PI: **GALARZA, JOSE M**	Title: Broadly protective (universal) virus-like particle (VLP) based influenza vaccine	
Received: 12/05/2012	FOA: PA12-088	Council: 05/2013
Competition ID: ADOBE-FORMS-B2	FOA Title: PHS 2012-02 OMNIBUS SOLICITATION OF THE NIH, CDC, FDA AND ACF FOR SMALL BUSINESS INNOVATION RESEARCH GRANT APPLICATIONS (PARENT SBIR [R43/R44])	
1 R43 AI106145-01A1	Dual: HL,NR	Accession Number: [redacted]
IPF: 10004226	Organization: TECHNOVAX, INC.	
Former Number:	Department:	
IRG/SRG: ZRG1 IMM-N (12)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Jose Galarza Ph.D.	TechnoVax Inc.	PD/PI
Diana Dalfo Ph.D	Technovax Inc.	Other (Specify)-Staff Scientist
Innocent Mbawuike Ph.D	Baylor College of Medecine	Co-Investigator
Ruben Donis Ph.D	CDC	Other (Specify)-Collaborator

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION
☐ Pre-application ☒ Application ☐ Changed/Corrected Application

2. DATE SUBMITTED 12/05/2012

Applicant Identifier

4. a. Federal Identifier [redacted]

b. Agency Routing Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** [redacted]

* Legal Name: TechnoVax Inc.

Department: | Division:

* Street1: 765 Old Saw Mill River Rd.

Street2:

* City: Tarrytown | County / Parish: Westchester

* State: NY: New York | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 10591-6702

Person to be contacted on matters involving this application

Prefix: Mr. | * First Name: Hector | Middle Name:

* Last Name: Munoz | Suffix:

* Phone Number: [redacted] | Fax Number: [redacted]

Email: [redacted]

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* [redacted]

7. * TYPE OF APPLICANT: R: Small Business

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:
☐ New ☒ Resubmission
☐ Renewal ☐ Continuation ☐ Revision

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

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Broadly protective (universal) virus-like particle (VLP) based influenza vaccine that can neutralize a broad spectrum of influenza A virus subtypes.

12. PROPOSED PROJECT:
* Start Date: 07/01/2013 | * Ending Date: 06/30/2015

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT** NY-018

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. | * First Name: Jose | Middle Name: M.

* Last Name: Galarza | Suffix: Ph.D.

Position/Title: CEO & Founder

* Organization Name: TechnoVax Inc.

Department: | Division:

* Street1: 765 Old Saw Mill River Rd.

Street2:

* City: Tarrytown | County / Parish: Westchester

* State: NY: New York | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 10591-6702

* Phone Number: [redacted] | Fax Number: [redacted]

* Email: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	[redacted]
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	[redacted]
d. Estimated Program Income	0.00

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

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

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

☒ *** I agree**

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

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: Mr. * First Name: Hector Middle Name:

* Last Name: Munoz Suffix:

* Position/Title: Chief Financial & Corp. Development Officer

* Organization: TechnoVax Inc.

Department: Division:

* Street1: 765 Old Saw Mill River Rd.

Street2:

* City: Tarrytown County / Parish: Westchester

* State: NY: New York Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 10591-6702

* Phone Number: [redacted] Fax Number: [redacted]

* Email: [redacted]

*** Signature of Authorized Representative**: Jose M. Galarza

*** Date Signed**: 12/05/2012

20. Pre-application Add Attachment | Delete Attachment | View Attachment

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

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: TechnoVax Inc.

DUNS Number: [redacted]

* Street1: 765 Old Saw Mill River Rd.

Street2:

* City: Tarrytown County: Westchester

* State: NY: New York

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 10591-6702 * Project/ Performance Site Congressional District: NY-018

Project/Performance Site Location 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Baylor College of Medecine

DUNS Number: [redacted]

* Street1: One Baylor Plaza

Street2:

* City: Houston County:

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 77030-3411 * Project/ Performance Site Congressional District: TX-007

Additional Location(s) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

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

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

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

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract 1235-TVx_UFlu_ABSTRACT_120512.pdf Add Attachment | Delete Attachment | View Attachment

8. * Project Narrative 1236-TVx_UFlu_Narrative_120512.pdf Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited 1237-TVx_UFlu_Bibliography_120512.pdf Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources 1238-TVx_Facilities and_Resources_120 Add Attachment | Delete Attachment | View Attachment

11. Equipment Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

1. ABSTRACT / SUMMARY

We propose to develop a broadly neutralizing, possibly universal influenza vaccine based on virus-like particles (VLPs) displaying remodeled HA molecules which present otherwise cryptic epitopes. These remodeled HAs will be expressed in forms lacking the dominant hypervariable epitopes and instead display distinctly conserved subdominant antigenic sites known to elicit an antibody response that will neutralize a broad spectrum of influenza viruses. The protective scope of current influenza vaccines is restricted to homologous viruses or closely related variants and vaccine efficacy wanes following the fast antigenic evolution of the influenza virus. Most protective antibodies target highly variable and dominant sites on the globular head of the HA molecule, although more conserved and less immune-recognized conformational antigenic sites are also present in the stem (HA2) and between the globular head (HA1) and stem portions of HA. Isolated human antibodies directed toward these sites have been found to neutralize a broad spectrum of influenza viruses. It seems reasonable therefore to prepare and test vaccines that display these highly conserved subdominant antigenic sites and determine if they stimulate a broad antibody response which is minimal in a natural influenza infection or following vaccination with formulations containing whole HA molecules. Incorporation of remodeled HA molecules into influenza virus-like particles (VLPs) should provide an excellent opportunity to develop a broadly neutralizing vaccine. VLPs are generated by the co-expression of four structural influenza proteins (M1, M2, HA and NA) and do not contain viral genetic material and are therefore unable to replicate or cause infection. VLPs displaying different remodeled HA molecules will be produced, characterized and tested for the presence of conserved epitopes by immunoprecipitation with specific antibodies recognizing these sites. The neutralizing activity of VLP immunized mice sera will be assessed by an in-vitro micro-neutralization assay using three antigenically distinct viruses. The protective efficacy and immunogenicity stimulated by a single or combined candidate vaccine will be further investigated in lethal challenge studies with three antigenically diverse viruses. Further development of the most promising VLP(s) will be pursued by a phase II SBIR proposal.

Narrative

Development of a broadly protective (universal) vaccine able to withstand antigenic variation and sustain efficacy for an extended time should have a major impact on influenza prevention. To achieve this goal, we propose to create virus-like particles (VLPs) displaying remodeled HA molecules revealing distinctly conserved subdominant antigenic sites known to elicit an antibody response that will neutralize a broad spectrum of influenza viruses.

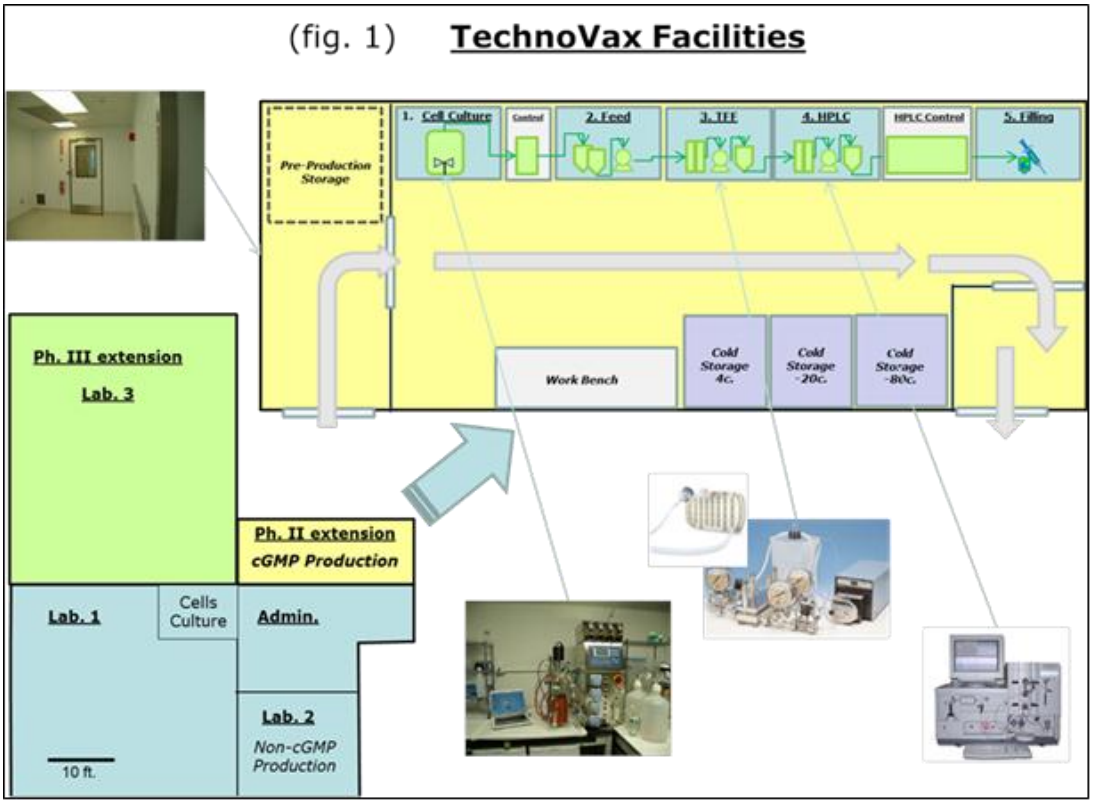
Facilities and Resources at TechnoVac Inc.

TechnoVax is located in Tarrytown, NY and occupies space in the BioMed Realty Trust campus, one of the largest biotech facilities in the state of NY. Most of the tenants are life science, biotech or pharmaceutical oriented businesses providing an excellent infrastructure, environment and synergy for the successful operation of companies. TechnoVax facilities comprise laboratory and offices space to accommodate our scientific team and management.

Laboratories:
Currently operating space for research and development comprises an area of approximately 2000sq. ft. divided into two independent fully equipped laboratory facilities including a tissue culture room featuring negative pressure air circulation and HEPA filtration for handling BSL2+ agents (inspected and approved by the USDA).

Equipment:
TechnoVax laboratories are equipped with three biosafety cabinets, inverted and fluorescence microscopes, micro-centrifuges (standard and refrigerated), one Beckman ultracentrifuge, electrophoresis units (DNA/protein), plate washer, ELISA reader (colorimetric), ELISA reader (fluorescence and chemiluminescence), spectrophotometer, thermocycler machines, autoclave, refrigerators, -20°C and -80°C freezers, CO2 incubators, shakers, transilluminator, digital documentation system, protein purification system, five liter fermenter with control unit, pH meter, autoclave, developer, liquid N2 storage, etc.

(fig. 1) **TechnoVax Facilities**



Two extension programs are currently planned for 1st quarter and 2nd half of 2013.

Pase II Extension - cGMP Production:
In order to produce cGMP vaccines for its own upcoming toxicology studies and clinical trials, TechnoVax is planning to lease additional space to set-up its own cGMP pilot plant. This production area will fully comply with FDA regulations (Guidance for the Industry CGMP for Phase 1 Investigational Drugs" under regulation 21 CRF 201.2(c) (referred to as phase 1 investigational drugs); U.S. Department of Health and Human Services, Food and Drug Administration, July 2008) and will allow TechnoVax to produce, for itself and third parties, biological vaccines and materials for Phase I clinical testing. The pilot plant will be operational towards mid of 2013 and will occupy an area of ~500-1000 sq. ft.

Phase III Extension:
TechnoVax is also planning to expand its current laboratory, production and administrative space in end 2013 by leasing an adjacent area of ~1800sq. ft.

Administration:
Fully equipped office space with printers, fax and copier machines occupies another ~1,000 sq. ft. TechnoVax, Inc. personnel have access to the NYMC library and via internet to e-journals. In addition the BMRLandmark campus provides conference rooms and shared facilities.

Qualifications:
The core competencies of TechnoVax, in addition to vaccine research and development, include genetic engineering and recombinant protein production utilizing eukaryotic and prokaryotic expression systems. Expertise and capabilities on protein design and reengineering permits us to generate molecules with modified or altered properties such as incorporating two different antigens in the same VLP, expressing critical antigens with cryptic neutralizing epitopes and expressing otherwise dangerous pathogen antigens. Our scientific team combines knowledge from biology, structural biology, biochemistry, molecular biology, immunology and cell biology to investigate properties of new protein for vaccine development and other applications. Protein purification and characterization are routinely performed in our laboratory implementing efficient protein purification strategies and complementary analytical/characterization methodologies. Assay development is an important investigative toll that form part of our core capabilities.

In addition, our company carries out animal studies to evaluate immunogenicity and efficacy of different vaccines and formulations. These preclinical evaluations require expertise on planning, study design, execution, data gathering and analysis all competencies that TechnoVax has been practicing since 2004. Furthermore, the combined experience of managers and scientists of TechnoVax covers a broad range of expertise including GLP and GMP manufacturing, preclinical and clinical pharmaceuticals development, formulations, regulatory issues, and project management.

TechnoVax participates in collaborative research and development programs with academic institutions and companies as well as manages SBIR funded projects. Our combined expertise and experience prepare us well to lead a VLP vaccine development and manufacturing projects.

Process Development:
During the last eight years of operations conducting vaccine research and development, TechnoVax has gained significant experience in devising and implementing methods for making products from laboratory scale to 5L fermentation capacity. These undertakings involve developing strategies as well as processes to scale up production and evaluate effectiveness of methods following refinements of parameters, protocols and conditions in order to maximize

yield and quality of the manufacture product. As part of the company activities, our team performs molecular biology work on diverse cloning strategies, gene expression and optimization studies in prokaryotic and eukaryotic systems (bacteria, insect and mammalian cells.). Cell lines development for transient or stable production of vaccine products or recombinant proteins antigens essential for preclinical studies or assay development are routinely created, selected and evaluated in our laboratory applying specifically devised procedures. Multiple gene recombinant vaccines or single proteins are being produced utilizing vectored expression methodologies such as baculovirus recombinants/ insect cells expression system. Candidate products developed utilizing this system include influenza virus-like particle (VLP) vaccines for epidemic and pandemic strains as well as subunits proteins antigens (HA, NA, NP). Similarly, stable transfected CHO, Vero and MDCK cells have been developed to manufacture VLP vaccines in a continuous basis utilizing standard fermentation techniques. TechnoVax has established upstream and downstream processes for the production of VLP vaccines utilizing recombinant baculovirus/insect cell system or stable transfected mammalian cells. Process development from tissue culture flasks volumes to 5L fermentation scale up processes are currently performed in the laboratory. Tangential flow filtration (TFF) processing systems, chromatographic methods, and gradient centrifugation are part of our bio-separation, recovery and purification strategies implemented to obtain candidate VLP vaccine product.

Quality Assurance:
TechnoVax has a fully developed and comprehensive “Laboratory Quality Assurance Plan” used to participate in consortium managed biddings for government contracts. The overall objective of our quality assurance programs is to generate defensible data that will meet the quality objectives of the user.

Other:
Dr. Jose M. Galarza is an Adjunct Professor, lecturer and graduate advisor at the Department of Microbiology of New York Medical College. This Institution is a good resource to access new College graduate seeking positions.

Facilities and Resources at Baylor College of Medicine

Department of Molecular Virology and Microbiology

Laboratory:
Sufficient laboratory space is available within the Department of Molecular Virology and Microbiology to carry out all proposed studies. Dr Mbawuike has his office and laboratory equipped for microbiological and immunological studies. Dedicated central tissue culture rooms, in situ technology and RT-PCR measurement rooms are part of each laboratory. He has free access to the facilities and equipment of the department of Molecular Virology and Microbiology. Dr. Mbawuike occupies rooms, 244E (600 sq. ft., main laboratory and office), 252E (146 sq. ft., gamma counter room) and 209C (200 sq. ft., postdoctoral fellows office and laboratory) and shares equipment storage space in rooms 264E (Cyrostat) and 212C (Flow Cytometer). The main laboratory has a dedicated tissue culture room with two laminar flow hoods and a two compartment CO2 incubator. Also, *in situ* technology and RT-PCR measurement rooms are part of each laboratory.

Dr Mbawuike office is staffed with shared support staff: an administrator, two secretaries and a clerk.

Animal Facilities: BCM BSL-3/3E Satellite Facility:
This is a ~3700 square feet, 13 room facility (rooms K101 – K117). The facility was constructed for biosafety level-3 (BSL-3) work, using both traditional and enhanced level pathogens. Due to the increased level of awareness needed for work with BSL-3 agents, the facility operates as a satellite facility under the anagement of the Biological Safety Officer, who reports to the Office of Environmental Safety. Seven of the 13 rooms (rooms K104, K106, K107, K108, K116B, K116C, and K116D) are flexible lab suites designed to be used as either animal or tissue culture rooms depending on the needs of the investigators. The remaining 6 rooms are supportive in nature and include a dedicated equipment room (K114), 2 gown-in rooms (K101, K116), a gown out/ shower room (K116F), 2 decontamination/autoclave rooms (K103, K116E) and a clean exit room (K117). An internal portion of the facility (K116-K116F) comprised of 6 rooms (3 flexible lab suites [K116B, K116C, K116D], gown-in room [K116], the gown-out/shower room [K116F], and a decontamination/autoclave room [K16E]) constitutes ~1250 square feet and has the ability to operate as an “enhanced” BSL-3 facility, without affecting operations in the BSL-3 suite. This enhanced section is referred to as the BSL-3E suite. The entire area including both the BSL-3 suite and BSL-3E suite is referred to as the BSL-3/3E Facility. The facility was designed to house small animal models, thus the design of the labs includes containment standards and requirements defined in the CDC/NIH publication “Biosafety in Microbiological and Biomedical Laboratories (BMBL)”, 5th Edition, 2007 for animal BSL-3 (ABSL-3) space plus enhancements (ABSL-3E) including.

Data Analysis:
Computers, laptops and servers fully equipped with various statistical (e.g. SAS, STATVIEW and SLIDEWRITE) and graphical and office processing software.

Equipment:
A new Beckman-Coulter MCL-XL Automated four color fluorescein activated cell sorting (FACS) instrument is located in Dr. Mbawuike’s laboratory for these studies. A multi-parameter research EPICS Model 753 Laser Instrument interfaced with a CICERO High Speed Cell Sorting System is available for our use. EXPO software for FACS data analysis is linked to the cytometers and to the ELISA plate reader. An ABI PRISM 7700Sequence Detection System (Applied Biosystems) for real time RT-PCR analysis is also available. Here is a list of other representative equipment available for the proposed research: Autoclaves, incubators, pipet plugged, centrifuges, spectrophotometers, automatic microtiter dilutor and dropper, phase contrast microscope, -70C freezers, Gamma counters with tape printout, vacuum pumps and gel destainer, micro-fractionaters, pH meters, sonifier, IBM compatible computers, manual multichannel pipettes, electrophoresis power supply, rotavaporator, portable compressors, -20C freezers, C02 freezers, electronic balances, liquid nitrogen tanks, ultracentrifuges, refrigerators. Vertis homogenizers, inverted microscopes, UV fluorescent microscope, light microscopes, scintillation counters, typewriters, Molecular Devices kinetic spectrophotometer and plate reader, MASH-11 cell harvester. Liquid nitrogen freezers and BioRad 600 confocal microscope.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Jose Middle Name: M.
* Last Name: Galarza Suffix: Ph.D.
Position/Title: CEO & Founder Department:
Organization Name: TechnoVax Inc. Division:
* Street1: 765 Old Saw Mill River Rd.
Street2:
* City: Tarrytown County/ Parish: Westchester
* State: NY: New York Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 10591-6702
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

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

Degree Type: PhD
Degree Year: 1981

***Attach Biographical Sketch** 1244-TVx_UFlu_JMG_biosketch_1 Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 1

Prefix: Dr. * First Name: Diana Middle Name:
* Last Name: Dalfo Suffix: Ph.D
Position/Title: Research Scientist Department:
Organization Name: Technovax Inc. Division:
* Street1: 765 Old saw Mill River Rd.
Street2:
* City: Tarrytown County/ Parish: Westchester
* State: NY: New York Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 10591-6702
* Phone Number: [redacted] Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Other (Specify) **Other Project Role Category:** Staff Scientist

Degree Type: PhD
Degree Year: 2005

***Attach Biographical Sketch** 1245-TVx_UFlu_Dalfo_biosketch Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: Dr. * First Name: Innocent Middle Name:

* Last Name: Mbawuike Suffix: Ph.D

Position/Title: Associate Professor Department:

Organization Name: Baylor College of Medecine Division:

* Street1: One Baylor Plaza

Street2:

* City: Houston County/ Parish:

* State: TX: Texas Province:

* Country: USA: UNITED STATES * Zip / Postal Code: 77030-3411

* Phone Number: [redacted] Fax Number:

* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Co-Investigator **Other Project Role Category:**

Degree Type: PhD

Degree Year: 1984

***Attach Biographical Sketch** 1246-TVx_UFlu_biosketch_Mbawu Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 3

Prefix: Dr. * First Name: Ruben Middle Name: O.

* Last Name: Donis Suffix: Ph.D

Position/Title: Associate Director Department: Influenza Division, OID, NCIRD

Organization Name: CDC Division: Influenza Division

* Street1: 1600 Clifton Rd.

Street2:

* City: Atlanta County/ Parish:

* State: GA: Georgia Province:

* Country: USA: UNITED STATES * Zip / Postal Code: 30333-0000

* Phone Number: [redacted] Fax Number:

* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Other (Specify) **Other Project Role Category:** Collaborator

Degree Type: PhD

Degree Year: 1986

***Attach Biographical Sketch** 1247-TVx_Bio_Donis_120512v2.p Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

To ensure proper performance of this form; after adding 20 additional Senior/ Key Persons; please save your application, close the Adobe Reader, and reopen it.

Tracking Number:GRANT11282977 Funding Opportunity Number:PA-12-088 Received Date:2012-12-05T17:27:04-04:00

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Galarza, Jose M.	CEO, TechnoVax, Inc. Adjunct Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
National College, Jose Hernandez, Buenos Aires	BS	12/1971	Science
University of La Plata, La Plata, Argentina	DVM	09/1977	Doctor Veterinary Medic.
University of La Plata, La Plata, Argentina	Doctorate	11/1981	Microbiology
Univ. of Utah Medical Center, Salt Lake City, UT	Post-Doc	1986/1991	Virology/Mol. Biology
Univ. of California Irvine, Irvine, CA	Res. Scient	1991/1995	Microbi./Molec. Genetics

A. Personal Statement

I have broad scientific and leadership experience conducting research in the areas of virology, molecular biology, immunology, and vaccine development over the course of 30 years. As a post-doctoral researcher, I investigated the fundamental molecular mechanisms of negative-sense (flu, VSV) viruses and positive-sense (polio) virus transcription/replication, its regulation and reconstitution of biochemical active complexes with recombinant proteins and synthetic templates. Furthermore, I studied the induction of cellular immune responses elicited by highly conserved recombinant viral proteins (CTL-vaccines) of rapidly evolving viruses. This academic scientific experience brought me to the pharma/biotech industry (Wyeth, currently Pfizer) as PI and leader where I pursue new lines of research and development in the field of vaccinology. During my tenure, I was involved in many multidisciplinary vaccine R&D projects, collaborations including influenza projects with external collaborators and a CRADA with the NIH to develop para-influenza and RSV vaccines, as well as regulatory and scientific advisory committee member but above all, in the management and leadership of a research team that ultimately discovered the formation of wild type and chimeric influenza virus-like particles (VLP), a platform technology for vaccine development. Furthermore, as member of the new technology for vaccine development advisory group participated in the evaluation of external and internal emerging technologies, HPV and Rotavirus VLPs, Alphavirus platform, Dengue vaccine development strategies, DNA based vaccines, conjugates, chimeric proteins as adjuvanted subunit vaccines, etc.

In 2003, I co-founded TechnoVax, Inc. and became its CEO. The main focus of our company is the R&D of vaccines based on the virus-like particle (VLP) technology. My research accomplishments and experience in the field of vaccine R&D, particularly in the VLP area, together with my managerial and leadership skills equip me with the knowledge, technical expertise and managerial experience that are required to effectively execute and develop a VLP-based vaccine development project. Development of a successful broadly protective influenza vaccine able to sustain efficacy for an extended time will have a major impact in the prevention and control of influenza. This project, in collaboration with Dr. Mbawuike, Baylor College of Medicine, generates extraordinary interest and enthusiasm.

Positions and Honors

Positions and Employment

1982-1986 Research Assistant; Immunology and Virology Section of the Federal Research Council (CONICET), Argentina

1985-1986 Adjunct Associate Professor; Dept. of Microbiology, Northeast University, Formosa, Argentina

1986-1991 Postdoctoral Research Associate, Department of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City, Utah

1991-1995 Research Scientist, Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, CA
1996-2002 Principal Scientist and Project Leader, Department of Vaccine Research and Development Wyeth, Pearl River, NY

2003-Present President, CEO and Founder, TechnoVax, Inc. Tarrytown, NY
Adjunct Associate Professor, Dept. of Microbiology and Immunology
New York Medical College, Valhalla, NY

Other Experience and Professional Memberships

1982-1985 Instructor, Department of Agriculture and Natural Resources. Courses in Animal Virology; Formosa, Argentina.
1985 Principal Organizer, Conference on Interferon; Northeast National University, Formosa, Argentina
Memberships: American Association for the Advancement of Science (1989-present); American Society for Virology (1990-present); American Society for Microbiology; The New York Academy of Sciences (2003-present)
2006-2011. NIH reviewer on study sections “Non HIV Microbial Vaccines”, “Bio-defense” and “Immunology and Virology Topics”.

Honors
1982 Exchange Scholarship, University of Glasgow
1986-1988 Organization of American States Fellowship

Patents
1. A Subunit Vaccine for Influenza Virus Containing NP Protein Produced in Sf9 Cells by Recombinant Baculovirus. Filing Date: July 1990; Inventors: Donald F. Summers, Jose M. Galarza, Innocent N. Mbawuike and Robert B. Couch
2. Reconstituted Influenza A Transcriptase Reaction Using Overproduced Influenza Proteins. Filing Date: August 23, 1991 Serial Number: 07/748,999; Inventors: Donald F. Summers and Jose M. Galarza
3. Nucleotide Sequence of Influenza A/Udorn/72 (H3N2) Genome. Filing Date: June 23, 2000; Inventors: Jose M. Galarza and Theresa Latham.
4. Assembly of Wild Type and Chimeric Influenza Virus-Like Particles (VLPs). Filing Date: June 23, 2000; Inventors: Jose M. Galarza and Theresa Latham.
5. Influenza Virus-Like Particles (VLP) Compositions Filing Date: April 30, 2007; Inventors: Jose M. Galarza and Demetrius Matassov.
6. Polyvalent Influenza Virus-Like Particles (VLP) Compositions Filing Date: April 30, 2007; Inventors: Jose M. Galarza and Demetrius Matassov.
7. Respiratory syncytial virus (RSV)-Like particles Vaccines Composed of Composed of chimeric RSV/influenza structural proteins. Inventors: J.M. Galarza and Demetrius Matassov, Filed June 2007; patent pending
8. Monovalent or divalent wild type respiratory virus (RSV)-like particles vaccines bearing viral surface antigens that stimulate an immune response that protects humans against one or both of the RSV groups (RSV-A and RSV-B). Inventors: J. M. Galarza and Demetrius Matassov. Filed date: June 2007, patent pending.

C. Selected Peer-reviewed publications

<u>Most relevant to current application</u>

1. Latham, T., **Galarza J.M.**, Formation of Wild-Type and Chimeric Influenza Virus-Like Particles (VLPs) Following Simultaneous Expression of Only Four Structural Proteins, Journal of Virology, 75: 6154-6165, 2001.

2. **Galarza, J.M**., Latham, T., Cupo, A., Virus-Like Particle (VLP) Vaccine Conferred Complete Protection against a Lethal Influenza Virus Challenge, Viral Immunology, 18: 244-251, 2005
3 Matassov, D., Cupo, A., **Galarza J.M.**, A Novel Intranasal Virus-Like Particle (VLP) Vaccine Designed to Protect Against the Pandemic 1918 Influenza A Virus (H1N1), Viral Immunology, Vol. 20, 447-452, 2007.

Additional publications of importance to the field

1. **Galarza, J.M**., Sowa, A., Hill, V.M., Skorko, R., Summers, D.F., Influenza A Virus NP Protein Expressed in Insect Cells by a Recombinant Baculovirus is Associated with a Protein Kinase Activity and Possesses Single Stranded RNA Binding Activity, Virus Research, 24: 91-106, 1992.
2. Mbawuike, I.N., **Galarza, J.M**., Summers, D.F., Couch, R.B., Baculovirus-Expressed Influenza A/Udorn (H3N2) Nucleoprotein Induces Protective T-cell Immunity Against Influenza A/H3N2 and A/H1N1 in Mice, Vaccine Research, 3: 211-227, 1994.
3. Neufelt, K., **Galarza, J.M**., Summers, D.F., Richards, O.C., Ehrenfeld, E., Identification of Terminal Adenylyl Transferase Activity of the Poliovirus Polymerase 3Dpol, Journal of Virology, 68: 5811-5818, 1994.
4. Shi, L., Peng, Q., Summers, D.F., **Galarza, J.M**., Influenza A Virus RNA Polymerase Subunit PB2 is the Endonuclease Which Cleaves Host Cell mRNA and Functions Only as Trimeric Enzyme, Virology, 208: 38-47, 1995.
5. Shi, L., **Galarza, J.M**., Summers, D.F., Recombinant-Baculovirus-Expressed PB2 Subunit of the Influenza A Virus RNA Polymerase Binds Cap Groups as an Isolated Subunit, Virus Research, 42:1-9, 1996.
6. Peng, Q., **Galarza, J.M**., Shi, L., Summers, D.F., Influenza A Virus RNA-Dependent RNA Polymerase Cleaves Influenza mRNA in vitro, Virus Research, 42: 149-158, 1996.
7. **Galarza, J.M**., Peng, Q., Shi, L., Summers, D.F., Influenza A Virus RNA-Dependent RNA Polymerase: Analysis of RNA Synthesis in vitro, Journal of Virology, 70: 2360-2368, 1996.

D. Research Support

Ongoing Research Support

2R44AI063830-03A1 (Galarza, PI) 05/01/2005 – 08/31/2012
NIAID, NIH
Title: Development of Influenza Virus-Like Particle (VLP) Vaccines
The parent Phase I SBIR award created and tested in preclinical studies several VLP vaccine candidates designed to protect against highly pathogenic pandemic strains of the influenza virus such as H1N1-1918, H5N1 and H7N7 avian influenza viruses. The goal of this SBIR II is to construct and produce the VLP vaccines in Sf9 & mammalian cells. Protective efficacy against avian H5N1, H7N7 and human H1N1-1918 influenza viruses will be tested.

Completed Research Support

1 R43 AI063830-01 (Galarza, PI) 2005-2007
NIH/NIAID, SBIR Grant to TechnoVax, Inc.
Title: Influenza Virus-Like Particles as Vaccines
Role: PI

NIH Service Grant # 5R01A12316. (Summers, PI) 1991-1995
Title: Influenza RNA-Dependent RNA Polymerase
Role: Co-Investigator

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Dalfo, Diana	Research Scientist
eRA COMMONS USER NAME (credential, e.g., agency login)	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Barcelona (Spain)	Bachelor	1999	Biology
University of Barcelona (Spain)	Master	2001	Genetics
University of Barcelona (Spain)	Ph. D.	2005	Genetics
Columbia University (NY, USA)	Postdoctoral	2005	Medicine
New York University (NY, USA)	Postdoctoral	2007	Developmental Genetics

A. Personal Statement

Our main goal is to produce highly immunogenic and non-infectious virus-like particle (VLP) vaccines using a cell-based manufacturing system. This technology allows the development of single or polyvalent vaccines that carry the antigen(s) to protect against influenza. These virus-like particles will contain a combination of different proteins: hemagglutinin (HA), neuraminidase (NA), matrix (M1) and M2 proteins. We aim to broad coverage against circulating viruses not included in the currently licensed vaccine and enhance vaccine potency for the elderly population.

B. Positions Honors and Grants.

Positions and Employment

1998 Undergraduate student, Department of Genetics, University of Barcelona
1998-1999 Investigation contract to the European project, UE Biotechnology programme Bio4-97-2123, Department of Genetics, University of Barcelona
1999-2000 Assistant Professor of Molecular Genetics, Department of Genetics, University of Barcelona
2000-2003 Predoctoral fellowship from Ministerio de Educacion y Cultura of Spain
2004 Collaboration fellowship with the Department of Genetics, University of Barcelona
2005 Postdoctoral Research Scientist, Medicine Department, Columbia University, NY
2007 Postdoctoral Fellow, Developmental Genetics Program, New York University, NY
2011 Research Scientist, TechnoVax, Inc.

Honors

2003 University of Barcelona Travel Grant Award to participate at the 7th Evolutionary Biology meeting in Marsella, France
2010 Sackler Institute Travel Grant Award to participate at the *C. elegans*: Development and Gene Expression Meeting, June 17-20 in Heidelberg, Germany
2008 Reviewer for the journal Cell Research
2008 Undergraduate Honors Thesis Committee member at Biology Department, NYU

Grants

2000 Spanish National Institute of Education and Culture grant for a stage of one month in the international amphioxus laboratory, Observatoire Oceanologique du Banyuls, France
2000-2003 Ph.D. fellowship supported by Spanish government (AP99)

C. Publications

1. **Dalfo D.,** Cañestro C., Albalat R., Gonzàlez-Duarte R. (2001) Characterization of a microsomal retinol dehydrogenase gene from amphioxus: retinoid metabolism before vertebrates. Enzymology and molecular Biology of Carbonil metabolism: 359-370. Edited by Elsevier Science (book chapter)
2. **Dalfo D**., Cañestro C., Albalat R., Gonzàlez-Duarte R. (2001) Characterization of a microsomal retinol dehydrogenase gene from amphioxus: retinoid metabolism before vertebrates. Chem-Biol Interact 130: 359-370
3. **Dalfo D**., Albalat R., Molotkov A., Duester G., Gonzàlez-Duarte R. (2002) Retinoic acid synthesis in the prevertebrates amphioxus involves retinol oxidation. Dev Genes Evol 212: 388-393
4. **Dalfo D**., Permanyer J., Gonzàlez-Duarte R., Albalat R. (2003) SDR-RDH enzymes in lower chordates. An evolutionary approach into the retinoic acid metabolism. P. 12th Inter. Congress on Genes, gene families and isozymes 185-188
5. Fuentes M., Schubert M., **Dalfo D**., *et al.* (2004) Preliminary observations on the spawning conditions of the European amphioxus (*Branchiostoma lanceolatum)* in captivity. J Exp Zoolog B Mol Dev Evol 302 (4): 384-391
6. [redacted]
7. **Dalfo D.,** Marques N., Albalat R. (2007) Analysis of the NADH-dependent retinaldehyde reductase activity of amphioxus retinol dehydrogenase enzymes enhances our understanding of the evolution of the retinol dehydrogenase family. FEBS J 274 (14): 3739-3752
8. Setty Y., **Dalfo D.,** Korta D., Hubbard EJ., Kugler H. (2011) A model of stem cell population dynamics: in-silico analysis and in-vivo validation. Development (in press)
9. [redacted]
10. [redacted]

D. Research Support

R01 DK068437 (NIH) PI: Dr. Blaner (Columbia University Medical School) 2003-2007
TITLE: Characterization of β-carotene cleavage enzymes in the mouse.
GOALS: Retinoic acid (RA) regulates relevant vertebrate physiological processes such as anterior-posterior pattern formation, cell proliferation, tissue differentiation, embryonic development and vision. The main source of RA derives from the enzymatic cleavage of dietary ß-carotenes. An excess of RA during early pregnancy has been associated with a significant increase in birth defects and in several kind of cancer. Understanding how RA is synthesized from the dietary ß-carotens would help to fight against cancer and thus, would have a significant effect in public health.
ROLE: I was the primary member working on these studies. I conceived, designed, performed and analyzed all the experiments (including making ischemic/reperfusion in mouse model and all the biochemical studies).

R01 GM061706 (NIH) PI: Dr. Hubbard (NYU, Medical School) 2005-2009
TITLE: RNAi-based identification of genes involved in the germline proliferation in *C. elegans*
GOALS: The goal of this project was to understand the development and molecular basis for soma-germline interactions that influence germline proliferation and differentiation. The germ cells of most animals, including mammals, proliferate extensively either before or during the initial phases of differentiation, building an adequate progenitor pool for adult gamete production. The conservation of disease pathways between *C. elegans* and higher organisms, together with its simplicity make of this organism an important model for human diseases.
ROLE: I was the primary member working on these studies. I conceived, designed, performed and analyzed all the experiments. This study has led to 1 peer-reviewed research articles submitted, 1 book chapter, 2 public talks and 4 poster presentations in international conferences.

R03 HD066005-01 (NIH) PI: Dr. Hubbard (NYU, Medical School) 2008-2010
TITLE: TFGß signalling role in the control of germline proliferation in *C. elegans*
GOALS: Transforming growth factor ß (TGFß) signaling is a key player in development including axis formation, body patterning, tissue specification and morphogenesis. At the cellular level, TGFß exerts its effects through several different mechanisms such as inhibition of cell cycle progression, stimulation of

differentiation and triggering of apoptosis. TGFß signaling has been best studied in *C. elegans* for its role in dauer formation (dauers are resistant to environmental conditions and do not age). However, a possible role for the TGFß pathway in cell proliferation in the *C. elegans* germ line has not been previously investigated
ROLE: I was a key member of this project and I was responsible of the design, performance and analysis of all experiments. In addition, I was absolutely involved in the process of writing the application for the NIH R03 grant. This study has led to 1 peer-reviewed research articles in preparation, 3 public talks and 4 poster presentations in international conferences.

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Mbawuike, Innocent	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Boston State College (Univ. Massachusetts)	B.S.	1978	Biology
Boston University	Ph.D.	1984	Microbiology
Georgetown University, Washington, D.C.	Post-Doc	1984-1986	Immunology
Baylor College of Medicine, Houston, Texas	Post-Doc	1986-88	Viral Immunology

A. Personal Statement

The focus of my research in the past thirty years has been to define the role of cell mediated immune (CMI) responses in the control of influenza viruses in both human and animal models. We were among the first to characterize the human IFN-γ ELISPOT assay for influenza in humans and since extended that to RSV and smallpox. In recent collaborative work with Dr. Sette in La Jolla Institute for Allergy and Immunology, we identified repertoire of T-cell specificities for influenza A virus in humans using the IFN-γ ELISPOT assay. We have also developed influenza A/H5N1 and A/H7N7 VLP reagents, each expressing the M1, M2, HA and NA proteins of the respective viruses (collaboration with Dr. Galarza of Technovax) and demonstrated their ability to elicit responses IFN-γ and GrB ELISPOT assays in human PBMC. With our research groups' participation in NIAID-sponsored clinical trials of influenza A/H5N1 and A/H7N7 vaccines and our expertise in influenza CMI, we are in a unique position to execute the proposed study successfully. In addition, an ongoing pandemic 2009 H1N1 influenza study presents a unique opportunity to directly assess the role of CMI in protection against influenza disease. My laboratory remains the CMI site for evaluation of CTL responses to numerous biodefense agent vaccines (including smallpox and pandemic influenza viruses) being tested at the VTEU and VRPRU at BCM. With two long term collaborators (Galarza and Zheng) and clinical perspective from Dr. Couch (Consultant), our research team is well positioned to successfully execute this project and generate data to provide important insights into how CMI fights influenza in humans.

B. Positions and Honors.

Positions and Employment

1984-1986 Postdoctoral Fellow in Immunology, Department of Microbiology, Georgetown University School of Medicine and Dentistry, Washington, D.C.
1986-1988 Research Associate, Influenza Research Center, Department of Microbiology & Immunology, Baylor College of Medicine
1988-1991 Instructor (Viral Immunologist), Influenza Research Center, Department of Microbiology & Immunology, Baylor College of Medicine
1991-1994 Research Assistant Professor, Department of Microbiology and Immunology, Baylor Coll. of Med
1994-2004 Assistant Professor, Department of Molecular Virology and Microbiology, Baylor Coll. of Medicine
2004-Present Associate Professor, Department of Molecular Virology and Microbiology, Baylor Coll. of Med.

Other Experience and Professional Memberships

Adhoc Reviewer, Immunity and Host-Defense (IHD) Study Section, NIAID, NIH, 2010
Member, Vaccines Against Microbial Diseases (VMD) Study Section, NIAID, NIH, 2005-2009.
Member, Special Emphasis Panel for NIAID: Immune Defense Mechanisms at the Mucosa, 2009.
Consultant, WHO Consultation on Immunological Assays To Evaluate Efficacy of Influenza Vaccines, 2005.
Member, Special Review Committee for NIAID, "Multicomponent Vaccine Development, 1994.

Ad hoc Reviewer, Journal of Virology, 1997-present.
Ad hoc Reviewer, Journal of Infectious Diseases, 1998-present.
American Association of Immunologists
American Association for the Advancement of Science
American Society for Microbiology
Clinical Immunology Society

Honors

1992-1994 Shannon Fellow, National Institute on Aging, National Institutes of Health
1994 National Science Foundation Award for the DNA LITERACY PROGRAM, 1994.
1994 National Institute on Aging and the Gerontological Society of America Award to The Third Annual Summer Training Course in Experimental Aging Research, San Francisco, CA
1995 American Association of Immunologists Travel Award to the 9th International Congress of Immunology
2002 American Association of Immunologists Faculty Travel Award, Experimental Biology

C. Selected Peer-reviewed publications

Most relevant to current application

1. DiFabio S, **Mbawuike IN**, Fujihashi K, Couch RB, McGhee JR, Kiyono H. Quantitation of human influenza-specific CTLs: Correlation of cytotoxicity and perforin synthesis with increased numbers of interferon gamma producing $CD8^+$ T cells. Int Immunol 6:11-19, 1994 (PMID: 8148319).
2. **Mbawuike, I.N**., C. L. Acuna, K.C. Walz, R. L. Atmar, S.B. Greenberg and Robert B.Couch.1997. Cytokines and impaired $CD8^+$ CTL activity among elderly persons and the enhancing effect of IL-12 . Mech. Age Dev. 94:25-39, 1997 (PMID: 9147358).
3. **Mbawuike, IN.,** K. Fujihashi, S. DiFabio, S. Kawabata, J. R. McGhee, R. B. Couch and H. Kiyono. 1999. Human IL-12 enhances interferon-γ- producing influenza memory CD8+ CTLs. J. Infect. Dis. 180:1477-1486 (PMID: 10515806).
4. **Mbawuike, I.,** Zang, Y., and Couch, R.B., Humoral and Cell-Mediated Immune Responses of Humans to Inactivated Influenza Vaccine with or without QS21 Adjuvant Vaccine. Vaccine. 25(17):3263-9, 2007 (PMID: 17280748).
5. Bui, H-B, Peters, B., Assarsson, E., **Mbawuike, I.**, and Sette, A. Ab and T cell epitopes of influenza A virus, knowledge and opportunities. PNAS 104: 246-251, 2006 **(PMCID: PMC1765443).**
6. **Mbawuike IN,** Zhang Y**,** Couch RB**.** Control of mucosal virus infection by influenza nucleoprotein-specific CD8+ cytotoxic T lymphocytes. Respir Res. 8:44-51, 2007 (**PMCID: PMC1914056)**.
7. Assarsson E, Bui HH, Sidney J, Zhang Q, Glenn J, Oseroff C, **Mbawuike IN**, Alexander J, Newman MJ, Grey H**,** Sette A. Immunomic analysis of the repertoire of T-cell specificities for influenza A virus in humans. J Virol. 82(24):12241-51, 2008 (**PMCID: PMC2593359)**.
8. Zheng B, Zhang Y, He H, Marinova E, Switzer K, Wansley D, **Mbawuike I**, Han S. Rectification of age-associated deficiency in cytotoxic T cell response to influenza A virus by immunization with immune complexes. J Immunol. 2007 Nov 1;179(9):6153-9. Free Article: http://www.jimmunol.org.ezproxyhost.library.tmc.edu/cgi/content/full/179/9/6153

Additional recent publications of importance to the field

1. **Mbawuike, I.N.,** Lange AR, Couch RB. Diminished influenza virus specific MHC class I-restricted cytotoxic T lymphocyte activity among elderly persons. Viral Immunol 6:55-64, 1992 (PMID: 8476508).
2. **Mbawuike, I.N**., Piedra, P, Cate, T.R. and Couch, R.B. Cytotoxic T lymphocyte (CTL) response of unprimed infants to natural influenza A virus infection and live or inactivated vaccine, J. Med. Virol. 50:105-111, 1996 (PMID: 8915874).
3. **Mbawuike, I.N.** S. Pacheco, C. L. Acuna, K. C. Switzer, Y. Zhang and G. R. Harriman. Mucosal Immunity To Influenza Without IgA: An IgA Knockout Mouse Model. J. Immunol. 162: 2530-2537, 1999. Free Article: http://www.jimmunol.org.ezproxyhost.library.tmc.edu/cgi/content/full/162/5/2530

4. **Mbawuike, I.N. ,** J. Wells[1], R. Byrd, S. G. Cron, W. P. Glezen and P. A. Piedra. HLA-Restricted $CD8^+$ CTL, IFN-γ and IL-4 Responses to Respiratory Syncytial Virus Infection in Infants And Children *J. Infect. Dis.* 183: 687-696, 2001 (PMID: 11181144).
5. **Mbawuike I.N.,** ZHANG, Y., WANG, Y and Song L. Cationic Liposome-Mediated Enhanced Generation Of Human HLA-Restricted RSV-Specific $CD8^+$ CTL. J. Clin. Immunol. 22: 164-175, 2002 (PMID: 12078858).
6. Zhang, Y, Y. Wang, X. Gilmore, K. Xu, M. Chen, P. Tebebi and **I.N. Mbawuike.** Apoptosis And Reduced Influenza A Virus Specific $CD8^+$ T Cells in Aging Mice. Cell Death and Differentiation, 9: 651-660, 2002 (PMID: 12032674).
7. Orson, F., B. M. Kinsey, C. L. Densmore, T. Nguyen, Y. Wu, **I. N. Mbawuike**, P. R. Wyde. Protection against influenza infection by cytokine-enhanced aerosol genetic immunization. J. Gene Medi.: 8, 488-497, 2006 (PMID: 16389596).

D. Research Support

Ongoing Research Support

NO1-AI-030039 (Couch, PI) 08/01/2003-07/31/2010
NIAID, NIH
TITLE: Viral Respiratory Pathogens Research Unit
GOALS: The major goal of this project is to examine the interaction of respiratory pathogens - viruses and bacteria. New methods to development protection will be explored.
Role: Project PI and Director: “Epitope-Based Multi-Peptide Vaccines For Influenza”

2R44AI063830-03A1 (Galarza, PI) 05/01/2005 – 08/31/2012
NIAID, NIH
Title: Development of Influenza Virus-Like Particle (VLP) Vaccines
The parent Phase I SBIR award created and tested in preclinical studies several VLP vaccine candidates designed to protect against highly pathogenic pandemic strains of the influenza virus such as H1N1-1918, H5N1 and H7N7 avian influenza viruses. The goal of this SBIR II is to construct and produce the VLP vaccines in mammalian cells. Protective efficacy against avian H5N1, H7N7 and human H1N1-1918 influenza viruses will be tested in mice and ferrets.
Role: Co-investigator

1R01AI062917-01A1 (Zheng, Biao, Q, PI) 06/01/05-02/28/11
NIAID, NIH
TITLE: Fc Receptor Signaling in Vaccine Design for the Elderly
The goal of this project is to study the mechanisms of immune modulation by manipulating Fc receptor signaling on antigen-presenting cells and lymphocytes. This proposal will try to identify novel immunization strategy to provide effective protection to immune compromised population against infectious diseases.
Role: Co-investigator

N01 AI25465 (Keitel, PI) 11/01/2007-10/31/2014
NIAID, NIH
Vaccine and Treatment Evaluation Units (VTEUs). The purpose of this contract is to strengthen and expand DMID’s capacity to conduct clinical trials of promising candidate vaccines and therapies for infectious diseases. The unit will also conduct clinical trials in larger populations and to safely test vaccines in specific vulnerable populations, such as infants and the elderly.
Role: CMI Laboratory Director: To evaluate T cell immune responses to vaccines.

Completed Research Support

1-U01-AI056447-01 (Subcontractor; Van Nest, PI; Dynavax) 08/01/2003-07/31/2007
NIAID, NIH
ISS-Linked NP Vaccine to Control Pandemic Flu Outbreak. The goal of this proposal is to develop a novel influenza vaccine which includes nucleoprotein (NP) linked to an immunostimulatory oligonucleotide (ISS) to induce protective immunity against divergent and potentially pandemic influenza strains.
Role: Subcontractor to Dynavax

1 R21 AI53454-01 (Mbawuike, PI) 09/01/2002-08/31/2005
NIAID, NIH
Vulnerability to Smallpox Due to Declining CTL Immunity. The major objectives of study are to compare the sero-prevalence and memory $CD8^+$ CTL activity status against vaccinia in representative young adult and elderly populations, and to identify easily detectable and quantifiable surrogates of CTL competence against vaccinia that may be used as a surrogate of CTL immunity to smallpox infection. A secondary objective is to explore the immunological basis for complications from vaccinia vaccination.
Role: PI

R01 AG14351 (Mbawuike, PI) 04/01/1997-05/31/2003
National Institute on Aging, NIH
TH1 cytokines and impaired CD8+ CTL in elderly humans. The major goal of this project is to determine the mechanism of reduced CD8+ CTL responses in elderly humans and how to correct it.
Role: PI

RO1 AG10057 Mbawuike (PI) (Mbawuike, PI) 09/30/1996-08/31/2000
National Institute on Aging, NIH
Influenza Nucleoprotein as a Probe for Low CTL in Aging. The major goals of this project are to test the hypothesis that the diminished ability of aged mice to clear influenza virus infection and recover from the disease is due to deficient $CD8^+$ cytotoxic T lymphocyte (CTL) activity and to identify the deficient mechanisms responsible for the deficient CTL activity. Novel influenza "T cell vaccines", namely, influenza plasmid NP DNA and recombinant NP baculovirus vaccines will be utilized as probes for $CD8^+$ CTL in aging.
Role: PI

NO1-A1-65316 (Glezen, PI) 09/30/96 - 12/31/03
NIAID, NIH
Maternal Immunization for the Prevention of Infectious Diseases in Neonates and Infants. The major goal of this project is to test vaccines in pregnant women to promote passive immunity in the neonate and young infant. The priority agents are RSV, group B streptococcus, pneumococci and Hemophilus influenzae type b.
Role: Co-investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Ruben O. Donis, Ph.D.	**Associate Director, Influenza Division, CDC**
eRA COMMONS USER NAME [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Buenos Aires, Argentina	M.V.	1972-1978	Veterinary Medicine
Cornell University, Ithaca, NY	Ph.D.	1980-1986	Virology
St. Jude Children's Research Hospital	Post-Doc	1986-1989	Molecular Virology

A. Personal Statement

The major emphasis of our research is to understand the virulence and evolution of influenza virus in avian and mammalian hosts to design public health intervention strategies. Reverse genetics and organismal approaches are being exploited to understand molecular determinants of genetic reassortment and its role on interspecies transmission and the emergence of pandemic influenza viruses. Studies on the virulence of influenza virus inform risk assessments and also prioritize development of vaccine candidates. An important focus of our work entails leveraging our expertise on antigenic drift of the hemagglutinin to develop structure-based approaches that expand the breath of immunity to seasonal and pandemic influenza vaccines. This information is being applied to development of broadly neutralizing therapeutic monoclonal antibodies and universal vaccines.

B. Positions and Honors

Professional Positions

1980-1981 Assistant Research Scientist, Division of Laboratories and Research, New York State Department of Health, Albany, New York

1986-1989 Postdoctoral Fellow, St. Jude Children's Research Hospital, Memphis, Tennessee

1989-2003 Assistant/Associate/Professor, Dept. of Veterinary and Biomedical Sciences, University of Nebraska

2003-2006 Chief, Molecular Genetics Section, Influenza Branch, CDC

2004-present Adjunct Professor, Dept. of Microbiology and Immunology, Emory University, Atlanta, Georgia

2006-2012 Chief, Molecular Virology and Vaccines Branch, Influenza Division, CDC

2012-present Associate Director, Influenza Division, CDC

Honors and Awards

1983-1986 Cornell University, NYS College of Veterinary Medicine Rotating Fellowship

2007 Distinguished Service Award, United States Secretary of Health and Human Services

2008 Leveraging Collaboration Award, Food and Drug Administration, US DHHS

2009 Charles Shepard Science Award, Centers for Disease Control and Prevention

C. Representative Peer-Reviewed Publications (2009-2012) from a total of 153

1. Schat KA, Bingham J, Butler JM, Chen LM, Lowther S, Crowley TM, Moore RJ, Donis RO, Lowenthal JW (2012) Role of position 627 of PB2 and the multibasic cleavage site of the hemagglutinin in the virulence of H5N1 avian influenza virus in chickens and ducks. PLOS One 7:e30960
2. Tong S, Li Y, Rivailler P, Conrardy C, Castillo DA, Chen LM, Recuenco S, Ellison JA, Davis CT, York IA, Turmelle AS, Moran D, Rogers S, Shi M, Tao Y, Weil MR, Tang K, Rowe LA, Sammons S, Xu X, Frace M, Lindblade KA, Cox NJ, Anderson LJ, Rupprecht CE, Donis RO (2012) A distinct lineage of influenza A virus from bats. Proc Natl Acad Sci U S A 109:4269

3. Yamada S, Shinya K, Takada A, Ito T, Suzuki T, Suzuki Y, Le QM, Ebina M, Kasai N, Kida H, Horimoto T, Rivailler P, Chen LM, Donis RO, Kawaoka Y (2012) Adaptation of a duck influenza A virus in quail. J Virol 86:1411
4. Barlow PG, Svoboda P, Mackellar A, Nash AA, York IA, Pohl J, Davidson DJ, Donis RO (2011) Antiviral activity and increased host defense against influenza infection elicited by the human cathelicidin LL-37. PLOS One 6:e25333
5. Chen LM, Rivailler P, Hossain J, Carney P, Balish A, Perry I, Davis CT, Garten R, Shu B, Xu X, Klimov A, Paulson JC, Cox NJ, Swenson S, Stevens J, Vincent A, Gramer M, Donis RO (2011) Receptor specificity of subtype H1 influenza A viruses isolated from swine and humans in the United States. Virology 412:401
6. Christman MC, Kedwaii A, Xu J, Donis RO, Lu G (2011) Pandemic (H1N1) 2009 virus revisited: an evolutionary retrospective. Infect Genet Evol 11:803
7. Cox N, Donis R, Barr JR (2011) Exposure science for viral diseases: 2009 H1N1 pandemic influenza virus. J Expo Sci Environ Epidemiol 21:1
8. Feeley EM, Sims JS, John SP, Chin CR, Pertel T, Chen LM, Gaiha GD, Ryan BJ, Donis RO, Elledge SJ, Brass AL (2011) IFITM3 inhibits influenza A virus infection by preventing cytosolic entry. PLoS Pathog 7:e1002337
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OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TechnoVax Inc.

Delete Entry *** Start Date:** 07/01/2013 *** End Date:** 06/30/2014 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Jose	M.	Galarza	Ph.D.	PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2.	Rev.	Diana		Dalfo	PhD	Staff Scientist	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
												Total Senior/Key Person	[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate (Master level)	12.00			[redacted]	[redacted]	[redacted]
1	Research Intern	12.00			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TechnoVax Inc.

Delete Entry *** Start Date:** 07/01/2013 *** End Date:** 06/30/2014 **Budget Period 1**

C. Equipment Description

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

	Equipment item	* Funds Requested ($)
1.	Misc. small equipment maintenance/replacement	[redacted]
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	[redacted]

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs

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

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

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TechnoVax Inc.

Delete Entry * Start Date: 07/01/2013 * End Date: 06/30/2014 Budget Period 1

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	[redacted]
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Total Direct Costs	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				
	Total Indirect Costs			[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)
	[redacted]

K. * Budget Justification 1239-TVx_UFlu_Budget_Justification_120 Add Attachment Delete Attachment View Attachment

(Only attach one file.)

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

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TechnoVax Inc.

Delete Entry *** Start Date:** 07/01/2014 *** End Date:** 06/30/2015 **Budget Period** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Jose	M.	Galarza	Ph.D.	PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2.	Dr.	Diana		Dalfo	PhD	Staff Scientist	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
												Total Senior/Key Person	[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	8.00			[redacted]	[redacted]	[redacted]
1	Research Intern	6.00			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TechnoVax Inc.

Delete Entry *** Start Date:** 07/01/2014 *** End Date:** 06/30/2015 **Budget Period 2**

C. Equipment Description

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

	Equipment item	* Funds Requested ($)
1.	Misc. small equipment maintenance/replacement	[redacted]
2.		
3.		
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9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	[redacted]

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

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

E. Participant/Trainee Support Costs

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

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

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: TechnoVax Inc.

Delete Entry * Start Date: 07/01/2014 * End Date: 06/30/2015 Budget Period 2

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Total Direct Costs	[redacted]	[redacted]	[redacted]
2.			
3.			
4.			
Total Indirect Costs			[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)
	[redacted]

K. * Budget Justification 1239-TVx_UFlu_Budget_Justification_120 Add Attachment Delete Attachment View Attachment

(Only attach one file.)

BUDGET JUSTIFICATION

GENERAL CONSIDERATIONS

In order to conduct the proposed project consisting of producing VLP vaccines and testing these vaccines in animals we have planned to budget our efforts over a two years period.

Scientific personnel salaries and related expenses represent 52% of TechnoVax expenses or 41% of Total Requested Funds. High salaries costs are driven by two main factors: 1) the project duration and 2) the higher cost of salaries in the New York metro area. To attract adequate talent TechnoVax needs to offer competitive salaries, however in order to maintain financial balance, our salaries are still about +30% lower than those offered at other larger biotech companies located within a 10 miles radius (e.g. Regeneron, Progenics, Acorda/Astellas, Pfizer, etc.). Therefore we believe that our budget includes adequate staffing level as well as reasonable other direct and indirect costs to support a successful outcome for this project.

Our sub-awardee, Dr. Innocent Mbawuike at Baylor College of Medicine, will perform the animal studies and is budgeting $[redacted] or about 20% of total requested funds. This cost is justified by the fact that these studies will need to take place in BSL-2 and "BSL-3 enhanced" level facilities.

TECHNOVAX PERSONNEL

Dr. Jose M. Galarza, D.V.M., Ph.D., CEO and President of TechnoVax will serve as PI of the project. His primary role will be oversight of all aspect of the project and direct hands-on participation in the creation, production, characterization and testing of Flu VLP vaccines. The PI will maintain constant communication with the Program Director, collaborators and external advisors to address scientific issues, progress and evaluate results. PI will allocate 25% of his time to the execution of this project. Proportional annual salary requested is [redacted] and [redacted] for periods 1 and 2 respectively.

Dr. Diana Dalfo, PH.D., is a TechnoVax Staff Scientist for VLP vaccines projects and will perform experiments and guide a Research Associate on the different aspects of the project. Dr. Dalfo will allocate 60% of her time to the execution of this project. Proportional annual salary requested is [redacted] and [redacted] for periods 1 and 2 respectively.

One Research Associate, M.S. (with 2-3 year experience), will be involved in cell culture maintenance, cloning, plasmid preparation and purification. Production and characterization of large scale DNA. Immunological assays for evaluating the immune response elicited by the Universal Flu VLP vaccine candidate (serum neutralization test). Research associate will allocate 100% of his/her time to the execution of this project. Annual salary requested is $[redacted] and $[redacted] for periods 1 and 2 respectively as a larger effort will occur during the first year.

Research Intern (M.S. graduate), will provide technical support in the lab to the research team on a full time basis for 18 months. Annual salary requested is [redacted]

Fringe Benefits: Based on current payroll data, Fringe Benefits amount to 25% of Base Salary Costs.

Salary and Costs increases: a 3% annual increase has been budgeted for salaries in period 2.

EQUIPMENT

Miscellaneous Equipment: as current lab equipment is mostly fully utilized by current projects, various small equipment items will be needed for day to day operations (fridge, -20C freezer, etc.). [redacted] has been budgeted annually to cover purchases and maintenance costs.

TRAVEL

Domestic Travel: $[redacted] in year 1 is budgeted for attending a major scientific conference and scientific meetings as follows: PI or Staff Scientist: [redacted] ($[redacted] for registration and $[redacted] for travel)

OTHER DIRECT COSTS

Material and supplies: General laboratory supplies including plastic-ware, reagents, chemicals, tissue culture media and protein analysis and purification material, Western blot reagent, disposables are requested for vaccine production, characterization and testing. This total budget of $[redacted] over 2 years is based on a current comparable project for the production of an influenza VLP vaccine and can be broken down into two general categories comprising:

	Year 1	Year 2
Plasticware, glassware and misc.:	[redacted]	[redacted]
Reagents, cell lines, Ab and chemicals:	[redacted]	[redacted]
Total Material and supplies	[redacted]	[redacted]

Equipment or Facility Rental/User Fees: Electron-Microscopy images will be needed after each production batch; we currently use City College of New York (CUNY) EM equipment for a cost of [redacted] per session.

INDIRECT COSTS

F&A costs: as awarded in previous SBIR grants, we are requesting a 25% indirect cost rate applied to direct costs to cover F&A costs.

FEE

TechnoVax requests 6.99% fixed fee for re-investment into company infrastructure and Operating costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	4	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		[redacted]
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	[redacted]	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	[redacted]	
6. Equipment or Facility Rental/User Fees	[redacted]	
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		[redacted]

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2013 *** End Date:** 06-30-2014 **Budget Period: 1**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1. Dr.	Innocent		Mbawuike		Co-PI		2.00			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	3.00			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Tracking Number: GRANT11282977

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2013 *** End Date:** 06-30-2014 **Budget Period: 1**

C. Equipment Description

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

Equipment Item	*** Funds Requested ($)**

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel **Funds Requested ($)**

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost

E. Participant/Trainee Support Costs **Funds Requested ($)**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant/Trainee Support Costs**

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2013 *** End Date:** 06-30-2014 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Supplies and Services	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. MTDC	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency DHHS, Arif Karim, 214-767-3261
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 1243-BAYLOR_Budget Justification_120512.pdf (Only attach one file.)	Mime Type: application/pdf

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **Budget Period: 2**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Innocent		Mbawuike		Co-PI		[redacted]			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	3.00			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **Budget Period: 2**

C. Equipment Description

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

Equipment Item	*** Funds Requested ($)**

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel **Funds Requested ($)**

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost

E. Participant/Trainee Support Costs **Funds Requested ($)**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant/Trainee Support Costs**

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Supplies and Services	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. MTDC	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency DHHS, Arif Karim, 214-767-3261
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 1243-BAYLOR_Budget Justification_120512.pdf (Only attach one file.)	Mime Type: application/pdf

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

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	[redacted]
Total Number Other Personnel	2
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	
1. Domestic	
2. Foreign	
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	[redacted]
9. Other 2	
10. Other 3	
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	

BUDGET JUSTIFICATION – Baylor College of Medecine

PERSONNEL

Innocent N. Mbawuike, Ph.D. (BCM PI) (about 2 months/year FT effort) is Associate Professor of Molecular Virology and Microbiology. Dr. Mbawuike has extensive experience in the evaluation of immunological responses related to influenza in animal models and humans. As BCM co-investigator on this SBIR Phase I proposal, he will conduct immunogenicity studies with mice immunized with influenza VLP vaccines. He will supervise influenza challenge studies in mice to be conducted in the BSL-2 facility. Dr. Mbawuike will monitor the day to day experiments conducted at BCM and will be responsible for preparing reports and manuscripts for studies generated at this site.

To be named. (Research Assistant I, about 3 months/year FT effort). An experienced senior research assistant will be hired or assigned to perform the animal immunization experiment and conduct virus and antibody assays under the supervision of Dr. Mbawuike. The research Assistant will participate and perform the influenza virus challenge studies in the BCM BSL-2 and BSL-3 facilities.

OTHER DIRECT COSTS

Animal purchase and housing: Approximately 250 young adult mice will be purchased for [redacted] ($[redacted] each). The cost for housing animals in the biohazard suite of BCM vivarium is based on $[redacted] for mice (4 mice per cage) per diem respectively. It is estimated that the animals will be housed for approximately 60 days during each experiment for a cost of [redacted]

Supplies: General Laboratory supplies including cell culture reagents and plastic ware were requested for laboratory tests. Laboratory test include virus titration, virus neutralization and hemagglutination inhibition (HI) for specimens obtained from mice and ELISA assays for antigen-specific IgG, IgA and IgM antibodies. A budget of $[redacted] is allocated over the two year period.

INDIRECT COSTS

As per negotiated rate of 56.50% with DHHS.

SBIR/STTR Information

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

*** Program Type (select only one)**

☒ SBIR ☐ STTR

☐ Both *(See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)*

*** SBIR/STTR Type (select only one)**

☒ Phase I ☐ Phase II

☐ Fast-Track *(See agency-specific instructions to determine whether a particular agency participates in Fast-Track)*

Questions 1-7 must be completed by all SBIR and STTR Applicants:

☒ Yes ☐ No	* 1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?
	* 1b. Anticipated Number of personnel to be employed at your organization at the time of award. 10
☐ Yes ☒ No	* 2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies? * If yes, insert the names of the Federal laboratories/agencies:
☐ Yes ☒ No	* 3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov
☒ Yes ☐ No	* 4. Will all research and development on the project be performed in its entirety in the United States? If no, provide an explanation in an attached file. * Explanation: Add Attachment Delete Attachment View Attachment
☐ Yes ☒ No	* 5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work? * If yes, insert the names of the other Federal agencies:
☒ Yes ☐ No	* 6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?
	* 7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions. * Attach File: Add Attachment Delete Attachment View Attachment

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

☒ Yes ☐ No	* 8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment. * Attach File: 1234-TVx_Commercialization_Hist Add Attachment Delete Attachment View Attachment
☒ Yes ☐ No	* 9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

☐ Yes ☐ No	* 10. Please indicate whether the answer to BOTH of the following questions is TRUE: (1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND (2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?
☐ Yes ☐ No	* 11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?

COMMERCIALIZATION HISTORY

TechnoVax has received one SBIR Phase II award in 2009:

Grant Number: [redacted]

Project Title: Development of Influenza Virus-Like Particle (VLP) Vaccines

As requested, a commercialization plan was provided at the time of the grant application. Currently this project is on-going and is in pre-clinical phase.

TechnoVax has not received any other SBIR Phase II award since 2009.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

Prefix:	Dr.
* First Name:	Jose
Middle Name:	M.
* Last Name:	Galarza
Suffix:	Ph.D.

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

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

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:	Mr.
* First Name:	Hector
Middle Name:	
* Last Name:	Munoz
Suffix:	
* Phone Number:	[redacted]
Fax Number:	[redacted]
Email:	[redacted]
* Title:	Chief Financial & Corp. Development Officer
* Street1:	765 Old Saw Mill River Rd.
Street2:	
* City:	Tarrytown
County/Parish:	Westchester
* State:	NY: New York
Province:	
* Country:	USA: UNITED STATES
* Zip / Postal Code:	10591-6702

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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:GRANT11282977 Funding Opportunity Number:PA-12-088 Received Date:2012-12-05T17:27:04-04:00

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)	1240-TVx_UFlu_INTRODUCTION_	Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1241-TVx_UFlu_SPECIFIC_AIMS_	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1242-TVx_UFlu_Research_Plan_	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		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1248-TVx_UFlu_VERTABRATES_AN	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research	1249-TVx_UFlu_Select_Agents_	Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements	1250-Mbawuike_LOI.pdf	Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1251-TVx_UFlu_Letters_Suppor	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	1252-TVx_UFlu_Sharing_Plan_	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

1. INTRODUCTION

This is a resubmission of a proposal to develop a broadly protective (universal) influenza VLP vaccine. We thank the reviewers for their insightful comments. We utilize VLP technology, since it permits modifications that would prevent virus replication for a viral vaccine. Broadly neutralizing antibodies have been isolated from humans and are directed against domains in the stem and other less immunogenic regions of HA. The isolation of such broadly neutralizing antibodies indicates that they are immunogenic and our goal is to determine if neutralizing antibody can be generated by VLPs modified to eliminate the immunodominant, hypervariable epitopes comprising the head region of HA. If so, they could be used in the case of an influenza pandemic, as a supplement to current vaccines to broaden protection as well as elicit longer duration of immunity. Previous reviewers found our proposal- "innovative", "highly significant", "a rational approach", "outstanding expertise in influenza vaccinology", "outstanding environment". However, weaknesses identified by the prior reviewers are discussed here and changes were made in the research plan. ***"Proposal is over ambitious for the two year project period":*** We have experience producing and characterizing VLP vaccines. We utilize CHO cells, which express the M1 and M2 structural proteins of the virus to which HA and NA are transfected to simplify and shorten VLP production time. We express the VLPs in CHO cells, which allows for adequate yield for characterization, immunological analysis with the broadly neutralizing human antibodies that we have assessed, as well as for immunogenicity and efficacy studies in animals. **"If the remodeled HA do not perform as expected the rest of the experiment cannot be completed":** We show as preliminary data that at least some of our remodeled HAs can be produced and have elicited neutralizing antibodies. However, to mitigate risk and investigate structural factors enhancing immunogenicity, we have designed several different constructs including 5TA which models a wild type HA, able to fold properly and form trimers but contains specific protease cleavage sites which allow for removal of the globular domain by protease treatment after the VLP is produced and purified exposing conserved subdominant epitopes in the HA2 (stem) and the remainder of HA1. Thus we expect to be able to produce, characterize and test this VLP as well as others. ***"Acknowledge but no alternate plans are presented"*:** VLPs are composed of a scaffold of the M1 and M2 proteins to which various remodeled HAs and NAs can be incorporated. To strengthen the design process, we have enlisted Dr. Zhongtoa Zhang a structural biologist/protein chemist in the Department of Biochemistry, New York Medical College (see letter). "***The preliminary results are not described clearly, and as presented, do not provide convincing evidence of feasibility for this project":*** We have revised the preliminary data section and provide a more detail explanation of what is presented in the sera neutralization assay shown in figure 4. "***The VLP vaccine may be expensive and laborious to produce"*** Initially, until the candidates that afford broad protection are identified, we will transiently transfect remodeled HA plasmids into a cell line that constitutively expresses the M1/M2 scaffold, allowing VLP production. Once candidates are identified, a constitutively producing vaccine cell line can be created. In fact, this strategy should be faster, less laborious, and more cost-effective than current methods of manufacturing seasonal flu vaccines. ***Rationale for using ELISA to quantify antibodies is not clear" "ELISA primarily measures binding Ab, which presumably these vaccines will not induce"*:** Our first method to evaluate antibody response is virus neutralization, ELISA is proposed as a secondary method to determine whether other antibodies are being elicited and can be measured by ELISA, particularly using virus as target antigen. The revised Research Plan addresses other reviewers' comments.

2. SPECIFIC AIMS

We propose to develop a broadly neutralizing influenza vaccine utilizing virus-like particles that display on their surfaces remodeled HA molecules. Most antibodies against HA target predominantly immunodominant but highly variable antigenic sites on the globular portion of the molecule, whereas the conserved subdominant antigenic sites, particularly those in the stem region are poorly recognized by the immune system. Recently identified human antibodies directed against these conserved antigenic sites have been isolated, and have demonstrated the ability to neutralize a broad range of influenza virus strains. It is reasonable, therefore to envision developing a vaccine that primarily displays highly conserved conformational antigenic sites able to elicit an antibody response recognizing these sites and obtain protection against a broad spectrum of antigenically distinct influenza viruses. To attain this goal and test this hypothesis, we propose to re-engineer influenza HA molecules and display these structures on the surface of influenza virus-like particles (VLPs). We will create remodeled HA by removing regions of the molecule that form the immune-dominant and highly variable antigenic sites and repositioning subdominant conserved and broadly protective antigenic regions. Broadly neutralizing influenza VLP vaccines displaying on their surfaces such remodeled HA molecules that reveal the subdominant conserved antigenic sites will be produced in mammalian cells. The immunogenicity and efficacy elicited by candidate VLP vaccines revealing remodeled HA molecules will be assessed in an influenza mouse model. To achieve these goals, we propose the following specific aims:

Specific aim #1: Construct several remodeled HA expression constructs devoid of highly variable immunodominant antigens sites but preserving conserved subdominant antigenic regions and sub-clone them into an appropriate plasmid for VLP creation. Produce and characterize virus-like particles (VLPs) displaying remodeled HA molecules on their surfaces. Evaluate the presence of conserved subdominant antigenic sites in HA molecules displayed on purified VLPs by immuno-precipitation using antibodies that exclusively recognized these sites.

Specific aim #2: Assess the immune response elicited in mice following immunization with candidate VLPs displaying alternative remodeled HA protein on their surfaces. The capacity of immunized mouse sera to neutralize different influenza A virus strains will be measured by an in-vitro micro-neutralization assay. Those VLP vaccines capable of inducing broadly neutralizing antibodies, as demonstrated by partially or completely neutralizing a panel of antigenically distinct influenza viruses, will be further tested in in-vivo efficacy and immunogenicity studies in mice alone and in combination with VLPs containing unaltered HA to detect synergy or competition.

Specific aim #3: Conduct efficacy and immunogenicity studies of single or combination HA remodeled VLP vaccines in a lethal influenza mouse model. Groups of immunized mice will be challenged with lethal doses of at least three antigenically distinct influenza viruses (HA5, HA1 and HA3). Further testing of the most promising candidate vaccines in ferrets will be pursued in a Phase II SBIR proposal, which will also seek enabling data for an IND application with the FDA.

3. RESEARCH PLAN

3.1. Significance and Impact

We propose to develop a broadly protective virus-like particle (VLP) based influenza vaccine that can neutralize a spectrum of influenza A virus subtypes by eliminating the major epitopes on HA to expose the more highly conserved universal and less available epitopes in the molecule.

Seasonal influenza infections continue to pose a significant burden in the US and around the world. Influenza A viruses cause respiratory infections that afflict more than 60 million Americans of all ages every year causing some 200,000 hospitalizations and about 36,000 deaths per year [1,2]. In addition to seasonal circulating viruses, pandemic strains periodically emerge with dire consequences [3,4]. For example, the H5N1 avian virus, continues to circulate in birds and causes occasional human infection with high mortality [5,6]. If this virus acquires the capacity to effectively transmit amongst humans, the consequences are likely to be severe [7,8]. In recent studies, airborne transmission of the H5N1 occurred in ferrets after four mutations in the HA and one mutation of the PB2 protein[9,10]. In addition, outbreak of flu in chickens, as happened recently in Mexico, could disrupt conventional production of vaccines.

The rapid antigenic evolution of influenza virus driven by the continuous accumulation of mutations (antigenic drift) or by gene swapping through reassortment (antigenic shift) overcomes the immune responses elicited by natural infection or vaccination. This necessitates periodic reformulation of vaccines with the predominant circulating strains and annual re-immunization to upgrade vaccine composition and to improve efficacy. Furthermore, the large number of avian and mammalian species susceptible to influenza virus provides additional sources of emerging virus that have the potential to give rise to a pandemic. Creation of a vaccine able to protect against both drifting viruses and those emerging from gene-shifting has been a major objective in the influenza vaccine field. Significant efforts have been applied to develop a universal vaccine, but achieving it has been elusive. However, the recent identification of broadly neutralizing antibodies from infected individuals [11,12] provides new possibilities for the design of vaccines able to elicit broad neutralizing protection. These broadly neutralizing antibodies predominantly bind to highly conserved sites on the stem regions of the hemagglutinin (HA), blocking virus infection by the steric inhibition of membrane fusion rather than receptor binding. Proper presentation of these highly conserved but subdominant (cryptic) antigenic sites in a vaccine composition could elicit broadly neutralizing antibodies. To achieve this goal, we propose to utilize VLPs displaying remodeled HAs that mainly exhibit these conserved epitopes. Development of a broadly protective (universal) vaccine able to withstand antigenic variation and sustain efficacy for an extended time should have a major impact on influenza prevention. Used not only as a stand-alone vaccine, but also as a component of seasonal vaccines, it could reduce both the incidence and severity of infection.

3.2. Innovation

We have shown that we can produce Virus-Like Particles (VLPs) with high immunogenicity and efficacy by expressing 4 flu proteins M1, M2, HA and NA simultaneously [31, 20, 21]. Also, we have produced VLPs with 2 different HAs in the same particle as well as chimeric molecules indicating the versatility of the system. The dominant immunogenic epitopes in HA are located in the globular domain necessary for binding to cellular receptors. If these are removed the virus would no longer be infectious and able to multiply. Current concepts suggest that these domains block immunogenic recognition of other regions of the HA molecule by steric hindrance and by immunodominance. The discovery of broadly neutralizing antibodies in formerly infected individuals indicates that these other epitopes are immunogenic and elicit a broad and effective immune response [11, 13]. We have designed a series of modified HA molecules to express in our VLP expression system. Preliminary studies with two of these reengineered HAs indicate that they are incorporated into VLPs produced in CHO cells, and elicit a immune response after immunization as tested in micro-neutralization assays of flu virus.

While these VLPs are produced by transient transfection in CHO cells expressing the M1 and M2 proteins, we have also created another VLP production system using continuous cell lines suitable for laboratory 5 liters scale up. Here we describe our preliminary findings and our proposal to produce several differently modified HAs to assess assembly, immunogenicity and efficacy that they would elicit against diverse flu strains. This cannot be done with egg based or cell based virus production methods.

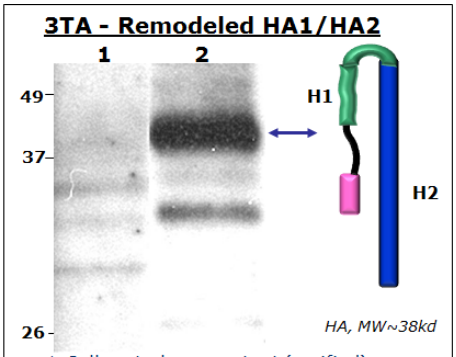


***Fig.1**: IGVirus-like particles (VLPs) produced in CHO cells expressing influenza proteins M1, M2, NA and a remodeled HA molecule. Analysis of purified VLPs by Western blot using an anti-HA2 antibody.*

Our rationale for creating a broadly protective vaccine is based on developing VLPs displaying remodeled HA molecules (VLP-HA-Rem) that expose widely conserved subdominant antigenic sites while devoid of dominant and hypervariable epitopes. Conserved antigenic sites in the HA molecule may comprise linear or conformational determinants (epitopes); and both configurations would be suitable targets for specific antibody elicitation. Most of the HA conserved epitopes map to the HA2 fragment or the HA1 and HA2 interface, distant from the receptor binding site. The stem region of the trimeric HA is formed by the HA2 fragments of each monomer and contains the fusion peptide. Antibodies directed to this region should neutralize virus entry by blocking membrane fusion sterically, as shown with the recently identified broadly neutralizing human antibodies [11, 12]. To better display these sites, we remodeled the HA molecules by deleting regions of the HA1 fragments containing dominant variable sites, rearranging disulfide bridges and/or introducing hinge linkers to reposition the highly conserved epitopes and making them predominant to elicit production of broadly protective neutralizing antibodies. These remodeled HAs are incorporated and displayed on the surface of native influenza virus-like particles [31]

VLP vaccines are highly immunogenic because of their particulate nature and display of a repetitive array of antigens (polyvalency). Structured macromolecular antigens such as VLPs facilitate cross-linking of multiple antigen receptors enhancing activation of B lymphocyte and priming T helper cells. It is anticipated that a VLP-HA-Rem vaccine will stimulate a robust and long lasting immune response and hopefully broad protection. To our knowledge, although attempts to develop a universal vaccine are being pursed[14-16], including the most recent work that grafted A-helix peptide on a icosahedral particle [30], the strategy proposed here for creating this type of vaccine is novel and if successful, could have a significant impact on the prevention and control of influenza.

3.3. Approach

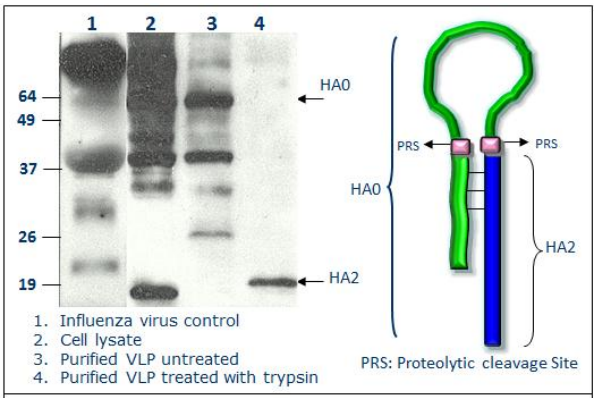


***Fig 2** : Virus-like particles (VLPs) were produced in CHO cells and purified by ultracentrifugation; then half of sample was treated with trypsin to remove the globular portion of the molecule. The HA fragments present in virus control (1), cell lysate (2), untreated (3) and protease treated VLPs were analyzed by Western blot using an anti-HA2 polyclonal antibody.*

Progressive accumulation of mutations, due to the high error frequency of the influenza polymerase, drives the rapid antigenic evolution of the HA molecules of these viruses. Furthermore, gene swapping between influenza A strains by reassortment brings about sudden and more pronounced antigenic changes (antigenic shift). These mechanisms allow influenza viruses to evade antibody responses elicited by natural infection or vaccination. Therefore, development of an influenza vaccine able to overcome antigenic evolution and afford broad and sustained protection is one of the most desirable goals in influenza vaccine development. Here, we propose a strategy to develop a broadly protective vaccines based on VLPs displaying on their surface remodeled HA molecules that primarily present highly conserved subdominant antigenic sites. Human antibodies directed at these sites have demonstrated the ability to neutralize a broad spectrum of antigenically distinct influenza viruses [11].

To generate remodeled HA, we have re-designed five distinct HA genes, which represent alternative structural conformations of the HA molecule. Computer and structural analysis was used to guide remodeling, molecular design and sequence selection [11,17-19]. Four of these constructs contain alternative truncations of the HA1 fragment, preserving or repositioning disulfide bridges and signal domains (Fig.1 & 5). The 3TA construct also contains a 12aa peptide linker that bridges non-deleted portions of the HA1, which connects to the HA2 fragment allowing for conformational epitopes to form (Fig. 1). A construct, 5TA comprises a full length HA molecule containing two unique protease cleavage sites that, following protein folding and display on the VLP vaccine, will allow for the enzymatic cleavage of the top portions of the molecule to expose the conserved determinants in the HA2-stem and stem/HA1 interphase (Fig. 2). Evaluation of their ability to be incorporated into VLPs and the potency and breadth of the immune response they generate should further enable universal vaccine design.

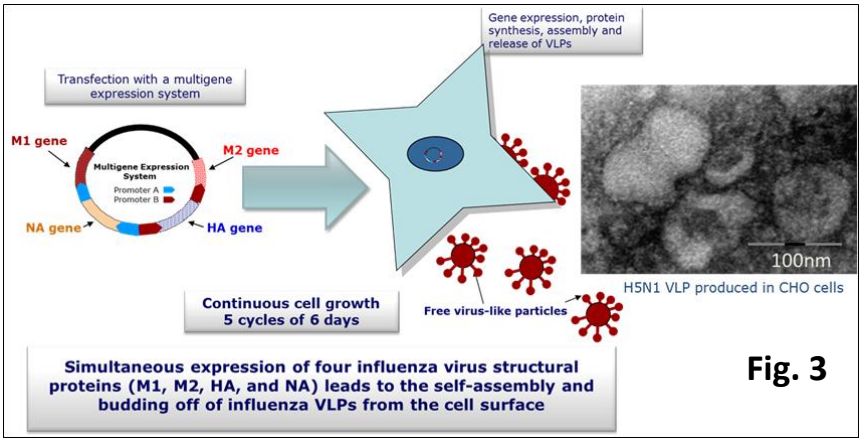


Fig. 3

TechnoVax has pioneered research in influenza virus-like particles (VLPs) (Latham T. and J. M. Galarza, 2001, J. Virol. 75:6154-6165) and the development of vaccines based on this technology. VLP vaccines have demonstrated immunogenicity and have been shown to provide protection against an otherwise lethal influenza virus challenge when administered via either intranasal or intramuscular routes [20,21] including 100% protection against a lethal challenge by influenza A/Vietnam/1203/2004 (H5N1) virus. VLP vaccines are produced in cell-based recombinant systems as structural and biochemical mimics of the wild type virus. These VLPs lack viral genetic material and are unable to replicate or cause infection. Vaccine inactivation is not required, better maintaining antigenic epitopes and immunogenicity. Because of the flexibility, speed and safety of the technology, vaccines can be generated rapidly and without risk of disseminating infectious material. Other VLP vaccines have proven successful in preventing HPV caused cervical cancers (Guardasil, Cervarix) and hepatitis B induced liver disease [22,23]. Our flu VLP manufacturing system is based on generating stably engineered mammalian cell lines. Candidate VLP vaccines are produced utilizing engineered cell lines that express the polypeptides required for the self-assembly and release of virus-like particles from the cell surface. Our plan is to produce the VLP vaccines in stably transfected cells (CHO) following the expression of M1-M2-NA and remodeled HAs. This approach eliminates the need for vectors or viruses to drive gene expression, minimizing downstream purification and safety concerns. We have produced H5N1 VLP using this strategy (figure 3). Additionally, we have created CHO and Vero lines that constitutively express the M1 and M2 proteins, which form a scaffold for VLP assembly. Addition of remodeled HA and NA genes would allow for the generation of VLPs displaying remodeled HA revealing conserved epitopes common to multiple subtypes. Furthermore, utilization of these cell lines for vaccine development will facilitate the regulatory process, as these cells are being extensively used in the biopharmaceutical industry for the manufacturing of many FDA approved products.

3.3.1 Preliminary Studies

Our initial studies were performed with two of the synthesized remodeled HAs to determine whether immunizing mice with VLPs displaying these molecules elicited antibodies capable of neutralizing a homologous influenza virus strain. Two remodeled HAs (3TA and 5TA-see fig.1 and 2) derived from the influenza A/Vietnam/1023/2004 (H5N1) were subcloned into our expression vector that also contains the M1, M2 and the NA construct. Design of this HA was based on the results reported with an identified human broadly neutralizing antibody [11] Our construct was transiently transfected into CHO cells together with a plasmid expressing the M1 and M2 proteins. Five days post-transfection, VLP material was collected from the culture medium, purified by ultracentrifugation and analyzed by Western blot utilizing an HA2 fragment (stem region) specific antibody. We detected these proteins in the purified material (Fig. 1 and 2), and then immunized four mice with each VLP preparation (3TA-VLP and 5TA VLP-protease treated) twice, two weeks

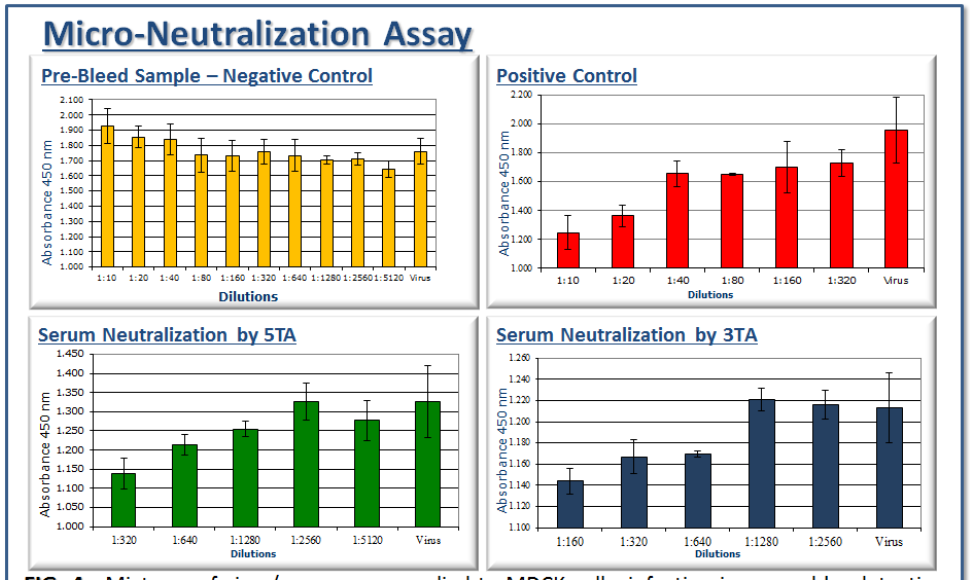
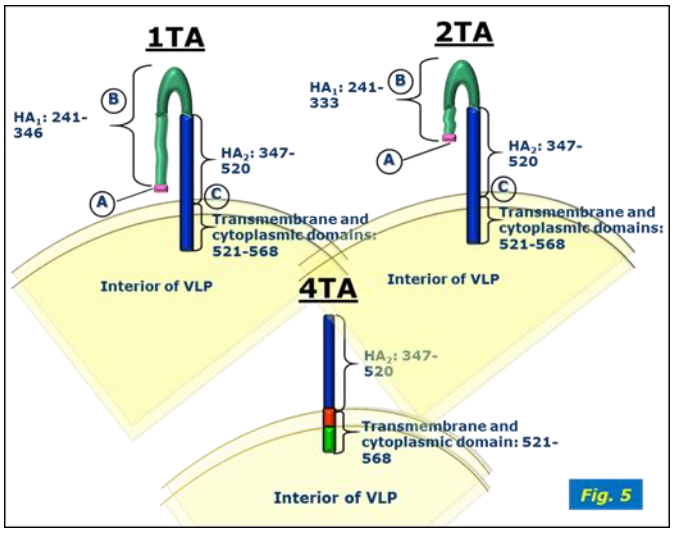


FIG. 4 - Mixtures of virus/serum were applied to MDCK cells; infection is assayed by detecting expression of influenza NP protein using an ELISA test [21]. Pre-bleed sample did not show virus neutralization as compared to virus control (right column-same panel), whereas 5TA and 3TA samples, as well as positive serum control, showed reduction of infection at lower dilution as compared to virus control (right column in each panel)

apart via intramuscular route, without adjuvant. Three weeks after the booster immunization, serum samples from each VLP immunized group were pooled and tested in an in-vitro micro-neutralizing assay [21] for their ability to inhibit the replication of a homologous influenza virus strain (Flu A-reassortant VN-H5N1-PR8/CDC-RG reference strain) in MDCK cells. Pre-immunization serum samples and inactivated-VN-H5N1-PR/CDC-RG reference strain immunized mice were used as negative and positive controls. Both VLP constructs, (3TA and 5TA VLPs) induced antibodies able to neutralize the homologous virus strains (Fig. 4 panels 5TA and 3TA) suggesting that these structures displayed the proper conformation of protective antigenic sites necessary for inducing an antibody neutralizing response. These preliminary studies indicates that it is feasible to remodel/re-engineer HA molecules by deleting the dominant antigenic regions and after immunizing mice elicit a neutralizing response which reduced flu virus infection of MDCK cells. While these results do not allow a candidate to be selected, they do provide proof of concept of the approach. In pursue of this objective, we propose three specific aims that are outlined below.

3.3.2 Experimental Design and Methods

Aim 1: *Generate alternative constructs of remodeled HA and sub-clone them into appropriate construct for VLP creation. Produce and characterize virus-like particles (VLPs) displaying remodeled HA molecules on their surfaces. Evaluate the presence of conserved subdominant antigenic sites in HA molecules displayed on purified VLP by immune-precipitation using antibodies that exclusively recognized these sites.*



In addition to the constructs described in the preliminary data, we plan to subclone into our expression vector three additional remodeled HA molecules (1TA, 2TA and 4TA) (Fig. 5). These constructs represent alternatively remodeled HAs, which differ in the size of HA1 deletion, retained sequences and cysteine residues involved in disulfide bridges. 1TAs N-terminal sequence contains D17 to Y23 to promote the formation of an inter-chain disulfide bond between C20-C483 and an HA1 deletion 24-240 while retaining residues 241 to 346 linked to the complete sequence of the HA2 fragment, which includes the trans-membrane and cytoplasmic domains. The 1TA construct also contains a mutation of C290 to G to prevent potential interference with C294-C318 disulfide bond formation, (aa numbering based on Uniprot KB database reference Q6DQ33). The 2TA construct encompasses a similar structure with a smaller deletion within the HA1 fragment. The 4TA construct encompasses the entire HA2 fragment compressing the extracellular (347-520), transmembrane, and cytoplasmic (521-568) domains (Fig. 5).

Virus-Like Particle (VLP) Production

Cell lines (CHO and Vero) that constitutively express the M1 and M2 proteins of the influenza virus have been constructed and will be used here. These M1 and M2 proteins are the scaffold elements for the assembly and release of VLP vaccines. To produce VLPs displaying remodeled HA molecules on their surfaces, these cells will be initially transiently transfected with plasmid vectors expressing one or another remodeled HA and NA genes. Those VLP constructs that demonstrate broadly protective effectiveness in the animals studies proposed below will be used to generate stably transfected cell lines for the continuous production of these type of vaccines

VLP Vaccine Purification and Characterization

Experimental VLPs will be purified from the culture medium five days post-transfection. We will first separate cells from the VLP bulk material and then concentrate the vaccine material to 1/20 of its original volume by tangential filtration. Concentrated vaccine material will be further purified by gradient ultracentrifugation. Alternative chromatographic methods including affinity and size exclusion processes are currently under development. Vaccine characterization and assessment of purity will be analyzed by Western blot, Coomassie blue and silver staining. The total protein content in the VLP vaccine will be determined using the Bradford assay and the HA content quantified by ELISA using a purified rHA5 as standard references and an anti-HA2 specific antibody. The presence of a specific conserved and broadly neutralizing epitope in the purified VLP-remodeled HA vaccines will be further assessed using immune-precipitation (IP) assays with two alternative

antibodies, one that recognizes a unique conserved site on the HA stalk- nAbs to the fusion peptide (kindly provided by our collaborator Dr. Ruben Donis, CDC-[11]-see letter) and a second polyclonal that exclusively reacts with the stem portion of the HA2 fragment. This analysis will confirm not only the presence of the expected remodeled HA on the VLP structure but also the presence of active antigenic sites important in eliciting a broadly protective response. Laboratory expression of other VLPs with non-optimized production systems has shown yields in the range of 50-100 mg/liter.

Aim 2: *Assess the immune response elicited in mice following immunization with candidate VLPs displaying alternative remodeled HA proteins on their surfaces. The capacity of immunized mouse sera to neutralize different influenza A virus strains will be measured by an in-vitro micro-neutralization assay. Those VLP vaccines capable of inducing broadly neutralizing antibodies, as demonstrated by partially or completely neutralizing a panel of antigenically distinct influenza viruses will be further tested by in-vivo efficacy and immunogenicity studies in mice.*

Those VLP constructs that show a positive Western blot for remodeled HA and reactivity in IP with at least one of the two testing antibodies will be further evaluated for the induction of neutralizing antibodies in mice. Groups of five BALB/C mice, eight weeks old, will be immunized twice, two weeks apart, via the intramuscular route with single VLP-remodeled HA or combination of VLP-HA-Rem vaccines. Three weeks after the booster, serum samples will be collected, pooled and tested in a micro-neutralization assay [21]. Serum samples from mice immunized with inactivated virus (see preliminary data) and pre-VLP immunization will serve as positive and background controls, respectively. To assess elicitation of neutralizing antibodies (nAbs), serum samples from immunized mice and controls will be incubated with homologous [influenza A/Vietnam/1203/2004 (H5N1) and Indonesia/05/2005 (H5N1), both PR8 reassortants and BSL2 agents] and heterologous viruses [influenza A/PR/8/34 (H1N1), A/swine/Iowa/30 (H1N1) A/Udorn/72 (H3N1), A/HK/68 (H3N2) and A/Mallard/Netherland/12/2000 (H7N7). Contemporaneous circulating influenza viruses [A/Perth/16/2009 (H3N2) and A/California/07/2009 (H1N1), also available in our laboratory, could be used to further establish the neutralizing ability of the most promising vaccines. Selected viruses include representatives of the two major phylogentic groups, within the 16 HA subtypes, and correlates with the basic structure of the stalk of HA [13-15]. Those VLP-Rem-HA vaccines that neutralize homologous and heterologous virus (cross-subtype neutralization) will be further tested for protective efficacy and immunogenicity in lethal challenge mouse model experiments. We expect that anti-HA nAbs will block infection by disrupting membrane fusion rather than preventing receptor binding, which is the prevailing mechanism and is readily overcome by antigenic variation.

Aim 3: *Conduct efficacy and immunogenicity studies of single or combination of VLP-HA Rem vaccines in a lethal influenza mouse model. Groups of immunized mice will be challenged with lethal doses of at least three influenza viruses subtypes (HA5, HA1 and HA3). Further testing of the most promising vaccine candidates in ferrets will be pursued in a Phase II SBIR proposal, which will also seek enabling data for an IND application with the FDA.*

Table 1. Efficacy Studies of VLP-Rem-HA Vaccine in Mice

Grp	N [a]	Vaccine	# Doses [b]	Route	Challenge [c]
1	15	VLP-Rem-HA	2	IM	Flu Virus*
2	15	Inact.-virus control	2	IM	Flu Virus*
3	15	Placebo (PBS)	2	IM	Flu Virus*

a: Five mice of each group will be used for measuring CMI;
b: HA content per dose to be determined based on results of aim 2;
c: 10LD50 lethal dose discharge via intranasal route;
IM: intramuscular; PBS: phosphate buffered saline.
* Three efficacy studies will be performed and challenged with H5N1, H1N1, and H3N2 influenza viruses (see text.)

Those VLP-Rem-HA vaccines that demonstrate elicitation of broadly nAbs in aim 2 will be tested for protective efficacy. These studies are designed to measure the ability of the vaccine to afford protection against a lethal challenge with at least three different influenza A virus subtypes. One example of the tree experiments follows: Forty-five female BALB/c mice (6-8 weeks old) will be randomized into 3 experimental treatment groups of 15 mice each; 1) VLP-HA Rem experimental vaccine, 2) Inactivated virus immunized control, and 3) placebo. Mice in all groups will receive two immunizations two weeks apart. The dose of the VLP-Rem-HA vaccine will be determined based on the results from aim 2. Blood samples for immunogenicity analysis will be collected before vaccination and three weeks after the booster shot. Subsequently, mice in all groups will be challenged with a lethal dose (10LD50) of test virus; one for each of the tree experiemtns: [A/Vietnam/1203/04 (H5N1), A/swine/Iowa/30 (H1N1), A/Udorn/72 (H3N2)].

Efficacy: The protective efficacy afforded by the VLP-Rem-HA or control vaccines will be evaluated by

monitoring clinical symptoms of influenza disease and protection from death. Body weight measurements and clinical signs of disease will be monitored daily for 18 days post-challenge at which time percent survival within each group will be recorded. Animals that experience greater than 20% body weight decrease or show severe symptoms of influenza such as labored breathing or paralysis will be euthanized. All challenge experiments will be performed in the animal facility of Baylor College of Medicine. This Institution has a CDC certified BSL3 containment facility, which is required to conduct experiments with influenza A/Vietnam/1203/04 (H5N1). Challenge experiments with A/swine/Iowa/30 (H1N1), A/Udorn/72 (H3N2) or related strains will require BSL2 containment, also available at Baylor. We will comply with all institutional, local, state and federal regulations for the execution of this work.

Immunogenicity: The level of serum antibody elicited by the VLP-HA Rem and control immunizations will be evaluated by micro-neutralization [25] and ELISA assays [21, 24]. A hemagglutination inhibition (HAI) test will not be performed because VLP-HA Rem vaccines were designed to block membrane fusion rather than receptor binding, which is measured in HAI. ELISA will measure antibody titers elicited by the vaccine that should recognize subdominant conserved sites present in the wild type virus. Furthermore, the anti-neuraminidase response elicited by VLP vaccination will be evaluated by measuring the neuraminidase inhibition activity of serum samples [26]. Evaluation of the humoral immune response is very important because virus-neutralizing antibodies are the correlate of protection from influenza. Recombinant H5 protein or influenza A reassortant VN-H5N1-PR8/CDC-RG reference virus will be used for assessing the humoral response. Serum samples from two mice of each group will be pooled to assure sufficient material for the serological determinations which will be performed prior to immunization (pre-immunization sample) and before challenge (post-immunization sample.) The IgG subtypes will be assessed by ELISA and characterized as to the type of immune response (Th1/Th2) elicited by VLP- HA Rem vaccine and virus control. This study will provide information on the magnitude of the systemic response elicited by the VLP-HA Rem vaccine, in comparison to inactivated virus vaccines.
Cell-mediated immunity assays will further evaluate the magnitude of the effector T-cell population stimulated by the different VLP-Rem-HA vaccines and controls. Interferon γ (IFN-γ) production in splenocytes of five mice per group will be measured by ELISPOT assay [27]. Reassortant virus or purified antigen will be used to re-stimulate splenocytes during the performance of the ELISPOT assay. There is considerable precedence for the significance of this measurement, which not only provides further information about the type of immune stimulation elicited, but also reflects on the induction of effector T-cell function [28,29]. Also, nasal and bronchial lavage will be collected to assess the presence of antibody (methods above) in the mucosal surface.

3.4. Potential Problems and Alternative Solutions

While our constructs elicit nAbs, they may not be sufficiently potent to be clinically useful as standalone vaccines. If necessary we will test whether VLPs enhance protection elicited by a standard flu vaccine. Testing this vaccine together with an adjuvant also will be considered. Proper folding and expression of all remodeled HAs is a concern; however, WB and IP assays aim to prove that VLPs display remodeled HA revealing conserved antigenic sites. Also, Dr. Zhang, a structural biologist and protein chemist in the Department of Biochemistry, New York Medical College, will use a structural and computational modeling approach to create new remodeled HA or improve the properties of the one being study. Selection of the HA5 molecule for remodeling is based on the data obtained with the isolated human broadly nAbs [11]. This work shows that the most broadly neutralizing Ab does not block infection with all 16HAs; therefore we expect to recreate several conserved epitopes in one or more of the VLP constructs in order to cover more serotypes. Furthermore, other HAs (e.g. H3, H7), which were no neutralized by the human antibody could be remodeled to broaden the neutralizing power of a VLP vaccine. Success with this strategy will provide the foundation to implement a similar approach to develop a broadly protective flu B vaccine. Completion of these studies and identification of candidate vaccines will provide the data to support a Phase II SBIR application.

Activities & Milestones

Task	Year 1				Year 2			
	Q3-13	Q4-13	Q1-14	Q2-14	Q3-14	Q4-15	Q1-15	Q2-15
Sub-Cloning/Sequencing	■	■						
VLP Production/Characterisation		■	■	■				
IP Analysis			■					
Initial Microneut. Assay			■	■				
Animal/Efficacy Studies				■	■	■		
Immunogenicity Evaluation						■	■	
Data Analysis							■	■
Prep. of SBIR II Application								■

3.5. Timelines

As per the Activities & Milestones chart.

Vertebrate Animals

Immunogenicity and efficacy studies proposed in this application will be carried out in the animal facility of Baylor College of Medicine, which includes a BSL-3E Enhanced Satellite Facility. This enhanced BSL-3 facility is designed to house small animal models with containment standards and requirements defined in the CDC/NIH publication "Biosafety in Microbiological and Biomedical Laboratories (BMBL)", 5th Edition, 2007 for animal BSL-3 (ABSL-3) space plus recommended enhancements (ABSL-3E). The facility is certified by the CDC and APHIS Agricultural Select Agents Programs and the BCM Select Agent Registration on file with the CDC. Dr. Mbawuike, Co-Investigator in this project and his staff have access to the BCM BSL-3/3E Select Agent facility and have completed the required BSL-3 training program offered by UTMB Environmental Health Safety Office. Dr. Mbawuike has completed federally mandated security risk assessment, and has been added to the Select Agent Registration on file with the CDC. Work with the influenza A/Vietnam/1203/04 (H5N1) will be carried out in this facility by Dr. Mbawuike and his team.
Other influenza viruses plan to be used in these studies [influenza A/Swine/Iowa/30 (H1N1) virus, A/Udorn/72 (H3N2), or possibly A/HK/68 (H3N2), A/Mallard/Netherland/12/2000 (H7N7), A/Perth/16/2009 (H3N2) and A/California/07/2009 (H1N1)], are BSL-2 agent and the BCM Facility also meet requirement for handling this agent.

The facility is fully accredited by the American Association for Laboratory Animal Science (AALAS). Veterinarians and staff provide oversight of animal health and well being guidance and assistance with veterinary medical and surgical techniques, and the services of disease surveillance, diagnosis and treatment, animal husbandry and nutrition, zoonosis control, hazard containment, and equipment and room sanitation. Additional staff assistance includes consultation with researchers on handling, restraint, anesthesia, analgesia and euthanasia.

Animals will be euthanized in accordance to the IACUC approved guidelines established by the American Veterinary Medical Association, so as to minimize pain or discomfort in the animal, which results in the rapid unconsciousness followed by cardiac or respiratory arrest and ultimate loss of brain function. Euthanasia of animals is expected if animals demonstrate these clinical conditions (not inclusive): rapid, shallow, and labored respiration, prolonged inappetence, pyrexia, loss of 20-25% body weight, and central nervous system disturbances.

Mouse model
BALB/c mice (6-8 weeks old, female) will be used to evaluate immunogenicity and efficacy of VLP-HA Rem vaccines. Groups of mice (as described in the research plan) will receive two doses of VLP-HA Rem or controls via IM route. Prior to any procedures, animals will be anesthetized with a combination of ketamine (70mg/kg) and xylazine (6mg/kg) (~volume 100µl) administered by intramuscular injection. Blood samples will be collected from the submandibular vein (as suggested by a prior reviewer). Mice will be euthanized by inter-peritoneal (IP) administration of sodium pentobarbital (50-90mg/kg) and cervical dislocation as a secondary procedure to ascertain death.

Prior to the efficacy studies (Aim: 3), we will assess the immune response elicited in mice following immunization with experimental VLP–HA Rem vaccines (Aim: 2). Immunization and serum samples collection will be performed at Baylor and micro-neutralization test at TechnoVax, Inc.

The total number of mice proposed in these studies is: **~250** (25 mice for aim 2 and 225 for aim 3)

Statistical Analysis

In the design of this study, considerations for determining animal sample size were based on several factors to minimize the number of animals required to achieve the objectives of the study while maintaining the statistical significance of the data. The appropriate number of animals for aim 3 was based on the following factors: the difference in means between two groups (effect size), standard deviation (variability of data), significance level (α) and power (1- β). The significance level was set at alpha = 0.05 and the desired power was set at 80% (β = 0.2). The other factors (effect size and deviations) were based on our previous studies. Student *t* test (two tailed, unpaired) in the Excel program will be used to compare antibody levels between two groups. In addition, ANOVA test will used to perform simple analysis of variance to test the means of antibody titers among several sample groups. For all analyses, a *P* value of less than 0.05 derived from a two-tailed test will be considered significant.

Select Agents Research

Avian influenza H5N1 challenge studies will be conducted BCM BSL-3E Enhance Satellite Facility located in the basement of Garage 6. This enhanced BSL-3 facility is designed to house small animal models with containment standards and requirements defined in the CDC/NIH publication “Biosafety in Microbiological and Biomedical Laboratories (BMBL)”, 5th Edition, 2007 for animal BSL-3 (ABSL-3) space plus recommended enhancements (ABSL-3E). The facility is certified by the CDC and APHIS Agricultural Select Agents Programs and the BCM Select Agent Registration on file with the CDC. Dr. Mbawuike and his staff have access to the BCM BSL-3/3E Select agent facility and have completed the required BSL-3 training program offered by UTMB Environmental Health Safety Office. Dr. Mbawuike has an approved rDNA and/or HA/HC protocol, have completed federally mandated security risk assessment, and have been added to our Select Agent Registration on file with the CDC

Experiments with the Influenza A/Swine/Iowa/30 (H1N1), and A/Udorn/72 (H3N2), will be performed at the Baylor College of Medicine Animal Facility under BSL-2 bio-containment.

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RESOURCES SHARING PLAN

It is the intent of TechnoVax to share the data generated during this research with NIH and by publishing in peer reviewed journals. Prior to disclosure, protection of patentable information will be established.

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier: [redacted]

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☒

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

* Previously Reported: Yes ☐ No ☐

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

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

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

5. * Disclosure Permission Statement

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

☒ Yes ☐ No