615996

52. Kwong PD, Doyle ML, Casper DJ, Cicala C, Leavitt SA, Majeed S, Steenbeke TD, Venturi M, Chaiken I, Fung M, Katinger H, Parren PW, Robinson J, Van Ryk D, Wang L, Burton DR, Freire E, Wyatt R, Sodroski J, Hendrickson WA, Arthos J. (2002). HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. *Nature* 420:678-682. PM:12478295

53. Schrader N, Kim EY, Winking J, Paulukat J, Schindelin H, Schwarz G. (2004). Biochemical characterization of the high affinity binding between the glycine receptor and gephyrin. *J Biol. Chem* 279:18733-18741. PM:14976213

54. MacDonald AJ, Cao L, He Y, Zhao Q, Jiang S, Lustigman S. (2005). rOv-ASP-1, a recombinant secreted protein of the helminth Onchocercavolvulus, is a potent adjuvant for inducing antibodies

to ovalbumin, HIV-1 polypeptide and SARS-CoV peptide antigens. *Vaccine* 23:3446-3452. PM:15837368

55. Du L, Zhao G, Lin Y, Sui H, Chan C, Ma S, He Y, Jiang S, Wu C, Yuen KY, Jin DY, Zhou Y, Zheng BJ. (2008). Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection. *J Immunol* 180:948-956. http://www.jimmunol.org/cgi/content/abstract/180/2/948

56. Du L, Zhao G, Lin Y, Chan C, He Y, Jiang S, Wu C, Jin DY, Yuen KY, Zhou Y, Zheng BJ. (2008). Priming with rAAV encoding RBD of SARS-CoV S protein and boosting with RBD-specific peptides for T cell epitopes elevated humoral and cellular immune responses against SARS-CoV infection. *Vaccine* 26:1644-1651. http://www.sciencedirect.com/science/article/B6TD4-4RRXN1X-7/2/0a6c4590212d1ca23b6051ae4b6b3f4a

57. Yoshikawa N, Yoshikawa T, Hill T, Huang C, Watts DM, Makino S, Milligan G, Chan T, Peters CJ, Tseng CT. (2009). Differential virological and immunological outcome of severe acute respiratory syndrome-coronavirus (SARS-CoV) infection in susceptible and resistant transgenic mice expressing human angiotensin-converting enzyme 2. *J. Virol.* 83:5451-5465. http://jvi.asm.org/cgi/content/abstract/JVI.02272-08v1

58. Bisht H, Roberts A, Vogel L, Bukreyev A, Collins PL, Murphy BR, Subbarao K, Moss B. (2004). Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. *Proc. Natl. Acad. Sci. USA* 101:6641-6646. PM:15096611

59. Stadler K, Roberts A, Becker S, Vogel L, Eickmann M, Kolesnikova L, Klenk HD, Murphy B, Rappuoli R, Abrignani S, Subbarao K. (2005). SARS vaccine protective in mice. *Emerg. Infect. Dis.* 11:1312-1314. PM:16110580

60. Chou TC, Hayball MP. (1991). CalcuSyn: Windows software for dose effect analysis. BIOSOFT, Ferguson, MO 63135, USA.

61. Reed LJ, Muench H. (1938). A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.* 27:493-497

62. Roberts A, Deming D, Paddock CD, Cheng A, Yount B, Vogel L, Herman BD, Sheahan T, Heise M, Genrich GL, Zaki SR, Baric R, Subbarao K. (2007). A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS Pathog* 3:e5. PM:17222058

63. Tseng CT, Huang C, Newman P, Wang N, Narayanan K, Watts DM, Makino S, Packard MM, Zaki SR, Chan TS, Peters CJ. (2007). Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human Angiotensin-converting enzyme 2 virus receptor. *J Virol* 81:1162-1173. PM:17108019

64. Deming D, Sheahan T, Heise M, Yount B, Davis N, Sims A, Suthar M, Harkema J, Whitmore A, Pickles R, West A, Donaldson E, Curtis K, Johnston R, Baric R. (2006). Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS Med* 3:e525. PM:17194199

65. Lokugamage KG, Yoshikawa-Iwata N, Ito N, Watts DM, Wyde PR, Wang N, Newman P, Kent Tseng CT, Peters CJ, Makino S. (2008). Chimeric coronavirus-like particles carrying severe acute respiratory syndrome coronavirus (SCoV) S protein protect mice against challenge with SCoV. *Vaccine* 26:797-808. PM:18191004

66. Chinese SARS Molecular Epidemiology Consortium. (2004). Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. *Science* 303:1666-1669. PM:14752165

67. Goud GN, Bottazzi ME, Zhan B, Mendez S, Deumic V, Plieskatt J, Liu S, Wang Y, Bueno L, Fujiwara R, Samuel A, Ahn SY, Solanki M, Asojo OA, Wang J, Bethony JM, Loukas A, Roy M, Hotez PJ. (2005). Expression of the Necator americanus hookworm larval antigen Na-ASP-2 in Pichia pastoris and purification of the recombinant protein for use in human clinical trials. *Vaccine* 23:4754-4764. PM:16054275

68. Roper RL, Rehm KE. (2009). SARS vaccines: where are we? *Expert Rev Vaccines* 8:887-898. PM:19538115

69. Yang ZY, Werner HC, Kong WP, Leung K, Traggiai E, Lanzavecchia A, Nabel GJ. (2005). Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. *Proc. Natl. Acad. Sci. USA* 102:797-801. PM:15642942

70. Olsen CW. (1993). A review of feline infectious peritonitis virus: molecular biology, immunopathogenesis, clinical aspects, and vaccination. *Vet. Microbiol* 36:1-37. PM:8236772

71. Weiss RC, Scott FW. (1981). Antibody-mediated enhancement of disease in feline infectious peritonitis: comparisons with dengue hemorrhagic fever. *Comp Immunol Microbiol Infect Dis* 4:175-189. PM:6754243

72. Du L, Zhao G, Li L, He Y, Zhou Y, Zheng BJ, Jiang S. (2009). Antigenicity and immunogenicity of SARS-CoV S protein receptor-binding domain stably expressed in CHO cells. *Biochem. Biophys. Res. Commun.* 384:486-490. http://www.sciencedirect.com/science/article/B6WBK-4W741WB-S/2/1eb0eef08ca9a149c20bc67ea466eaa7

73. Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JSM, Poon LLM. (2003). Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China. *Science* 302:276-278

74. Subbarao K, McAuliffe J, Vogel L, Fahle G, Fischer S, Tatti K, Packard M, Shieh WJ, Zaki S, Murphy B. (2004). Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. *J Virol* 78:3572-3577. PM:15016880

COLLABORATIVE/CONTRACTUAL ARRANGEMENTS

Sub-awards

Two organizations will be subawarded: New York Blood Center (NYBC) and University of Texas Medical Branch (UTMB).

At **NYBC**, Dr. Shibo Jiang, Head of Viral Immunology Laboratory and Sara Lustigman, Head of the Laboratory of Molecular Parasitology will plan, direct and execute the majority of the studies in Aim 1 in close involvement and discussion with Dr Hotez and Dr Bottazzi at BCM. NYBC will also be the liaison for the efficacy studies in collaboration with Dr. Chien-Te K. Tseng at the Department of Microbiology and Immunology, University of Texas Medical Branch (UTMB).

At **UTMB**, Dr Chien-Te K. Tseng, PhD, Associate Professor, Department of Microbiology and Immunology, Center for Biodefense and Emerging Diseases, Institute of Human Infection and Immunity, Sealy Center for Vaccine Development, and Galveston National Laboratory (GNL) at UTMB-Galveston, will serve as principal investigator. Dr. Tseng's will focus on defining the host innate antiviral responses against and the pathogenesis of the severe acute respiratory syndrome (SARS) coronavirus (CoV). Dr. Tseng will be the lead investigator in SARS pathogenesis with the animal model for the SARS research, including several strains of gene knockout (KO) mice and wild-type (wt) mice. Dr. Tseng will be fully engaged in all phases of this project, including coordinating, planning and designing, and together with his staff, executing and supervising the proposed experiments. He will also be responsible for the interpretation of the data, preparing the reports and manuscripts as required for publications.

The allocations for these sub-grants are as follows:

Organization	Direct Costs per year				
	Year 1	Year 2	Year 3	Year 4	Year 5
NYBC	$275,000	$ 300,000	$ 175,000	$ 75,000	$ 50,000
UTMB	$125,000	$ 200,000	$ 150,000	$ 0	$ 0

Contracts

Baylor College of Medicine will enter into a contractual agreement with **Sabin Vaccine Institute** for activities to be performed under Specific Aim 2 and 3. A fee-for-service contract will be provided to the Sabin Vaccine Institute to cover activities related to Quality assurance, regulatory and to manage the contract cGMP manufacturing, GLP toxicology and purchase of the adjuvant. The contract will be distributed as follows: Year 1: $75,000; Year 2: $575,000 and Year 3: $600,000

Sabin Vaccine Institute will manage the fee-for-service contracts and activities related to the GMP manufacture and the GLP toxicology testing for the RBD recombinant protein based vaccine for SARS. In addition, it will implement its Quality Management System (QMS) to provide quality assurance (QA) function for the process development, manufacturing, and toxicology testing activities as outlined in the grant proposal.

This system includes the operation of:
• Policy of compliance with applicable regulations and ethical obligations
• Standard Operating Procedures to assure the standardization of relevant procedures, which includes, but is not limited to, laboratory techniques, documentation practices, and the maintenance of laboratory equipment.
• Documentation system which enables the efficient origination, completion, and storage of documents that contain information on both regulated activities and other activities that are conducted in support of downstream product manufacture
• Conduct of Quality Assurance (QA) audits
• Program for risk assessment and risk management

Finally Sabin will purchase the toxicology-grade aqueous or stable emulsion formulation of Gluco-Pyranosylphospho-Lipid A (GLA-AF, a synthetic lipid A derivative), an immunostimulant for use in the GLP toxicology study from Immune Design Corporation (I DC).

New York *Blood Center*
Lindsley F. Kimball Research Institute

Office of Sponsored Programs
310 East 67 Street.
New York, NY 10065-6275
Phone: 212-570-3034
Fax: 212-737-4506
Email: bgreene@nybloodcenter.org

May 10th, 2011

Baylor College of Medicine
Department of Pediatrics
6621 Fannin
Suite A150, MC 1-3420
Houston, TX 77030

Re: Statement of Intent to Establish a Consortium Agreement with Baylor College of Medicine

This letter is to confirm that the New York Blood Center agrees to participate in the study entitled "RBD recombinant protein-based SARS vaccine for biodefense preparedness". The principal investigator for the New York Blood Center is Dr. Shibo Jiang. The total costs for New York Blood Center will be $1,436,750, for a Period of Performance of May 1st, 2012 – April 30th, 2017. This includes the on-site facilities and administrative costs of 64.2%. The DUNS number for the New York Blood Center is 073271827.

The appropriate programmatic and administrative personnel of Baylor College of Medicine will establish written inter-institutional agreements to ensure compliance with all pertinent federal regulations and policies in accordance with the *PHS Grants Policy Statement,* PHS 398 *Application for Public Health Service Grant*, and the NIH *Guidelines for Establishing and Operating Consortium Grants.*

Sincerely,

Barry A. Greene, M.P.A.
Executive Director
Office of Sponsored Programs

utmb Health
Microbiology & Immunology

301 University Blvd.
Galveston, TX 77555-0158

May 10, 2011

Baylor College of Medicine
Department of Pediatrics
6621 Fannin, Suite A150, MC 1-3420
Houston, TX 77030

Re: Statement of Intent to Establish a Consortium Agreement with Baylor College of Medicine

This letter is to confirm that the University of Texas Medical Branch agrees to participate in the study entitled "*RBD recombinant protein-based SARS vaccine for biodefense preparedness.*. The principal investigator for UTMB is Chiente Tseng, Ph.D. The total costs for UTMB will be $726,750 for a Period of Performance of 5-1-12 through 4-30-16. This includes the off-campus facilities and administrative costs of 53%. The DUNS number for UTMB is *8007711490000.*.

The appropriate programmatic and administrative personnel of Baylor College of Medicine will establish written inter-institutional agreements to ensure compliance with all pertinent federal regulations and policies in accordance with the *PHS Grants Policy Statement,* PHS 398 *Application for Public Health Service Grant*, and the NIH *Guidelines for Establishing and Operating Consortium Grants.*

Sincerely,

Connie Barton
Associate Director, Office of Sponsored Programs

SABIN
VACCINE INSTITUTE

May 5, 2011

Baylor College of Medicine
Department of Pediatrics
6621 Fannin, Suite A150, MC 1-3420
Houston, TX 77030

Re: Letter of Support in response to NIH/NIAID Partnerships for Biodefense (R01) RFA-AI-11-014 Regarding Contract The Sabin Vaccine Institute.

This letter is to confirm that The Sabin Vaccine Institute agrees to participate in the study entitled "RBD recombinant protein-based SARS vaccine for biodefense."

As part of its scope of work, The Vaccine Development Team at the Sabin Vaccine Institute (Sabin) will perform three main functions. First, Sabin will manage the fee-for-service contracts and activities related to the GMP manufacture of and the GLP toxicology testing for the RBD recombinant protein-based vaccine for SARS. Second, Sabin will implement its Quality Management System (QMS) to provide quality assurance (QA) function for the process development, manufacturing, and toxicology testing activities as outlined in the grant proposal. And third, Sabin will purchase the toxicology-grade aqueous or stable emulsion formulation of Gluco-Pyranosylphospho-Lipid A (GLA-AF, a synthetic lipid A derivative), an immunostimulant for use in the GLP toxicology study from Immune Design Corporation (IDC).

The Vaccine Development Team at Sabin currently manages sub-grantees on five continents to support the development of vaccines for human hookworm and schistosomiasis. These include academic collaborators, governmental entities (in Brazil and China), and fee-for-service contractors. Sabin has successfully managed and completed three GMP manufacturing campaigns and three full GLP toxicology studies including study design and sponsor-level QA.

The Vaccine Development Team at Sabin has installed and manages a fully operational Quality Management System (QMS) to ensure that all work completed at the Baylor College of Medicine/Texas Children's Hospital and other partners adhere to established benchmarks of quality. This system includes the operation of:

- Policy of compliance with applicable regulations and ethical obligations
- Standard Operating Procedures to assure the standardization of relevant procedures, which includes, but is not limited to, laboratory techniques, documentation practices, and the maintenance of laboratory equipment.
- Documentation system which enables the efficient origination, completion, and storage of documents that contain information on both regulated activities and other activities that are conducted in support of downstream product manufacture
- Conduct of Quality Assurance (QA) audits
- Program for risk assessment and risk management

We understand that the appropriate programmatic and administrative personnel of Baylor College of Medicine will establish written inter-institutional agreements to ensure compliance with all pertinent federal regulations and policies in accordance with the PHS Grants Policy Statement, PHS 398

Sabin Vaccine Institute
2000 Pennsylvania Ave, NW, Suite 7100 Washington, DC 20006
Phone (202) 842-5025 Fax (202) 842-7689 www.sabin.org

Application for Public Health Service Grant, and the NIH Guidelines for Establishing and Operating Consortium Grants.

The attached proposal estimates the costs necessary to implement the project.

Sincerely,

(b)(6)

Brian R. Davis
Chief Operating Officer
Phone: (b)(6)
Email:

SABIN
VACCINE INSTITUTE

May 5, 2011

Baylor College of Medicine
Department of Pediatrics
6621 Fannin, Suite A150, MC 1-3420
Houston, TX 77030

Re: Letter of Support in response to NIH/NIAID Partnerships for Biodefense (R01) RFA-AI-11-014 Regarding Contract The Sabin Vaccine Institute (Sabin)

Thank you for the opportunity to submit a cost proposal for Sabin's services in support of the grant proposal entitled, "RBD recombinant protein-based SARS vaccine for biodefense."

Activity	Year 3 (05/14-04/15)	Year 4 (05/15-04/16)	Year 5 (05/16-04/17)	Total
Sabin Services:				
Quality assurance and regulatory support for process development	$75,000			$75,000
Quality assurance, management and oversight of GMP manufacturing campaign		$51,000		$51,000
Quality assurance, management and oversight of GLP Toxicology Study			$238,550	$178,550
Contracted Services:				
GMP Manufacturing Campaign		$524,000		$524,000
GLP Toxicology Study			$186,450	$186,450
Gluco-Pyranosylphospho-Lipid A (GLA, a synthetic lipid A derivative)			$175,000	$235,000
Total:	**$75,000**	**$575,000**	**$600,000**	**$1,250,000**

Sincerely,

(b)(6)

Brian R. Davis
Chief Operating Officer
Phone: (b)(6)
Email:

4 May 2011

Michael W. Marine
Chief Executive Officer
Sabin Vaccine Institute
2000 Pennsylvania Avenue, Suite 7100
Washington, DC 20006
(b)(6)

Dear Mr. Marine:

COUNTERSIGNED LETTER OF COMMITMENT - RFA-AI-11-014

In response to the NIH/NIAID Partnerships for Biodefense (R01) RFA-AI-11-014, I write to advise that I am delighted to be involved as a consultant in support of the project entitled **"RBD recombinant protein-based SARS vaccine for biodefense preparedness"** that is currently being prepared for submission by the Sabin Vaccine Institute. I will serve as a Quality Assurance consultant for preclinical activities in support of Sabin Vaccine Institute's vaccine development programs undertaken at the Baylor College of Medicine/Texas Children's Hospital or other locations as needed. In this capacity, I will assess and monitor the project in compliance with Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) and conduct periodic inspections, data audits, protocol reviews, write and revise standard operating procedures (SOPs), reports, and other quality assurance-related activities upon request. The consulting rate will be $90.00 per hour with a maximum of twenty (20) hours per week plus mileage expenses as allowed by IRS, or other transportation expenses incurred for travel to and from required worksites.

Yours sincerely,
(b)(6)

LINDA SCHELLHAAS, BA, RQAP-GLP
QUALITY REVIEWS, INC.
130 TRAVELLER ROAD
FALLING WATERS, WV 25419

With the concurrence of The Sabin Vaccine Institute:

Brian R. Davis
Chief Operating Officer