PI: **Liu, Yingru**	Title: Experimental Gonococcal Vaccine	
Received: 08/24/2016	FOA: PA16-302	Council: 01/2017
Competition ID: FORMS-D	FOA Title: PHS 2016-02 OMNIBUS SOLICITATION OF THE NIH, CDC, FDA AND ACF FOR SMALL BUSINESS INNOVATION RESEARCH GRANT APPLICATIONS (PARENT SBIR [R43/R44])	
2 R44 AI115877-02	Dual: DK,HD	Accession Number: 3964434
IPF: 4529801	Organization: THERAPYX, INC.	
Former Number:	Department: TherapyX Inc.	
IRG/SRG: ZRG1 IMM-R (12)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 2: [redacted] Year 3: [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:*
Yingru Liu	1450566070000	PD/PI
Dominick Auci Ph.D	TherapyXinc	Co-PD/PI
Stacia Furtado	TherayXinc	Other Professional-Senior Scientist
Edith Mathiowitz	Brown University	Other Professional-Collaborating Scientist
Nejat Egilmez	University of Louisville	Other Professional-Collaborating Scientist
Michael Russell	TherapyXinc	Consultant

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier
R43AI115877

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT12235554

5. APPLICANT INFORMATION

Organizational DUNS*: 1450566070000

Legal Name*: TherapyX Inc.
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*:
ZIP / Postal Code*:

Person to be contacted on matters involving this application
Prefix: Dr. First Name*: Dominick Middle Name: Last Name*: Auci Suffix:
Position/Title: Vice President
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(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*
Experimental Gonococcal Vaccine

12. PROPOSED PROJECT
Start Date* 01/01/2017
Ending Date* 12/31/2018

13. CONGRESSIONAL DISTRICTS OF APPLICANT
KY-003

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Yingru Middle Name: Last Name*: Liu Suffix:
Position/Title: Senior Research Scientist
Organization Name*: 1450566070000
Department: TherapyX Inc.
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
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*	$0.00
d. Estimated Program Income*	$[redacted]

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

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

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

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: Nejat Middle Name: Last Name*: Egilmez Suffix:
Position/Title*: Executive Vice President
Organization Name*: TherapyX Inc.
Department:
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*
Dominick Auci

Date Signed*
08/24/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1252-CoverLetterGCVaxPhaseII.pdf

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

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name: TherapyXinc
Duns Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: NY-027

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: TherapyXinc
DUNS Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: KY-003

File Name

Additional Location(s)

Funding Opportunity Number: PA-16-302. Received Date: 2016-08-24T11:01:24.000-04:00

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

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

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

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number A 3354-01

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

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

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

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

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1247-ABSTRACTvaccinePhaseII FINAL.pdf
8. Project Narrative*	1248-narrativephaseIIvaccine.pdf
9. Bibliography & References Cited	1249-REFERENCES GC Final.pdf
10.Facilities & Other Resources	1250-Facilities and Other Resources NKE.pdf
11.Equipment	1251-EQUIPMENT.pdf

Funding Opportunity Number: PA-16-302. Received Date: 2016-08-24T11:01:24.000-04:00

ABSTRACT

Genital tract infection with *Neisseria gonorrhoeae* (gonorrhea) does not induce a state of specific protective immunity and it can be acquired repeatedly. Despite public health measures, the disease persists at an unacceptably high frequency; there is no vaccine against it, and resistance even to the latest generations of antibiotics continues to emerge. Recent findings have revealed that *N. gonorrhoeae* subverts the immune system for its own benefit by eliciting innate responses that it can survive and by suppressing specific adaptive responses that would eliminate it. However, this induced immunosuppression can be reversed by treatments that effectively redirect the immune response against *N. gonorrhoeae*. Proof-of-principle has been established for a novel strategy of prophylaxis against genital gonococcal infection using a mouse model that is accepted as the only currently available animal model. The current vaccine prototype, GvaX12® (a combinatorial formulation of our proprietary sustained-release nanoparticulate interleukin-12 and gonococcal outer membrane vesicles) induces anti-gonococcal T-cell and antibody responses, and generates protection against homologous and heterologous strains for up to 6 months. In this Phase II application, we will define, optimize, and validate a vaccination regimen including route, aiming for intranasal administration. We will determine the basis of cross-protection against diverse strains of naturally occurring *N. gonorrhoeae*, and begin preliminary pharmacokinetics and toxicology studies in preparation for subsequent toxicological testing in nonhuman primates. Along with the preclinical data, toxicology results and future plans will be incorporated into a briefing package that will be submitted to the FDA in a request for a Type C Meeting.

NARRATIVE (PUBLIC HEALTH RELEVANCE STATEMENT)

Gonorrhea is the second-most-frequent, notifiable infectious disease in the United States; the Centers for Disease Control report ~350,000 cases annually, and world-wide incidence is estimated at 78 million new infections per year. No vaccine is available and the emergence of multiple-drug-resistant strains now raises serious and urgent concerns over future treatment options. This proposal seeks to develop a novel strategy for prophylactic vaccination against gonorrhea by directing the immune response to generate lasting protective immunity.

FACILITIES AND OTHER RESOURCES

Office and Laboratory Space

TherapyX^{Inc} recently moved its administrative offices to Louisville, KY in pursuit of potential new investment opportunities and state-supported programs for small-business development. Our research and development facilities remain in Buffalo, NY where we have an existing facilities use agreement with the State University of NY. All research work outlined in Aims 1 and 2 will be performed in our Buffalo, NY location (primary site). Dr. Yingru Liu and all research personnel work at the Buffalo facility. Dr. Dominick Auci, with the assistance of Ms. Samina Raza, works out of the Louisville office (site 1) but travels to the Buffalo facility once a month.

Our administrative location in Louisville, KY consists of 600 square foot rental office space equipped with 2 Dell E520 desktop computers networked to the internet, a laser printer, photocopier, scanner, file cabinets, 3 desks and telephones.

Our research office and laboratory space is located in Sherman Hall and our BSL2 and conventional animal facilities are located in the Biomedical Education Building at SUNY at Buffalo Medical School. These facilities include a total of 1200 sq. ft., of laboratory, 200 sq. ft. of office space and a 600 sq. ft. animal room. The office and laboratory in the Buffalo location are equipped with 3 Dell E520 computers, and 1 Dell Dimension computer, a networked laser printer and Ethernet connection to the internet with full access to the SUNY at Buffalo library and electronic journal subscriptions. The office is equipped with filing cabinets, office furniture, computers, FAX/Copier and telephones. An agreement entered into by TherapyX^{Inc} and SUNY at Buffalo also allows for employees access to common equipment rooms.

Our research facility is adjacent to the Witebsky Center for Microbial Pathogenesis and Immunology, an interdepartmental center occupying two floors of the Biomedical Research Building on the State University of New York at Buffalo (UB) South Campus. The Center brings together a diverse group of 20 active, extramurally funded faculty who share a common interest in various aspects of the constituent sub-disciplines, including bacteriology, virology, parasitology, mycology, infectious diseases, and immunity to infection and to tumors. TherapyX^{Inc} has ongoing collaborations with a number of members of the Witebsky Center and the Department of Microbiology and Immunology at UB.

Animal Care Facilities

Research animal facilities, including BSL-2 suites, are available through the UB Laboratory Animal Facility, which is adjacent to the TherapyX^{Inc} Laboratories in Sherman Hall. The company has a dedicated 600 sq ft room in the animal facility that is maintained by the LAF staff for a fee. This facility is accredited by AAALAC, and complies with all applicable Federal and State laws and guidelines. The program provides procurement facilities, routine animal care, health surveillance, and veterinary support.

Other Resources

The Health Sciences Library is situated nearby on the UB South Campus. Inter-library loan service, photocopying, and both internal and external (Medline) computerized literature search and full-text retrieval services are available to TherapyX^{Inc} An inter-institutional agreement with SUNY at Buffalo allows full access to the Confocal Microscopy and Flow Cytometry core facility at the Witebsky Center at SUNY at Buffalo. Histology services for H & E staining of paraffin-embedded tissue sections as well as immuno- histochemical staining of frozen sections are available from the Histology services laboratory of the SUNY at Buffalo Medical School.

The Confocal Microscopy and Flow Cytometry Facility is a University instrument laboratory operated by the School of Medicine and Biomedical Sciences for the benefit of the research community of the University at Buffalo. TherapyX^{Inc} has a facilities use agreement that allows for use of the equipment and the expertise and aid of the facility director Dr. Wade J. Sigurdson and Flow Cytometry administrator Dr. Ray Kelleher.

Biohazard Protections

This project includes work with *Neissseria gonorrhoeae*. The following section describes the special Standard Operating Procedures* that are followed while working with these biohazardous organisms.

SUNY at Buffalo Occupational Health Monitoring Program and the Use of Hazardous Agents

The Occupational Health Monitoring Program

The Occupational Health Monitoring Program (OHMP) at SUNY at Buffalo is mandatory for all persons in contact with research animals or their unfixed tissues. The current program requires that all personnel working with animals must participate in, and must be trained in animal care and in safety. All personnel who are to handle animals, or to be in facilities where animals are kept, must complete a questionnaire describing their proposed activities, and must also provide information relevant to their health. These two components (activities and health) are reviewed by an occupational health physician, Dr. David Hughes, Occupational Physician at ECMC who then provides an assessment of the risk of the proposed activities (which is returned to IACUC administrator), and makes recommendations to the individual for vaccines etc. All costs for enrolling in the OHMP are covered by the University.

Use of Hazardous Agents

All researchers using potentially hazardous agents or materials such as infectious and cancerous agents, highly toxic chemicals or recombinant DNA molecules falling under NIH guidelines have an obligation to inform the University Biosafety Committee by registering their research program. Animal use is regulated by the Institutional Animal Care and Use Committee, in collaboration with these groups. It is the Principal Investigator's responsibility to provide appropriate information on these materials to all those who may be exposed to these materials and/or their hazardous degradation products and inform the CM-LAF Director or Manager of when these projects will start. This information shall include but not be limited to (a) written hazard descriptions, such as material safety data sheets (MSDS); (b) hazard specific and documented training of personnel including both research workers and support personnel such as animal care workers; and (c) the posting of appropriate standardized signage. The CMLAF will arrange training of LAF personnel, posting of signage on the holding room doors, appropriate housing of animals and cage tags.

The Office of Environmental Health and Safety Services can provide appropriate registration documents and NIH/CDC Guidelines, assist in obtaining appropriate MSDS information sheets, standardized signage and generic training and information on the establishment of a Chemical Hygiene Plan and/or a Blood Borne Pathogen Program (as required by OSHA).

* The SUNY at Buffalo CM-LAF Standard Operating Procedure for research with *Neisseria gonorrhoeae* is included in the Biohazard attachment.

BIOHAZARD PROTECTIONS

SUNY at Buffalo Occupational Health Monitoring Program and the Use of Hazardous Agents

The Occupational Health Monitoring Program

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Use of Hazardous Agents

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The Office of Environmental Health and Safety Services can provide appropriate registration documents and NIH/CDC Guidelines, assist in obtaining appropriate MSDS information sheets, standardized signage and generic training and information on the establishment of a Chemical Hygiene Plan and/or a Blood Borne Pathogen Program (as required by OSHA).

* The SUNY at Buffalo CM-LAF Standard Operating Procedure for research with *Neisseria gonorrhoeae* is included here for reference.

COMPARATIVE MEDICINE
LABORATORY ANIMAL FACILITIES

STANDARD OPERATING PROCEDURE
for
NEISSERIA GONORRHOEAE SAFETY PRECAUTIONS

1.0 **PURPOSE:**
This SOP applies to all Researcher staff and LAF employees working in BSL 2 facility with this organism

2.0 **SCOPE:**
This SOP outlines the Occupational Health and Safety Guidelines when working with *Neisseria gonorrhoeae*. Gonorrheal organisms are infectious exclusively to humans. The greatest risk maybe the direct contact of excreta on bedding to mucous membranes (eyes, mouth, cuts) *N. gonorrhoeae* is not a life threatening infection and the strains used are fully susceptible to antibiotics. No vaccine is available. The organism is not transmissible from mouse to mouse. Organisms do not persist in the environment for long periods of time, and are susceptible to common disinfectants. However all material and mice that are in contact with gonorrhea must be treated as potentially infectious.

3.1 PROCEDURE:

3.2 Personnel must enroll in the Occupational Health Medical Program at UB.

3.3 All work with this organism will be conducted in the BSL Level 2 Facility.

3.4 Standard entry, gowning, and personal hygiene procedures of BSL2 will be followed

3.5 NIOSH N95 face masks will be worn

3.6 Safety glasses will be worn in the animal holding room and when cage changing as an additional precaution.

3.7 Cages & bedding will be autoclaved prior to removal from BSL2.

3.8 After autoclaving, bedding will be discarded into Red Biohazard bags, and disposed as Certified Medical Waste.

3.9 Autoclaved cages, water-bottles, lids etc will be taken to the cagewash area and sanitized in a routine manner

3.10 All equipment and supplies must be autoclaved or disinfected with alcohol or 10% Bleach prior to removal from BSL2.

3.11 If a contaminated needle-stick or mucous membrane exposure occurs, follow **SOP #3.F.1: Exposure to Blood and Other Body Fluids, Bites, Scratches and Needle Punctures**

A Notify your supervisor

B Call ECMC 898-4153 and ask to see a doctor

C Inform workers compensation at 645-5000 X1025 and ask for Annette Lozo

D After-hours, proceed to ECMC and explain your are enrolled in the OHMP at UB and you were hurt at work

E Medical attention should be sought within 24 hours of the exposure or injury

3.12 Carcasses must be treated as infectious and incinerated.

EQUIPMENT

The TherapyX^{Inc} laboratories are equipped with standard biomedical laboratory apparatus, including:

- Bio-Tek FLx800 multi-detection Microplate Reader
- Tissue culture biosafety hood,
- Tissue culture incubator (CO_2)
- Microbiological incubator (CO_2)
- Stereo low-power microscope Inverted microscope
- Mx3005P QPCR System
- Image analysis system with digital camera for use with microscopes (Image Pro Plus ver. 4)
- Conventional open column chromatography equipment with fraction collector, pump, UV monitor in a refrigerated cabinet, recorder, and selection of columns
- FPLC system (Pharmacia) with 2 pumps, GP-250 controller, UV (280nm) monitor, fraction collectorMono Q 10/10, Mono S 10/10, various Superose, Sephacryl, Superdex, and HiTrap affinity columns
- Polyacrylamide gel electrophoresis and electroblotting equipment
- Stratagene Mx3005P QPCR System quantitative RT-PCR machine
- Ultracold (-80°C) freezer, liquid nitrogen freezer
- Refrigerators, freezers, water-baths, balances, pH meters, microcentrifuges, pipettors
- Magnetic cell sorter apparatus (MACS)
- High-speed centrifuge
- Light microscope
- Chemical fume hood

Microparticle, production, (as well as analysis and validation):

- Silverson L4RT-A Shear Mixer and 4 overhead mixers
- Waters 2695 Alliance System with 996 PDA detector
- Virtis 4KBTZL-105 lyophilizer
- Shimadzu GC-17A gas chromatograph with a flameionization detector and Tekmar 7000HT autosampler
- Coulter LS230 particle size analyzer
- Shimadzu GPC system
- Perkin Elmer Differential Scanning Calorimeter Puris 1
- Perkin Elmer Differential Scanning Calorimeter Puris 7
- Perkin Elmer FT-IR Spectrometer 1725 X,
- GBC Cintra 40 UV-Vis Spectrophotometer
- Shimadzu UV-2501PC UV-Vis Recording Spectrophotometer

Common equipment in the Witebsky Center available to Therapyx, Inc.:

- Reagent-grade water system (Milli-Q)
- Walk-in cold room (4°C)
- UV/visible spectrophotometer
- Spectrofluorimeter
- Luminometer
- High-speed and ultra-centrifuges
- Bacteriological shaker/incubators and autoclaves

- Li-Cor Odyssey CLx imager

Confocal Microscopy and Flow Cytometry core facility equipment available to Therapyx, Inc. employees:

- Epi-fluorescence and confocal microscopes fitted with digital cameras (Zeiss)
- 4-color flow cytometer (FACSCalibur)
- 7-color flow cytometer/cell sorter (FACSAria)
- Leica AS/LMD laser capture microdissection system
- ELISPOT plate-reader
- Becton-Dickinson FACS Array Bioanalyzer multiplex bead and cellular assay system

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Yingru Middle Name Last Name*: Liu Suffix:

Position/Title*: Senior Research Scientist
Organization Name*: 1450566070000
Department: TherapyX Inc.
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

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: 2007

	File Name
Attach Biographical Sketch*:	1235-Bio-sk. Liu Y.pdf
Attach Current & Pending Support:	1236-LIU Current Research Support.pdf

Funding Opportunity Number: PA-16-302 . Received Date: 2016-08-24T11:01:24.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Dominick Middle Name Last Name*: Auci Suffix: Ph.D

Position/Title*: Vice President, Research and Development
Organization Name*: TherapyXinc
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number: [redacted] E-Mail*: [redacted]

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

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

Degree Type: PhD Degree Year: 1990

	File Name
Attach Biographical Sketch*:	1237-Auci NEW NIH bio Phase II vaccine.pdf
Attach Current & Pending Support:	1238-Auci current Funding.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Stacia Middle Name Last Name*: Furtado Suffix:

Position/Title*: Senior Research Scientist
Organization Name*: TherayXinc
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number: [redacted] E-Mail*: [redacted]

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

Project Role*: Other Professional Other Project Role Category: Senior Scientist

Degree Type: PhD Degree Year: 2008

	File Name
Attach Biographical Sketch*:	1239-Furtado biosketch phaseIIvaccinePhase2.pdf
Attach Current & Pending Support:	1240-FurtadoCurrentSupport.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Edith Middle Name Last Name*: Mathiowitz Suffix:

Position/Title*: Professor of Medical Sciences and Engineeri
Organization Name*: Brown University
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

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

Project Role*: Other Professional Other Project Role Category: Collaborating Scientist

Degree Type: Ph.D. Degree Year: 1985

	File Name
Attach Biographical Sketch*:	1241-Mathiowitz_biosketch.pdf
Attach Current & Pending Support:	1242-mathiowitzCurrent Support.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Nejat Middle Name Last Name*: Egilmez Suffix:

Position/Title*: Professor and Chair
Organization Name*: University of Louisville
Department: Microbiology and Immunology
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

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

Project Role*: Other Professional Other Project Role Category: Collaborating Scientist

Degree Type: Ph.D. Degree Year: 1986

	File Name
Attach Biographical Sketch*:	1243-Egilmez bio vaccine Phase II FINAL.pdf
Attach Current & Pending Support:	1244-Egilmez new CurrentSupport.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Michael Middle Name Last Name*: Russell Suffix:

Position/Title*: Advisor
Organization Name*: TherapyXinc
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

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

Project Role*: Consultant Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1973

	File Name
Attach Biographical Sketch*:	1245-Russell bio vaccine PhaseII FINAL.pdf
Attach Current & Pending Support:	1246-Current support MWR.pdf

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Yingru Liu	Senior Research Scientist, TherapyX, Inc.
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
Beijing Medical Univ., Beijing, China	B.Med. (=M.D.)	08/90	Medicine
Univ. Brit. Columbia, Vancouver, BC, Canada	M.S.	05/03	Neuroscience
Univ. Brit. Columbia, Vancouver, BC, Canada	Ph.D.	11/07	Neuroimmunology
Harvard Medical School, Boston, MA, USA	Postdoc	08/08	Neurology, Immunology
Univ. at Buffalo, Buffalo, NY, USA	Postdoc	12/10	Immunology, Microbiology

A. Personal Statement

Since completing my medical studies at Beijing Medical University, China, in 1990, I have been working on research in immunology and microbiology for more than 20 years, in both clinical and basic research institutes. My Ph.D. project at the University of British Columbia explored the immunomodulatory activities of bilirubin in rodent models of multiple sclerosis. I continued with immunological research at Brigham and Women's Hospital, Boston MA (2007-8), and later at Roswell Park Cancer Institute, Buffalo NY (2008-9). Through all these years of study I solidified my medical knowledge and acquired a wide range of experimental techniques relating to cellular and molecular biology, immunology, and microbiology. In May 2009 I joined Dr. Michael Russell's laboratory at the University at Buffalo, where I brought my expertise in T cell and cytokine biology to ongoing projects on immunity to *Neisseria gonorrhoeae (Ng)*, resulting in novel understanding of the mechanism whereby this organism manipulates the immune system to its own advantage.

In 2013 I joined TherapyX^{Inc} to lead their vaccine program. The current application focuses on the development of a vaccine against *Ng*. To this end, I have been the PI on 2 different SBIR projects focusing on novel Ng therapies at TherapyX^{Inc}. In the past 3 years we have shown how anti-Ng immunity can be counter-manipulated to provide a potential new treatment for gonorrhea, and have developed a new approach to vaccine development. Based on my background and recent accomplishments I am uniquely qualified to continue directing this research and development program.

B. Positions and Honors

1990 – 1993 **Neurological Resident**, Department of Neurology, Neuroimmunology Lab, The Third Hospital of Beijing Medical University, Beijing, China

1993 – 1996 **Doctor in Charge**, Department of Neurology, The Third Hospital of Beijing Medical University, Beijing, China

1996 – 1999 **Clinical Assistant Professor**, Department of Neurology, Neuroimmunology Lab, The Third Hospital of Beijing Medical University, Beijing, China

1999 – 2007 **Graduate Research Assistant**, Brain Research Center, University of British Columbia, Vancouver BC, Canada

2007 – 2008 **Postdoctoral Research Fellow**, Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

2008 – 2009 **Postdoctoral Research Fellow**, Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY

2009 – 2010 **Postdoctoral Associate**, Department of Microbiology & Immunology, University at Buffalo, Buffalo, NY

2010 – present **Research Assistant Professor**, Department of Microbiology & Immunology, University at Buffalo, Buffalo, NY

2013 – present **Senior Research Scientist**, TherapyX ^{Inc}., Buffalo, NY

Awards/Credentials

1987 – 1990	**University Scholarship** from Beijing Medical University, Beijing, China.
1996	**Distinguished Doctor**, The Third Hospital of Beijing Medical University, Beijing, China.
2005 – 2007	**Senior Graduate Studentship Award** from MICHAEL SMITH FOUNDATION FOR HEALTH RESEARCH, British Columbia, Canada.
2010	**NIH Travel Scholarship** to attend the International Pathogenic Neisseria Conference, Banff, AB, Canada, 2010.

C. Contributions to Science

My contributions have been mainly in exploring new therapies for immunological and infectious diseases and novel approaches to the development of effective vaccines.

1. During my MSc and PhD work at the University of British Columbia, I investigated the mechanisms of axon and neuron damage in multiple sclerosis, as this process contributes directly to disease progression and permanent neurologic damage. Using experimental autoimmune encephalomyelitis (EAE), an animal paradigm of multiple sclerosis, I investigated and exploited a new oxidative defense system that involves the conversion of heme to bilirubin. Although it had been shown that bilirubin was a powerful antioxidant substance both *in vitro* and *in vivo*, my study was the first to systematically explore its immunosuppressive activities, and demonstrated that bilirubin possesses various beneficial immunomodulatory effects, including its protective actions against autoimmunity, hemolysis, and antibody-dependent cell-mediated cytotoxicity. Although in traditional Oriental medicine animal biles have proved useful in the treatment of many human disorders, the potential utility of bilirubin in diseases is limited due to its insolubility and its toxicity at high concentrations. My PhD research was the first to identify biliverdin reductase, a water-soluble and nontoxic natural biological enzyme, as a novel strategy for treatment of stress- and immune-related diseases. My results demonstrated that biliverdin reductase can regenerate bilirubin in a catalytic cycle and amplifies the action of bilirubin without significantly increasing its biological concentration. All these findings were published in highly respected journals, such as *Journal of Immunology*, and were highly cited in PubMed.

   a. **Liu Y**, Zhu B, Luo L, Li P, Paty DW, Cynader MS. Heme oxygenase-1 plays an important protective role in experimental autoimmune encephalomyelitis. *Neuroreport* 12: 1841-1845 (2001).
   b. **Liu Y**, Zhu B, Wang X, Luo L, Li P, Paty DW, Cynader MS. Bilirubin as a potent antioxidant suppresses experimental autoimmune encephalomyelitis: implications for the role of oxidative stress in the development of multiple sclerosis. *J. Neuroimmunol.* 139: 27-35 (2003).
   c. **Liu Y**, Liu J, Tetzlaff W, Paty DW, Cynader MS. Biliverdin reductase, a major physiologic cytoprotectant, suppresses experimental autoimmune encephalomyelitis. *Free Radical Biol. Med.* 40: 960-967 (2006).
   d. **Liu Y**, Li P, Lu J, Xiong W, Oger J, Tetzlaff, and Cynader MS. Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis. *J. Immunol.* 181: 1887-1897 (2008).

2. After I completed my graduate study in 2007, I accepted a postdoctoral research fellow position at Center for Neurologic Diseases, Harvard Medical School where I continued to look for new therapeutic options for a potentially disabling disease. To date, multiple sclerosis treatments have demonstrated effectiveness in reducing relapses but have had limited efficacy in slowing disease progression. At Harvard, I and Dr. Tanuja Chitnis identified a fusion protein, CD200, which could specifically protect neurons against inflammatory attack and halt symptom accumulation in chronic EAE. This finding has significant implications for the treatment of multiple sclerosis, as well as other neuroinflammatory diseases. The work was published in *The Journal of Neuroscience*.

   a. **Liu Y**, Bando Y, Vargas-Lowy D, Elyaman W, Khoury SJ, Huang T, Reif K, Chitnis T. CD200R1 agonist attenuates mechanisms of chronic disease in a murine model of multiple sclerosis. *J. Neurosci.* 30(6):2025-38 (2010). PMCID: 2837938

3. After moving to Buffalo NY in 2009, I joined Dr. Michael Russell's laboratory at the University at Buffalo and later, in 2013, began working at TherapyX^{Inc} in continuing collaboration with Dr. Russell. Dr. Russell is an internationally recognized expert in mucosal immunology, and is well known for his work on human immune responses to *Neisseria gonorrhoeae*. Gonorrhea is widespread globally and is a major cause of reproductive tract damage in women. In addition, it is well known that gonorrhea can be acquired repeatedly with apparently no development of protective immunity against reinfection, and no vaccine is available. Dr. Russell's group had previously demonstrated that *N. gonorrhoeae* selectively induces Th17-driven innate immune responses that it can survive, and concomitantly suppresses Th1/Th2-driven adaptive immune responses. My projects here address a very important, long-lasting question in gonococcal infection: how *N. gonorrhoeae* evades or inhibits host specific protective immunity that would eliminate it. My research at Buffalo has demonstrated that the induction of IL-10/Tr1 cells and TGF-β plays a critical role in the suppressive effect of *N. gonorrhoeae* on host adaptive immunity. Furthermore, my results showed for the first time that the inhibitory effect of this pathogen on Th1/Th2-mediated specific immune responses can be overcome by therapeutic intervention. Blockade of IL-10, TGF-β, or both, significantly enhanced specific protective immunity against *N. gonorrhoeae*, and resulted in the generation of immune memory and production of specific antibodies in a mouse model of vaginal gonococcal infection. In addition, we developed a novel technology of microencapsulated IL-12 or anti-IL-10 for local intravaginal treatment of *N. gonorrhoeae* infection, with minimal toxic potential. We have further demonstrated that microencapsulated IL-12 serves as an effective adjuvant for a prophylactic gonococcal vaccine administered intravaginally. The present studies are aimed at developing this finding further to provide the preclinical basis for a novel prophylactic vaccine against gonorrhea.

a. **Liu Y**, Russell MW. Diversion of the immune response to *Neisseria gonorrhoeae* from Th17 to Th1/Th2 by treatment with anti-TGF-β antibody generates immunological memory and protective immunity. *mBio* 2: e00095-11 (2011). PMCID: 3101786.
b. **Liu Y**, Islam E, Jarvis GA, Gray-Owen S, Russell MW. *Neisseria gonorrhoeae* selectively suppresses the development of Th1 and Th2 cells, and enhances Th17 cell responses, through TGF-β-dependent mechanisms. *Mucosal Immunol.* 5: 320-31 (2012). PMCID:3328619
c. **Liu, Y**, Liu, W, and Russell, MW. Suppression of host adaptive immune responses by *Neisseria gonorrhoeae*: role of interleukin 10 and type 1 regulatory T cells. *Mucosal Immunol.* 7: 165-176 (2014). PMCID:3812424.
d. **Liu, Y**, Egilmez, NK, and Russell, MW. Enhancement of adaptive immunity to *Neisseria gonorrhoeae* by local intravaginal administration of microencapsulated IL-12. *J. Infect. Dis.* 208: 1821-1829 (2013). PMCID:3814831.
e. **Liu, Y**, Hammer, L, Hobbs, MM, Zielke, RA, Sikora, AE, Jerse, AE, Egilmez, NK, and Russell, MW. Experimental vaccine induces Th1-driven immune responses and protection against *Neisseria gonorrhoeae* in a murine model. MS submitted to *Mucosal Immunol.* (Aug. 2016)

Complete List of Published Work

MyBibliography: http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/49301078/public

D. Research Support

Current Research Support

R44-AI-104067-02 (SBIR Phase II) Y. Liu (PI) 02/01/13 – 05/31/17
NIH/NIAID
Therapy and prophylaxis for genital tract infection
The goal of phase II is to develop a novel approach to treatment of genital gonococcal infection by the local administration of microencapsulated IL-12. Role: PI.

R43-AI-115877-01 (SBIR Phase I) Y. Liu (PI) 12/01/14 – 11/30/16

NIH/NIAID
Experimental gonococcal vaccine
The goal of this project is to establish proof-of-principle for a novel vaccine against genital gonococcal infection. Role: PI.

Completed Research Support

R43-AI-104067-01 (SBIR Phase I) Y. Liu (PI) 02/01/13 – 01/14/14
NIH/NIAID
Therapy and prophylaxis for genital tract infection
The goal of phase I is to establish proof-of-principle for a novel treatment of genital gonococcal infection by the local administration of microencapsulated IL-12. Role: PI.

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Dominick L. Auci, MBA, Ph.D	Vice President, Research and Development
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
SUNY at Stony Brook, NY	B.S.	1984	Biology and Philosophy
SUNY at Brooklyn, NY	Ph.D.	1990	Pathology
Baruch College	MBA	1999	Executive Management

A. Personal Statement

I was recruited to TherapyX with over twenty years of pharmaceutical experience that includes leadership of research and development programs focused on infectious and immune mediated inflammatory diseases. As Director of Allergy, Autoimmunity and Inflammation at Hollis-Eden Pharmaceuticals, I led all aspects of the discovery and development of novel compounds through phase II clinical trials. At TherapyX, I have successfully lead the development of scale-up methods for TGFβNanoCap, a nanoparticulate encapsulated form of TGFβ for oral delivery. I have coordinated efforts from multi-disciplinary project teams that lead to several successful IND applications and phase I/II study protocols. My role in this project will be to oversee production and validation of drug product, animal studies (including toxicology studies at Comparative Biosciences), sample analysis, as well as data interpretation, evaluation and presentation. I will consult with Drs. Liu, Egilmez, Furtado and Mathiowitz to address any emergent difficulties. I will also continue to work closely with consultants at Regulatory Professionals to prepare a briefing package and request for a type C meeting with the FDA.

B. Positions and Honors.

2014- Present	**Vice President, R&D**, TherapyXinc, Louisvillle, KY
2011-2013	**Cancer Vaccine Program Manager**, University of Washington, Seattle, WA
2001-2011	**Director, Allergy, Autoimmunity, Inflammation,** Hollis-Eden Pharmaceuticals, San Diego, CA
2000-2001	**Scientist/Manager Biomedical Laboratories,** Pall Corporation, East Hills, N.Y.
1996-1998	**Associate Director, Plasma and Blood Cell Repository, Women's Interagency AIDS Study,** SUNY Health Science Center, Brooklyn, NY
1992-2000	**Assistant Professor,** Department of Pathology, SUNY Health Science Center, Brooklyn, NY
1992-2000	**Adjunct Assistant Professor,** Department of Natural Sciences, York College, The City University of New York, Jamaica, NY
1992-1999	**Associate Director, Clinical Immunology Laboratory/Flow Cytometry,** SUNY Health Science Center, Brooklyn, NY
1990-1991	**Postdoctoral Fellow, Immunopharmacology,** Joint appointments in the laboratories of Peter Dukor, M.D., Head, Sandoz Forschungsinstitut, Vienna, Austria, and Helen G. Durkin, Ph.D., Department of Pathology, SUNY at Brooklyn, NY

C. Contributions to Science

A full list of my published work can be found at: http://www.ncbi.nlm.nih.gov/pubmed/?term=Auci+Dominick

1. Control of IgE responses

My early postdoctoral work focused on the ontogeny of IgE bearing B cells and IgE forming cells in humans and mice. We found that unlike B cells bearing other isotypes, IgE bearing B cells never cloned in germinal centers, but appeared first in Peyer's patches and Mesenteric Lymph nodes about a week after immunization. Later they were found in T cell dependent areas of spleen. These studies led to the development of in vivo and in vitro (IgE ELISPOT) assays to test pharmaceuticals aimed at turning off allergic responses at the source, at the level of the IgE producing B cell. I remained at Downstate Medical Center in Brooklyn as Assistant Professor of Pathology for the remainder of the decade exploring regulation of IgE responses and providing clinical immunology services during the HIV outbreak.

I. **Auci, D.L.,** Carucci, J.A., Chice S.M., Smith, M.C., Dukor, P., and Durkin, H.G., Control of IgE responses II. (1993). Isotype specific suppression of peak BPO specific IgE antibody forming cell responses in BPO-KLH sensitized mice after oral administration of muramyldipeptide or murabutide. *J. Immunopharm. 26:157*

II. **Auci, D.L.,** Kleiner, G.I., Shaikh, A., Acosta, E., Athanassiades, T.J., and Durkin, H.G., (1993). Cytokine-induced suppression and potentiation of hapten specific immediate hypersensitivity responses. *Immunol. Invest. 22:203*

III. **Auci, D.L.,** Chice, S.M., Heusser, C., Athanassiades, T.J., and Durkin, H.G., (1992). Origin and fate of IgE bearing lymphocytes II. Gut associated lymphoid tissue as sites of first appearance of IgE bearing B lymphocytes and hapten specific IgE antibody forming cells in mice immunized with benzylpenicilloyl-keyhole limpet hemocyanin by various routes: relation to asialo Gm 1 ganglioside+ cells and IgE/CD23 immune complexes. *J. Immunol. 149:2241*

IV. **Auci, D.L.**, Smith, T.N., Jagoo, A.D., Kleiner, G.I., Bluth, H.M., Chice, S.M., Bard, E., Anderson, V., Zevallos, E., and Durkin, H.D. (1997). IgE-Bearing Cells and Epsilon-Specific mRNA in Lymphoid Organs of Two Children with AIDS. *Ped. AIDS HIV Infect. Fetus to Adolesc. 8:102-107*

2. The Immunobiology and Therapeutic Potential of Novel Androstene Metabolites and Synthetic Derivatives.

My subsequent work in industry focused on the discovery and development of anti-inflammatory and immune modulating steroid hormones derived from the dihydroepiandrosterone (DHEA) metabolome for use as immune therapy. Part of this work involved the discovery and development of natural, and novel synthetic DHEA derivatives for treatment of infectious diseases. Compounds were screened in vitro and in vivo for immune modulating activity and tested in rodent models.

I. Ahlem, C., **Auci, D.L.,** Nicoletti, F., Pieters R., Kennedy, M., Page, T., Reading C., Enioutina, E., and Frincke., J.M., (2011). Pharmacology and immune modulating properties of androst-5-ene-1 3β,7 β,17 β -triol, a 2 DHEA metabolite in the human metabolome. J Steroid Biochem Mol Biol. 2011 Sep;126(3-5):87-94

II. Conrad, D., Wang, A., Pieters, R., Nicoletti, F., Mangano, K., van Heeckeren, A.M., White, S.K., Frincke, J.M., Reading, C.L., Stickney, D., and **Auci, D.L.,** (2010) HE3286, an oral synthetic steroid, treats lung inflammation in mice without immune suppression. *Journal of Inflammation, 7:52.*

III. Nicoletti, F., Conrad, D., Wang, A., Pieters, R., Mangano, K., van Heeckeren, A., White, S.K., Frincke, J., Reading, C.L., **Auci, D.L**., and Stickney, D. (2009). 16α-Bromoepiandrosterone (HE2000) limits non-productive inflammation and stimulates immunity in lungs. *Clin. Exp. Immunol. 158:308-316*

3. Development of encapsulated cytokines as treatment for immune mediated inflammatory and infectious disease.

I joined TherapyX in May of 2014 and have been focused on the development of nanoparticulate enpasulated cytokines and small molecules such as all trans retinoic acid, TGFβ, IL-12, and IL-10 as oral or mucosal treatments for various inflammatory and infectious diseases. I successfully lead the development of scale up spray drying methods for TGFβNanoCap, a nanoparticulate encapsulated form of TGFβ for oral delivery in collaboration with the team at Bend Research, Bend, OR.

I. Conway, T.F., Hammer, L., Furtado, S., Mathiowitz, E., Nicoletti, F., Mangano, K., Eglimez, N.K., and **Auci, D.L.** Oral Delivery of Particulate Transforming Growth Factor Beta 1 and All-Trans Retinoic Acid Reduces Gut Inflammation in Murine Models of Inflammatory Bowel Disease. J Crohns Colitis. (2015) Aug;9(8):647-58.

D. Research projects ongoing or completed in the past 3 years

Ongoing:

"Therapy and Prophylaxis for Genital Tract Infection". NIH 2R44-AI104067-02. 7/1/2014-6/30/2016.
Role: Co-PI (Liu, PD/PI), 5% effort. Total costs: $ 1,498,000.

"Delivery of nanoencapsulated TGFbeta and ATRA for the Treatment of IBD". NIH 2R44-AI080009-02A1. 4/1/2011-3/31/2016.
Role: PI, 45% effort. Total costs: $ 2,999,994.

BIOGRAPHICAL SKETCH

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

NAME**: Stacia (Chmura) Furtado**

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE**: Senior Scientist**

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Brown University, Providence, RI	Sc.B.	1995	Psychology
Brown University, Providence, RI	Sc.M.	2001	Biology
Brown University, Providence, RI	Ph.D.	2008	Biology

A. Personal Statement

My primary field of study is the development of novel polymeric drug delivery systems by utilizing various methods of microencapsulation as well as the in vitro and in vivo characterization of controlled drug delivery systems. For this grant, I will be responsible for the encapsulation of IL-12 as well as for performing the physico-chemical analytical methods in characterizing the drug product *in vitro* and *in vivo* as needed.

B. Positions and Honors

2008-present Senior Research Scientist, TherapyX^{inc,} Buffalo, New York.
2008-2009 Visiting Scientist, Brown University, Providence, RI.
2007-2008 Senior Scientist, Freedom2, Inc., Providence, RI.

C. Contribution to Science

My current research interests include the development and characterization of novel microsphere based delivery systems specifically for local delivery of proteins such as insulin and growth factors as well as in the development of nanotechnology approaches for therapeutic applications such as infectious diseases, cancer and autoimmune or immune mediated inflammatory diseases.

1. Conway, T.F., Hammer, L., Furtado, S., Mathiowitz, E., Nicoletti, F., Mangano, K., Eglimez, N.K., and Auci, D.L. Oral Delivery of Particulate Transforming Growth Factor Beta 1 and All-Trans Retinoic Acid Reduces Gut Inflammation in Murine Models of Inflammatory Bowel Disease. J Crohns Colitis. (2015) Aug;9(8):647-58.
2. Chung AY, Li Q, Blair SJ, De Jesus M, Dennis KL, LeVea C, Yao J, Sun Y, Conway TF, Virtuoso LP, Battaglia NG, Furtado S, Mathiowitz E, Mantis NJ, Khazaie K, Egilmez NK. Oral interleukin-10 alleviates polyposis via neutralization of pathogenic T-regulatory cells. Cancer Res. 2014 Oct 1;74(19):5377-85.
3. Yuval Ramot, Raul Brauner, Kongbin Kang, John V. Heymach, Stacia Furtado and Abraham Nyska. Quantitative Evaluation of Drug-induced Microvascular Constriction in Mice Kidney Using a Novel Tool for 3D Geometrical Analysis of Ex vivo Organ Vasculature. Toxicol Pathol. 2014 Mar 26;42(4):774-783.
4. Jingxuan Shan, Shoba DSousza, Sasha Bakhru, Eman Al-Azwani, Maria Ascierto, Konduru, Sastry, Shahinaz Bedri, Dhanya Kizhakayil, Idil, Aigha, Joel Malek, Issam Al-Bozom, Salah, Gehani, *Stacia Furtado*, Edith Mathiowitz, EnaWang, Francesc Marincola, and Lotfi Chouchane. TNRC9 down-regulates BRCA1 expression and promotes breast cancer aggressiveness. Cancer Research. 2013. May 1;73(9):2840-9

5. Sasha H. Bakhru, *Stacia Furtado*, A. Peter Morello & Edith Mathiowitz. Oral delivery of proteins by biodegradable nanoparticles. Advanced Drug Delivery Reviews. 2013 Jun 15;65(6):811-21.
6. Bryan Laulicht, Alexis Mancini, Nathanael Geman, Daniel Cho, Kenneth Estrellas, *Stacia Furtado*, Russell Hopson, Anubhav Tripathi, Edith Mathiowitz. Bioinspired Bioadhesive Polymers: Dopa-Modified Poly(acrylic acid) Derivatives. Macromol Biosci. 2012 Nov 12 (11):1555-65.
7. Patel RS, Cho DY, Tian C, Chang A, Estrellas KM, Lavin D, *Furtado S*, Mathiowitz E. Doxycycline delivery from PLGA microspheres prepared by a modified solvent removal method. J Microencapsul. 2012;29(4):344-52.
8. Lavin DM, Zhang L, *Furtado S*, Hopkins RA, Mathiowitz E. Effects of protein molecular weight on the intrinsic material properties and release kinetics of wet spun polymeric microfiber delivery systems. Acta Biomater. 2012 Aug 16.
9. Lavin DM, Stefani RM, Zhang L, *Furtado S*, Hopkins RA, Mathiowitz E. Multifunctional polymeric microfibers with prolonged drug delivery and structural support capabilities. Acta Biomater. 2012 May;8(5):1891-900.
10. *Stacia Furtado*, Danielle Abramson, Roxanne Burrill, Gloria Olivier, Celinda Gourd, Emily Bubbers, and Edith Mathiowitz, Oral Delivery of Insulin Loaded Poly(Fumaric-co-sebacic) Anhydride Microspheres, International Journal of Pharmaceutics, Volume 347, Issue 1-2, January 2008, Pages 149-55.
11. *Stacia Furtado*, Danielle Abramson, Liat Simhkay, Daniel Wobbekind and Edith Mathiowitz, Subcutaneous Delivery of Insulin Loaded Poly(fumaric-co-sebacic anhydride) Microspheres to Type 1 Diabetic Rats, European Journal of Pharmaceutics and Biopharmaceutics, Volume 63, Issue 2, June 2006, Pages 229-236.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

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

NAME: Mathiowitz, Edith

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor of Medical Science and Engineering

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Tel Aviv University, Israel	B.Sc.	1973	Chemistry
Weizmann Institute of Science, Israel	M.Sc.	1979	Physical Chemistry
Weizmann Institute of Science, Israel	Ph.D.	1985	Physical Chemistry

A. Personal Statement

Dr. Edith Mathiowitz is a full Professor of Medical Science and Engineering at Brown University in the Department of Molecular Pharmacology, Physiology, and Biotechnology. She is also the Director of the Biotechnology Graduate Program. Her extensive experience includes development of polymers that are used in drug and gene delivery systems and tissue engineering applications, including stem cell recruitment. She holds an undergraduate degree in chemistry from Tel Aviv University and a master's and PhD in physical chemistry from the Weizmann Institute of Science. Dr. Mathiowitz trained as a postdoctoral associate with Professor Robert Langer at the Massachusetts Institute of Technology. She is a Member of the National Academy of Inventors and an AIMBE fellow. Dr. Mathiowitz work resulted in major publications, but also translated into two start-up companies, Spherics, Inc. and Perosphere, Inc., which focus their efforts on developments to improve patient compliance. She has advised about 30 Graduate students about the same number of master and honor students over 100 undergraduate students and three postdoctoral fellows.

Dr. Mathiowitz' role will be to supervise in the encapsulation of cytokine as well advise and oversee the physico-chemical analytical methods used in the analysis of the encapsulated protein. She has a long-standing collaborative relationship with TherapyX^{Inc} and will continue to offer advice for the nanoparticle production, scale-up, stability and consistency portions of this project.

B. Positions and Honors

Positions and Employment

1975-1979 Research Chemist at the Israel Institute of Biological Research, Nes Ziona, Israel, Polymer Department, in collaboration with the late Dr. A. Raziel, on applied polymer research.
1984-1986 Postdoctoral Fellow at the Department of Applied Biological Sciences, with Prof. R. Langer, Massachusetts Institute of Technology.
1987-1989 Research Associate at the Department of Surgery, Children's Hospital, Harvard Medical School.
1989-1991 Senior Research Scientist in Drug Delivery. Enzytech, Inc.
1989-1991 Visiting Scientist at the Department of Chemical Engineering, Massachusetts Institute of Technology
1991-1994 Assistant Professor of Medical Science, Director of Graduate Students, Division of Biology and Medicine, Section of Artificial Organs, Biomaterials and Cellular Technology.
1994-1999 Associate Professor of Medical Science and Engineering, Department of Molecular Pharmacology, Physiology & Biotechnology, Director of Graduate Program - Artificial Organs, Biomaterials and Cellular Technology
2000- Professor of Medical Science and Engineering, Department of Molecular Pharmacology, Physiology & Biotechnology, Director of Graduate Program - Biotechnology

Other Experience and Professional Memberships

1987-89 Research associate at the Department of Surgery, Children's Hospital, Harvard Medical School
Controlled Release Society 2008-2011 Founder and CEO of Perosphere Inc.
1997-99 President of start up company, Spherics
1999-2005 Chairwomen and consultant, Spherics

American Chemical Society (Polymer Division)
American Association for the Advancement of Science
Material Research Society Biomaterials Society
American Society for Artificial Internal Organs (ASAIO)

Honors

1973 Distinction Prize for B.Sc. students (Tel Aviv University, Israel).
1979-84 Feinberg Fellowship, Weizmann Institute of Science, Israel.
1982 Delek Prize for distinctive research work (Weizmann Institute of Science, Israel).
1985-87 Bantrell Postdoctoral Fellowship (MIT).A competitive award from MIT in the field of surface science.
1991-93 Whitaker Foundation Award.
1994 Recognition Award for Excellence in Guiding Graduate Student Research. Controlled Release Society - Procter & Gamble. Awarded in Nice, France.
1997 Fellow AIMBE
2000 The Eurand Award for Excellence in Research in the Area of Oral Drug Delivery Systems.
2013 Fellow of the National Academy of Inventors
2015 Fellow of the Controlled Release Society

C. Contribution to Science

My current research interests include development and characterization of novel bioadhesive delivery systems, oral delivery of proteins such as insulin and growth factors, development of nanotechnology approaches for therapeutic applications, such as cancer, development of bone repair delivery systems, development of drug eluting vascular grafts, use of progenitor cell to redirect healing of vascular grafts and treatment of obesity, development of novel liquid crystals as smart sensory devices, and gene/DNA delivery. I have published over 120 research articles, acquired 75 patents and acted as a consultant to biotech companies.

1. The work related to novel bioadhesive delivery systems ranged from basic engineering of fundamental science to the measurement of tensile forces between tissue/materials interactions, to designing smart delivery systems including the bioadhesive novel polymers. The overall aim was to develop an effective oral delivery system for peptide and proteins based on safe, biocompatible, biodegradable nanoparticles; specifically, to develop engineered bioerodible nanoparticles that, once delivered to the mucosal tissue, are capable to penetrate the barrier, reach the epithelium, penetrate the cell, and distribute to internal organs. We have achieved this by developing an encapsulation technique, which first stabilizes sensitive proteins for delivery after oral administration; the polymer then degrades, thereby releasing the encapsulated protein. Additionally, nanoparticles were designed using a very specific bioadhesive coating that is capable of delivering the nanoparticles to intestinal tissue in order to enhance penetration and control the uptake. Recently, two major publications (PNAS, 2013 and JCR, 2013) describe this work. My lab demonstrated that the relatively high degree of microsphere uptake in the absorptive and non-absorptive epithelium indicates that endocytosis as well as phagocytotic mechanisms are responsible for the uptake of microspheres in the small intestine. These findings may potentially guide research aimed at delivering specific molecules to target organs. In addition, the methods and in vivo model that were used may also be useful to toxicologists who are interested in determining the fate or tissue distribution of microparticles, including whether such particles can cross the blood-brain barrier.
    a. E. Mathiowitz, J. Jacob, Y. Jong, G. Carino, D. Chickering, P. Chaturvedi, C. Santos, K. Vijayaraghavan, S. Montgomery, M. Bassett and C. Morrell, "Biologically Erodable Microspheres as Potential Oral Drug Delivery Systems," *Nature* 386, 410-414, 1997.

b. Laulicht B, Gidmark NJ, Tripathi A, Mathiowitz E. Localization of magnetic pills. Proc Natl Acad Sci U S A. 2011 Feb 8;108(6):2252-7. doi: 10.1073/pnas.1016367108. Epub 2011 Jan 21. PubMed PMID: 21257903; PubMed Central PMCID: PMC3038721
c. Laulicht B, Tripathi A, Schlageter V, Kucera P, Mathiowitz E. Understanding gastric forces calculated from high-resolution pill tracking. Proc Natl Acad Sci U S A. 2010 May 4;107(18):8201-6. doi: 10.1073/pnas.1002292107. Epub 2010 Apr 19. PubMed PMID: 20404209; PubMed Central PMCID: PMC2889561
d. Joshua J. Reineke, Daniel Y. Cho, Yu-Ting Dingle, A. Peter Morello III, Jules Jacob, Christopher G. Thanos, Edith Mathiowitz a Unique insights into the intestinal absorption, transit, and subsequent biodistribution of polymer-derived microspheres. PNAS, vol. 110 no. 34, 13803–13808, 20 PubMed PMID: 23922388; PubMed Central PMCID: PMC3752225

2. In the area of microencapsulation, my work focuses on using basic concepts of polymer phase separation phenomenon to design self-assembled microspheres, vascular grafts, and fiber reinforced by solvent induced crystallization. One example is the development of multi-walled microspheres. (*Nature* 1994) Many approaches for the controlled release of drugs involve incorporation of the drug molecules into the matrix of microscopic polymer spheres or capsules. Those existing methods for preparing such microparticles do not, however, always guarantee a constant release rate. For example, drug molecules may be trapped preferentially at the surface; they have to diffuse through an increasing thickness of polymer when the particles are non-eroding, or the surface area changes for eroding particles. In other situations, pulsed release may be required—an application to which simple polymer microspheres do not readily lend themselves. Our work on how to engineer multi-walled microspheres might solve some of these problems. In addition we have developed a one-step process for preparing double-walled polymer microspheres based on phase separation between polymer mixtures. With an appropriate choice of interfacial tensions and evaporation rates, a spherical droplet of one polymer becomes coated with a highly uniform layer of the other.
    a. Pekarek, J. Jacob and E. Mathiowitz “Double-walled Microspheres for Controlled Drug Release,” *Nature*, 367, 258-260, January 20, 1994.
    b. K. Pekarek, J. Jacob and E. Mathiowitz “One-step preparation of double-walled microspheres,” *Advanced Materials,* 6, No. 9, 684-687, 1994.
    c. Morello AP 3rd, Burrill R, Mathiowitz E. Preparation and characterization of poly(methyl methacrylate) - iron (III) oxide microparticles using a modified solvent evaporation method. J Microencapsul. 2007 Aug;24(5):476-91. PubMed PMID: 17578736..
    d. Laulicht B, Cheifetz P, Mathiowitz E, Tripathi A. Evaluation of continuous flow nanosphere formation by controlled microfluidic transport. Langmuir. 2008 Sep 2;24(17):9717-26. 2008 Aug 6
3. Nanomedicine: Bioadhesive delivery systems ultimately led to the development of bioadhesive nanoparticles. Research in my laboratory started by developing novel bioadhesive polymers, which are different, then the traditional hydrogels. It was the understanding that polymers such as polyanhydrides are more adhesive in general and better delivery systems for certain drugs, since they retain hydrophilic and hydrophobic drugs more effectively than hydrogels such as poly acrylic acid. Specifically, we observed that bioadhesion with those new polymers enhanced the residence time in rats, dogs, and pigs as well as enhanced the bioavailability of hydrophobic drugs. The question my group then asked was what will now happen if we made nanoparticles and deliver insulin and DNA. The results were astonishing and were initially received with a lot of criticism, yet *Nature* did accept the evidence and publish the data. From here, my group has focused on studding the mechanism of action and more specifically what type of polymers will be both useful and non-toxic. By utilizing bioadhesive nanoparticles, we increase the residence time of these nanoparticle delivery systems in the GI tract, thereby producing a greater chance of uptake of these particles by both Peyer’s Patches as well as by the enterocytes of the intestine with subsequent release into the blood.
    a. Conway TF, Hammer L, Furtado S, Mathiowitz E, Nicoletti F, Mangano K, Egilmez NK, Auci DL. Oral Delivery of Particulate Transforming Growth Factor Beta 1 and All-Trans Retinoic Acid Reduces Gut Inflammation in Murine Models of Inflammatory Bowel Disease. J Crohns Colitis. 2015 Aug;9(8):647-58. doi: 10.1093/ecco-jcc/jjv089. Epub 2015 May 18. PubMed PMID: 25987350; PubMed Central PMCID: PMC4817304
    b. Chung AY, Li Q, Blair SJ, De Jesus M, Dennis KL, LeVea C, Yao J, Sun Y, Conway TF, Virtuoso LP, Battaglia NG, Furtado S, Mathiowitz E, Mantis NJ, Khazaie K, Egilmez NK. Oral interleukin-10 alleviates polyposis via neutralization of pathogenic T-regulatory cells. Cancer Res. 2014 Oct

1;74(19):5377-85. doi: 10.1158/0008-5472.CAN-14-0918. Epub 2014 Sep 16. PubMed PMID: 25228656; PubMed Central PMCID: PMC4322772. N.Egilmez, Y.Jong, J.Jacobs, C.Santos, E.Mathiowitz, Y.Iwanuma, R.Bankert, "Cytokine immunotherapy of cancer with controlled release biodegradable microspheres in a human tumor xenograft/SCID mouse model, *Cancer Immunology Immunotherapy,* 1998 Mar; 46(1):21-4.

c. M.Kuriakose, F-A.Chen, N.Egilmez, Y.Jong, E.Mathiowitz, M.Delacure, "W.Hicks, T.Loree, R.Bankert, Interleuckin-12 delivered by biodegradable microspheres promotes the antitumor activity of human peripheral blood lymphocytes in a human head and neck tumor xenograft/SCID mouse model", *Head and Neck*. 2000 Jan;22(1):57-63.

4. Tissue engineering: The idea behind drug-eluting vascular grafts was to develop a process for the inclusion of polymer microspheres in microporous polyurethane tubes and membranes. Our laboratory developed a very unique way to make porous vascular grafts. This work focused on the development of small diameter vascular grafts by optimizing the process based on phase separation phenomenon, one of my favorite topics. These composites were fabricated via a spray, phase-inversion technique using polyurethane, and either spray-dried poly (d,l-lactide-co-glycolide 50:50) microspheres or commercially available fluorescent polystyrene-latex microspheres. We intended to release heparin to prevent clotting as this is a major hindrance with the development of small diameter vascular grafts. This project reflected my desire to not only work on traditional dosage forms but to develop complex systems for tissue engineering.

   The last topic in this area relates to obesity. The first clinically-accepted, autologous cell-based therapy (Carticel^{TM}) aimed at augmenting a cell population via harvest, expansion, and re-implantation of chondrocytes into cartilage defects. Applying a similar principle to increase the brown adipocyte population could be a highly effective therapeutic strategy to combat obesity; however, the invasiveness of extraction and difficulties facing expansion of brown adipocytes complicate a straightforward extraction and expansion approach. Instead, here we propose recruiting adipose stem cells using microencapsulated soluble factors to concentrate a more easily expandable population of cells in an easily accessible subcutaneous pouch. In the past, our group has successfully recruited hematopoietic stem cells into subcutaneous pouches by eluting soluble factors within a single week. The proposed study is using the same principle of polymer microencapsulation elution of soluble factors to recruit adipose stem cells for *in vitro* expansion.

   a. Shan J, Dsouza SP, Bakhru S, Al-Azwani EK, Ascierto ML, Sastry KS, Bedri S, Kizhakayil D, Aigha II, Malek J, Al-Bozom I, Gehani S, Furtado S, Mathiowitz E, Wang E, Marincola FM, Chouchane L. TNRC9 downregulates BRCA1 expression and promotes breast cancer aggressiveness. Cancer Res. 2013 May 1;73(9):2840-9. doi: 10.1158/0008-5472.CAN-12-4313. Epub 2013 Feb 27. PubMed PMID: 23447579N.
   b. Egilmez, Y.Jong, M.Sabel, J.Jacob, E.Mathioiwitz, R.Bankert, "*In Situ* Tumor Vaccination with Interleukin-12-encapsulated Biodegradable Microspheres:Induction of Tunor Regression and Patent Antitumor Immunity", Cancer Research 60, 3832-3837, July, 2000
   c. Lavin DM, Zhang L, Furtado S, Hopkins RA, Mathiowitz E. Effects of protein molecular weight on the intrinsic material properties and release kinetics of wet spun polymeric microfiber delivery systems. Acta Biomater. 2013 Jan;9(1):4569-78. doi: 10.1016/j.actbio.2012.08.005. Epub 2012 Aug 16. PubMed PMID: 22902813
   d. Laulicht B, Cheifetz P, Tripathi A, Mathiowitz E. Are in vivo gastric bioadhesive forces accurately reflected by in vitro experiments? J Control Release. 2009 Mar 4;134(2):103-10. doi: 10.1016/j.jconrel.2008.11.012. Epub 2008 Nov 27. PubMed PMID: 19087887.

5. I am not only interested in publications, but also in translating my work into two start-up companies, Spherics, Inc. and Perosphere, Inc., which focus their efforts on developments to improve patient compliance. Spherics, Inc. was based on my patents in the area of bioadhesion and was focused on developing a long-acting drug for the central nervous system, specifically for Parkinson's disease. Perosphere. Inc. is a specialty pharmaceutical company with a strong foundation in rescue molecules. The lead drug candidate is a reversal agent for new oral anticoagulants (NOACs). There is currently no approved reversal agent for these NOACs. I have submitted as co-inventor seven patent applications in the area of risk molecules, vaccines, and charge masking agents for oral delivery.

   I was lucky to have motivated outstanding students in the Biotechnology and BME programs. Each one of my graduate students took a unique course, Experimental Surgery, which is taught at Brown by a dedicated team.

Thus, almost each one of my graduate students was able to perform preclinical studies that depended on funding from institutions such as NIH and JDRF. Surgeries were performed on mice, rats, dogs and pigs. The transfer from the lab to a startup company went smoothly because of the experience my graduate students had in performing proficient animal studies.

My publication list is available following the links:
http://www.ncbi.nlm.nih.gov/sites/myncbi/18KSYwaci_o5w/bibliography/48079688/public/?sort=date&direction=ascending

A. Research Support

Ongoing Research Support

Therapy X Mathiowitz (PI) 06/01/11-05/31/17
Oral Delivery of Nanoencapsulated TGF1 and All-trans-Retinoic Acid for the Treatment of Inflammatory Bowel Disease
The major goal of this project is to complete pre-clinical formulation and treatment optimization studies in the murine adoptive T-cell transfer model of established IBD

[redacted]

[redacted]

[redacted]

Completed Research Support

[redacted]

7R01CA100656-11 Mathiowitz (PI) 05/01/2014 – 04/30/2016
NIH/University of Louisville (Subaward)
Integrating Innate and Adaptive Immunity in Cancer Therapy
The major goal of this project to encapsulate cytokes for eradicating cancer.

[redacted]

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Egilmez, Nejat K.	Professor and Chairman
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 Minnesota, Minneapolis/St. Paul	B.S.	1980	Biochemistry
State University of New York at Buffalo	M.A.	1983	Cell & Molec. Biology
State University of New York at Buffalo	Ph.D.	1986	Cell & Molec. Biology
Louisiana St. Univ. Med. Ctr., New Orleans, LA	Postdoc	1986-88	Biochem. & Molec. Biol.

A. Personal Statement

I currently hold the position of Chairman in the Department of Microbiology and Immunology, School of Medicine, University of Louisville. I have led an independent research program since 2001 focusing on the study of tumor immunity, immune therapy and immune regulation. During this period I authored/co-authored 52 peer-reviewed original research and invited review articles, and trained 10 pre- and postdoctoral students. My laboratory originally pioneered the use of sustained-release biodegradable nanoparticulate cytokine formulations, particularly that of IL-12, in cancer therapy. More recently, in collaboration with TherapyX, Inc. and the Russell laboratory at SUNY Buffalo, we have extended the use of particulate IL-12 adjuvants to the treatment of genital tract infections. Therefore I am uniquely situated to assist TherapyX^{Inc} in advancing the studies proposed in this phase II SBIR application. My role in this project will be to provide scientific guidance and oversight of the planned studies.

B. Positions and Honors.

1988-1994 Assistant Professor, Robert College/Department of Biology, Istanbul, Turkey
1994-2001 Cancer Research Scientist I, II, Assistant Member, Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY
2001-2003 Research Assistant Professor, Department of Microbiology and Immunology, State University of New York, Buffalo, NY
2003-2006 Assistant Professor, J.G. Brown Cancer Center and Department of Microbiology and Immunology, University of Louisville, Louisville, KY.
2006-2012 Associate Professor, Department of Microbiology and Immunology, School of Medicine and Biomedical Sciences, State University of New York, Buffalo, NY.
2012-2013 Professor, Department of Microbiology and Immunology, School of Medicine and Biomedical Sciences, State University of New York, Buffalo, NY.
2013-present Professor and Chairman, Department of Microbiology and Immunology, School of Medicine, University of Louisville, Louisville, KY.

Ad-hoc member NIH/TTT study section, October 2005.
Ad-hoc member NIH/TME study section, February 2008, October 2008, October 2009, June 2010.
Ad-hoc member NIH/ZATI SM13 study section, March 2009.
Ad-hoc member NIH/ZRG1 OTC-F study section, June 2010.
Ad-hoc member NIH/ZRG1 BCMB-A 51R study section, March 2014.
Ad-hoc member NIH/ZRG1 OTC-C(02)M study section, October 2014
Ad-hoc member NIH/ZRG1 F09B-B920) study section, March 2015
Ad-Hoc member NIH/ZAT1 SM (37) L study section June 2015
Chartered member NIH/TME study section 7/2010 – 6/2014.

Editor-in-Chief, International Journal of Immunology and Immunotherapy

Associate Editor: Frontiers in Immunology, Immunological Investigations, Journal of Immunology Research

Member American Association of Immunology
American Association for Cancer Research
International Society for Biological Therapy of Cancer
American Association for the Advancement of Science

C. Contributions to Science

A full list of my published work can be found at: http://www.ncbi.nlm.nih.gov/pubmed/?term=egilmez+n

1. Molecular factors that contribute to cellular aging.

   My early postdoctoral work focused on delineating the cellular factors that contributed to replicative lifespan in unicellular organisms. These studies led to the advancement of the notion that certain age-associated factors are preferentially retained in the progenitor cells conferring the "senescent" phenotype. This notion was subsequently validated by numerous laboratories with the discovery of several such factors including extrachromosomal rDNA circles and sirtuins.

   Egilmez, N.K., J.B. Chen and S.M. Jazwinski. 1990. Preparation and Characterization of Old Yeast Cells. J. Gerontol., 45:B9-17.
   Egilmez, N.K., J.B. Chen and S.M. Jazwinski. 1989. Specific Alterations in Transcript Prevalence During the Yeast Life-span. J. Biol. Chem. 264:14312-14317.
   Egilmez, N.K. and S.M. Jazwinski. 1989. Evidence for the Involvement of a Cytoplasmic Factor in the Aging of the Yeast Saccharomyces cerevisiae. J. Bacteriol. 171:37-42.

2. Human tumor/TIL-SCID mouse xenograft models for the study of tumor immunity and immune therapy.

   My subsequent postdoctoral training was in tumor immunology and immune therapy. Part of this work involved the development and optimization of intact human tumor/TIL xenograft models for evaluation of cancer immune therapy as a bridge model between purely mouse models and clinical studies. Our studies established and validated the use of autologous PBL/tumor and more importantly intact human tumor biopsy with autologous TIL xenografts in SCID mice as novel in vivo models of human cancer for study of human tumor immunity and immunotherapy.

   Hess, S.D., Egilmez, N.K., Bailey, N., Anderson, T.M., Mathiowitz, E., Bernstein, S. H. and Bankert, R.B. 2003. Human CD4+ T cells present within the microenvironment of human lung tumors are mobilized by the local and sustained release of IL-12 to kill tumors in situ by indirect effects of IFN-gamma. J Immunol. 170(1):400-12.
   Egilmez, N.K., Hess, S.D., Chen, F-A, Takita, H., Conway, T. and Bankert, R.B. 2002. Human CD4+ Effector T-Cells Mediate an Indirect IL-12 and IFN-γ-Dependent Suppression of Autologous Lung Tumor Xenografts in SCID Mice. Cancer Res. 62:2611-2617.
   Egilmez, N.K., Jong, Y.S., Hess, S.D., Jacob, J.S., Mathiowitz, E. and Bankert, R.B., 2000. Cytokines delivered by biodegradable microspheres promote effective suppression of human tumors by human peripheral blood lymphocytes in the SCID/Winn model. J. Immunother. 23(2):190-195.
   Egilmez, N.K., R. Cuenca, S.J. Yokota, Sorgi, F. and R.B. Bankert. 1996. In vivo Cytokine Gene Therapy of Human Tumor Xenografts in SCID Mice. Gene Therapy 3:607-614.

3. Sustained-release Micro/nanoparticulate protein adjuvants for the treatment of inflammatory disease.

   In the past decade and a half my laboratory has pioneered the development and use of injectable micro/nanoparticulate sustained-release formulations of immune-modulatory macromolecules in disease therapy. Our earlier work focused primarily on the development of these novel formulations for cytokine-based therapy of cancer, utilizing numerous cytokines including IL-2, IL-12, GM-CSF and

TNFα as an alternative approach to gene therapy. We have published extensively on the utility of these reagents in numerous transplantable and spontaneous murine as well as human tumor/SCID mouse xenograft models. These reagents are currently under development for clinical trials in cancer patients.

Recent work evaluated the potential of such formulations as oral therapeutics in the treatment of gut-associated inflammatory disorders including gastrointestinal cancers and IBD. These studies not only established the utility of microparticulate IL-10 and TGFβ in therapy of gut dysplasia, but provided unique insight into the immunological basis of their activity.

Finally, we demonstrated that intragenital application of particulate cytokine or antibody can act as a therapeutic vaccine. Specifically, this strategy induced the eradication of established disease and provided long-term protection from subsequent challenge in a murine model of Neisseria gonorrheae infection, extending the clinical utility of these reagents to infectious disease.

[redacted]

Chung AY, Li Q, Blair SJ, De Jesus M, Dennis KL, LeVea C, Yao J, Sun Y, Conway TF, Virtuoso LP, Battaglia NG, Furtado S, Mathiowitz E, Mantis NJ, Khazaie K, Egilmez NK. Oral Interleukin-10 Alleviates Polyposis via Neutralization of Pathogenic T-Regulatory Cells. Cancer Res. 2014 Oct 1;74(19):5377-85.

Liu Y, Egilmez NK, Russell MW. 2013. Enhancement of Adaptive Immunity to Neisseria gonorrhoeae by Local Intravaginal Administration of Microencapsulated Interleukin 12. J Infect Dis. 208(11):1821-9.

Hill, H.C., Conway, T.F., Sabel, M.S., Jong, Y.S., Mathiowitz, E., Bankert, R.B. and Egilmez, N.K. 2002. Cancer Immunotherapy with Interleukin-12 and Granulocyte-Macrophage Colony-Stimulating Factor-encapsulated microspheres: Coinduction of innate and adaptive immunity and cure of disseminated disease. Cancer Res. 62:7254-7263.

4. Role of regulatory rebound in defining the long-term efficacy of immune therapy.

In the past decade our mechanistic focus was on a central conundrum of cancer immune therapy, i.e. the general failure of immune-based therapies to achieve durable tumor regression in cancer patients despite the ability to induce potent antitumor T-cell activity. We approached this problem from the unique perspective of a specific therapeutic, i.e. IL-12, a potent antitumor cytokine with robust activity in murine models but only transient effects in patients. Our studies delineated the post-IL-12 cellular immune events that occur in the tumor microenvironment and the associated immune structures revealing the critical role of feedback regulation in short-circuiting IL-12-induced antitumor T-cell cytotoxicity. We found that IFNγ, the secondary effector cytokine downstream of IL-12, drives not only the cytotoxic but also the regulatory mechanisms that ultimately shut down the effector response. Specifically, we defined the role of IL-12-IFNγ-Indoleamine 2, 3 Dioxygenase axis in promoting the post-treatment T-regulatory cell rebound that ultimately restored suppressive homeostasis in tumors. We are currently dissecting the molecular mechanisms that drive the immunogenic (IL-12+ IDO-) to tolerogenic (IL-12- IDO+) switch in DC phenotype in post-IL-12 animals. Our findings are conceptually in line with the recent recognition of the critical role of T-cell co-inhibitory/regulatory molecules (now termed immune checkpoint molecules) in pre-mature termination of antitumor T-cell cytotoxicity, a paradigm that has revolutionized the field of cancer immunotherapy.

[redacted]

Harden JL, Gu T, Kilinc MO, Rowswell-Turner RB, Virtuoso LP and Egilmez NK. 2011. Dichotomous effects of IFNγ on Dendritic cell function determine the extent of Interleukin-12-driven antitumor T-cell immunity. J Immunol. 187(1):126-32.

Gu T, Rowswell-Turner RB, Kilinc MO and Egilmez NK. 2010. Central role of IFNγ-Indoleamine 2,3-dioxygenase in regulation of Interleukin-12-mediated antitumor immunity. Cancer Res. 70(1):129-138.

Nair RE, Kilinc MO, Jones SA and Egilmez, NK. 2006. Chronic immune therapy induces a progressive increase in intra-tumoral T-suppressor activity and a concurrent loss of tumor-specific CD8+ T-effectors in her-2/neu transgenic mice bearing advanced spontaneous tumors. J. Immunol. 176(12):7325-34.

D. Research projects ongoing or completed in the past 3 years

Ongoing:

"Integrating Innate and Adaptive Immunity in Cancer Therapy", NIH, 2R01-CA100656-01A1, 7/1/10 – 4/30/16. NCE
Role: Principal Investigator, 10% effort. Total direct costs: $[redacted]

"Delivery of nanoencapsulated TGFbeta and ATRA for the Treatment of IBD". NIH 2R44-AI080009. 4/1/2011-3/31/2016.
Role: Co-I (Auci PD/PI), 5% effort. Total costs: $[redacted]

Completed:

"Oral Immune-modulatory Adjuvants for Treatment of Colorectal Carcinoma", NIH 1-R21-AI092133-01A1. 7/1/2011 - 6/30/2014.
Role: Principal Investigator, 10% effort. Total direct costs $[redacted]

Intratumoral T-suppressor cell Homeostasis in Breast Cancer, DOD Breast Cancer Program / Pre-doctoral fellowship.
BC093325 (Rowswell-Turner, PI) 12/15/09-12/14/12
Role: Mentor, 5% effort. Total direct costs $[redacted]

BIOGRAPHICAL SKETCH

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

NAME
RUSSELL, Michael W.

eRA COMMONS USER NAME (credential, e.g., agency login)
[redacted]

POSITION TITLE
Professor Emeritus

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)*	Completion date MM/YYYY	FIELD OF STUDY
Cambridge University, England	B.A.	06/1966	Biochemistry
Cambridge University, England	M.A.	05/1970	
Reading University, England	Ph.D.	06/1973	Microbiology
Guy's Hospital Medical and Dental Schools, London, England	Post-doc	05/1979	Immunology

A. PERSONAL STATEMENT

I have >40 years of research experience in mucosal immunology especially in relation to the generation and functions of IgA antibodies, and immunity to infections of the mouth and genital tract. I have published 140 peer-reviewed papers and 88 book chapters or conference reports, which have garnered a total of >9200 citations (ResearchGate; *h*-index = 54). My current research interests focus on immunity to *Neisseria gonorrhoeae*. Previous investigations on the human immune response to *N. gonorrhoeae* led to a novel approach in the study of immunity to gonorrhea, and to the hypothesis that this well-adapted pathogen suppresses adaptive immune responses that might eliminate it and instead elicits innate responses that it can resist. My lab has demonstrated that IL-17 and Th17-related immune responses are generated in a mouse model of vaginal gonococcal infection, and that clearance of the infection is dependent in part upon signaling through the IL-17 receptor, whereas Th1/Th2 immunity is suppressed by a mechanism dependent on TGFβ, IL-10, and the generation of type 1 regulatory T cells. More recently, in collaboration with TherapyX^{inc}, we found that this immunosuppression can be reversed by the intravaginal delivery of the company's nanoparticulate IL-12 formulation, which permits the development of protective immune responses. We are now pursuing a prophylactic vaccine approach accomplishing the same objective that should be safer for human use. Although now formally retired, I continue to maintain a research laboratory at SUNY Buffalo, and in my role as consultant, will continue to provide scientific and technical advice and oversight for this Phase II application. Our accomplishments over the past few years lead us to believe that we are at the cutting edge of this endeavor.

1. Liu, Y., Feinen, B., and Russell, M.W. New concepts in immunity to *Neisseria gonorrhoeae*: innate responses and suppression of adaptive immunity favor the pathogen, not the host. Front. Microbiol. 2: 52 (2011). PMCID:3153028
2. Woof, J.M., and Russell, M.W. Structure and function relationships in IgA. Mucosal Immunol. 4: 590-597 (2011). PMID:21937984
3. Jerse, A.E., Bash, M.C., and Russell, M.W. Vaccines against gonorrhea: current status and future challenges. Vaccine 32: 1579-1587 (2014). PMCID:4682887
4. Russell, M.W. Thinking globally, acting locally: harnessing the immune system to deal with recalcitrant pathogens. mBio 6: e00382-15 (2015). PMCID:4436072

B. POSITIONS AND HONORS

Employment

1968-1972 Research Student, Department of Bacteriology, National Institute for Research in Dairying, and Department of Microbiology, University of Reading, UK.
1972-1979 Postdoctoral Associate (1978-9, Lecturer), Department of Oral Immunology and Microbiology, Guy's Hospital Medical and Dental Schools, London, UK.
1979-1981 Visiting Investigator, Institute of Dental Research, University of Alabama at Birmingham (UAB).
1982-1986 Research Assistant Professor of Microbiology; Investigator, Inst. of Dental Research, UAB.
1986-1992 Research Associate Professor of Microbiology, UAB.
1987-1988 Visiting Associate Professor, Dept. of Oral Biology, Royal Dental College, Aarhus, Denmark.
1992-2000 Research Professor of Microbiology and Oral Biology, UAB.
2000-2016 Professor of Microbiology & Immunology, and Oral Biology, University at Buffalo (UB)
2016- Professor Emeritus

Other Experience and Professional Memberships

1976- Member, British Society for Immunology
1976-2014 Member, International Association for Dental Research (IADR)
1980- Member, American Society for Microbiology (ASM)
1982- Member, American Association of Immunologists
1987- Member, Society for Mucosal Immunology
2004-2006 Director, Witebsky Center for Microbial Pathogenesis and Immunology, UB
2004- Member, American Association for the Advancement of Science
2007- Member, American Society for Reproductive Immunology
2010 Research Scholar, Department of Medical Microbiology and Immunology, University of Wisconsin, Madison (on sabbatical leave from UB, June – November).

Honors and Awards (selected)

1963-1965 Scholar of St. John's College, University of Cambridge, UK
1996-1998 WHO/IUIS Nomenclature Committee, Subcommittee on IgA nomenclature
1999-2010 R37 MERIT award, NIH/NIDCR
2000 Fellow, American Academy of Microbiology
2007 Distinguished Scientist Award for Research in Oral Biology, IADR

C. Contributions to Science

My contributions, spanning >40 years, have been predominantly in the field of mucosal immunity, especially with respect to the biological functions of IgA antibodies, immunity to infections of the mouth and genital tract, and novel approaches to the development of mucosal vaccines, including the use of heat-labile enterotoxins as adjuvants and vaccine delivery systems. Most recently my work has focused on immunity to *Neisseria gonorrhoeae,* novel approaches to therapy and development of a prophylactic vaccine against gonorrhea. This has been pursued in collaboration with the early-stage research and development company, TherapyX, Inc. (Buffalo, NY), for which I serve on the Board of Scientific Advisors and as a Consultant.

1. As a postdoctoral scientist at Guy's Hospital, London, I was a member of a group working on a vaccine against dental caries using a rhesus monkey model. While there I discovered and characterized protein antigens (AgI/II and AgIII) of the oral bacterium, *Streptococcus mutans*. AgI/II came to be the prototype of a family of streptococcal surface proteins, and was the first such protein to be crystographically resolved. In addition, I was tasked with purifying monkey IgM, IgG, and IgA, and the production of specific antibody reagents against them. This allowed us also to demonstrate the transudation of circulating Igs into the oral cavity through the gingival crevices, and to determine the circulating half-lives of Igs in this species. After moving to UAB, I continued to work on immunity to dental caries and my lab showed that oral or intranasal immunization with AgI/II was effective in generating protective mucosal antibody responses against *S. mutans* in rodent and monkey models. We also demonstrated the ability of S-IgA antibodies to inhibit *S. mutans* adherence to tooth-like sufaces.

   a. Russell, M.W., Bergmeier, L.A., Zanders, E.D., and Lehner, T. Protein antigens of *Streptococcus mutans*: Purification and properties of a double antigen and its protease-resistant component. Infect. Immun. 28: 486-493 (1980).
   b. Lehner, T., Russell, M.W., Caldwell, J., and Smith, R. Immunization with purified protein antigens from *Streptococcus mutans* against dental caries in rhesus monkeys. Infect. Immun. 34: 407-415 (1981).

c. Hajishengallis, G., Nikolova, E., and Russell, M.W. Inhibition of *Streptococcus mutans* adherence to saliva-coated hydroxyapatite by human secretory immunoglobulin A (S-IgA) antibodies to cell surface protein antigen I/II: reversal by IgA1 protease cleavage. Infect. Immun. 60: 5057-5064 (1992).
d. Katz, J., Harmon, C.C., Buckner, G.P., Richardson, G.J., Russell, M.W., and Michalek, S.M. Protective salivary immunoglobulin A responses against *Streptococcus mutans* infection after intranasal immunization with *S. mutans* antigen I/II coupled to the B subunit of cholera toxin. Infect. Immun. 61: 1964-1971 (1993).

2. In contrast to the role of secretory IgA antibodies at mucosal surfaces, the biological significance of circulating IgA antibodies has remained incompletely understood. Intrigued by this on moving to UAB, I first demonstrated the ability of serum polymeric IgA to transport bound antigen through the liver into bile, thereby revealing a novel function of IgA antibodies in the non-inflammatory elimination of non-degradable antigens. Later, while on sabbatical in Denmark, I showed that complement activation by human IgA was essentially artifactual, being dependent on the interfacial denaturation of IgA, whereas IgA antibody-antigen complexes failed to activate complement. In contrast, human IgA antibodies, or their Fab fragments, inhibited complement activation by IgG antibodies, thereby affirming the essentially anti-inflammatory nature of IgA. Further elaboration of these studies on my return to UAB indicated that the glycan chains of interfacially aggregated IgA contributed to activation of the alternative complement pathway, and that the ability of IgA antibodies to promote phagocytosis by neutrophils depended on their activation status and expression of FcαR.

a. Russell, M.W., Brown, T.A., and Mestecky, J. Role of serum IgA: Hepatobiliary transport of circulating antigen. J. Exp. Med. 153: 968-976 (1981).
b. Russell, M.W., and Mansa, B. Complement-fixing properties of human IgA antibodies: alternative pathway complement activation by plastic-bound, but not by specific antigen-bound IgA. Scand. J. Immunol. 30: 175-183 (1989).
c. Russell, M.W., Reinholdt, J., and Kilian, M. Anti-inflammatory activity of human IgA antibodies and their Fabα fragments: inhibition of IgG-mediated complement activation. Eur. J. Immunol. 19: 2243-2249 (1989).
d. Nikolova, E.B., and Russell, M.W. Dual function of human IgA antibodies: inhibition of phagocytosis in circulating neutrophils and enhancement of responses in IL-8-stimulated cells. J. Leukocyte Biol. 57: 875-882 (1995).

3. Holmgren's group at the University of Göteborg, Sweden, developed the first generation oral cholera vaccine (Dukoral) which consists of killed cholera vibrios plus the nontoxic B subunit of cholera toxin (CT). To determine whether other vaccine antigens could be "piggybacked" on to CTB, I embarked in collaboration with this group on studies of chemically coupling *S. mutans* AgI/II to CTB to generate a novel mucosal vaccine, exploiting the model of dental caries in rodents. We subsequently applied this to intranasal immunization and investigated the cellular immune responses in nasal lymphoid tissues, and further demonstrated the effectiveness of intranasal immunization for inducing disseminated mucosal immune responses in primates. We also developed a novel recombinant genetic approach to create nontoxic chimeric mucosal immunogens from AgI/II and CT.

a. Czerkinsky, C., Russell, M.W., Lycke, N., Lindblad, M., and Holmgren, J. Oral administration of a streptococcal antigen coupled to cholera toxin B subunit evokes strong antibody responses in salivary glands and extramucosal tissues. Infect. Immun. 57: 1072-1077 (1989).
b. Wu, H.-Y. and Russell, M.W. Induction of mucosal immunity by intranasal application of a streptococcal surface protein antigen with the cholera toxin B subunit. Infect. Immun. 61: 314-322 (1993).
c. Hajishengallis, G., Hollingshead, S.K., Koga, T., and Russell, M.W. Mucosal immunization with a bacterial protein antigen genetically coupled to cholera toxin A2/B subunits J. Immunol. 154: 4322-4332 (1995).
d. Russell, M.W., Moldoveanu, Z., White, P.L., Sibert, G.J., Mestecky, J., and Michalek, S.M. Salivary, nasal, genital, and systemic antibody responses in monkeys immunized intranasally with a bacterial protein antigen and the cholera toxin B subunit. Infect. Immun. 64: 1272-1283 (1996).

4. The development of mucosally administered vaccines, which are needed especially for infections of the gastrointestinal, respiratory, and genital tracts, is limited by the lack of suitable adjuvants for use by these routes. In collaboration with Connell's group at UB, we first demonstrated that type II heat labile enterotoxins possessed similar adjuvant properties to CT, but with significant differences that were amenable to exploitation for vaccine development. Further development of these studies after I had moved to UB revealed the basis of the adjuvanticity of these type II enterotoxins and their mutants, and led to the discovery that their B subunits activated Toll-like receptor 2.

   a. Martin, M.H., Metzger, D.J., Michalek, S.M., Connell, T.D., and Russell, M.W. Distinct cytokine regulation by cholera toxin and the type II heat-labile enterotoxins involves differential regulation of CD40 ligand on $CD4^+$ T cells. Infect. Immun. 69: 4486-4492 (2001).
   b. Hajishengallis, G., Tapping, R.I., Martin, M.H., Nawar, H., Lyle, E.A., Russell, M.W., Connell, T.D. Toll-like receptor 2 mediates cellular activation by the B subunits of type II heat-labile enterotoxins. Infect. Immun. 73: 1343-1349 (2005). PMCID:1064972
   c. Nawar, H., Arce, S., Russell, M.W., Connell, T.D. Mucosal adjuvant properties of mutant LT-IIa and LT-IIb enterotoxins that exhibit altered ganglioside-binding activities. Infect. Immun. 73: 1330-1342 (2005). PMCID:1064923
   d. Arce, S., Nawar, H.F., Muehlinghaus, G., Russell, M.W., Connell, T.D. *In vitro* induction of IgA- and IgM-secreting plasma blasts by cholera toxin depends upon T cell help and is mediated by CD154 up-regulation and inhibition of IFN-γ synthesis. Infect. Immun. 75: 1413-1423 (2007). PMCID:1828582

5. For the past 20 years I have investigated immunity to *Neisseria gonorrhoeae*. This infection is widespread globally and is a major cause of reproductive tract damage in women. Moreover it can be contracted repeatedly as there is no induction of protective immunity against reinfection, and no vaccine is available. The continuing emergence of antibiotic resistance is raising concerns that gonorrhea could become untreatable. Initially at UAB we found that antibody and cytokine responses to uncomplicated genital tract infection in both men and women were minimal, suggesting that *N. gonorrhoeae* had the ability to interfere with the normal development of immune responses. Subsequent studies at UB have sustained this hypothesis, and we have demonstrated in a mouse model of genital tract infection that *N. gonorrhoeae* selectively induces Th17-driven innate immune responses that it can survive, and concomitantly suppresses Th1/Th2-driven adaptive immune responses by mechanisms dependent on the cytokines TGF-β and IL-10, and involving type 1 regulatory T cells. Our further studies have identified novel approaches to the treatment of gonococcal infection by the local administration of agents that reverse the induced immunosuppression, thereby affording a new approach to the treatment of infectious disease. These findings have also informed a novel approach to the development of a vaccine against gonorrhea, utilizing microencapsulated IL-12 as a Th1-driving adjuvant for a gonococcal outer membrane vesicle vaccine, which we are pursuing in this project.

   a. Liu, Y., and Russell, M.W. Diversion of the immune response to *Neisseria gonorrhoeae* from Th17 to Th1/Th2 by treatment with anti-TGF-β antibody generates immunological memory and protective immunity. mBio 2: e00095-11 (2011). PMCID:3101786
   b. Liu, Y., Islam, E., Jarvis, G.A., Gray-Owen, S., and Russell, M.W. *Neisseria gonorrhoeae* selectively suppresses the development of Th1 and Th2 cells, and enhances Th17 cell responses, through TGF-β-dependent mechanisms. Mucosal Immunol. 5: 320-331 (2012). PMCID:3328619
   c. Liu, Y., Egilmez, N.K., and Russell, M.W. Enhancement of adaptive immunity to *Neisseria gonorrhoeae* by local intravaginal administration of microencapsulated IL-12. J. Infect. Dis. 208: 1821-1829 (2013). PMCID:3814831.
   d. Liu, Y., Liu, W., and Russell, M.W. Suppression of host adaptive immune responses by *Neisseria gonorrhoeae*: role of interleukin 10 and type 1 regulatory T cells. Mucosal Immunol. 7: 165-176 (2014). PMCID:3812424
   e. Liu, Y., Hammer, L., Hobbs, M.M., Zielke, R.A., Sikora, A.E., Jerse, A.E., Egilmez, N.K., Russell, M.W. (2016) Experimental vaccine induces Th1-driven immune responses and protection against *Neisseria gonorrhoeae* in a murine model. MS submitted to Mucosal Immunol. (August 2016).

Complete List of Published Work (140 peer-reviewed publications and 88 book chapters)

MyBibliography: http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/41133423/public/

D. RESEARCH SUPPORT

Current Research Support

R44-AI-104067-02 (SBIR Phase II) Y. Liu (PI) 02/01/13 – 05/31/17
NIH/NIAID (No-cost extension)
Therapy and prophylaxis for genital tract infection
The goal of phase II is to develop a novel approach to treatment of genital gonococcal infection by the local administration of microencapsulated IL-12.
Role: collaborating scientist.

R43-AI115877-01 (SBIR Phase I) Y. Liu (PI) 12/01/14 – 11/30/16
NIH/NIAID (No-cost extension)
Experimental gonococcal vaccine
The goal of this project is to establish proof-of-principle for a novel vaccine against genital gonococcal infection.
Role: collaborating scientist.

Completed Research Support

R21-AI092348 M.W. Russell (PI) 12/03/10 – 11/30/13
NIH/NIAID
Imaging antigen and adjuvant uptake for enhancing response to mucosal vaccines
The major goals of this project were to characterize the cells and tissues involved in the uptake and processing of enterotoxin adjuvants and recombinant chimeric immunogens based on enterotoxins when administered by mucosal routes, utilizing imaging flow cytometry.
Role: PI

Liu Current Support

Current Research Support

R44-AI-104067-02 (SBIR Phase II) Y. Liu (PI) 02/01/13 – 05/31/16
NIH/NIAID
Therapy and prophylaxis for genital tract infection
The goal of phase II is to develop a novel approach to treatment of genital gonococcal infection by the local administration of microencapsulated IL-12.
Role: PI.

R43-AI-115877-01 (SBIR Phase I) Y. Liu (PI) 12/01/14 – 11/30/16
NIH/NIAID
Experimental gonococcal vaccine
The goal of this project is to establish proof-of-principle for a novel vaccine against genital gonococcal infection.
Role: PI.

D. Research projects ongoing or completed in the past 3 years

Ongoing:

"Therapy and Prophylaxis for Genital Tract Infection". NIH 2R44-AI104067-02. 7/1/2014-6/30/2016.
Role: Co-PI (Liu, PD/PI), 5% effort. Total costs: $ [redacted]

"Delivery of nanoencapsulated TGFbeta and ATRA for the Treatment of IBD". NIH 2R44-AI080009-
02A1. 4/1/2011-3/31/2016.
Role: PI, 45% effort. Total costs: $ [redacted]

Furtado, Current Support

ACTIVE

Therapy X NIH 2R44-AI104067-02 06/01/2011 – 05/31/2017
Oral Delivery of Nanoencapsulated TGF1 and All-trans-Retinoic Acid for the Treatment of Inflammatory Bowel Disease
The major goal of this project is to complete pre-clinical formulation and treatment optimization studies in the murine adoptive T-cell transfer model of established IBD Role: Senior Scientist.

NIH/University of Louisville (Subaward 7R01CA100656-11 NCE) 05/01/2014 – 04/30/16
Integrating Innate and Adaptive Immunity in Cancer Therapy
The major goal of this project to encapsulate cytokes for eradicating cancer. Role:Senior Scientist

ACTIVE

(Mathiowitz) 06/01/2011 – 05/31/2017
Therapy X
Oral Delivery of Nanoencapsulated TGF1 and All-trans-Retinoic Acid for the Treatment of Inflammatory Bowel Disease
The major goal of this project is to complete pre-clinical formulation and treatment optimization studies in the murine adoptive T-cell transfer model of established IBD

NPRP 4-748-2-277 (Mathiowitz) 03/15/2012 – 03/14/2015
Qatar National Research Foundation
Nanotechnologies and Treatment of Obesity: From Polymeric Nanoparticle-Based Recruitment of Brown Adipose Stem cells Toward Autologous Cell-Based Therapy.
The major goal of this project is to use the principle of polymeric nanoencapsulation and elution of soluble factors to recruit adipose stem cells for in vitro expansion.

(Mathiowitz) 03/25/2014 – 03/24/2016
Takeda Pharmaceuticals, Inc.
Oral Formulation of Antibody for Gut Disease
The major goal of this project is developing oral delivery for large proteins…

(Mathiowitz) 07/21/2014 – 7/20/15
TADGEP, LLC
Characterization of Algae Delivery Systems Developed by TADGEP
The major goal of this project to elucidate the mechanism of action of proprietary technology developed by the company.

7R01CA100656-11 (Mathiowitz) 05/01/2014 – 04/30/16 (NCE)
NIH/University of Louisville (Subaward)
Integrating Innate and Adaptive Immunity in Cancer Therapy
The major goal of this project to encapsulate cytokes for eradicating cancer.

(Mathiowitz) 12/01/2014 – 11/30/2017
Bio-Tree Systems, Inc.
Characterization of Vascular Casts Created using Polymeric Materials
The major goal of this project is to develop scaffold for vascular imaging….

D. Research projects ongoing or completed in the past 3 years

Ongoing:

"Integrating Innate and Adaptive Immunity in Cancer Therapy", NIH, 2R01-CA100656-01A1,
7/1/10 – 4/30/16. NCE
Role: Principal Investigator, 10% effort. Total direct costs: $[redacted]

"Delivery of nanoencapsulated TGFbeta and ATRA for the Treatment of IBD". NIH 2R44-AI080009.
4/1/2011-3/31/2016.
Role: Co-I (Auci PD/PI), 5% effort. Total costs: $[redacted]

Completed:

"Oral Immune-modulatory Adjuvants for Treatment of Colorectal Carcinoma", NIH 1-R21-AI092133-01A1.
7/1/2011 - 6/30/2014.
Role: Principal Investigator, 10% effort. Total direct costs $[redacted]

Intratumoral T-suppressor cell Homeostasis in Breast Cancer, DOD Breast Cancer Program / Pre-doctoral fellowship.
BC093325 (Rowswell-Turner, PI) 12/15/09-12/14/12
Role: Mentor, 5% effort. Total direct costs $[redacted]

CURRENT SUPPORT

Michael W. Russell

Current Research Support

R44-AI-104067-02 (SBIR Phase II) — Y. Liu (PI) — 02/01/13 – 05/31/16
NIH/NIAID
Therapy and prophylaxis for genital tract infection
The goal of phase II is to develop a novel approach to treatment of genital gonococcal infection by the local administration of microencapsulated IL-12.
Role: collaborating scientist.

R43-AI115877-01 (SBIR Phase I) — Y. Liu (PI) — 12/01/14 – 11/30/16
NIH/NIAID — (No-cost extension)
Experimental gonococcal vaccine
The goal of this project is to establish proof-of-principle for a novel vaccine against genital gonococcal infection.
Role: collaborating scientist.

Completed Research Support

R21-AI074791 — M.W. Russell (PI) — 02/01/09 – 01/31/13
NIH/NIAID
Gonococcal inflammatory immune responses
The major goals of this project were to determine the significance of IL-17 in host responses to *Neisseria gonorrhoeae* in a mouse vaginal infection model.
Role: PI

R21-AI092348 — M.W. Russell (PI) — 12/03/10 – 11/30/13
NIH/NIAID
Imaging antigen and adjuvant uptake for enhancing response to mucosal vaccines
The major goals of this project were to characterize the cells and tissues involved in the uptake and processing of enterotoxin adjuvants and recombinant chimeric immunogens based on enterotoxins when administered by mucosal routes, utilizing imaging flow cytometry.
Role: PI

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TherapyX Inc.

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Yingru		Liu		PD/PI	[redacted]	6.00			[redacted]	[redacted]	[redacted]
2 .		Dominick		Auci		Co-PI	[redacted]	3.00			[redacted]	[redacted]	[redacted]
3 .		Stacia		Furtado		Senior Scientist	[redacted]	9.00			[redacted]	[redacted]	[redacted]
4 .		Michael		Russell		Consultant	[redacted]	2.40			[redacted]	[redacted]	[redacted]
5 .		Nejat		Eglimez		Collaborating Scienist	[redacted]	0.60			0.00	0.00	0.00
6 .		Edith		Mathiowitz		Collaborating Scientist	[redacted]	0.60			0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laura Hammer (Senior Research Technician)	6.00			[redacted]	[redacted]	[redacted]
1	Julianny Perez (Research Technician)	12.00			[redacted]	[redacted]	[redacted]
1	Samina Raza	1.20			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-302 . Received Date: 2016-08-24T11:01:24.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: TherapyX Inc.

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **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:

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)

Tracking Number: GRANT12235571 Funding Opportunity Number: PA-16-302 . Received Date: 2016-08-24T11:01:24.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: TherapyX Inc.

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **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	[redacted]
7. Alterations and Renovations	
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 . Overhead	40.00	[redacted]	[redacted]
		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*	File Name: 1234-BudgetJustificationVaccinePhase II.pdf (Only attach one file.)

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

Tracking Number: GRANT12235571 Funding Opportunity Number: PA-16-302 . Received Date: 2016-08-24T11:01:24.000-04:00

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TherapyX Inc.

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Yingru		Liu		PD/PI	[redacted]	6.00			[redacted]	[redacted]	[redacted]
2 .		Dominick		Auci		Co-PI	[redacted]	3.00			[redacted]	[redacted]	[redacted]
3 .		Stacia		Furtado		Senior Scientist	[redacted]	6.00			[redacted]	[redacted]	[redacted]
4 .		Michael		Russell		Consultant	[redacted]	2.40			[redacted]	[redacted]	[redacted]
5 .		Nejat		Eglimez		Collaborating Scienist	[redacted]	0.60			0.00	0.00	0.00
6 .		Edith		Mathiowitz		Collaborating Scientist	[redacted]	0.60			0.00	0.00	0.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laura Hammer (Senior Research Technician)	6.00			[redacted]	[redacted]	[redacted]
1	Julianny Perez (Research Technician)	12.00			[redacted]	[redacted]	[redacted]
1	Samina Raza	1.20			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-302 . Received Date: 2016-08-24T11:01:24.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: TherapyX Inc.

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 2**

C. Equipment Description

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

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

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
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)

Tracking Number: GRANT12235571 Funding Opportunity Number: PA-16-302 . Received Date: 2016-08-24T11:01:24.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: TherapyX Inc.

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
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 . overhead	40.00	[redacted]	[redacted]
		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*	File Name: 1234-BudgetJustificationVaccinePhase II.pdf (Only attach one file.)

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

THERAPYX, INC. BUDGET JUSTIFICATION

We feel that the number of personnel and the proposed effort are needed to complete the anticipated studies in the allotted 2 year period. The justification for each individual and their proposed percent effort is shown below.

Personnel

Yingru Liu, M.D., Ph.D., P.I. (Effort: 50% Year 1, 50% Year 2) Dr. Liu was the PI on the original Phase I award which produced the results presented in this Phase II application. He is an experienced scientist with expertise in a wide range of experimental techniques relating to cellular and molecular biology, immunology, and microbiology. His training in Dr. Michael Russell's laboratory at the University at Buffalo, which combines microbiological and immunological aspects, makes him uniquely qualified to continue the development of our novel prophylactic vaccine against gonorrhea, GvaX12®. Dr. Liu will work closely with Drs Furtado and Auci to coordinate microsphere production and validation. He will supervise Ms. Hammer and Mr Perez, and along with them, perform the *in vivo* mouse inoculations, microparticle therapy, tissue collection and the *in vitro* studies proposed here. He will also be responsible for data interpretation/evaluation and will address any emergent issues. He will also contribute to the production of regulatory documentation for submission to the FDA, participate in the type C meeting, and contribute to continued development plans.

Dominick L. Auci MBA, Ph.D., Co-P.I. (Effort: 25% Year 1, 25% Year 2) Dr. Auci is an experienced immunologist with expertise in immune therapy of infectious disease, cancer, and immune mediated inflammatory diseases. He has extensive industrial and academic experience with drug development, IND creation and working with fee for service contract regulatory organizations. He has effectively directed the development of small molecules and biologicals, including phase I/II clinical trials. While at TherapyX^{Inc}, he has directed the successful completion of the SBIR phase I and phase II studies employing microparticles to treat cancer and Inflammatory Bowel Disease (IBD). He has overseen the proof of principle studies, optimization, toxicology and scale up of the company's TGFβ and all-trans retinoic acid micro-particle formulations for the treatment of IBD. He has led the company in its past dealings with the FDA, working with consultants from Regulatory Professionals and experts from Charles River Laboratories and Comparative Biosciences to plan and execute toxicology studies and produce regulatory documentation and meeting requests for the FDA. Dr. Auci has decades of experience managing and coordinating international research and development efforts at multiple locations. He will provide administrative direction and will assist Dr. Liu to ensure that the programmatic and scientific goals of this project are met as described in the application. Along with consultants, he will monitor toxicology studies, lead the production and submission of a type C meeting request to the FDA, the associated briefing package, interrogatives, and other regulatory documentation. He will lead the company's team at the meeting and the subsequent formulation of development plans in response to agency recommendations.

Stacia M Furtado, Ph.D. Senior Scientist (Effort: 75% Year 1, 50% Year 2) Dr. Furtado joined TherapyX^{Inc} in June 2008 and has contributed to several SBIR projects involving the development of oral microparticulate peptide/protein formulations for the treatment of diabetes and inflammatory bowel disease. She has extensive experience in microparticle design, production, and testing. She has produced the IL-12 microparticles used in the associated phase I studies. Dr. Furtado will perform all microparticle production and analytical testing (i.e., HPLC, gas chromatography, DSC, particle sizing, and FTIR) for drug product validation.

Dr. Michael W. Russell, Ph.D., Consulting Scientist (Effort: 20% Year 1, 20% Year 2) Dr. Russell has over 40 years of research experience in mucosal immunology especially in relation to infections of the mouth and genital tract. While working with Dr. Russell, Dr. Liu has elucidated the mechanisms of suppression of Th1/Th2 immunity in gonococcal infection. These studies have led to the proposal of the novel approach to the treatment of gonococcal infection by redirecting host immunity using IL-12 microparticles. Dr. Russell will spend one day a week at TherapyX$^{Inc.}$ and will serve as a consulting scientist on the both the murine studies (*in vivo* and *in vitro*) and toxicology plans proposed in this application, as well as in the preparation of regulatory documents and meetings with regulatory agencies.

Nejat K. Egilmez, Ph.D., Executive Vice-President. (Effort: 5% Year 1, 5% Year 2) Dr. Egilmez has pioneered the development of the cytokine-encapsulated proteins for use in immunotherapy and has published in this area extensively with his collaborator Dr. Mathiowitz. Dr. Egilmez's laboratory, which collaborates with TherapyX^{Inc} uses many of the cellular/molecular immune assays that will be used in the proposed studies and he will be available for consultation and technical advice when needed.

Edith Mathiowitz, Ph.D., Collaborating Scientist. (Effort: 5% Year 1, 5% Year 2). Dr. Mathiowitz is an internationally recognized leader in the field of nano-encapsulation and drug delivery. She has led many projects involving the encapsulation of small molecule drugs, and proteins and is a leader and innovator in nano/microparticle formulation development. The formulation development studies detailed in the preliminary results section were designed and performed under her direction and supervision. TherapyX^{Inc} and Dr. Mathiowitz have a long standing relationship with past collaborations on nanoparticle-based delivery of cytokines for the therapy of cancer, nano-particulate vaccine adjuvant development and nano-particulate therapeutics. Dr. Mathiowitz will continue to offer guidance and support, particularly for drug product manufacturing and validation portions of this project as well as in the preparation of regulatory documents and meetings with regulatory agencies.

Laura Hammer, B.S., Senior Research Technician. (Effort: 50% Year 1, 50% Year 2) Ms. Hammer has over 25 years' of experience in molecular biology, pharmacology and immunology. Ms. Hammer has been with TherapyX^{Inc} for 9 years and along with developing in vitro bioassays for numerous cytokines including IL-12, she has worked extensively in FACS analysis, the *in vitro* testing of proteins and microparticle release assays. Ms. Hammer has contributed to previous TGFβ microparticle studies by conducting in vitro release bio-assays as well as FACS analysis and ELISA. She assisted Dr. Liu with the phase I *in vivo* and *in vitro* work and will continue to work closely with him and Mr. Perez to execute the proposed studies.

Julianny Perez, B.S., Research Technician. (Effort: 100% Year 1, 100% Year 2) is a recent graduate with laboratory experience in Western Blotting, PCR, RNA isolation and several cell-based assays. He will assist Dr. Liu with the *in vivo* mouse inoculations, microparticle therapy and tissue collection. He will also assist Ms. Hammer with the *in vitro* microparticle studies including, bio-assays and ELISA.

Samina Raza, Ph.D. Director of Operations (Effort: 10% Year 1, 10% Year 2) has been with the company since its inception in 2000. She will continue to oversee operations, administration and management efforts for the period of the award. She will work closely with Dr. Auci in budgeting, planning, accounting, as well as documentation and compliance with all state, federal and local regulatory agencies.

Animals, Materials and Supplies

The materials and supplies for the project consist of recombinant IL-12, microparticle polymer, HPLC Columns, β-Estradiol, Ceftriaxone, bacterial and cell culture materials, antibodies for FACS analysis and immunofluorescence, intracellular staining kits, ELISA kits and mice to conduct the studies proposed in the research design section. A detailed breakdown of expenses can be found below.

Animal Care and Use:	Balb/c mice (792 x $30.00)	$[redacted]
	Animal Care (14,256 days x $0.58/day)*	$[redacted]
		$[redacted]
Materials and Reagents:	Recombinant mIL-12 (10 mg)	[redacted]
	Microparticle Polymers and Solvents	[redacted]
	HPLC Columns, vials, inserts etc.	[redacted]
	Antibodies (Fluorochrome Labeled)	[redacted]
	Intracellular Staining Kits	[redacted]
	ELISA kits	[redacted]
	Antibiotics, Estradiol	[redacted]
	Agar, Culture Media, buffers etc.	[redacted]
	Plastic ware, pipettes, syringes etc.	[redacted]
	Gonococcal Ag, Prep. and ELISA	[redacted]
		$[redacted]
	Total for animals, materials and reagents	**$[redacted]**

*As per agreement with the Division of Comparative Medicine Laboratory Animal Facilities (CM-LAF) at the State University of New York at Buffalo per diem charges for BSL2 mice housed in Micro-isolator cages are $0.58 per mouse per day. Please see the vertebrate animal section for the justification for the numbers of mice used.

Equipment or Facility Rental/User Fees

Fluorescence Microscopy and flow cytometry
As per agreement with the Confocal Microscopy and Flow Cytometry core facility at the Witebsky Center at SUNY at Buffalo charges are $80.00 per hour for use of the Becton Dickinson FACSCalibur 4-color flow cytometer and the Zeiss LSM 510 Meta NLO Confocal Microscope ($[redacted] x 40 hours = $[redacted]

Histology Fees
Histology services to processing and serial sectioning of samples for the studies described in aim 1A-II cost $32 per sample for outside concerns (20 mice x 4 tissues each = 80 x $[redacted] = $[redacted]

$[redacted] + [redacted] + [redacted] = $146,[redacted]

Equipment Purchase

We are requesting funds to purchase a replacement for an aging cell culture hood. We propose to purchase a CLASS II A2 4 115V package from Fisher Scientific at a cost of $6,872.62.

YEAR 2

The materials and supplies needed for the second year of the project consist of recombinant IL-12, microparticle polymer, β-Estradiol, Ceftriaxone, bacterial and cell culture materials, antibodies for FACS analysis and immunofluorescence, intracellular staining kits, ELISA kits and mice to conduct the studies proposed in the research design section. A detailed breakdown of expenses can be found below.

Animals, Materials and Supplies

Animal Care and Use:	Balb/c mice (604 x $30.00)	$ [redacted]
	Animal Care (21,744 days x $0.58/day)*	[redacted]
		$ [redacted]
Materials and Reagents:	Recombinant mIL-12 (5mg)	[redacted]
	Microparticle Polymers and Solvents	[redacted]
	Intracellular Staining Kits	[redacted]
	ELISA kits	[redacted]
	Antibiotics, Estradiol	[redacted]
	Agar, Culture Media, buffers etc.	[redacted]
	Plastic ware, pipettes, syringes etc.	[redacted]
	Gonococcal Ag, Prep. and ELISA	[redacted]
		$ [redacted]
	Total for animals, materials and reagents	$ [redacted]

*As per agreement with the Division of Comparative Medicine Laboratory Animal Facilities (CM- LAF) at the State University of New York at Buffalo per diem charges for BSL2 mice housed in Micro-isolator cages are $0.58 per mouse per day. Please see the vertebrate animal section for the justification for the numbers of mice used.

Aim 2B Standard teratogenicity toxicity in rats (see Appendix 1)

Subcontracted to Comparative Biosciences……$ [redacted]

Aim 2 C Standard Reproductive Toxicology

Subcontracted to Comparative Biosciences……$ [redacted]

TOTAL Subcontract costs……$ [redacted]

Consultant Fees ……$ [redacted]

A pre-pre-IND meeting will be requested during last half of the second year of the project. The type C meeting will pose questions to the FDA concerning, the design of primate toxicology studies, and to confirm FDA expectations for data to support the Phase I clinical study, primarily with respect to safety. The consultants at Regulatory Professionals have extensive expertise in the preparation of clinical (IND) and marketing applications for various drug formulations, including biologics and polymeric drug delivery systems, and are especially well qualified to guide us through this process (see Appendix 2).

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	6	
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	[redacted]	
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]

Funding Opportunity Number: PA-16-302 . Received Date: 2016-08-24T11:01:24.000-04:00

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

SBIR/STTR Information

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:

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?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 10

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

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?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

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?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

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)?* ● Yes ❍ No

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:* 1259-Commercalization Plan GC FINAL.pdf

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

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.

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.* ● Yes ❍ No

Attach File:* 1260-COMMERCIALIZATION STATEMENT.pdf

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ● Yes ❍ No

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.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ❍ Yes ❍ No

(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?

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?* ❍ Yes ❍ No

COMMERCIALIZATION PLAN

The rapid spread of antimicrobial resistance throughout the U.S. and the world is considered a major threat to public health and national security. The President has emphasized that tackling this issue is a priority; in September 2014, the White House introduced the President's National Strategy to Combat Antibiotic Resistant Bacteria (CARB), released concurrently with the President's Council of Advisors on Science and Technology (PCAST) report and recommendations to the President on combating antibiotic resistance.[1] Together, these reports identify priorities and guide coordination across U.S. government agencies for 1) better prevention and response to the spread of antibiotic resistance; 2) increased surveillance of emerging antibiotic resistance in humans, animals, and the environment; 3) improved capability for detection and diagnostics; 4) accelerated development of new products, including new classes of antibiotics, therapeutics, and vaccines; and 5) enhanced international collaboration.[39] The federal commitment to addressing antimicrobial resistance was further emphasized by a Presidential budget request to Congress for $1.2 billion to support these efforts.[2]

One disease that is particularly resistant, *Neisseria gonorrhoeae* (i.e., gonorrhea) is known to be acquired repeatedly with apparently no development of protective immunity resulting from previous infection. The reasons for this are poorly understood, but the generally accepted view is that this pathogen has an extraordinary capacity of for antigenic variation, involving most of its surface antigens. Coupled with multiple mechanisms for resisting complement, *N. gonorrhoeae* can evade whatever adaptive immune responses the host develops against it. Although these considerations imply that the development of a successful vaccine may be improbable, studies using a mouse model of vaginal gonococcal infection have led to a different view: that N. gonorrhoeae (Ngo) selectively elicits innate host defenses that it can survive and concomitantly suppresses adaptive immune responses that would eliminate it. These findings put comprehension of immunity to N. gonorrhoeae in a completely new light, and moreover reveal novel approaches to treatment and prevention based on the manipulation of cytokines that control Th1, Th2, and regulatory immune responses.

TherapyX^{inc}'s goal is to generate safe and effective sustained-release nano-particulate immunotherapeutics for the treatment of inflammation and infectious disease**.** Our lead product, GvaX12®, is a nanoencapsulated interleukin 12 (IL-12) plus outer membrane vesicles (OMV) formulated for mucosal delivery**.** This vaccine is intended to enhance specific adaptive immune responses that provide lasting, protective immunity against genital tract infection with heterologous strains of *N. gonorrhoeae*. The goals of this commercialization plan are to provide information regarding US gonorrhea infections, identify the target market for vaccine commercialization, address market strategy, discuss industry outreach efforts, and measure the value such a vaccine would have for both health organizations and TherapyX^{Inc}.

1. Gonorrhea Overview

Sexually transmitted diseases (STDs) affect people in all walks of life. More than half of all Americans will be infected with an STD at some point in their lifetime.[3] STDs are a substantial health challenge facing the United States, as an estimated 110 million US men and women have contacted STDs.[4] Because the majority of infections may go undiagnosed and unreported, the true burden of STD's in America is likely far greater than reporting would indicate. Reported cases of the three nationally notifiable STDs – chlamydia, gonorrhea, and syphilis – have increased for the first time since 2006, according to data published by the Center for Disease Control and Prevention (CDC) in the 2014 STD Surveillance Report. Each of these infections is a threat to an individual's immediate and long-term health and well-being. America's worsening STD epidemic is a clear sign that better diagnosis, treatment, and prevention techniques are urgently needed.

Young people aged 15-24 are particularly at risk for STDs, accounting for half of the nearly 20 million new cases that occur each year, according to the CDC. In this age segment, STDs account for nearly $16 billion in health care costs.[3] Thus, preventing STDs among youth is a key priority.

[1] The White House. "National Action Plan for Combating Antibiotic Resistant Bacteria." 2015
[2] President's Council of Advsiors on Science and Technology. Report to the President on Combating Antibiotic Resistance. 2014.
[3] Koutsky L. (1997). Epidemiology of genital human papillomavirus infection. American Journal of Medicine, 102(5A), 3-8.
[4] Satterwhite CL, et al. Sexually transmitted infections among U.S. women and men: Prevalence and incidence estimates, 2008. Sex Transm Dis 2013; 40(3): pp. 187-193

Gonorrhea, an STD caused by the *N. gonorrhoeae* bacterium, infects the mucous membranes of the reproductive tract, including the cervix, uterus, and fallopian tubes in women, and the urethra in women and men.[5] It can also infect the mucous membranes of the mouth, throat, eyes, and rectum. Complications from gonorrhea infections are frequent and debilitating, and disproportionately affect women.

The surge in antimicrobial resistant strains of the gonorrhea organism is threatening the effectiveness of treatment both in the United States and internationally. While medication for gonorrhea has been available for decades, the bacteria have grown resistant to every drug ever used to treat it. To ensure that available antibiotics remain effective for as long as possible, a multi-faceted approach will be required. However, the role of vaccines in preventing bacterial infections (as well as the potential role of vaccines (direct and indirect) in reducing the overall use of antibiotics) is surprisingly under-represented in both discussion and action to date. Vaccines not only prevent infectious diseases before they occur, thereby eliminating the need for antibiotic treatments and unnecessary contacts with the healthcare system, but also decrease circulation of the pathogen, which protects the community under the well-established principle of "herd immunity".

Complications: The first symptoms are usually discrete and occur over a period of two to ten days after sexual intercourse with an infected partner. Often, no symptoms develop in people already infected with gonorrhea: 10 to 15% of men and up to 80% of women do not show any symptoms at all with new infections.[6] In sexually active individuals, gonorrhea can cause clinically unapparent mucosal infections, symptomatic urethritis and cervicitis, and upper urogenital tract infections. Systemic or disseminated gonococcal infections (DGI) are infrequent (0.5–3%), occur mainly in women, and include a characteristic gonococcal arthritis-dermatitis syndrome, suppurative arthritis, and (rarely) endocarditis, meningitis or other localized infections.[7] In men ~15% of untreated urethritis cases progress to epididymitis, a common cause of male infertility.[8] In women, untreated cervical infections commonly progress to the upper reproductive tract, which contributes to pelvic inflammatory disease (PID) and HIV:[5]

- *Pelvic Inflammatory Disease (PID)* is the most common complication associated with gonorrhea, resulting in an estimated 26% of cases; ~4.2% of U.S. women report being treated for PID in their lifetime.[9] PID results from the ascension of microorganisms from the cervix and vagina to the upper genital tract. The wide variation in symptoms and signs associated with PID can make diagnosis challenging; when symptoms are mild, PID can go unnoticed by women and their health care providers. No single historical, physical, or laboratory finding is both sensitive and specific to the diagnosis of PID.[7] Direct medical expenditures for PID and its sequelae have been estimated at $2.7 billion.[7]

- *Infertility:* PID can lead to infertility and permanent damage of a woman's reproductive organs. While recurrent episodes of PID are associated with a greater risk of infertility,[10] even subclinical PID has been associated with infertility.[11] Perhaps the most significant burden attributed to PID comes from the long-term reproductive sequelae of tubal infection: tubal factor infertility, ectopic pregnancy, and pelvic adhesions, which can lead to chronic pelvic pain. Prompt antibiotic treatment can prevent severe damage to the reproductive organs, but is not guaranteed. Women diagnosed with PID are six times more likely to have ectopic pregnancy, are 18% more likely to experience chronic pelvic pain after a single episode, and are increasingly likely to suffer from tubal factor infertility, with incidence ranging from 8% after the first episode to as high as 40% after three episodes.[7] At least 15 percent of all American women who are infertile can attribute it to tubal damage caused by PID.[12]

[5] Center for Disease Control. "Gonorrhea – CDC Fact Sheet" (2016)
[6] Center for Disease Control. " Pelvic Inflammatory Disease (PID) – CDC Fact Sheet" (2016)
[7] Jerse, Ann, Margaret C. Bash, Michael W. Russell. "Vaccines against Gonorrhea: Current status and Future Challenges" Vaccine. 2014 Mar 20; 32(14): 1579–1587
[8] Chesson, Harrell *et al*. "The estimated Direct medical cost of sexually transmitted diseases among American youth, 2000" Perspectives on Sexual and Reproduction Health Volume 36, Issue 1 January/February 2004.
[9] Satterwhite CL, et al. Sexually transmitted infections among U.S. women and men: Prevalence and incidence estimates, 2008. Sex Transm Dis 2013; 40(3): pp. 187-193
[10] Westrom L, Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. Am J Obstet Gynecol 1980; 138:880-892.
[11] Wiesenfeld HC, Hillier SL, Meyn LA, Amortegui AJ, and Sweet RL. Subclinical pelvic inflammatory disease and infertility. Obstet & Gyn, 2012; 120:37-43.
[12] Ness RB et al. (2004). Condom use and the risk of recurrent pelvic inflammatory disease, chronic pelvic pain, or infertility following an episode of pelvic inflammatory disease. American Journal of Public Health, 2004, 94:1327-1329.

- *Gonorrhea & HIV.* Untreated gonorrhea can increase a person's risk of acquiring or transmitting HIV, the virus that causes Acquired Immune Deficiency Syndrome (AIDS). Untreated gonorrhea can make a person with HIV more infectious, because untreated STD's can increase the HIV viral load in genital fluids.[13] Having gonorrhea also can make it more likely that an HIV-negative person will be infected with HIV if they are exposed to the virus.[11]

In summary, it is extremely important to prevent or treat gonorrhea in the early stages of the disease, especially in high-risk populations.

Occurrence: In 2014, 350,062 cases of gonorrhea were reported; however, the CDC estimates that approximately 820,000 new gonorrheal infections occur in the United States each year. The discrepancy between the two numbers is due to infections being undetected (asymptomatic) and/or unreported.[14] This makes gonorrhea the second most commonly reported STD behind only chlamydia (~1.4M). The overall US gonorrhea infection rate has increased 5.1% since 2013, and increased 10.5% since 2010.[15] Furthermore, the World Health Organization (WHO) estimates that globally 106 million new gonorrhea cases occur among adults each year, representing a 21% increase in just three years.[16] Gonorrhea disproportionately affects certain groups of individuals more than others when factors of age, sex, location, race and socioeconomic status are considered.:[2]

- *Age:* 2014 CDC data (Figure 1) shows that youths are at the highest risk of acquiring an STD, accounting for half of the estimated 20 million new STDs diagnosed each year. This is especially true for gonorrhea:[17] despite being a relatively small portion of the sexually active population, young people between 15 and 24 accounted for nearly two-thirds of gonorrhea cases, estimated at ~570,000.[15] Young individuals are at a greater risk for several reasons: young women's bodies are biologically more susceptible to STDs because of increased cervical ectopy; some young people do not get the recommended STD tests; many young people are hesitant to talk openly and honestly with a doctor or nurse about their sex lives; the absence of insurance or transportation can make it more difficult for young people to access STD testing; and some young people have more than one sex partner.[18]

- *Sex:* In 2014 (Figure 2) the rate of reported gonorrhea cases among men (120.1 cases per 100,000 males) was higher than the rate among women (101.3 cases per 100,000 females).[15] Between 2013 and 2014, the gonorrhea rate among men increased 10.5%, while the rate among women decreased 0.4%. During 2010–2014, the rate among men increased 27.9%, while the rate among women decreased 4.1%.[15] "The magnitude of the increase among men compared with a decrease among women suggests either increased transmission or increased case ascertainment among gay, bisexual, and other men who have sex with men (collectively MSM). However, most jurisdictions do not routinely report the sex of the partner or the site of infection for gonorrhea cases; hence, trends in gonorrhea rates among MSM over time cannot be assessed."[15]

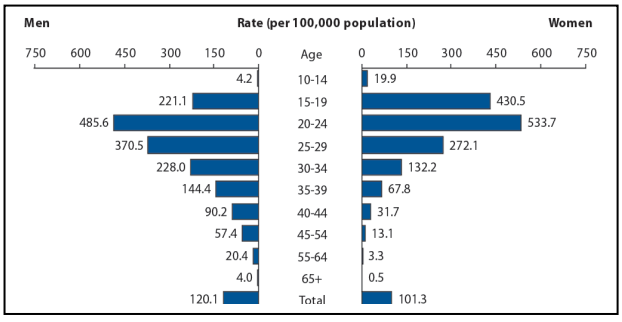

Figure 1: Rates of Reported Cases by Age and Sex, United States, 2014

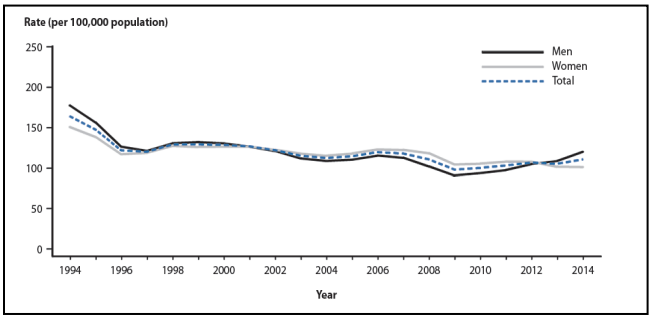

Figure 2: Rates of Reported Cases by Sex, United States, 1994–2014

[13] Fleming ic health policy and pra ses to sexual transmission of HIV infection. Sex Transm DIs, 75(1), 3–17 (1999).
[14] Centers for Disease Control and Prevention. "Sexually Transmitted Disease Surveillance 2014"
[15] Satterwhite CL, et al. Sexually transmitted infections among U.S. women and men: Prevalence and incidence estimates, 2008. Sex Transm Dis 2013; 40(3): pp. 187-193
[16] Magnus Unemo and Robert A Nicholas. "Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea." Future Microbiol. 2012 Dec; 7(12): 1401–1422
[17] Center for Disease Control. "Reported STDs in the United States: 2014 National Data for Chlamydia, Gonorrhea, and Syphilis" (2014)
[18] Center for Disease Control. "Information for Teens & Young Adults: Staying Healthy and Preventing STDs."

- *Location:* In 2014, rates of reported gonorrhea cases per 100,000 in population ranged by state (Figure 3), from a low of 13.4 in Vermont to a high 194.6 in Louisiana. In the District of Columbia the gonorrhea rate was 291.3 per 100,000 population.[15] Between 2013 and 2014, gonorrhea rates increased in 70% (35/50) of the states and decreased in 30% (15/50) of the states and the District of Columbia.[15] In 2014, as in previous years, the South had the highest rate of reported gonorrhea cases (averaging 131.4 cases per 100,000 population) among the four US regions, followed by the Midwest (106.6 cases per 100,000 population), West (101.1 cases per 100,000 population), and Northeast (84.7 cases per 100,000 population).[15] Between 2013 and 2014, the gonorrhea rate increased 22.2% in the West and 3.1% in the South, but decreased 1.5% in the Midwest and 0.6% in the Northeast.[19]

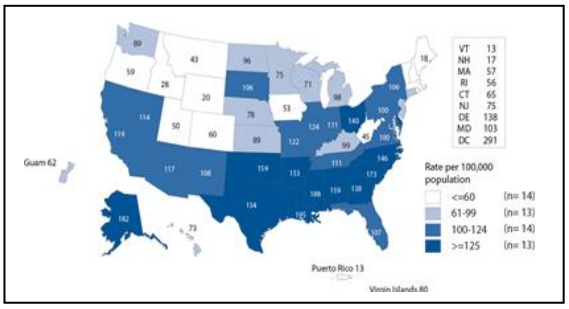

Figure 3: Rates of Reported Cases by State, United States and Outlying Areas, 2014

The overall rate of reported gonorrhea cases in the 50 most populous metropolitan statistical areas (MSAs) was 122.8 cases per 100,000 population in 2014, representing a 5.0% increase compared with 2013 (117.0 cases per 100,000 population).[17] In 2014, 60.6% of reported gonorrhea cases were reported by these MSAs.[17] Since 2010, the gonorrhea rate among women in the 50 most populous MSAs has been lower than the rate among men. In 2014, the rate among women in these MSAs was 102.0 cases per 100,000 females, while the rate among men was 144.1 cases per 100,000 males.[17]

- *Race*: In 2014, among the 48 states that submitted data on race and ethnicity (Figure 4), the rate of reported gonorrhea cases remained highest among blacks (405.4 cases per 100,000 population), 10.6 times the rate among whites (38.3 cases per 100,000 population). The gonorrhea rate among American Indians/Alaska Natives (159.4 cases per 100,000 population) was 4.2 times that of whites, the rate among Native Hawaiians/Other Pacific Islanders (102.1 cases per 100,000 population) was 2.7 times that of whites, the rate among Hispanics (73.3 cases per 100,000 population) was 1.9 times that of whites, and the rate among Asians (19.3 cases per 100,000 population) was 0.5 times that of whites.[17] During 2010–2014, among the 43 states that submitted race and ethnicity, the gonorrhea rate increased among American Indians/Alaska Natives (100.3%), whites (59.3%), Hispanics (51.2%), Asians (45.3%), and Native Hawaiians/Other Pacific Islanders (44.2%). During this same time period, the gonorrhea rate decreased 8.2% among blacks.[17]

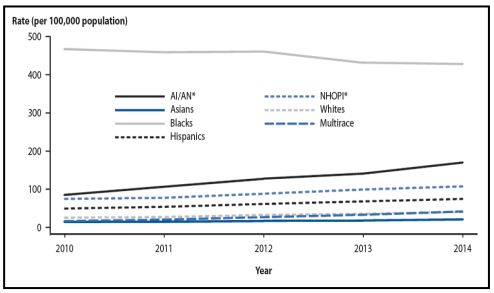

Figure 4: Rates of Reported Cases by Race/Ethnicity, United States, 2010–2014 Socioeconomic Status

- *Socioeconomic Status:* Gonorrhea infections tend to cluster in geographically definable risk spaces, often located in low socioeconomic status (SES) urban neighborhoods, suggesting that socio-cultural determinants may influence the clustered spatial pattern observed for the infection.[20] Prevalence of the infection has a direct impact on the incidence of infection. Low SES can impair timely access to services in risk spaces, thereby increasing the duration of infection and, ultimately, the prevalence of infection within a sexual network. Neighborhoods representing the lowest SES quartile accounted for 58% of reported gonorrhea cases, and individuals from the lowest SES neighborhoods were more than four times more likely to acquire infection than those from the highest SES neighborhoods[21]

[19] Center for Disease Control. "Reported STDs in the United States: 2014 National Data for Chlamydia, Gonorrhea, and Syphilis"
[20] Ashleigh B Sullivan *et al* "Are neighborhood socio-cultural factors influencing the spatial pattern of gonorrhea in North Carolina." Ann Epidemiol. 2011 Apr; 21(4): 245–252.
[21] Lacey CJ, Merrick DW, Bensley DC, Fairley I. "Analysis of the sociodemography of gonorrhoea in Leeds, 1989-93" (1997) BMJ. 1997 Jun 14;314(7096):1715-8.

Diagnosis: Physicians and other health care professionals typically use three methods of diagnosing gonorrhea: biological samples from the infected area with staining (gram staining), detection of bacterial genes in urine, and bacteria growth in laboratory cultures.[22] Many doctors prefer to use dual testing to increase the accuracy of the diagnosis. Although gram staining is a very accurate test for diagnosing men, it is not effective in women: only one woman out of two infected with gonorrhea will show an identifiable trace. Most often, doctors use urine and cervical secretions to detect bacterial genes. These tests give even better results than culturing the bacteria and therefore are widespread. Culture tests involve placing a sample of the secretion in a culture medium and incubating it for up to two days, to allow the bacteria to grow and multiply.[23] The sensitivity of this test depends on the area from which the sample is taken: cultures of cervical samples detect infection in about 90 percent of the cases. It should be noted that cultures also are used to determine the drug resistance of the bacteria, which increases the overall usage of this method.[21]

Treatment: Gonorrhea can currently be cured with the right treatment. However, in order to eliminate reinfection, both sex partners must be treated, because typically both are infected. The CDC now recommends dual therapy (using two drugs) for the treatment of gonorrhea. On the basis of experience with other microbes that rapidly develop antimicrobial resistance, a theoretical basis exists for combination therapy, in which two antimicrobials with different mechanisms of action are used to improve treatment efficacy and potentially slow the emergence and spread of antimicrobial resistance.[24] The current treatment recommendation is Ceftriaxone (250 mg intramuscular dose) plus Azithromycin (1g orally in single dose).[22] Cefixime (400 mg in a single dose) can be used if Ceftriaxone is not available.[22] Several other antimicrobials are active against *N. gonorrhoeae*, but none have substantial advantages over the recommended regimen, and efficacy data (especially for pharyngeal infection) is limited.[22] In up to one-half of infected patients, chlamydia occurs simultaneously with gonorrhea; for such patients, oral tetracycline, doxycycline, minocycline, or erythromycin is prescribed for a period of 7 days, in addition to the Ceftriaxone and Azithromycin.[22]

Resistance to Treatment: Unfortunately, gonorrhea treatment is complicated by the ability of *N. gonorrhoeae* to develop resistance to antimicrobials:

- *Antimicrobial resistance:* In 1986, the Gonococcal Isolate Surveillance Project (GISP), a national sentinel surveillance system, was established to monitor trends in antimicrobial susceptibilities of urethral *N. gonorrhoeae* strains in the United States.[25] This system annually collects approximately 5-6,000 N. gonorrhoeae samples from men with urethral gonorrhea at STD clinics in approximately 25-30 U.S. cities and measures the concentration of various antimicrobials needed to stop the bacteria's growth in the laboratory. A minimum inhibitory concentration (MIC) is the lowest concentration of drug needed to stop growth and is an indication of how susceptible the bacteria is to treatment with an antibiotic.[24] The higher the MIC, the greater the dose required for effective treatment. If MICs become too high, the antibiotic will not work at all.

- *Historical Trends in Drug Resistance:* In response to the ongoing threat of drug resistance, the CDC has repeatedly revised its gonorrhea treatment guidelines to phase out the use of antibiotics that have become less effective in treating the infection, due to shifts in antimicrobial resistance patterns. *N. gonorrhoeae* has progressively developed resistance to all of the antimicrobials used for treatment:[26]
  - 1930s: Introduction of sulfanomide antimicrobials to treat gonorrhea
  - 1940s: Due to increasing resistance, sulfanomides no longer recommended for treatment; penicillin becomes treatment of choice
  - 1980s: Due to increasing resistance, penicillin and tetracycline no longer recommended
  - 1990s: Fluoroquinolones become predominant treatment
  - 2007: Fluoroquinolones no longer recommended; cephalosporins (including injectable ceftriaxone and oral cefiximine) become backbone of treatment

[22] Jerse, Ann, Margaret C. Bash, Michael W. Russell. "Vaccines against Gonorrhea: Current status and Future Challenges" Vaccine. 2014 Mar 20; 32(14): 1579–1587

[23] Jerse, Ann, Margaret C. Bash, Michael W. Russell. "Vaccines against Gonorrhea: Current status and Future Challenges" Vaccine. 2014 Mar 20; 32(14): 1579–1587

[24] Lacey CJ, Merrick DW, Bensley DC, Fairley I. "Analysis of the sociodemography of gonorrhoea in Leeds, 1989-93" (1997) BMJ. 1997 Jun 14;314(7096):1715-8.

[25] Center for Disease Control. "2015 STD Treatment Guidelines: Gonococcal Infections"

[26] Center for Disease Control. "Addressing the Threat of Drug-Resistant Gonorrhea- CDC Fact Sheet." (2016)

- 2012: Cefixime no longer recommended as a first-line regiment, leaving ceftriaxone-based dual treatment as recommended treatment
- 2015: Cetraixone plus azithromycin is the only recommended treatment

Recently labeled a "superbug," *N. gonorrhoeae* has retained resistance to antimicrobials previously recommended for first-line treatment and has now demonstrated its capacity to develop resistance to the extended-spectrum cephalosporin (ceftriaxone), the last remaining option for first-line empiric treatment of gonorrhea.[27] Alarmingly, society may have reached the point where untreatable infections can be expected. Such fears have become a reality in Australia, France, Norway, Sweden and the UK, as some infections can no longer be treated with current antibiotic classes.[26] Unfortunately, few antibiotic options remain that are simple, well-studied, well-tolerated, and highly effective. The combination of persistently high gonorrhea morbidity in some populations, along with cephalosporin-resistant gonorrhea, reinforces the need for more effective methods of prevention, particularly vaccines.

Treatment Centers: The number of gonorrhea cases reported by STD clinics declined between 2005 and 2014.[28] In 2014 (Figure 5), 15.1% of gonorrhea cases were reported by STD clinics, a decrease from 2013 when 16.3% of gonorrhea cases were reported by STD clinics.[27] In 2014, among women, private physicians or health maintenance organizations (HMOs) were the most common reporting source (25.1%), followed by family planning clinics (8.5%), STD clinics (8.2%), other health department clinics (6.6%), and emergency rooms (5.5%). Among men, private physicians/HMOs (20.5%) and STD clinics (17.2%) were the most common reporting sources. Other reporting sources for men included other health department clinics (8.7%), emergency rooms (5.9%), and family planning clinics (4.7%)[29] Research aimed at characterizing the client demographics of STD clinics found that most clinics see young (51% under 25 years of age), minority (64% nonwhite), and poor (43% make less than $10,000 per year) individuals with high STD rates (66% diagnosed with 1 or more STDs). Roughly half had previously visited the STD clinic, and STD symptoms were cited as the reason for the visit in 63% of cases.[30] This research supports the continuing need for publicly funded, categorical STD clinics in urban areas with high STD morbidity and the importance of easily accessible, confidential, expert STD services.[29]

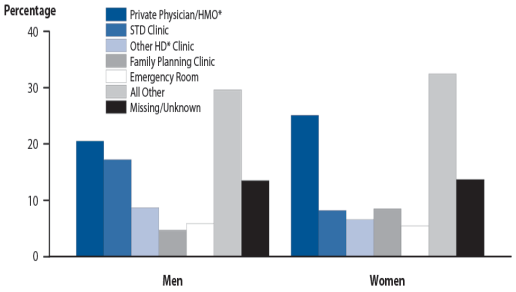

Figure 5: Percentage of Reported Cases by Sex & Reporting Sources, United States, 2014

Economic Burden of Treatment: In 2014, 163,208 women and 86,943 men were treated for gonorrhea in the US. These treatments resulted in $43.4M and $9.9M in direct medical care payments for men and women, respectively, a total of $53.3 million. When considering that the CDC estimates that 820,000 new cases of gonorrhea occur annually, total medical care expenditures have the potential to nearly triple to $124.8M, with women totaling $101.6M and men totaling $23.2M. These costs can be broken down between the costs of screening, the costs of diagnosis and treatment, and the costs of sequelae resulting from untreated acute infections or from delayed or improper treatment:

- *Screening Costs:* Screening tests usually are administered during visits for non-gonorrhea related issues in which gonorrhea screening is provided.[31] The cost for cases detected through screening – covering expenses for office visits (including treatment visits, where appropriate), diagnostic testing and treatment – range from $36 to $69.[30] The lower estimate is for screening in a correctional setting, and the higher estimate reflects a private setting.[30]

[27] Magnus Unemo and Robert A Nicholas. "Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea." Future Microbiol. 2012 Dec; 7(12): 1401–1422
[28] Centers for Disease Control and Prevention. "Sexually Transmitted Disease Surveillance 2014"
[29] Center for Disease Control. "Reported STDs in the United States: 2014 National Data for Chlamydia, Gonorrhea, and Syphilis"
[30] CL Celum, *et al.* "Patients attending STD clinics in an evolving health care environment. Demographics, insurance coverage, preferences for STD services, and STD morbidity." Sex Transm Dis. 1997 Nov;24(10):599-605.
[31] Marrazzo JM et al., Performance and cost-effectiveness of selective screening criteria for Chlamydia trachomatis infection in women, Sexually Transmitted Diseases, 1997, 24(3):131-141.

- *Diagnostic and Treatment Costs:* Other cases are detected through a diagnostic visit initiated by patients with symptoms. For these cases, costs range from $69 to $125.[32] The cost per case would be lower if most symptomatic men were treated on the basis of symptoms or a clinic-performed gram stain (without diagnostic laboratory-based testing), but research has found high rates (83-92%) of expensive diagnostic testing among private providers.[33] The $69 estimate was determined to be the average cost per acute asymptomatic case or symptomatic case treated.[34] Data on the proportion of gonorrheal infections that are asymptomatic or that do not have recognized symptoms vary. Women are generally more likely than men to have asymptomatic infections, but even among men, asymptomatic infections and infections without recognized symptoms are estimated at approximately 34%.[35] (It is noted that the medication to treat gonorrhea is relatively inexpensive: the price of Ceftriaxone is between $9.50 and $23.00, while Azithromycin is priced between $25.00 and $30.00, depending on the pharmacy. However, the public sector typically receives price breaks for use in low-cost clinics; for example, Azithromycin costs only $11.50 for public providers.[36])
- *Costs of Sequelae:* Although untreated acute infections for which patients never seek care entail no direct costs, treating their sequelae can be costly. Data for chlamydia (due to higher data availability) can be used to determine the gonorrhea-related costs of epididymitis in men and PID in women (because no evidence exists that costs vary significantly according to the organism involved).[37] Estimated rates, less than 15%, of progression from chlamydial infection to epididymitis were used to estimate those for acute gonorrhea. PID develops approximately 26% of the time following cervical Gonococcal infection.[38] As PID develops in an estimated 20% of women with untreated acute Gonococcal infections and in 6% of those with successfully treated infections.[36]

Taking all of these costs into account, the average cost per case for men was $53 and $266 for women. These costs were calculated by assuming that acute infections were untreated in 29% of men and 72% of women.[39] In women, 81% of the cost per case is attributable to sequelae, whereas in men, 92% of the cost per case is attributable to acute infection treatment.[37]

2. Market Analysis

Current gonorrhea control measures are inadequate and are seriously threatened by the rapid emergence of pathogens with antimicrobial resistance. Unfortunately, virtually no promising candidates are in sight for either therapeutic or prophylactic vaccines. In fact, the role of vaccines in preventing bacterial infections, as well as their potential role in reducing the overall use of antibiotics, has been under-represented in current approaches. Nonetheless, an appreciation for the role vaccines, as opposed to focusing solely on the development of novel antibiotics, is growing. Although vaccines will not be a practical or feasible solution for all antibiotic resistant bacteria, supporting vaccine development against some of these pathogens, particularly gonorrhea, can have a major impact in decreasing the burden of resistant infections. For some infections, vaccines represent the most logical strategy for protecting high-risk patients who are repeatedly exposed to resistant bacterial pathogens, due to frequent interactions with the healthcare system, demographic risk factors, and non-modifiable host factors.[41] If there is a bright side, it is that U.S. and world policymakers have focused efforts on gonorrhea treatment, prevention, and the clear need for a vaccine like never before.

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[32] Begley CE, McGill L and Smith PB, The incremental cost of screening, diagnosis, and treatment of gonorrhea and chlamydia in a family planning clinic, Sexually Transmitted Diseases, 1989, 16(2):63-67

[33] Ratelle S et al., Management of urethritis in health maintenance organization members receiving care at a multispecialty group practice in Massachusetts, Sexually Transmitted Diseases, 2001, 28(4):232-235.

[34] Marrazzo JM et al., Performance and cost-effectiveness of selective screening criteria for Chlamydia trachomatis infection in women, Sexually Transmitted Diseases, 1997, 24(3):131-141.

[35] Turner CF et al., Untreated gonococcal and chlamydial infection in a probability sample of adults, Journal of the American Medical Association, 2002, 287(6):726-733.

[36] Blandford JM1, Gift TL." The cost-effectiveness of single-dose azithromycin for treatment of incubating syphilis." Sex Transm Dis. 2003 Jun;30(6):502-8.

[37] Washington AE, Johnson RE and Sanders LL, Jr., Chlamydia trachomatis infections in the United States: what are they costing us? Journal of the American Medical Association, 1987, 257(15):2070-2072

[38] Westrom L and Eschenbach D, Pelvic inflammatory disease, in: Holmes KK et al., eds., Sexually Transmitted Diseases, New York: McGraw-Hill, 1999, pp. 783-809

[39] St. Lawrence JS et al., STD screening, testing, case reporting, and clinical and partner notification practices: a national survey of US physicians, American Journal of Public Health, 2002, 92(11):1784-1788.

Corporate Investment in Vaccines: In recent years, major pharmaceutical companies have poured most of their research dollars into highly profitable medicines to fight cancer, metabolic and immune mediated diseases, and prevalent infectious disease such as HIV and hepatitis C. These drugs not only command high prices but also are typically used for far longer periods of time than vaccines.

Many factors confound the production of vaccines, which are more risky and less lucrative for larger pharmaceutical companies:

- The cost of developing a vaccine, from research and discovery to product registration, is estimated to be between $200 million and $500 million.[40] It costs roughly the same amount of money to bring new cancer drugs to market, which can command $100,000 or more per year per patient.
- Low-cost existing vaccines can serve as a disincentive for developing improved vaccines that will be considerably more costly to advance than those already on the market. Pharmaceutical companies often cannot make an economic case for investing in superbugs, as they have a fiduciary duty to maximize shareholder profit.
- Live vaccines are troublesome to manufacture and are closely regulated by the Food and Drug Administration (FDA) for quality control.
- For decades, vaccines have been a neglected corner of the drugs business, relying on old technology, little investment and abysmal profit margins for both the firm producing them and the practices deploying them, at times doing so at a loss.[41]
- The anti-vaccine movement has grown. This movement typically relies on an old, discredited study linking vaccination to autism and the theory that unscrupulous doctors and pharmaceutical companies stand to make huge profits on vaccinations. Yet, after review, that argument is historically unfounded.

Altogether, a combination of high production costs, low market prices, and heavy regulation contribute to the shortage of novel vaccines. In the absence of a new ways of compensation, the production of vaccines does not make economic sense. Even though the vaccine industry is likely more profitable now than in previous years, the result of global market forces, the economic truth is that pharmaceutical companies need financial support or incentives to keep producing vaccines. Yet, regardless of company profits, the economic and social benefits of vaccination are unsurpassed; wide spread vaccination is the only strategy that has ever come close to eradicating a pathogen. Thus, it is up to governments and foundations to spearhead efforts to combat the problem, which includes supporting innovative small businesses with promising approaches to tackling resistant superbugs.

Market Size: Estimates put the vaccine market value at $24 billion; while huge, it is a mere 2 to 3 percent of a trillion-dollar worldwide pharmaceutical industry.[43] The most recently marketed vaccine for an STD was for the Human Papillomavirus (HPV). The first HPV vaccine (Gardasil) was approved by the FDA in June of 2006 and became part of the free Vaccines for Children (VFC) program within in year of approval.[42] The private sector cost of treatment is ~$540 as 3 doses must be given, each costing $180. However, the CDC purchases this same vaccine for $120 per dose, resulting in a $360 cost of treatment, a 33% discount.[43]

Given that antimicrobial-resistant gonorrhea is a top priority at the CDC a premium can be expected for a single dose vaccine. It is estimated the market would be willing to pay $350 (similar to two doses of the private sector HPV vaccine cost), with a conservative estimate of $250 (similar to two doses of public sector HPV vaccine cost) and an aggressive estimate of $450 (less than the cost of HPV treatment in the private sector, but more than the public sector). With this information in mind, it can be estimated that the revenue potential of the gonorrhea vaccine will be somewhere between $250 and $350. If the vaccine is selling to the private sector for $350, it will be sold to the public sector at a 33% discount (~$235). In addition, the CDC has indicated that gonorrhea is a high priority target; therefore, it is likely that the CDC would purchase a substantial percentage of the vaccines produced, perhaps more than 50%.

When estimating the market size of the gonorrhea vaccine, it is probable that the estimate will fall between the CDC reported (350,000) and estimated total rate of infection (over 800,000). It is expected that once the

[40] André FE. “How the research-based industry approaches vaccine development and establishes priorities.” Developmental Biology (Basel). 2002
[41] Bourree, Lam. “Vaccines are profitable, so what?” The Atlantic; February 10, 2015
[42] Center for Disease Control. “Vaccines for Children Program: Price List”
[43] Center for Disease Control. “CDC Vaccine Price List”

vaccine becomes more known to the public, women will be more likely to seek treatment. Thus, the revenue potential might be estimated at $4.1M in year 1, $16.4M in year 5 and $17.9 in year 10. In our estimates of market value, we used the more conservative reported (known) rates (Table 1). Note that these estimates do not account for international sales.

Table 1. Market Penetration & Value

US Penetration & Value	Year 1	Year 5	Year 10
US Patient Population (known)	350,000	280,000	154,000
Decline in Patient Population (YoY)	0	20%	45%
Percentage Women	46.6%	46.6%	46.6%
Women Patient Population	163,100	130,480	71,764
Vaccination Rate	5%	25%	50%
Women Vaccinated	8,155.00	32,620.00	35,882.00
Sales: Price per vaccine @ $250	$2,038,750	$8,155,000	$8,970,500
Sales: Price per vaccine @ $350	$2,854,250	$11,417,000	$12,558,700
Sales: Price per vaccine @ $450	$3,669,750	$14,679,000	$16,146,900
Average: Price Per Vaccine @$300	$2,446,500	$9,786,000	$10,764,600

Assumptions. **1.** Price will be determined based on a best-estimate derived from similar vaccines along with "higher and lower" cost considerations to better understand the sensitivity of the price point; **2.** The treatment is for the female population only, equating to 46.6% of infections; **3.** After year 1 of market penetration, public agencies should begin to purchase the product at a volume discount; **4.** The overall rate of infection will decline overtime due to immunization; **5.** Patient population differences between CDC reported and estimated; **6.** General population growth; *7.* Rate of vaccination; **8.** Increase in vaccination rate due to public visibility; **9.** Cost and sales to the private sector; 1**0**. Cost and acceptance into public sector programs.

3. Company Overview

TherapyX^{Inc} is a privately held biopharmaceutical company, headquartered in Louisville Kentucky, with research facilities in Buffalo, NY. The company currently has 8 employees and remains focused on the development and commercialization of nanoparticulate protein-based immunotherapeutics for the treatment of infectious disease, inflammation and cancer.

TherapyX^{inc} and collaborators have developed a patented phase-inversion nano-encapsulation (PIN®) technology to create clinically targeted slow-release cytokine adjuvants for use in autoimmune and cancer therapies, and now bacterial infectious diseases. The process affords a mild encapsulation methodology with minimal loss of structural integrity or biological activity of macromolecules. Various cytokines – including IL-2, GM-CSF, TNFα, TGFβ-1, and IL-12 – have been successfully encapsulated and tested in murine systems, and encapsulation efficiency, release kinetics, and post-encapsulation specific activity of selected cytokines have been evaluated. Formulation studies have resulted in IL-12 encapsulated in poly-lactic acid microspheres, with 90% efficiency, fully-preserved cytokine bioactivity, and full stability for 2 years.

Recently, we developed proprietary scale up spray drying methods that can commercially produce kilogram sized batches suitable for non-clinical studies in primates and for clinical studies in humans. Batch-to-batch consistency and stability was demonstrated. The polymers used to create these formulations already have been approved for use in a number of applications by the FDA. The commercial production of bacterial outer membrane vesicles (OMVs) or specific bacterial peptides eventually will be outsourced to specialty microbiology companies.

Research at TherapyX^{Inc} has been funded primarily through the SBIR program of the NIH in the past 8 years (Table 2). In the Phase I SBIR project (R43-AI115877), we, proved the concept that our lead product GvaX12® – a combination of an interleukin-12 (IL-12) formulation plus gonococcal outer membrane vesicles (OMV) – could serve as prototype prophylactic vaccine. Our present proposal to exploit local application of GvaX12® is based on several factual considerations: (i) soluble IL12 is ineffective; (ii) systemic manipulation of cytokines in

humans can have adverse consequences; (iii) IL-12 counteracts IL-10 and other suppressive cytokines; (iv) local application of GvaX12® should avoid major systemic complications and direct favorable responses to the site of infection; (v) the combination of OMV and particulate IL-12 (GvaX12®) serves as a prototype prophylactic vaccine.

Table 2. Selected Awards.

SBIR	Description
R43-AI080009	Delivery of Nanoencapsulated TGFβ and ATRA for the Treatment of IBD. 7/20/2008-12/31/2009, Thomas Conway, P.I
R44-AI080009	Nanoencapsulated TGFβ and ATRA for the Treatment of IBD. 4/01/2012-3/31/2016, Dominick Auci, P.I.
R43-AI104067	Therapy and Prophylaxis for Genital Tract Infection. 02/01/2013 – 01/31/2015, Yingru Liu, P.I.
R44-AI104067	Therapy and Prophylaxis for Genital Tract Infection. 06/15/2014 – 05/31/2016, Yingru Liu, P.I.
R43-AI115877	"Experimental Gonococcal Vaccine" 01/01/2015 – 12/31/2015, Yingru Liu, P.I.

4. Intellectual Property Protection

Patents and other proprietary rights are critical to TherapyX's business. We file patent applications to protect our technology, inventions, and improvements to our inventions that we consider important to our product development. TherapyX^{inc} has exclusive worldwide licenses from Brown University for the protein micronization process and the PIN® technology used to create GvaX12®, its other lead product, TGFβNanoCap® for treatment of IBD, and other formulations targeting immune mediated inflammatory diseases and cancer. The method for the polymeric micro/nanoencapsulation of proteins by phase inversion nanoencapsulation (PIN®) process is covered under a number of patents. PIN® is unique in that, because the process does not require emulsification, it is gentler and less likely to denature sensitive proteins. Table 3 lists the PIN® technology patents included in the agreement with Brown:

Table 3. Patents

Title	Number	Country	Issue Date
Process for preparing microparticles through phase inversion phenomena	6,143,211	USA	2000
Process for preparing microparticles through phase inversion phenomena	6,235,224	USA	2001
Process for preparing microparticles through phase inversion phenomena	6,616,869	USA	2003
Process for preparing microparticles through phase inversion phenomena	12/171,275	USA	Pending
Process for preparing microparticles through phase inversion phenomena	718482	Canada	2009
Process for preparing microparticles through phase inversion phenomena	0844871	Europe	2004
Micronized freeze-dried particles	7,029,700	USA	2006
Micronized freeze-dried particles	2,397,404	Canada	2008
Micronized freeze-dried particles	1246609	Europe	2010
Compositions for stabilizing and delivering proteins	Provisional filed	USA	Pending

5. Commercialization Strategy

The CDC is urging the acceleration of research on new vaccines or drug combinations now, as it takes years to bring them to market. This puts TherapyX^{Inc} in a prime position to leverage these efforts in the development of its vaccine. The company's PIN® nano-encapsulation platform represents a general strategy for the immunotherapy of disease through the application of slow-release cytokines and drugs. The overall timing for development of a GvaX12®-based prophylactic vaccine to prevent gonorrhea is described below, based on the

successful completion by Q1/Q2 2019 of the proposed PK/PD preclinical studies and GLP nonclinical toxicology studies respectively:

- File an IND in late 2019; start Phase I/IIa clinical studies, complete the trial in late 2020, and assess the drug's safety in gonorrhea patients.
- In 2021, initiate Phase IIb/III clinical studies to demonstrate the safety and efficacy of the optimized vaccine to prevent infection with gonorrhea. The trial design will enable a Go/No-Go decision to convert the initial Phase IIb trial to a Phase III pivotal trial. The projected study completion date is late 2022.
- File the biologics license application (BLA) in the 2nd half of 2023 and receive FDA approval in the 2nd half of 2024.
- Immediately launch after approval in 2025.

The target market for launch of the vaccine will be to STD clinics as they serve young, minority, and poor individuals with high STD rates. The clinic demographic data syncs perfectly with the demographic of potential target patients who are at the highest risk of infection. There are currently 1,036 of the clinics across all 50 states with the vast majority of them being in California (92), Texas (52), New York (49), Florida (48), and Pennsylvania (41).[44] In addition, the company's efforts can be easily fast-tracked with bulk purchasing from the CDC, quickly putting vaccines into STD clinics across the US and allowing for rapid market penetration.

The company's current strategic direction is to complete the IND filing and the Phase II/IIb SBIR work, and then partner with major pharmaceutical and biotechnology companies to complete development to market registration. The ideal partner will have biologic drug expertise as well as an effective global sales and marketing organization. It is possible that the partner would conduct some, if not all of the production and marketing activities, which would serve to reduce capital investment requirements.

Such collaboration will produce key benefits for TherapyX^{Inc}, including obvious financial aspects, and aid the company with key expertise needed to develop its other pipeline products on its own. The company plans to make extensive use of outsourced manufacturing activities, coupled with strong internal quality management and assessment systems to ensure an adequate supply of high quality clinical and commercial products while minimizing capital investment. Overall the company's strategy has the potential to rapidly commercialize an innovative approach that will benefit patients worldwide. Strengths, weaknesses, opportunities and threats are summarized in Table 4.

Table 4. **SWOT Analysis**

Strengths	Opportunities
1) First mover advantage 2) Novel approach using biologics 3) Therapy can be combined with other medications 4) "Herd" immunity 5) Easily identifiable target market 6) One-time administration of vaccine 7) Vaccine is a prime target for licensing or partnership	1) Antibiotic resistance requires vaccine 2) Governmental support for vaccine necessary for new entrants 3) Emerging/developing country markets 4) Newly diagnosed cases each year 5) CDC bulk purchasing 6) Premium pricing 7) High concentrations of infected individuals
Weaknesses	**Threats**
1) Only addresses 46.6% of the market 2) Many infected individuals are asymptomatic or never seek treatment 3) No sales, marketing or distribution divisions in house 4) Pre-clinical trial phase 5) Small firm with significant funding needs to bring product to market	1) Vaccine effectiveness erodes long-term profitability 2) Distributed network of treatment centers 3) No validated effectiveness in curing the infection 4) Parental thoughts/beliefs hinder uptake in youth 5) Total market value is based on significant estimation by CDC

6. Key Operations Analysis

Finance Plan: In 2017, TherapyX^{inc} intends to raise an estimated $2.625 million through the sale of Series A Preferred Shares. These funds will be in addition to the SBIR award and will be used primarily to (i) initiate phase I/II studies of GvaX12® with a view to out-licensing the product to a partner, and (ii) for general corporate purposes. The first investor group targeted will be the Buffalo Angel Investor Network. While this group votes on investment opportunities, the investors do not pool their funds; rather, they participate as individual investors. To limit the number of new shareholders, we will limit the sale to 16 individuals at $100,000 each; the remaining $1.025 million will be sold to a seed funding venture capital firm (25 potential seed venture funds with life science investments in Western New York and the northeast U.S. have been targeted) or to any combination of the two investments that achieves the capital requirement for items (i) and (ii) .

[44] A list of contact information for all the publicly funded free and low-cost STD clinics can be found in support document "Free and Low Cost Providers Contact List."

Production and Marketing: TherapyX^{inc} plans to focus its operations on R&D for the foreseeable future. The company intends to outsource the manufacturing, distribution and sales of its products. In previous capacities the company has partnered with Bend Research, Inc. for all of its drug manufacturing. Bend Research, Inc. is now part of Capsugel's Dosage Form Solutions business unit headquartered in Morristown, NJ. This partnership has provided access to additional technologies, R&D capabilities, and a global manufacturing infrastructure. TherapyX^{inc} also has partnered with Neumedicines, Inc., a private biotechnology firm developing protein therapeutics that address unmet clinical and societal needs in oncology, hematology, and immunology. Our agreement with Neumedicines insures unique and continued access to clinical grade rhIL-12. The company operates from its headquarters in Pasadena, CA. Recently, we have begun discussions with researches at Allergan inc, regarding possible co-development of encapsulated peptides for oral delivery. We expect that any GvaX12® prophylactic vaccine partner would conduct some, if not all of the production and marketing activities, which serves to reduce development-associated capital requirements.

Revenue Stream: Based on our expected development timelines and other assumptions, we project a profit of ~$5 million for GvaX12® in 2030. License and milestone payments will primarily drive revenues through 2022. After 2022 (projected launch) royalties (projected at ~5%) on sales will drive top-line revenue growth. Projections are based upon (1) the known female patient populations indicated above; (2) vaccination rates of 5% in year 1, growing steadily to 25% by year 5 (2030); and an average cost of $300 per vaccine. The financial forecasts do not include the commercialization of additional pre-clinical stage projects that could impact the revenue growth of the company as early as 2020. Operating expenses include anticipated development costs.

($000s)	2020	2021	2022	2023	2025	2026	2027	2028	2029	2030
Licensing Fees & Royalties	$0	$0	$500	$500	$1000	$1,800	$3,500	$5,500	$7,500	$10,000
Grants	$500	$1,000	$1,000	$1,000	$400	$400	$850	$850	$850	$850
Investments	$0	$2,625	$0	$0	$0	$0	$0	$0	$0	$0
Revenue	$0	$3,625	$1,500	$1,500	$1,400	$2,200	$4,350	$6,350	$8,350	$10,850
Operating Expenses	$400	$1,000	$1,000	$1,000	$1,000	$1,500	$2,000	$3,000	$4,000	$5,000
EBIT	$100	$2,625	$500	$500	$400	$700	$2,350	$3,350	$4,350	$5,850

COMPANY COMMERCIALIZATION STATEMENT

Therapyx, Inc. has not received more than 15 SBIR Phase II awards from the Federal Government during the preceding five fiscal years.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

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

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

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

○ Yes ● No

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

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

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

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

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

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ○ No

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

*Previously Reported: ○ Yes ○ No

6. Change of Investigator / Change of Institution Section

☐ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12235571 Funding Opportunity Number: PA-16-302 . Received Date: 2016-08-24T11:01:24.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	1253-SPECIFIC AIMS.pdf
3. Research Strategy*	1254-Res plan NKE FINAL.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	1255-VERTEBRATE ANIMALS VACCINE phase II FINAL.pdf
10. Select Agent Research	
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13. Letters of Support	1256-VaccinePhaseII LOS.pdf
14. Resource Sharing Plan(s)	
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Appendix	
16. Appendix	1257-Appendix 1 Repo study designs and quotes.pdf 1258-Appendix 2 IND Budget Proposal Master-TherapyX.pdf

Funding Opportunity Number: PA-16-302. Received Date: 2016-08-24T11:01:24.000-04:00

SPECIFIC AIMS

The overall objective of this SBIR project is **to develop a prophylactic vaccine against *Neisseria gonorrhoeae*** (i.e., gonorrhea). In Phase I, using a murine model of genital gonococcal infection, we have established **Proof-of-Principle** that i.vag. immunization with a proprietary sustained-release particulate interleukin-12 (IL-12) formulation plus gonococcal outer membrane vesicles (OMV) - the combination we refer to as GvaX12® - induces durable Th1-driven adaptive immune responses that protect against genital tract infection with heterologous strains of *N. gonorrhoeae* [progress report]. In fulfilling the Phase I milestones we demonstrated (i) induction of gonococcus-specific Th1 cells, (ii) generation of anti-gonococcal antibodies in serum and genital tract secretions, (iii) protection against diverse strains of *N. gonorrhoeae*, (iv) recall of specific antibodies and reactivation of T cells after challenge infection, and (v) duration of protection against infection for 6 months. In addition we have shown that protection depends on both antibodies and IFNγ.

In Phase II, we will use the same murine model to identify alternative routes of immunization that may be more applicable to humans (potentially including males), and determine the basis of protective immunity against antigenically diverse clinical isolates of *N. gonorrhoeae*. In addition, we will perform preliminary pharmacokinetics and toxicity studies and prepare materials for submission to the FDA for a type C meeting.

The specific aims for Phase II are as follows:

Aim 1: Define, optimize, and validate a vaccination regimen. This aim will encompass three tasks:

A. Determine a route of administration, intranasal vs. intravaginal, with respect to: 1) protection against challenge with *N. gonorrhoeae* ; and 2) immune response correlates of protection (i.e., antibody titers, antibody specificity and T cell cytokine responses).
B. Determine the basis of cross-protective immunity against antigenically diverse clinical isolates of *N. gonorrhoeae*
C. Determine whether immunization with detergent-extracted OMV-containing GvaX12® induces protective immune responses against *N. gonorrhoeae*

Aim 2: Rodent pharmacokinetics and reproductive toxicology. This aim involves two tasks:

A. Bioavailability and pharmacokinetics of IL-12 following intravaginal and intranasal administration of GvaX12® in mice.
B. Conduct reproductive toxicology studies in rats (i.e., embryonic-fetal development and effects on pre- post-natal development)

Aim 3: Request a type C pre-pre-IND meeting with the FDA and prepare a briefing package with written questions. Upon successful completion of Aims 1 and 2, TherapyX^{inc} will request a type C pre-pre-IND meeting with the FDA and prepare a briefing package with written questions. At this meeting, we will seek agency guidance to shape future toxicological and clinical study designs

Milestones to be accomplished:
Year 1:
1. Demonstrate efficacy of intra-nasal versus intra-vaginal routes of vaccine delivery;
2. Elucidate the basis of cross-protection against antigenically diverse strains;

Year 2:
3. Determine whether detergent-extracted OMV are effective as a vaccine immunogen;
4. Define systemic exposure and the potential for reproductive toxicity;
5. Receive agency guidance that will shape toxicological and clinical study designs.

Upon successful completion of these aims TherapyX^{inc} will submit an SBIR Phase IIb grant application, which will seek to evaluate the pharmacokinetics and potential toxicity of this vaccine in non-human primates, and prepare for an IND submission.

RESEARCH STRATEGY

1. Significance

Despite public health control measures, gonorrhea remains an all-too-common disease, and is the second most frequent reportable infectious disease in the US. Although the reported incidence of this disease exceeds 350,000 cases per annum (1), the real incidence is estimated to be 820,000 cases per annum (2). World-wide incidence is now estimated at 78 million new cases per annum (3). Women bear the greater morbidity because untreated gonorrhea can lead to upper tract infection and pelvic inflammatory disease, with tubal scarring, infertility, and risk for ectopic pregnancy which can be life-threatening. Gonorrhea also increases the risk of acquisition and transmission of HIV up to 5-fold in both sexes (4). Because no vaccine is available (5), both its control and treatment depend upon antibiotics. However, *N. gonorrhoeae* has steadily developed resistance to each class of antibiotics deployed against it, including fluoroquinolones and most recently even extended-spectrum cephalosporins, raising serious concerns that gonorrhea could become untreatable (6). The CDC has listed antibiotic-resistant *N. gonorrhoeae* as one of the top three pathogens presenting "an immediate public health threat that requires urgent and aggressive action" (7). A recent WHO technical consultation on vaccines against sexually transmitted infections (to which our collaborator Dr. Russell contributed) called for renewed efforts to develop a vaccine against gonorrhea (8, 9). In June 2015, the NIAID convened a workshop to discuss the status of gonococcal vaccine development that both Dr. Russell and the PI attended (see report published in ref. 10).

Several previous attempts have been made to develop vaccines against gonorrhea, three in recent times although only two went into clinical trial (reviewed in 11, 12). The first, based on killed whole gonococci, proved ineffective as did a later and substantially larger effort based on gonococcal pili; the third based on porin protein was abandoned. These disappointments discouraged further efforts and engendered pessimism that vaccination against gonorrhea might not be feasible. Contributing to this situation is the well-known observation that uncomplicated gonorrhea can be acquired repeatedly with apparently no development of protective immunity resulting from previous infection. The reasons for this are poorly understood, but it is generally thought that extensive antigenic variation coupled with multiple mechanisms for resisting complement enable *N. gonorrhoeae* to evade whatever adaptive immune responses the host develops against it. Consequently, in the absence of a reproducible state of immunity, the determinants of human immune protection have not been defined.

Recent studies in our collaborator's laboratory using a mouse model of genital gonococcal infection have cast new light on this problem. It was shown that ***N. gonorrhoeae*** **selectively elicits innate host defenses that it can survive and concomitantly suppresses adaptive immune responses that would eliminate it, by mechanisms involving IL-10, TGFβ, and type 1 regulatory T cells (13-17).** These studies further showed that: 1) immunosuppression can be reversed by neutralizing TGFβ and IL-10 (14, 17); and 2) local intravaginal (i.vag.) treatment with TherapyX^{inc}'s sustained-release nanoparticulate IL-12 formulation (but not soluble IL-12) during gonococcal infection accelerates clearance of the infection and induces resistance to secondary infection several weeks later (18). This latter finding implies the development and recall of immune memory (18). We therefore inferred that i.vag. nanoparticulate IL-12 effectively turned the infection into a live vaccine, and consequently proposed that it could serve as an adjuvant for a non-viable prophylactic gonococcal vaccine.

This notion was directly tested in our Phase I work. The data demonstrated that prophylactic vaccination with a formulation containing particulate IL-12 plus gonococcal outer membrane vesicles (GvaX12®) administered i.vag resulted in long-term protection from subsequent challenge (see Phase I data below, 19). Thus we now have a candidate prophylactic vaccine for clinical development.

2. Innovation:

The innovative aspects of our proposal are two-fold. **First,** we now have a novel approach for developing a vaccine against *N. gonorrhoeae* based on a small animal model in which we can reliably induce a state of protective immunity against genital gonococcal infection. *This situation was never attained in the previous unsuccessful efforts to create a gonococcal vaccine and represents a major conceptual advance*. We have found that mice can be immunized i.vag. with GvaX12® to generate protection against subsequent challenge

with live *N. gonorrhoeae* (progress report). Having established Proof-of-Principle, we now hypothesize that protective immunity against genital gonococcal infection depends upon: 1) Th1-driven production of anti-gonococcal antibodies (Abs) in serum and genital secretions; and 2) immune memory that can be recalled on subsequent exposure to *N. gonorrhoeae*.

Second, the sustained-release nanoparticulate IL-12 formulation, a critical component of GvaX12®, represents an important technological innovation. Our patented phase inversion nanoencapsulation (PIN®) process provides a mild encapsulation methodology without loss of structural integrity or biological activity of macromolecules (20). Studies of encapsulation efficiency, release kinetics, and post-encapsulation specific activity studies established that excipient-stabilized macromolecules can be incorporated in poly-lactic acid (PLA) particles in a two-step process with 90% efficiency, fully-preserved bioactivity and storage stability for up to one year (21). Finally, recent scale-up work at TherapyX^{inc} has achieved commercial methods for the production of kilogram size batches of the formulation removing a major hurdle that has thwarted the development of similar products. **Thus GvaX12® is now ideally situated for development as a vaccine that induces Th1-driven protective immunity against *N. gonorrhoeae*.**

3. Approach

3.1 Phase I Results

In Phase I of this SBIR grant we established **proof-of-principle that i.vag. immunization with GvaX12® induces adaptive Th1-driven immune responses that protect against genital gonococcal infection**. Gonococcal outer membrane vesicles (OMV) were selected as the immunogen because they contain most gonococcal surface antigens (Ags) in native conformation, whereas in heat- or formaldehyde-killed cells some Ags may be denatured. Moreover, gonococcal OMV have been used to immunize mice intranasally, though with variable results (11, 12, 22), and meningococcal OMV vaccines have been developed against *N. meningitidis* and successfully used in humans (23).

We have attained the Milestones stated in the Phase I application as follows:

Milestone 1: Intra-vaginal immunization with GvaX12® protects from genital gonococcal infection by inducing IFNγ-secreting T cells specific for gonococcal antigen in the draining iliac lymph nodes (Fig. 1).

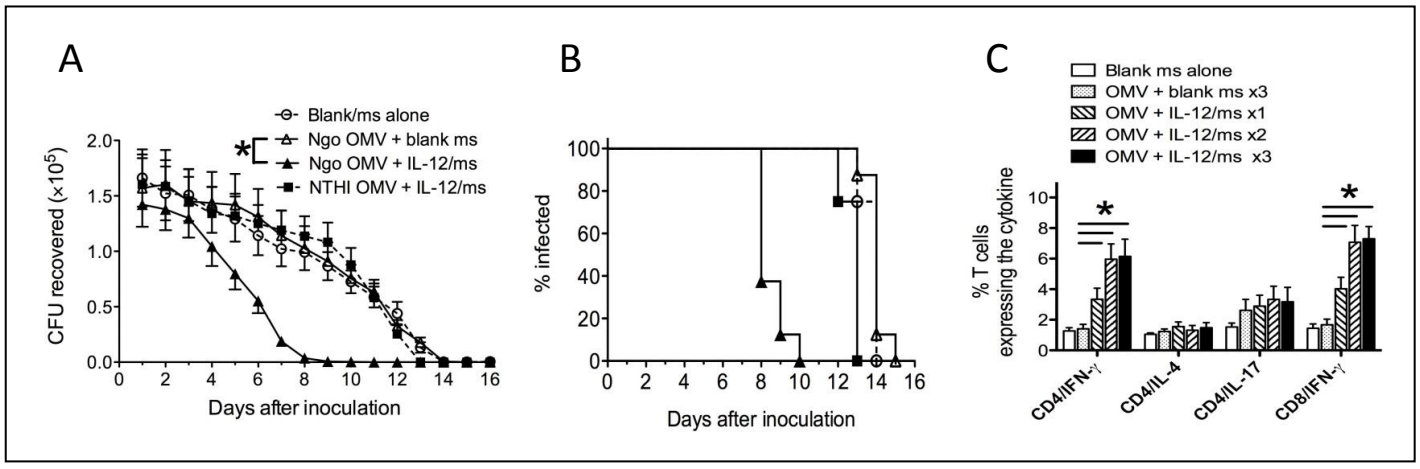


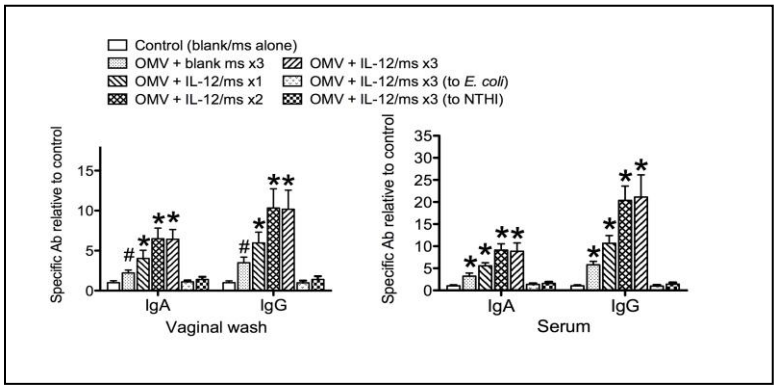
Figure 1: GvaX12® protects from Ngo challenge and induces Ngo-specific T-cell immunity in a murine model of Ngo infection. In this model (19) , young female mice in diestrus are treated with estradiol, placed on antibiotics to suppress the overgrowth of commensals, and then vaginally infected by instilling a suspension of freshly grown, virulent *N. gonorrhoeae*. Infection, monitored by vaginal swabbing and plating, typically clears in 2-3 weeks, and is accompanied by an influx of neutrophils. Vaccine efficacy is determined by the change in the rate of Ngo clearance after challenge. Mice (N=8) were immunized twice with gonococcal (Ngo; FA1090) OMV (50µg protein) plus blank or IL-12 particles (1µg IL-12); controls were sham-immunized with blank particles or with NTHI OMV plus IL-12 particles (GvaX12®). Two weeks later, all mice were challenged with Ngo strain FA1090. A: recovery of Ngo (mean ±SEM, CFU), P <0.01 (ANOVA) gonococcal OMV + IL-12 particles vs. blank particles; B: % of animals remaining infected at each time point, P <0.0001 (Kaplan-Meier, log-rank test) Ngo OMV + IL-12 particles vs. blank particles. C: T cell cytokine responses

induced by immunization with gonococcal OMV plus IL-12/ms, prior to gonococcal challenge. ILN cells were isolated 2 weeks after the last immunization with 1, 2, or 3 doses of gonococcal OMV (50µg protein) plus IL-12/ms (1µg IL-12). Control ILN were obtained from mice sham-immunized with blank ms (3 doses) and additional mice were immunized 3x with gonococcal OMV plus blank ms. Data shown as mean ±SEM % of $CD4^+$ or $CD8^+$ cells staining for each cytokine. * $P < 0.01$ (ANOVA) comparing immunization with IL-12/ms vs. blank ms, N=3 samples per group.

The above data establish that GvaX12® enabled mice to reject a bacterial challenge more effectively than vaccination with OMV/blank particles and that the clearance of bacteria was associated with a Th1 response.

Milestone 2: Anti-gonococcal Ab were generated in serum and vaginal secretions by i.vag. immunization with GvaX12® (Fig. 2).

Fig. 2: T-cell cytokine responses induced by immunization with gonococcal OMV plus IL-12, prior to gonococcal challenge. ILN cells were isolated 2 weeks after the last immunization with 1, 2, or 3 doses of gonococcal OMV (50µg protein) plus IL-12 particles (1µg IL-12). Control ILN were obtained from mice sham-immunized with blank controls (3 doses) and additional mice were immunized 3x with gonococcal OMV plus blank particles. Data shown as mean ±SEM % of $CD4^+$ or $CD8^+$ cells staining for each cytokine. * $P < 0.01$ (ANOVA) comparing immunization with IL-12 vs. blank, N=3 samples per group.



These results demonstrate that GvaX12® induced potent Ng-specific IgG and IgA responses both in the serum and in the mucosa and that maximal response required 2 immunizations.

Milestone 3: i.vag. immunization with GvaX12® induced protection against antigenically different strains of *N. gonorrhoeae* (Fig. 3).

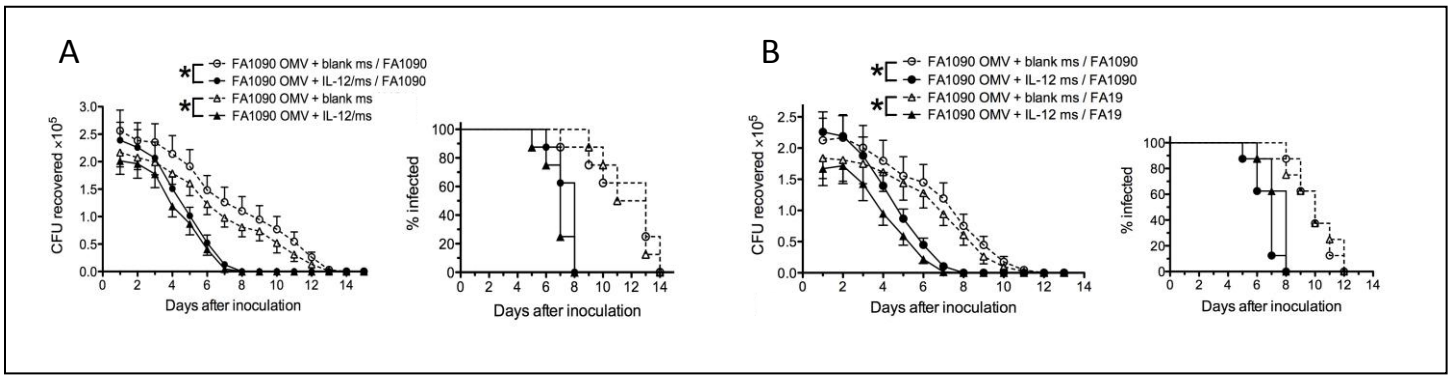


Fig. 3 . Protection against heterologous gonococcal challenge. A. Challenge with strain MS11. One month after immunization with FA1090 GvaX12® or control, mice were challenged with *N. gonorrhoeae* strains FA1090 or MS11. Left panel: recovery of *N. gonorrhoeae* (mean ±SEM, CFU), * P <0.001 (ANOVA) N=8; right panel: % of animal remaining infected at each time point), P <0.02 for FA1090 challenge, IL-12 vs. blank ; P <0.001 IL-12 vs. blank for MS11 challenge (Kaplan-Meier analysis, log-rank test) N=8. B: Challenge with strain FA19. One month after immunization with FA1090 GvaX12® or blank control, mice were challenged with *N. gonorrhoeae* strains FA1090 or FA19. Left panel: recovery of *N. gonorrhoeae* (mean ±SEM, CFU), * P <0.001 (ANOVA) N=8; right panel: % of animal remaining infected at each time point, P <0.02 for FA1090 challenge, IL-12 vs. blank; P <0.001 for FA19 challenge, IL-12 vs. blank (Kaplan-Meier analysis, log-rank test), N=8.

The above data reveal that GvaX12®-induced immunity to strain FA1090 provided cross-protection from strains MS11 and FA19.

Milestone 4: Immune responses (T cells and Ab) induced by i.vag. immunization with GvaX12® were recalled after challenge infection (Fig. 4). Note that IL-17-secreting T cells are induced by gonococcal infection alone, regardless of prior immunization or treatment (13, 16, 18).

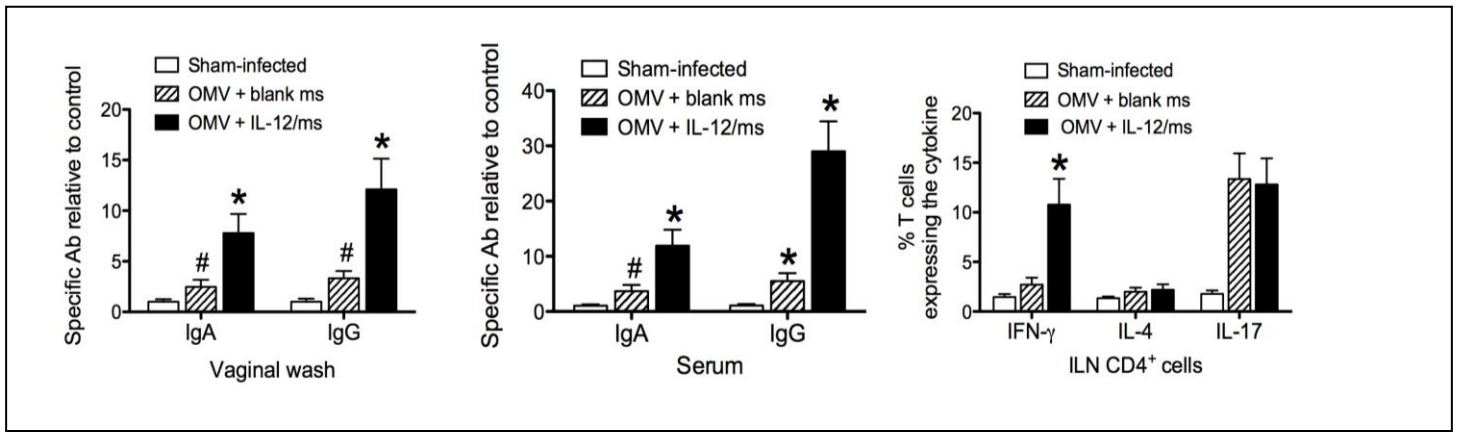


Figure 4: Recall of responses in mice after challenge with *N. gonorrhoeae* FA1090 6 months after immunization with FA1090 OMV plus IL-12. **A:** Vaginal wash antibodies (N=5 samples), **B:** serum antibodies (N=5 samples), **C:** cytokine production by $CD4^+$ ILN cells (N=3 samples). # *P* <0.05, * *P* <0.01 vs control samples from sham-infected mice (ANOVA).

These results establish that GvaX12® induced long-term Ng-specific Th1 and antibody responses.

Milestone 5: Protective immunity against genital infection with *N. gonorrhoeae* persisted for at least 6 months after i.vag. immunization with GvaX12® (Fig. 5) and that long-term protection required both Th1 and B-cell responses (Fig 6).

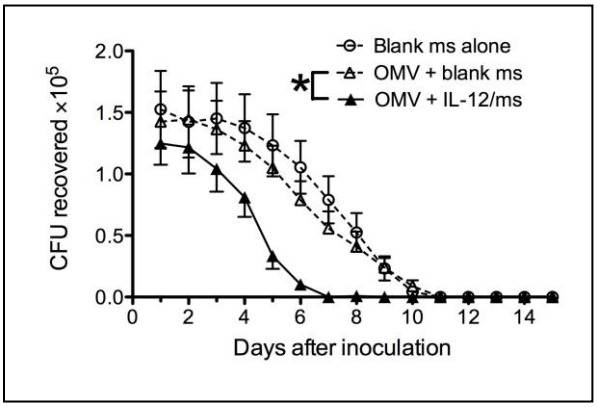


Fig. 5: Protection against gonococcal (FA1090) challenge persisted for at least 6 months after immunization with 2 doses of gonococcal (FA1090) OMV plus IL-12. Data shown as mean ±SEM CFU of *N. gonorrhoeae* recovered at each time point; ***** *P* <0.01, N=8 (ANOVA).

The enhanced clearance of a bacterial challenge after 6 months establishes that GvaX12® induced long-term memory. In addition we demonstrated that long-term protection against genital gonococcal infection depended on both B cells (presumably to produce Ab) and IFNγ (Fig. 6).

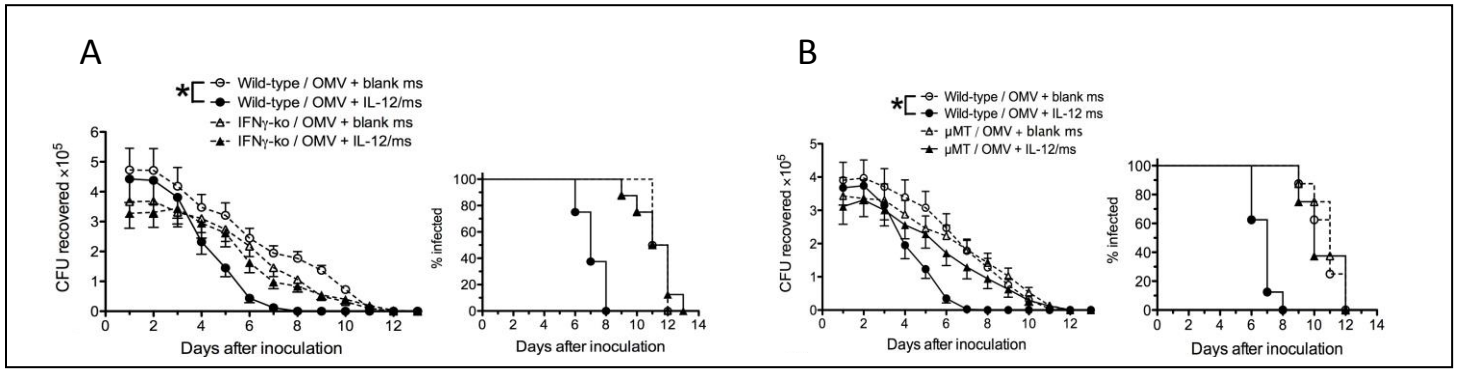


Fig. 6: Protection induced by immunization with GvaX12® depends on IFNγ and B-cells. **A:** Course of infection (FA1090) in IFNγ-ko vs wild-type mice immunized with FA1090 OMV GvaX12®. **B:** Course of infection (FA1090) in μMT (B-cell ko) vs wild-type mice immunized with FA1090 OMV GvaX12®. Left panels, recovery of *N. gonorrhoeae* (mean ±SEM, CFU) • *P* <0.01 (ANOVA); right panels, % of animal remaining infected at each time point, *P* <0.0001 for wild-type mice, IL-12 vs. blank (Kaplan-Meier analysis, log-rank test).

Publication: A manuscript reporting these and additional findings has been submitted to *Mucosal Immunology*. If accepted before this application is reviewed we will inform the SRO:
Liu, Y., Hammer, L., Liu, W., Hobbs, M.M., Zielke, R.A., Sikora, A.E., Jerse, A.E., Egilmez, N.K., Russell, M.W. (2016) Experimental vaccine induces Th1-driven immune responses and protection against *Neisseria gonorrhoeae* in a murine model.

3.2 The Phase II Project

The goal of this proposed Phase II SBIR project is to advance GvaX12® towards clinical trials by: 1) developing a more practicable vaccination regimen in terms of the route of administration (intranasal vs. intravaginal); 2) determining immune response correlates of protection: 3) determining the basis for protection against naturally occurring, antigenically diverse, clinical isolates of *N. gonorrhoeae*; 4) perform bioavailability, pharmacokinetic, and initial safety studies on GvaX12® in rats; and 5) prepare a briefing package with written questions for the FDA, requesting a pre-pre-IND meeting. The FDA's response will be used to guide continued development including OMV scale up, and immune response and toxicological studies in non-human primates. The results should also enable (i) defining the specificity and type of immune responses that need to be generated to elicit protective immunity, and (ii) framing hypotheses for testing in clinical trials.

The proposed studies will be limited to female mice. The female mouse is the only animal model currently available for experimental in vivo studies of genital gonococcal infections and immunity to them (24); there is no experimental animal model for male tract gonococcal infection. Moreover, women suffer the greater burden of morbidity from gonorrhea, because of the potential for damage to the reproductive tract that arises from ascending infection. This infection can lead to tubal scarring, hence infertility; increased risk of ectopic pregnancy; and painful and debilitating pelvic inflammatory disease. A developing fetus can be infected in utero, leading to pre-term birth or septic abortion, and an infant delivered through an infected birth canal risks ocular infection that can lead to blindness. Therefore, limiting our studies to female mice is not only unavoidable but also justifiable in addressing the greater problem in females.

3.2.1 Specific Aim 1: Define, optimize and validate a vaccine regimen

Task 1A: To determine an optimal route of immunization: intravaginal vs. intranasal

The current i.vag. immunization regimen, although shown to be effective in our Phase I studies, is thought to be impracticable for human prophylactic application, because i.vag. immunization likely will be unacceptable for adolescent girls and many women, and inapplicable for males. However, substantial literature, including studies in our collaborator's laboratory, shows that intranasal (i.n.) immunization elicits responses in the genital tract, in primates and in males as well as females (25-27), and may be even more effective than i.vag. immunization for generating genital antibody responses (28).

Compare protection against challenge with N. gonorrhoeae

Based on our previous gonococcal immunization and infection studies (13, 14, 16-18), including the phase I results shown above, we will use groups of 8-12 female mice, BALB/c or C57BL/6 strains, at 2-4 months of age. The mice will be prepared for gonococcal challenge (24) by injecting s.c. with 0.5mg estradiol (Premarin) on days –2, 0, and +2, placed on an antibiotic regime, and challenged by i.vag. instillation of $5x10^6$ CFU of freshly grown *N. gonorrhoeae*. The course of infection will be monitored by taking daily vaginal swabs for viable cell counting on selective agar (a GC agar base supplemented with IsoVitalex, hemin, and antibiotics: streptomycin, vancomycin, nystatin, colistin, and trimethoprim). Statistical analysis by 2-way ANOVA of CFU recovered at each time point or by Kaplan-Meier analysis of the number of mice remaining infected each day (clearance is defined as at least 3 successive days of negative gonococcal recovery) has revealed significant differences (P <0.05 or lower) between control and experimental groups, each with at least 8 animals completing the experiments. To guard against inadvertent loss of mice through accident (a rare event) or overgrowth of commensal microbiota under estrogen treatment, experiments will be commenced with groups of at least 10 animals. Experiments requiring challenge after a prolonged interval, e.g., up to 6 months will start with 12 animals. Experiments will be performed and gonococcal recovery will be enumerated by persons who are "blinded" to the group designations. All experiments will be repeated 2-3 times.

I.vag. immunization has been successfully achieved by instilling GvaX12® containing OMV prepared as described (19, 29) from *N. gonorrhoeae* strains FA1090, MS11, or FA19 (containing 40µg protein) plus IL-12 particles (1µg murine IL-12) and repeating the dose 2 weeks later (see above). We will compare this regimen with administering the same dose of GvaX12® intranasally (i.n.) to a group of 10-12 female mice, and giving a second similar dose 2-3 weeks later. Control groups will include sham-immunized mice given vehicle only by the same routes. Additional controls for the i.n. route will include mice immunized with OMV plus blank particles, or with IL-12 particles alone. As these latter controls have previously been performed repeatedly by

the i.vag. route and shown to be no different from sham-immunization, they will not be routinely repeated. Two weeks after the second immunization, mice will be challenged with *N. gonorrhoeae* of the same strain as used to prepare the OMV, and infection monitored by vaginal swabbing and plating. The results will be evaluated statistically using 2-way ANOVA of gonococcal CFU recovered, and Kaplan-Meier analysis of the number of mice remaining infected, to determine (a) if protection against gonococcal challenge is achieved by i.n. immunization with GvaX12® (compared to controls), and (b) whether i.n. immunization is as effective as i.vag. immunization.

Compare immune responses with i.n. versus i.vag. immunization

As in our previous studies, serum, saliva, and vaginal wash samples will be collected from mice prior to immunization, ~2 weeks after immunization and prior to challenge, and at termination (after clearance of infection) for assay of anti-gonococcal Abs (IgG and IgA; IgM Ab are low, variable, and uninformative) by ELISA. Western blot analysis of the Abs also will be performed using OMV subjected to SDS-PAGE to reveal the predominant Ag bands detected by these Abs.

- For the i.vag.-immunized mice, the iliac lymph nodes (ILN) will be excised from i.vag.-immunized mice to assess T cell responses in the genital tract-draining lymph nodes. In some instances, the mice will be euthanized after immunization and prior to challenge
- For the i.n.-immunized mice, we will instead use the superficial and deep cervical lymph nodes (CLN) which drain the nasal lymphoid tissues (NALT) (30-32). T cell responses will be assessed by: (a) intracellular staining and flow cytometry of ILN or CLN cells (co-stained for surface markers including CD4 and CD8) for the production of cytokines ex vivo (IFNγ, IL-4, and IL-17); (b) culture of ILN or CLN cells preloaded with carboxyfluorescein succinimide ester (CFSE) with or without gonococcal Ag (OMV) for 4 days followed by staining for surface markers (CD4 and CD8) and flow cytometry to assess proliferation by attenuation of CFSE staining (secreted cytokines can also be assayed by ELISA); and (c) RT-PCR assay of mRNA extracted from ILN or CLN cells for the key Th1, Th2, and Th17 transcription factors Tbet, GATA3, and RORγt, respectively.

Differences in the quantitative responses shown by different groups of mice will be analyzed statistically using Student's *t* tests (for 2 groups) or ANOVA (for multiple groups). All of these experimental and statistical methods have been used in our previous studies (14, 16-18).

Outcomes, potential difficulties, further experiments/alternatives, and significance/interpretation for Task 1A:

The primary objectives are to determine: 1) whether i.n. immunization with GvaX12® is as effective as i.vag. immunization in protection against i.vag. challenge with *N. gonorrhoeae*; and 2) whether similar immune responses are induced by these two routes. If i.n. immunization is effective, we would expect it to be associated with similar immune responses (i.e., anti-gonococcal Abs in serum and vaginal wash, and IFNγ-secreting CD4+ T cells in the draining lymph nodes). Evaluation of salivary IgA Ab responses would reveal the induction of a common mucosal immune response, which would be expected for i.n. immunization, but not for i.vag. immunization, because the genital tract does not contain the mucosal inductive site tissues found in the nasal passages of mice (28, 30). If protection is weak or ineffective, we expect it to be associated with inadequate immune responses, and in that case we will adjust the dose of vaccine (GvaX12®) given i.n., and/or the number of doses (e.g., 3 or more doses at 2-week intervals) to increase the immune responses and generate protection.

An important consideration will be the generation of Ab (IgG and IgA) in vaginal wash after i.n. immunization as presumably this will be critical for protection. Based on previous experience we expect i.n. immunization to be at least as effective in generating genital tract Ab responses as i.vag. immunization (25, 28). Discordance between protection and immune response induction by the two routes would require further investigation, and possibly provide insight into the mechanisms of protective immunity. If i.n. immunization effectively generates both protection and immune responses equivalent to i.vag. immunization, we will immunize male mice i.n. with the same GvaX12® regime to determine if they generate the same serum and salivary Ab responses as female mice. Although it is not possible to evaluate protective immunity in male mice, a positive result would broaden the potential applicability of GvaX12® to both sexes.

Given that vaginal IgG Abs are likely derived from the circulation, we will also compare the response to s.c. immunization with GvaX12® (with appropriate controls) because would expect this route to induce predominantly serum IgG Ab, as well as vaginal IgG Ab as reported previously (22). If this should be sufficient to generate protection against gonococcal challenge, it would be important to determine whether a Th1/IFNγ response were also induced, because we previously demonstrated this effect to be essential for protective immunity (see Phase I data above). However, GvaX12® is intended for local mucosal, not systemic application, because systemic release of IL-12 can be toxic. Therefore, an alternative Th1-driving adjuvant for systemic immunization with OMV may be necessary; however, that possibility is beyond the scope of this application.

We realize that the mouse model cannot fully predict the efficacy of the proposed vaccine in humans, which can only be determined by means of clinical trials. *N. gonorrhoeae* is an exclusively human pathogen and non-human primates are not susceptible to infection, with the exception of chimpanzees (33). However, despite its limitations the mouse model is the only available animal model for studying the response of an intact mammalian immune system to *N. gonorrhoeae*, and we have used it to good effect for this purpose for several years. Importantly it provides a means to elucidate the type(s) of immune response necessary for protective immunity against gonococcal infection, and may also identify potential surrogate markers of protection. Thus it can test hypotheses that cannot be tested in humans and help to frame the questions that will need to be addressed in humans.

Task 1B**:** Determine the basis of cross-protective immunity against antigenically diverse clinical isolates of *N. gonorrhoeae*

We have found that i.vag. immunization with GvaX12® containing OMV derived from any of three widely used "laboratory strains" (FA1090, MS11, and FA19) induces protection against challenge, not only against the homologous strain, but also against each other, despite their known antigenic differences (see above). In addition, serum and vaginal Ab showed strong cross-reactivity in ELISA against heterologous gonococcal strains (see above). Although these findings were wholly unexpected, they have been repeatedly reproduced. However, the extent to which these extensively subcultured strains are representative of naturally circulating *N. gonorrhoeae* strains is uncertain. Therefore, to further investigate these findings we will determine whether Ab cross-reactivity and associated cross-protection against challenge extends to antigenically diverse, minimally passaged *clinical isolates* of *N. gonorrhoeae* (in collaboration with Dr. Marcia Hobbs, University of North Carolina, Chapel Hill; letter attached).

Our preliminary findings showed: 1) that immunization of mice i.vag. with GvaX12® containing OMV prepared from strain FA1090 also protects against subsequent challenge (with two minimally passaged clinical isolates provided by Dr. Hobbs): and 2) that serum and vaginal Ab react in ELISA with these isolates, similar to what we observed with heterologous lab strains (data not shown). Although we can extend these experiments to include additional clinical isolates, the time and effort involved in such an extension would severely limit the number of strains that could be tested. Therefore we propose the following process:

1. First, we will perform screening assays by ELISA on a larger number (at least 30) of clinical isolates from diverse geographic locations in order to determine whether serum and vaginal Abs induced by immunization of mice with OMV from the laboratory strains cross-react with these clinical isolates in comparison to the homologous strains. For this purpose, we will immunize mice i.vag. with GvaX12® containing OMV prepared from gonococcal strains FA1090, MS11, and FA19 (at least 20 mice each), as described in Aim 1A above, and collect serum and vaginal wash 2 weeks after the last dose. Terminal bleeding (from the inferior vena cava or subclavian vein) of the mice will be performed under anesthesia in order to obtain as much serum as possible. Samples will be pooled to yield 5-10ml of pooled serum and 20ml or more of vaginal wash. Because this is a screening procedure and we already know that control immunizations are not protective and do not induce Ab, control serum and vaginal wash will be obtained from unimmunized mice. ELISA plates will then be coated with whole cells of *N. gonorrhoeae*, including strains FA1090, MS11, and FA19 as well as the clinical isolates, freshly grown overnight on "chocolate agar" plates incubated at 37°C in 5% CO_2 as described previously. Concentrations of gonococci in each harvested culture will be adjusted to the same level by OD at 600nm. The pooled serum and vaginal wash from immunized and control mice will be serially diluted and incubated on the plates in quadruplicate for 4-6 hours. Bound antibodies will be detected by

incubation with alkaline phosphatase-conjugated anti-IgG or anti-IgA reagents (Southern Biotechnology), followed by appropriate substrate development.

2. We will then perform western blots to identify gonococcal Ag bands detected by the Abs. For this purpose small-scale OMV preparations will be made from each isolate (by shearing cell suspensions in lithium acetate buffer and applying differential centrifugation as we have done previously (13), their protein concentrations will be determined by the Pierce bicinchoninic acid method (34). OMV (10-50µg protein) will be boiled in SDS sample buffer (with 2-mercaptoethanol), separated on SDS-polyacrylamide (10%) gels, blotted to nitrocellulose membranes and probed with suitably diluted serum or vaginal wash pools from immunized or control mice. Bound Ab will be detected using peroxidase-conjugated anti-IgG or anti-IgA reagents and chemiluminescence. A replicate gel will be stained with Coomassie blue to reveal the protein bands. Pre-stained MW markers will be included on each gel.
3. We will then test selected clinical isolates (up to 6, chosen at random, to include strains that do *not* react with the Ab in immunized mouse serum or vaginal wash, if any) in challenge-protection experiments. For this purpose, 2 groups (A and B) of 12 mice will be immunized i.vag. with GvaX12® containing OMV from strains FA1090, MS11 or FA19, as described above (Aim 1A). Two control groups (C and D) of 12 mice will be unimmunized. One month after immunization, one immunized and one control group (A and C) will be challenged i.vag. with the selected clinical isolate (transformed to be streptomycin-resistant by Dr. Hobbs), and the other two groups (B and D) will be challenged with the immunizing strain (FA1090/MS11/FA19). The course of infection will be monitored by vaginal swabbing and plating as described above. The recovery of gonococcal CFU and the numbers of mice remaining infected will be analyzed statistically by 2-way ANOVA and Kaplan-Meier methods, respectively, as described above. Each experiment will be repeated to verify the result.

Outcomes, potential difficulties, further experiments/alternatives, and significance/interpretation for Task 1B

We will first relate the ability of clinical isolates to react in ELISA with Ab in serum and vaginal washes of i.vag.-immunized mice to their porin and Opa types as determined by Dr. Hobbs. On the basis of limited information pertaining to the laboratory strains (FA1090, MS11, and FA19 are known to express different *porB* and *opa* genes), we do not expect to find any close concordance, but given that porin and Opa are major surface proteins, this will be examined. Examination of the sera and vaginal wash by western blots against OMV proteins should confirm lack of reactivity against bands corresponding to porin and Opa proteins, as noted already for FA1090, MS11, and FA19. However, caution is warranted because protein Ags are denatured in SDS-PAGE and may not be fully restored on blotting to nitrocellulose membranes, therefore any Ab reactive with them could be missed. An alternative approach would be to separate OMV proteins in non-denaturing gels for blotting; however the MW of the protein bands cannot be readily assigned and identification of the porin and Opa bands would be uncertain. It is also possible that the culture conditions used to prepare OMV, both for immunization and for Ab analysis, will affect the expression of various Ags, e.g., those regulated by iron or anaerobiosis. Thus it is important to grow *N. gonorrhoeae* under standard conditions; however, these conditions can be altered in light of findings to determine if, for instance, Ags selectively expressed under iron-limited or anaerobic conditions are relevant.

It is possible that Abs against lipooligosaccharide (LOS) contribute to protective immunity. Interest currently focuses on the 2C7 epitope which is expressed by >95% of clinical isolates under phase-variable control (35, 36). We will check the expression of 2C7 in the various strains of *N. gonorrhoeae*, using 2C7 Ab obtained from Dr. Peter Rice (University of Massachusetts; letter attached), to determine whether protective immunity and Ab reactivity accord with its expression. Otherwise, determining LOS type and structure is beyond the scope of the current proposal, and would require further collaborations with those who have the necessary expertise.

The more important question is whether i.vag. immunization with OMV from the laboratory strains induces protection against naturally occurring strains of *N. gonorrhoeae*. Although this question cannot be comprehensively assessed within this proposal, we will determine whether protection is shown against at least 6 randomly chosen clinical isolates, including any that do not recognize Ab in ELISA and/or western blot assays. If protection is dependent on vaccine-induced Ab that recognize the challenge strain, then any clinical isolates that fail to react with Ab should not be protected against, and a finding to the contrary would challenge the hypothesis. Conversely, if strains are found that are not protected against despite recognizing vaccine-induced Ab, this would negate the hypothesis that these Ab determine protective immunity. If we are to go

forward with a vaccine based on gonococcal OMV it will be important to know if there is a frequent occurrence of strains that are not protected against. If so, then a broader-based vaccine, perhaps incorporating OMV from multiple strains, or including selected additional Ags (as in the recently introduced 4CMenB vaccine against *N. meningitidis*) would become necessary.

If in Aim 1A it is found that i.n. immunization with GvaX12® results in protective immunity against homologous challenge (similar to that found with i.vag. immunization) and generates Ab (as detected by ELISA and western blotting against the homologous strain) it will be important to determine: 1) whether these Ab also recognize Ag in the clinical isolates; and 2) whether protective immunity against them is induced by this route of immunization. It is possible that different patterns of Ab reactivity may be induced by the two routes (because the nasal passages contain organized lymphoid tissue where mucosal immune responses are initiated, whereas such tissues are absent from the genital tract (30, 37)). If so, these differences could help elucidate the contribution of different Ab specificities to gonococcal immunity.

Task 1C**:** To determine whether immunization with GvaX12® containing detergent-extracted OMV induces protective immune responses against *N. gonorrhoeae*

A potential disadvantage of native OMV as a vaccine immunogen arises from reactogenicity caused by the presence of LOS. This effect has been observed with meningococcal OMV vaccines that are administered parenterally, although the mucosal routes of administration that we propose for our gonococcal OMV vaccine might prove less reactogenic. However, to forestall this potential problem, we will evaluate the ability of detergent-extracted gonococcal OMV (deOMV) to induce protection against *N. gonorrhoeae*, and to induce responses similar to those observed with native OMV.

For this purpose, we will modify our standard OMV (FA1090) preparation by extraction with 1% sodium deoxycholate in 1mM EDTA, 0.1M Tris buffer pH 8.5, followed by chromatography on a size-exclusion column (38). Mice will be immunized i.vag. with 2 doses (2-week interval) of GvaX12® containing deOMV (50µg protein) as described above. Control mice will be immunized with deOMV plus blank particles, and a comparison group of mice will be immunized with GvaX12® containing native OMV. Samples of serum and vaginal wash will be collected at 2 weeks after immunization for Ab assay. One month later mice will be challenged with *N. gonorrhoeae* FA1090, and the course of infection will be monitored by vaginal swabbing and plating, as described above. Further samples of serum, vaginal wash, and ILN will be collected at termination, for evaluation of Ab and cytokine responses, as described above.

We will compare protection against challenge, as well as Ab and cytokine responses induced by immunization with deOMV plus IL-12 particles with those induced by native OMV plus IL-12 particles, and with those induced by deOMV administered with blank particles. If the response to deOMV plus IL-12 particles is significantly less than that induced by native OMV plus IL-12 particles, we will repeat the experiment using increased doses of deOMV (e.g., 100-200µg protein), or by administering 3 or more immunizations. If i.n. immunization is successful with native OMV (Aim 1A above), we will further determine whether it works with deOMV.

Outcomes and significance for Task 1C

No difficulty is expected in performing experiments to evaluate the efficacy of deOMV as a vaccine immunogen in comparison with native OMV. On the basis of reports that meningococcal deOMV are less immunogenic than native OMV (attributed to the reduced LOS content), we may find that gonococcal deOMV are also less immunogenic; if so, we will determine whether increased dose or frequency of immunization can compensate adequately. The significance of successful results with deOMV may lie with diminished toxicity as revealed by the studies proposed in Aim 2 below

3.2.2 **Specific Aim 2:** Conduct studies of Rodent pharmacokinetics and reproductive toxicology

A. Task 2A: determine the bioavailability and pharmacokinetics of IL-12 following intravaginal and intranasal administration of GvaX12® in mice.

The objective of this study is to gain insight into the bioavailability, pharmacokinetics and potential acute toxicity of IL-12 following a single i.vag. or intranasal (i.n.) administration of GvaX12® or as a bolus intravenous (i.v.) dose of IL-12 to female mice. The study design is as follows:

Table 1 study design

Group	Test Item	Dose Route	Dose Level (mg/kg)	Dose Rate (mL/kg)	Dose Concentration (mg/mL)	No. of Animals Females
1	GvaX12® [1µg IL-12 + OMV (40µg protein)]	i.vag.	IL-12: 0.04 OMV protein: 1.6	1.6	IL-12: 25 OMV protein: 1.0	12
2	GvaX12® [1µg IL-12 + OMV (40µg protein)]	i.n.	IL-12: 0.04 * OMV protein: 1.6	1.6	IL-12: 25 OMV protein: 1.0	12
3	IL-12 [1µg] soluble	i.v.	0.04	4.0	0.01	12

* Dose of IL-12 given i.n. is based on i.vag. dose. If higher levels are found to be needed by this route (Specific Aim 1A), the dose used for these studies will be modified accordingly.

The following parameters and end-points will be evaluated in this study: clinical signs (i.e., hunched posture, ruffled fur, lethargy, loss of appetite, cool to touch), body weights, and toxicokinetic parameters (see below). Samples will be collected according to the following table:

Table 2 Schedule for Sample Collection

Group	Subgroup	No. of animals	Sample Collection Time Points (post start of dose)									
			5 min	15 min	30 min	1h	2h	4h	6h	12h	16h	24h
1	A	3	-	-	X	-	-	-	- X^T	-	-	-
	B	3	-	-	-	X	-	-	-	X^T	-	-
	C	3	-	-	-	-	X	-	-	-	X^T	-
	D	3	-	-	-	-	-	X	-	-	-	X^T
2	A	3	X	-	-	-	-	X^T	-	-	-	-
	B	3	-	X	-	-	-	-	X^T	-	-	-
	C	3	-	-	X	-	-	-	-	X^T	-	-
	D	3	-	-	-	X	-	-	-	-	-	X^T
3	A	3	X	-	-	-	-	X^T	-	-	-	-
	B	3	-	X	-	-	-	-	X^T	-	-	-
	C	3	-	-	X	-	-	-	-	X^T	-	-
	D	3	-	-	-	X	-	-	-	-	-	X^T

X = Sample collected via saphenous vein puncture ; X^T = Sample collected *via* terminal bleed on anesthetized mice from the inferior vena cava or subclavian vein;
- = Not applicable.

Blood will be collected into serum separation tubes (SST) tubes by saphenous vein puncture (target of 0.1 mL) or *via* the abdominal aorta (maximum possible volume) while under isoflurane anesthesia (terminal samples only). An additional terminal blood sample (target volume up to 1 mL) will be collected of 1 spare animal and used as a pre-dose sample. Samples will be frozen immediately over dry ice and transferred to a freezer set to maintain -20°C until use. Levels of IL-12 will be measured by ELISA. T_{max}, (time of maximum serum concentration), $T_{½}$ (half-life), C_{max} (maximum serum concentration), $C_{max/D}$ (maximum serum concentration divided by dose) and AUC (area under the curve) will be calculated. Levels of other pro and anti-inflammatory cytokines (e.g., IFNγ, TNFα, IL-10 and TGFβ) will also be measured.

Outcomes, potential difficulties, alternatives and significance For Task 2A

The primary outcome measure for the pharmacokinetic study will be the circulating level of IL-12 over time, according to the schedule in Table 2. Secondary measures include circulating levels of IFNγ. The absolute bioavailability will be calculated using the dose-corrected area under curve (AUC) for non-intravenous administration divided by the AUC for intravenous administration, according to classical pharmacology modeling. However, based on our previous experience we do not expect significant systemic exposure of IL-12 by either the i.n. or i.vag. route. Because the generation of IFNγ is a well-known effect of IL-12, we expect systemic IL-12 exposure also will result in increased IFNγ. The results will inform starting doses for subsequent IND enabling non-clinical studies in rodents.

No technical difficulties are anticipated in the performance of these studies. All the methods are in place and have been used previously. Extensive experience with GvaX12® administered i.vag. in doses up to 2µg of cytokine per mouse given at 2-day intervals for up to 5 doses, revealed no adverse effects. Whether similar doses given intranasally will result in adverse or toxic effects is unknown. GvaX12® is not designed for intravenous administration; however, standard pharmacokinetic and toxicological evaluations require comparisons with this route. Mice will be inspected at least twice daily for the following signs: hunched posture, ruffled fur, lethargy, loss of appetite, coolness to touch, and body weight. Animals displaying signs of distress after GvaX12® administration will be reported and monitored closely. A combination of 2 or more signs without evidence of recovery within 12h, or >10% loss of body weight, will be recorded as adverse reaction and the affected animals will be euthanized.

B. Reproductive toxicology studies in rats.

IL-12 is a pro-inflammatory cytokine. Because the proposed vaccine may be used in women of child-bearing age, reproductive toxicity is a concern. Therefore, early reproductive toxicology testing is proposed. Studies, including a GLP embryo-fetal development study and a study for effects on pre- and post-natal development in rats will be conducted at Comparative Biosciences. Complete study designs can be found in Appendix 1. If the i.n. route is found to be effective, reproductive toxicology will be delayed until our type C meeting (see below).

Outcomes, potential difficulties, and alternatives for Task 2B

GvaX12® might induce untoward effects at the proposed dose levels. However, a major goal of encapsulation is to limit systemic exposure and side effects. Therefore, we believe such a finding is unlikely. However, significant reproductive toxicity would trigger a no-go decision for the continued development of this product. We have identified several back-up drug substances as substitute for IL-12 (e.g., anti-IL-10 antibody), which are under development separately.

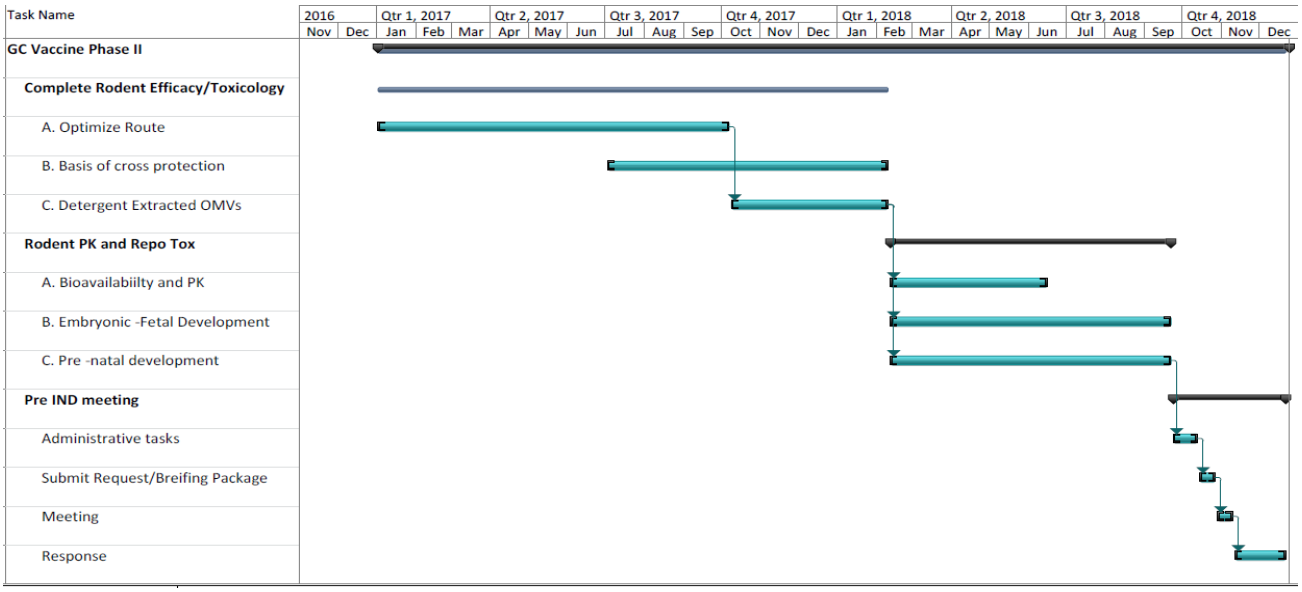
3.2.3 Specific Aim 3: To prepare and submit a briefing package, written questions and formal request for a pre-pre-IND (Type C) meeting with the FDA

The successful accomplishment of Aims 1 and 2 will incentivize and prioritize the development of our prophylactic gonorrhea vaccine. The early input of the FDA will be invaluable in guiding our IND enabling scale-up and toxicology study designs. Regulatory Professionals is already assisting TherapyX^{inc} in the preparation and submission of GvaX12® IND-related materials. The master services agreement and SOW related to the proposed studies is included in Appendix 2. The consultants at Regulatory Professionals have extensive expertise in the preparation of briefing packages, meeting requests, clinical (IND) and marketing applications for various drug formulations, including biologics and polymeric drug delivery systems. A briefing package and meeting request will be prepared and submitted along with specific questions in the areas of clinical, nonclinical, and Chemistry, Manufacturing and Control (CMC). The aim is to confirm FDA requirements for data to support Phase I/II clinical studies, primarily with respect to safety. Ultimately, the questions will be determined by the data collected from the studies conducted in Aims 1 and 2 and by our discussion with toxicology consultants at Regulatory Professionals.

Outcomes, potential difficulties, and significance

The FDA encourages investigators to initiate contact with the agency as early in the drug development process as possible. The stated goal is to allow investigators the opportunity to consider FDA recommendations in planning preclinical and clinical development programs. TherapyX^{inc} has recently completed a pre-pre-IND (Type C) meeting with the FDA for a separate project involving oral administration of TGFβ-loaded particles (TGFβNanoCap®). The preparation for this meeting included the creation of a briefing package which included specific questions requesting the FDA's opinion of the company's proposed Non-Clinical (toxicology), Quality (CMC), and Clinical (early clinical trial design) study plans. We expect that the preparation of the briefing package for this program will follow the same developmental path. If circumstances dictate, additional consulting expertise can be obtained. We do not expect there to be any difficulties that cannot be overcome with the assembled team of advisors.

TIMELINE


Task Name
2016
Qtr 1, 2017
Qtr 2, 2017
Qtr 3, 2017
Qtr 4, 2017
Qtr 1, 2018
Qtr 2, 2018
Qtr 3, 2018
Qtr 4, 2018
Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
GC Vaccine Phase II
Complete Rodent Efficacy/Toxicology
A. Optimize Route
B. Basis of cross protection
C. Detergent Extracted OMVs
Rodent PK and Repo Tox
A. Bioavailabiilty and PK
B. Embryonic -Fetal Development
C. Pre -natal development
Pre IND meeting
Administrative tasks
Submit Request/Breifing Package
Meeting
Response

VERTEBRATE ANIMALS

TherapyX^{Inc}. has an inter-institutional agreement in place with the University at Buffalo (UB) regarding these and other animal studies. All *in vivo* murine studies will be performed in the Laboratory Animal Facilities of the Jacobs School of Medicine and Biomedical Sciences at UB (Animal Welfare Assurance Number A3354-01). The care and use of laboratory animals at UB is in accordance with the principles and standards set forth in the Principles for Use of Animals (NIH Guide for Grants and Contracts), the Guide for the Care and Use of Laboratory Animals (DHEW, PHS, NIH Publ. No. 80-23, Rev 1978), the provision of the Animal Welfare Acts (P.L. 89-544 and its amendments), NYS laws for animal welfare and other applicable laws and regulations. Compliance is validated by the UB IACUC and regular inspections by USDA and NYS inspecting veterinarians. Assurances and the composition of the Institute Animal Care and Use Committee are on file with the Office of Protection from Research Risks, Office of the Director, NIH.

1. Proposed use of animals

Inbred wild-type mice (BALB/c) of 2-6 months age at the start and of female sex will be used. Small volumes of blood (50-100µl) will be obtained from the saphenous vein, or from the retro-orbital sinus under anesthesia. Larger volumes of blood will be collected as a terminal procedure under anesthesia. Spleens, genital tracts, lymph nodes, and other organs will be obtained from mice killed by cervical dislocation under anesthesia. IACUC approval for these studies is in place (protocol # MIC19074Y). For the gonococcal vaginal challenge model, 2-month old female mice will be injected subcutaneously with 0.5mg of 17-β estradiol (Premarin) on days –2, 0, and +2 relative to gonococcal challenge, then injected once daily i.p. with streptomycin sulfate (1.2mg) and vancomycin HCl (0.6mg), and provided drinking water containing trimethoprim sulfate (0.04g/100ml). On day 0, the mice are inoculated vaginally with *N. gonorrhoeae* (approx. $5x10^6$ CFU) suspended in PBS containing 0.5mM $CaCl_2$, 1mM $MgCl_2$, and 1% gelatin. Colonization is monitored daily for up to 14 days (or until the infection is cleared) by vaginal swabbing and plating. Animals are then euthanized and terminal blood samples and tissues harvested for laboratory investigation. These experiments will be performed in a separate BSL2 suite, and used bedding, cages, and wastes will be autoclaved or disinfected prior to disposal or re-use. Carcasses will be treated as infectious waste.

2. Justification

Mice are chosen for *in vivo* investigation of immune responses to *N. gonorrhoeae* because they constitute a well-established model for immunological studies, there is considerable background information on their immune systems, and a wide range of high-quality immunochemical and cellular reagents is available. It is not possible to investigate immune responses to infection other than by means of a live animal infection model, and the mouse vaginal gonococcal challenge model is the only available model of genital gonococcal infection. Human infection studies of this nature would be unethical. In-bred animals are required for *in vitro* cell culture experiments. Groups of animals used for gonococcal challenge (12) are as small as possible consistent with yielding statistically valid data.

Anticipated mouse numbers:

Year 1

Aim 1A. To determine an optimal route of immunization: Intravaginal vs Intranasal.

We will compare the efficacy of optimized intravaginal dose of GvaX12®+ OMVs with the intranasal route. Groups will consist of 12 mice.

1. Intra-vaginal route: GvaX12® + OMVs (12 BALB/c)
2. Intra-nasal route: GvaX12® + OMVs (12 BALB/c)
3. Sham intra-vaginal (12 BALB/c)
4. Sham intra nasal (12 BALB/c)
5. Blank (control) microparticles + OMVs intranasal (12 BALB/c)
6. GvaX12® alone intranasal (12 BALB/c)

Six groups X 12 per group = 72 X up to 3 repeats **= 216** BALB/c mice

Aim 1B. To determine the basis of cross-protective immunity against antigenically diverse clinical isolates of N. gonorrhoeae.

We will: (I) determine whether cross-reactivity of Ab and associated cross-protection against challenge extends to antigenically diverse, minimally passaged clinical isolates of N. gonorrhoeae; and (II) identify by means of proteomics technologies the gonococcal Ags detected by Abs induced in mice immunized with gonococcal OMV plus GvaX12®

For each clinical isolate examined:
1. Groups A and B immunized with FA1090/MS11/FA1 (24 BALB/c mice)
2. Groups C and D Unimmunized mice (24 BALB/c mice)

Four groups x 12 mice per group = 48 mice x up to 2 repeats = 96 BALB/c mice
X 6 clinical isolates to be examined = **576 mice**

Total Mice in Year 1…. 216 + 576 = up to 792 mice

Year 2

Aim 1C. To determine whether immunization with detergent-extracted OMV (plus GvaX12®) induces protective immune responses against *N. gonorrhoeae*

Mice will be immunized by the intravaginal route with detergent-extracted OMV + GvaX12® and challenged with the homologous gonococcal strain. Groups will consist of 12 mice

1. GvaX12® + deOMV (12 BALB/c)
2. Blank ms + de OMV (12 BALB/c)
3. GvaX12® + native OMV (12 BALB/c)

Three groups x 12 mice per group = 36 mice, x up to 6 repeats with doses adjusted as necessary = **216 mice**

Repeat experiments using intranasal immunization (if successful) = **216 mice**

Aim 2A. Determine the bioavailability and pharmacokinetics of IL-12 following intravaginal/intranasal administration of IL12/ms vaccine in mice.

To goal of this aim is to obtain preliminary data on bioavailability and pharmacokinetics of IL-12 following a single intravaginal/intranasal or bolus intravenous dose of IL-12 to BALB/c mice

1. Group 1 intra-vaginal (12 BALB/c)
2. Group 2 intra-nasal (12 BALB/c)
3. Group 3 soluble IL-12 given intravenously (12 BALB/c)

Three groups x 12 mice per group = 36 mice x up to 2 repeats = **72 BALB/c mice**

Total in Year : 216 + 216 + 72 = 604 mice

Aims 2B and 2C. Reproductive Toxicity in rats.

If necessary (i.e., if we need to use intra-vaginal administration), we will conduct routine reproductive toxicology, arriving at a potentially no-go decision node. This work will be subcontracted to Comparative Biosciences and completed under their IACUC (see Appendix 3 for details).

Aim 2B: Embryonic-Fetal Development Study

109 Sprague Dawley rats will be used at Comparative Biosciences, Sunnyvale, CA, to investigate potential effects on embryonic-fetal development. These are standard animals and standard numbers required for these routine reproductive toxicity assessments (See Appendix 3).

Aim 2C Effects on pre and post-natal development

260 Sprague Dawley rats will be used at Comparative Biosciences, Sunnyvale, CA, to investigate potential effects on pre- and post-natal development. These are standard animals and standard numbers required for these routine reproductive toxicity assessments (See Appendix 3).

Total: 109 + 260 **= 369 animals**

3. **Veterinary care and facilities**

The UB Laboratory Animal Facility is directed by full-time professional veterinarians and staffed by trained, qualified personnel, all of whom may be called upon for advice and assistance at any time. The facility is fully accredited by the American Association for the Accreditation of Laboratory Animal Care, and complies with NIH policy, the Animal

Welfare Act, and all applicable federal, state, and local laws. Mandatory training is provided for all laboratory and scientific personnel who are involved in procedures. Veterinary staff at UB include Dr. Lisa Martin, Director, Dr. Sasha Black, Clinical Veterianrian, and Dr. Jennifer Peirick, Clinical Veterinarian.

4. Provisions for animal welfare

Mice will be housed and maintained according to the prescribed standards for this species, under the supervision of the UB Laboratory Animal Facility. For blood collection from the saphenous vein or retro- orbital sinus, mice will be anesthetized by inhalation of isofluorane for 1 minute. For terminal bleeding from the subclavian vein, mice will be anesthetized with xylazine plus ketamine (1.5mg/100g and 10mg/100g, respectively, i.p.) and the animals will be killed (by cervical dislocation) while under anesthesia.

5. **Euthanasia**

Mice will be killed at the termination of experiments by cervical dislocation under anesthesia, or by CO_2 asphyxiation, or by overdose of sodium pentobarbital injected i.p. All of these are consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

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Department of Medicine
Division of Infectious Diseases
August 22, 2016

Dr. Yingru Liu
Senior Research Scientist
TherapyX Inc.
Farber 138
3435 Main St.
Buffalo, NY 14214

Dear Yingru,

I am pleased to continue my enthusiastic support of your work with Dr. Michael Russell at the University of Buffalo in preparation for the new SBIR Phase II grant application entitled "Experimental Gonococcal Vaccine." As you know, the development of new treatment and immunoprophylaxis strategies for genital tract infection with *Neisseria gonorrhoeae* is urgently needed, as increasing gonococcal antimicrobial resistance threatens the continued utility of current antibiotic treatments. Your preliminary success with administration of IL-12 microspheres during primary infection with *N. gonorrhoeae* strain FA1090 and protection against re-infection with the homologous strain, well-characterized lab strains and more recent clinical isolates from the archives of our UNC strain collection is very encouraging.

For a subcontract in the new grant, I agree to provide up to 30 clinical isolates of *N. gonorrhoeae* from a variety of geographic locations to screen for reactivity with serum and vaginal antibodies from vaccine-protected mice. In addition, we will transform up to 6 strains in my laboratory to streptomycin resistance for use in the murine model of female genital tract infection. Should your studies lead to the development of a product for Phase I clinical trial studies in humans, you know that we will be anxious to collaborate using the experimental human infection model of gonococcal urethritis, which is up and running here at the University of North Carolina at Chapel Hill.

Here's wishing you and Mike and your colleagues good luck with the grant. I look forward to tracking your continued success demonstrating the potential for a gonococcal vaccine, at long last!

Sincerely,

[redacted]
Marcia M. Hobbs, Ph.D.
Professor of Medicine, Microbiology & Immunology and Allied Health Sciences

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BROWN

DEPARTMENT OF MOLECULAR PHARMACOLOGY,
PHYSIOLOGY, AND BIOTECHNOLOGY

August 8th, 2015

Dominick L. Auci, Ph.D.
Vice President Research and Development
TherapyX Inc

Dear Dr. Auci,

I am happy to provide advice and technical support with regard to the preparation and characterization of PIN microspheres for the studies outlined in your NIAID SBIR phase II application entitled "*Experimental Gonococcal Vaccine*."

I have been working in the area of drug delivery, particularly with bioerodable oral polymeric particles for the delivery of biological macromolecules and small molecule drugs, for the past 3 decades. The Phase Inversion Nanoencapsulation technology was invented in my laboratory and has been tested for efficacy in numerous mammalian models over the past decade.

I believe the proposed studies represent a very exciting opportunity to bring the PIN particle technology closer to the clinic. I am looking forward to continuing our productive collaboration with TherapyX Inc. and Dr. Egilmez.

Sincerely yours,

[redacted]

Edith Mathiowitz, Ph.D.
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COMPARATIVE BIOSCIENCES, INC.
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH
786 Lucerne Drive . Sunnyvale, California 9408 . telephone: 408-738-9260 . fax: 408-738-9278

August 21st, 2016

Dr. Dominick Auci, MBA, Ph.D.
Vice President, Research and Development
TherapyX^Inc
138 Farber Hall
3435 Main Street
Buffalo, NY 14214

Dear Dr. Auci,

Re: "*Experimental Gonococcal Vaccine*."

Dear Dr. Auci,

Please accept this letter as indication that Comparative Biosciences, Inc. is committed to work on a fee for service basis with TherapyX^Inc. as indicated in the grant application to support the development of nanoencapsulated IL-12, Comparative Biosciences, Inc, Sunnyvale, CA is pleased to offer you assistance with your rodent toxicology and safety studies as you develop your therapeutic drug delivery system. Work on the referenced application will be valid for the duration of the grant award provided that the actual work is in compliance with the approved research proposal

CBI is a preclinical contract research organization with nonclinical safety assessment location in Sunnyvale, CA that delivers comprehensive drug development and toxicology services. We will support this grant application by providing preclinical safety and toxicology testing. Comparative Biosciences, Inc. has a nearly twenty-year history of providing assistance to companies performing preclinical studies. Included among the members of our scientific staff are eight individuals with doctoral degrees, two board-certified pathologists, a laboratory animal veterinarian and two ophthalmologists also; and we have a full time, independent quality assurance unit. We provide state of the art toxicology, pharmacokinetics, pharmacology, and efficacy studies, particularly in the area of wound healing, repair and dermal burns. Our histopathology laboratory provides GLP and research histology and pathology, including special stains, immunohistochemistry, *in situ* hybridization, and antibody cross reactivity studies. We specialize in working with small innovative companies but also work with larger drug companies and academic organizations.

We have a fully equipped rodent and large animal facility that will be an excellent asset as you pursue your studies. (OLAW Assurance No. A4269-01). As you know,

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your individual protocols will be reviewed by our duly constituted IACUC committee and receive its approval before your actual study can commence. Our IACUC has in place a duly approved IACUC proposal your studies. There are separate rodent and large animal holding rooms and a fully equipped surgery room and general procedure rooms, separate necropsy, and clean/dirty corridors with a pass through cage washer and autoclave as well as toxicology-related specialty equipment. We are AAALAC accredited, registered with the USDA, FDA, OLAW, have a duly constituted IACUC, and comply with the NIH Guide. Procedure rooms are completely equipped with gas anesthesia, surgical microscope and oxygen chamber. Comparative Biosciences, Inc. occupies a 16,000 sq ft facility in Sunnyvale, California. The facility includes an 8100 sq ft vivarium and a 1500 sq ft histology laboratory, with the balance dedicated to support functions (offices, conference rooms, archive room, etc). The vivarium includes a surgery, procedure room, gowning room, cage washing and service area, and 10 animal rooms. All histopathology is performed on-site, including a variety of special stains, immunohistochemistry, cryosectioning, plastic and paraffin embedding and sectioning, morphometry.

CBI is very experienced in the preparation of animals for toxicology studies. Our histology laboratory has considerable expertise in the preparation of burn tissue specimens, immunohistochemistry, histomorphometry, histopathologic assessments, photomicroscopy and report preparation.

We thank you for your interest in our facility and look forward to working with you on this project.

With kindest regards,

Carol Meschter, DVM, PhD, DACVP
Institutional Official
CEO
Comparative Biosciences, Inc.

RPI Regulatory Professionals, Inc.

August 8, 2016

Dr. Dominick Auci, MBA, Ph.D.
Vice President, Research and Development
TherapyX^{inc}
138 Farber Hall
3435 Main Street
Buffalo, NY 14214

Dear Dr. Auci,

Regulatory Professionals, Inc. (RPI) is happy to express its commitment to TherapyX^{inc} as indicated in their phase II SBIR application entitled " Experimental Gonococcal Vaccine."

RPI is happy to continue its long-standing relationship with TherapyX^{inc} that has included support for its other programs, as well as on-going assistance on this product aimed at genital tract infection. We will continue to provide consultation services and strategic assistance with associated toxicology program design, CMC (chemistry, manufacturing and controls) strategies, as well as preparation of type C meeting briefing packages and related questions in preparation for IND submission.

RPI is well qualified to provide the services as indicated in this phase II application. RPI is an experienced team of regulatory professionals dedicated to helping our clients advance the development and registration of their products with worldwide health authorities (US, Canada, EU and Japan). Our focus is to provide strategic and tactical support in all aspects of regulatory affairs, including nonclinical, CMC and clinical development for drugs, biologics, devices and combination products. Additionally, RPI has extensive knowledge base in the areas of regulatory affairs, electronic submission preparation, GXP compliance, technical and medical writing, advertising/promotional labeling.

We here at RPI are very excited about the potential of your vaccine and look forward to continuing working with TherapyX^{inc}.

[redacted]
President and CEO
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