PI: [redacted]	Title: [redacted]	
Received: 10/12/2009	FOA: PA09-043	Council: 05/2010
Competition ID: ADOBE-FORMS-A	FOA Title: MENTORED PATIENT-ORIENTED RESEARCH CAREER DEVELOPMENT AWARD (K23)	
[redacted]	Dual: AI	Accession Number: 3233857
IPF: [redacted]	Organization: [redacted]	
Former Number:	Department: Pediatrics, Neonatology [redacted]	
IRG/SRG: CHHD-A	AIDS: N Animals: N Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N	Expedited: N New Investigator: N Early Stage Investigator: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 132,390 Year 2: 133,140 Year 3: 133,912 Year 4: 134,708 Year 5: 135,528		

Senior/Key Personnel:	*Organization:*	*Role Category:*
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Reference Letters

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
10/12/2009	[redacted]
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

☐ Pre-application ☐ Application ☒ Changed/Corrected Application

4. Federal Identifier [redacted]

5. APPLICANT INFORMATION

*** Organizational DUNS:** [redacted]

* Legal Name: [redacted]

Department: [redacted] Division:

* Street1: [redacted]

Street2: [redacted]

* City: [redacted] County: [redacted]

* State: [redacted] Province:

* Country: [redacted] * ZIP / Postal Code: [redacted]

Person to be contacted on matters involving this application

Prefix: * First Name: [redacted] Middle Name:

* Last Name: [redacted] Suffix:

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

Email: [redacted]

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

7. * TYPE OF APPLICANT: [redacted]

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: [redacted]

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE: [redacted]

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT: [redacted]

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)* [redacted]

13. PROPOSED PROJECT:

* Start Date: 07/01/2010 * Ending Date: 06/30/2015

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant: 15 b. * Project: 15

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: * First Name: [redacted] Middle Name:

* Last Name: [redacted] Suffix:

Position/Title: [redacted]

* Organization Name: [redacted]

Department: [redacted] Division:

* Street1: [redacted]

Street2:

* City: [redacted] County: [redacted]

* State: [redacted] Province:

* Country: [redacted] * ZIP / Postal Code: [redacted]

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

* Email: [redacted]

OMB Number: [redacted]

Expiration Date: 04/30/2008

[redacted] [redacted]

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	723,252.00
b. * Total Federal & Non-Federal Funds	723,252.00
c. * Estimated Program Income	0.00

17. * 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

18. 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.***

19. Authorized Representative

Prefix: * First Name: [redacted] Middle Name: R

* Last Name: [redacted] Suffix:

* Position/Title: [redacted]

* Organization: [redacted]

Department: [redacted] 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]

*** Signature of Authorized Representative** [redacted]

*** Date Signed** 10/12/2009

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

21. Attach an additional list of Project Congressional Districts if needed.

Add Attachment | Delete Attachment | View Attachment

OMB Number: [redacted]
Expiration Date: 04/30/2008

[redacted] [redacted]

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

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: [redacted]

* Street1: [redacted]

Street2: [redacted]

* City: [redacted] County: [redacted]

* State: [redacted] Province:

* Country: [redacted] * ZIP / Postal Code: [redacted]

Project/Performance Site Location 1

Organization Name:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: * ZIP / Postal Code:

Additional Location(s) Add Attachment Delete Attachment View Attachment

OMB Number: [redacted]
Expiration Date: 04/30/2008

RESEARCH & RELATED Other Project Information

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

1.a If YES to Human Subjects

Is the IRB review Pending? ☒ Yes ☐ No

IRB Approval Date:

Exemption Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

Human Subject Assurance Number: 00002636

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

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

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

4.b. If yes, please explain:

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

4.d. If yes, please explain:

5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ☐ Yes ☒ No

5.b. If yes, identify countries:

5.c. Optional Explanation:

6. * Project Summary/Abstract abstract.pdf Add Attachment | Delete Attachment | View Attachment

7. * Project Narrative projplan.pdf Add Attachment | Delete Attachment | View Attachment

8. Bibliography & References Cited ref.pdf Add Attachment | Delete Attachment | View Attachment

9. Facilities & Other Resources Facilities_Upload.pdf Add Attachment | Delete Attachment | View Attachment

10. Equipment Major_Equipment_Upload.pdf Add Attachment | Delete Attachment | View Attachment

11. Other Attachments Add Attachments | Delete Attachments | View Attachments ☒

OMB Number: [redacted]
Expiration Date: 04/30/2008

Project Summary

[redacted] whose current investigative efforts are directed towards elucidating the pathogenic mechanisms underlying the development of bacterial vaginosis (BV). BV, the most common vaginal infection worldwide, is associated significant adverse sequelae including preterm birth and acquisition of sexually transmitted diseases. The association of BV with the onset of preterm labor is of major public health importance as BV has been estimated to cause 90,000 excess preterm births per year. Recently, maternal vitamin D deficiency has been linked to the development of BV during the first trimester of pregnancy. Vitamin D is an important regulator of innate immune response and deficiencies of this critical vitamin have been associated with an increased susceptibility to other infectious diseases. The role of vitamin D in the pathogenesis of BV may be of particular relevance in explaining the striking racial disparities in the prevalence of BV. African-American populations, which are at highest risk for vitamin D-deficiency, also have the highest prevalence of BV and BV-associated preterm birth. Identification of potentially modifiable risk factors for BV represents a unique opportunity to reduce the burden of this exceedingly prevalent disease and its associated morbidities. These observations have led the candidate to formulate the primary hypothesis of this research proposal: that vitamin D deficiency is a significant risk factor for the development of BV in non-pregnant, healthy adult women and that this effect is mediated by a deficiency of Vitamin D-responsive immune mediators. The primary aims of this proposal are 1) to investigate the role of vitamin D status in the development of BV in a cohort of non-pregnant, healthy adult women, and 2) to explore specific molecular mechanisms by which vitamin D signaling controls vaginal innate immune responses relevant to BV.

This research proposal represents a critical step in attaining the candidate's long-term goal: to transition to an independent research career focused on mechanistic and translational studies of preterm birth. Superb mentorship, participation in the Master's program in Patient-Oriented Research**,** and continued access to the abundant resources and rich intellectual environment across departments and schools at [redacted] leave this candidate well-positioned to achieve this goal. This Career Development Award will serve as a driving force in [redacted] success, providing the protected time and additional training required for this candidate to become an independent investigator.

Project Narrative

Bacterial vaginosis is the most common vaginal infection worldwide, and is associated with significant adverse sequelae, including preterm birth and acquisition of sexually transmitted diseases. This proposal is designed to explore the hypothesis that vitamin D deficiency is a significant risk factor for the development of bacterial vaginosis in non-pregnant, healthy adult women. Identification of potentially modifiable risk factors for bacterial vaginosis represents a unique opportunity to reduce the burden of this exceedingly prevalent disease and its associated morbidities.

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Facilities and Other Resources

Laboratory: The recently reconstructed (June 2006) laboratory is more than 400 sq. ft. of dedicated space in the [redacted], which also houses the research laboratories of the Departments of Pediatrics, Medicine, Physiology, Biochemistry, and others. The proximity of laboratories from several departments as well as the joint appointment and active involvement of [redacted] in the Department of Microbiology make this a fertile environment for collaboration. The lab contains all of the equipment necessary for molecular biology, bacteriological studies, tissue culture (dedicated laminar flow hood and CO2 incubators in the lab), and microscopy (upright, inverted, and stereoscopic microscopes) and shares adjacent core equipment space (including dark room facilities, newly purchased Tecan fluorescent microplate reader, ultracentrifuge, and flow cytometry). Additional basic equipment within the lab includes freezers (-80°C, -20°C), chromatography refrigerator (+4°C), refrigerated tabletop centrifuge and microcentrifuges, laminar flow hood and two CO2 incubators, SDS-PAGE/Western blot apparatus, agarose gel equipment and UV transilluminator with digital imaging system, microplate reader for ELISA, spectrophotometer, balances, pH meter, water baths, nutator, and incubator/shaker.

Computer: Ample computer facilities are present in the laboratory for the proposed work (including 4 networked Intel-based Macintosh computers and 2networked Pentium 4 PCs with up to date bioinformatics and image processing software, color laser printer, and scanner).

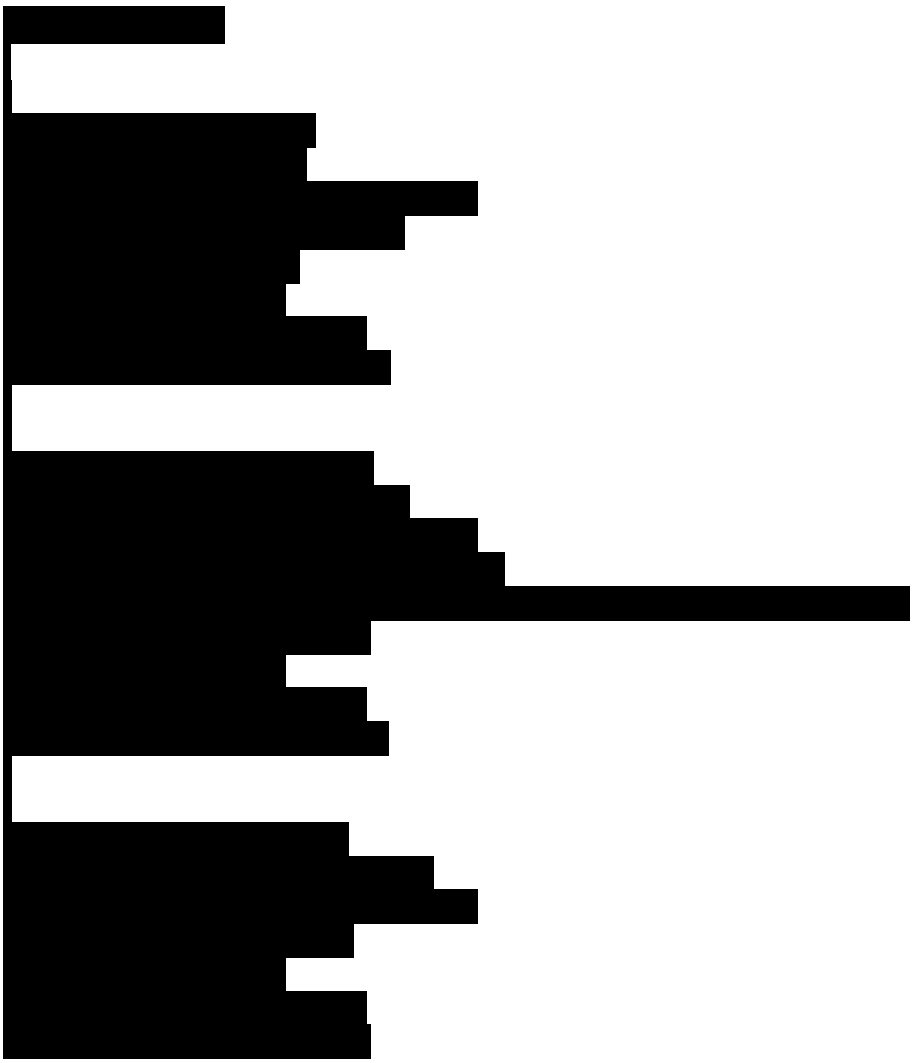
Office: All investigators and mentors are equipped with private offices on site. [redacted] office space is on the same floor as the laboratory.

Clinical: A cohort of non-pregnant, healthy adult women presenting for primary care at the [redacted] gynecology clinics will be recruited for this study. The extent of contact with the subject will be a single visit for the specimen collection.

Other: The core facilities at [redacted] are outstanding, including microarray and protein chemistry cores, the Genome Center, and the Irving Institute for Clinical and Translational Research (a[redacted]) which has core biostatistics, biomarker/sample storage, planning, regulatory, and execution support for translational research at [redacted]. The Division of Statistical Genetics has experience with large, SNP-based investigations and will be an active collaborator in this endeavor.

EQUIPMENT

In addition to standard equipment, the [redacted] laboratory has a dedicated real-time qPCR system (ABI StepOne Plus) as well as multiple conventional and gradient thermal cyclers (Eppendorf). There is an Agilent BioAnalyzer 2100 with DNA, RNA, and protein analysis capability within the laboratory as well as an Amaxa Nucleofector II for electroporation of eukaryotic cells. The laboratory has a new (purchased in 2007) dedicated system for live-cell fluorescent imaging (Zeiss AxioObserver Z1) with 10x, 20x, 40x (long working-distance), and oil-emersion 63x fluorescent objectives. This inverted microscope has a motorized z-axis, a grid array (Apotome) system, heated stage, CCD-based and high-speed CMOS cameras, and state-of-the-art imaging software (Axiovision) running on a dedicated high-speed computer.



RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: | * First Name: [redacted] | Middle Name:

* Last Name: [redacted] | Suffix:

Position/Title: [redacted] | Department: [redacted]

Organization Name: [redacted] | Division:

* Street1: [redacted]

Street2:

* City: [redacted] | County: [redacted]

* State: [redacted] | Province:

* Country: [redacted] | * Zip / Postal Code: [redacted]

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

* E-Mail: [redacted]

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

*** Project Role:** [redacted] | **Other Project Role Category:**

***Attach Biographical Sketch** [redacted] Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 1

Prefix: | * First Name: [redacted] | Middle Name:

* Last Name: [redacted] | Suffix:

Position/Title: | Department: [redacted]

Organization Name: [redacted] | Division:

* Street1: [redacted]

Street2:

* City: [redacted] | County: [redacted]

* State: [redacted] | Province:

* Country: [redacted] | * Zip / Postal Code: [redacted]

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

* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Other (Specify) | **Other Project Role Category:** Co-Mentor

***Attach Biographical Sketch** [redacted] Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support [redacted] Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: | * First Name: [redacted] | Middle Name: [redacted]

* Last Name: [redacted] | Suffix:

Position/Title: [redacted] | Department: [redacted]

Organization Name: [redacted] | Division:

* Street1: [redacted]

Street2: [redacted]

* City: [redacted] | County: [redacted]

* State: [redacted] | Province:

* Country: [redacted] | * Zip / Postal Code: [redacted]

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

* E-Mail: [redacted]

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

*** Project Role:** Other (Specify) | **Other Project Role Category:** Mentor

***Attach Biographical Sketch** [redacted] Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support [redacted] Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 3

Prefix: | * First Name: [redacted] | Middle Name: [redacted]

* Last Name: [redacted] | Suffix:

Position/Title: | Department: [redacted]

Organization Name: [redacted] | Division:

* Street1: [redacted]

Street2:

* City: [redacted] | County: [redacted]

* State: [redacted] | Province:

* Country: [redacted] | * Zip / Postal Code: [redacted]

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

* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Other (Specify) | **Other Project Role Category:** co-mentor

***Attach Biographical Sketch** [redacted] Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support [redacted] Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4

Prefix: | * First Name: [redacted] | Middle Name: [redacted]

* Last Name: [redacted] | Suffix:

Position/Title: | Department: [redacted]

Organization Name: [redacted] | Division:

* Street1: [redacted]

Street2:

* City: [redacted] | County: [redacted]

* State: [redacted] | Province:

* Country: [redacted] | * Zip / Postal Code: [redacted]

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

* E-Mail: [redacted]

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

*** Project Role:** Other (Specify) | **Other Project Role Category:** Co-Mentor

***Attach Biographical Sketch**	[redacted]	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support	[redacted]	Add Attachment	Delete Attachment	View Attachment

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)		Add Attachment	Delete Attachment	View Attachment
Additional Biographical Sketch(es) (Senior/Key Person)		Add Attachment	Delete Attachment	View Attachment
Additional Current and Pending Support(s)		Add Attachment	Delete Attachment	View Attachment

OMB Number: [redacted]
Expiration Date: 04/30/2008

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
[redacted]	[redacted]
eRA Commons User Name: [redacted]	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

C. Research Support

Ongoing

Division of Neonatology/Perinatology, [redacted]
The division has committed both financial support (salary and start-up funding for 3 years) as well as research time (85%) free from clinical responsibilities.

Completed

[redacted] **Young Investigator Grant** [redacted]
[redacted]
This project examined neurologic outcomes following resuscitation using either 100% oxygen or room air in asphyxiated neonatal mice.

NIH Pediatric Research Loan Repayment Award [redacted]
[redacted] oxygen or room air in asphyxiated neonatal mice
[redacted].
This study investigated the hypothesis that PI3K signaling regulates neutrophil migration into inflamed neuronal tissues following hypoxic-ischemic injury in neonatal mice.

Principal Investigator/Program Director (Last, First, Middle):

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
[redacted]	[redacted]
eRA COMMONS USER NAME	
[redacted]	

EDUCATION/TRAINING: INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

B.

1) [redacted]ron over [redacted]

9: 792-796, 2007.

Principal Investigator/Program Director (Last, First, Middle): [redacted]

[redacted]

Principal Investigator/Program Director (Last, First, Middle): [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
[redacted]	[redacted]
eRA COMMONS USER NAME	
[redacted]	

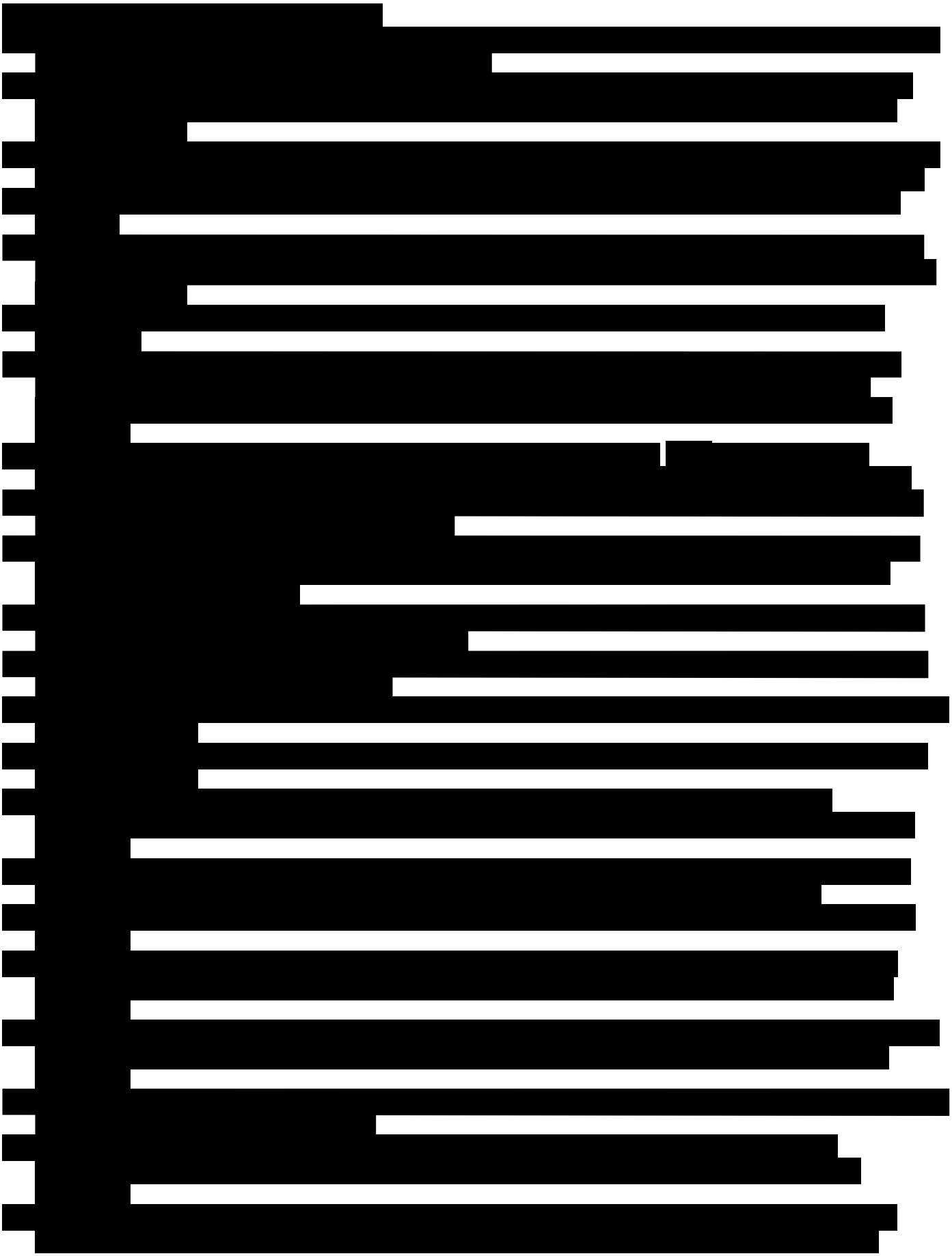
EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

Please refer to the application instructions in order to complete sections A, B, and C of the Biographical Sketch.

[redacted]

Principal Investigator/Program Director (Last, First, Middle): [redacted]



Principal Investigator/Program Director (Last, First, Middle): [redacted]

[redacted]

Principal Investigator/Program Director (Last, First, Middle): [redacted]

[redacted]

NIH/NICHD

[redacted]

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

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
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

A. Positions and Honors.

[redacted]

[redacted]

[redacted]

[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
[redacted]	[redacted]
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
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

C. Research Support

Ongoing Research Support

[redacted]
NIH
[redacted]
The major goal of the subcontract is to examine the usefulness of optical coherence tomography (OCT) for the evaluation of the safety of microbicides, and to conduct phase I clinical trials of combination microbicides.
Role: Co-Investigator

[redacted] are to assess the risk factors associated with CMV infection among adolescent girls and boys and to assess whether immunization with 3 doses of the CMV gB/MF59 vaccine will reduce the acquisition of a systemic CMV infection in healthy CMV seronegative adolescent females.
Role: PI on Subcontract

[redacted]
NIH
[redacted] immunization on adolescent girls behavior and risk of HPV subtypes.
Role: PI on Subcontract

[redacted]
The major goal is to evaluate the attitudes of women ages 26-55 years with regards ot the HPV vaccine.
Role: PI

[redacted])
[redacted]
Using health behavior theories and theories related to the effects of persuasive messages (i.e., inoculation theory), to systematically test the effects of brief persuasive message interventions on receipt of the first dose of HPV vaccine; and evaluate the effects of the interventions on follow-up with subsequent doses of vaccine (using reminder notices with persuasive message content).
Role: Co-Investigator

Selected Completed:

[redacted]
NIH
[redacted] women in the Gulf south region.
Role: PI on Subcontract

[redacted]
The major goal is to evaluate the relationship of parental sexual values and parenting style with decisions to have their daughter immunized for HPV.
Role: PI

[redacted]
[redacted]ajor goal of this project is to examine developmental factors associated with acceptability of microbicides by adolescents.
Role: PI

[redacted])
NIH
[redacted]
The major goal of the subcontract is to exam[redacted]e the acceptability of dendrimer microbicides used in clinical trials.
Role: PI on Subcontract

[redacted]
NIH
[redacted]
The major goal is to include medical students in the data collection regarding attitudes toward adolescent use of topical microbicides.
Role: PI

[redacted]
The major goal is to explore the developmental context of adolescents' perceptions of genital herpes and genital herpes screening.
Role: PI

[redacted]
The major goal is to determine the seroprevalence of HSV-2 among children who have a history of sexual abuse. To evaluate whether there are predictors of seropositivity among this sample of children who have been abused. To evaluate of sensitivity and specificity of commercially available kits (Focus) in the context of evaluating children with suspected abuse.
Role: PI

Current Research Support - [redacted]

[redacted]
Co-P.I,. [redacted] contract on New Technologies in Newborn Screening [redacted] (10% effort)
Direct – =$18,600 per year (total award $800,000 per year)

[redacted]
Consultant, [redacted]
Children [redacted] (8% effort)
Direct - $25,000

[redacted]
Co-Investigator, [redacted] (20% effort)
Direct: $ 33,200 (total award $11 million per year)

[redacted]
t - $143,000 per year

[redacted]", [redacted] ($500,000 total costs for one year – 10% effort)
Total direct: $320,000 per year

Current Research Support – [redacted]

[redacted]

This project investigates the mechanism of synergistic epithelial proinflammatory responses during polymicrobial stimulation.

Clinical Innovator Award [redacted]

7[redacted]
$100,000 direct costs/yr

This project examines the inhibition of airway epithelial defense against bacterial pathogens by cigarette smoke.

[redacted]

[redacted]0
$25,000 direct costs/yr

This project investigates the temporal regulation of proinflammatory signaling by epithelial cells during bacterial pneumonia.

[redacted]

7[redacted]
$40,000 direct costs/yr

[redacted]

This project examines mechanisms of infection-associated preterm birth.

[redacted]

Novel approaches to bacterial vaginosis: diagnostics and risk factors.
This project evaluates new diagnostic strategies for bacterial vaginosis.

Pending Support

[redacted] (proposed)
$250,000 direct costs/yr

Current Research Support – [redacted]

[redacted] (PI until 2/1[redacted] then co-investigator) [redacted] 9 [redacted] of a novel influenza vaccine strategy suitable for use in the developing world

[redacted] (Co-PI with [redacted]) [redacted] examines the epidemiology, immunology, pathogenesis and control of STDs. The project by [redacted] and [redacted] investigates toll like receptor agonists as a topical microbicide strategy.

[redacted] (PI) [redacted] This project focuses on the preclinical and clinical development of dendrimer-based combination microbicides. Our project focuses on the development and use of new optical imaging systems to assess microbicide safety in non-human primates and women.

[redacted] (PI) [redacted] factors associated with acceptability of microbicides by adolescents.

[redacted] Development and evaluation of Vivagel in the prevention of HIV infection in women.
This project deals with the clinical development of a dendrimer microbicide in preventing HIV transmission.

Current Research Support - [redacted]

[redacted] (PI) [redacted]
Subcontract [redacted] (PI)
[redacted]
The major goal of the subcontract is to examine the usefulness of optical coherence tomography (OCT) for the evaluation of the safety of microbicides, and to conduct phase I clinical trials of combination microbicides.
Role: Co-Investigator

[redacted] (PI) [redacted]
Subcontract [redacted] (PI)
[redacted] are to assess the risk factors associated with CMV infection among adolescent girls and boys and to assess whether immunization with 3 doses of the CMV gB/MF59 vaccine will reduce the
acquisition of a systemic CMV infection in healthy CMV seronegative adolescent females.
Role: PI on Subcontract

[redacted] [redacted]
Subco [redacted]
[redacted]
The major goal of the project is to examine the impact of HPV immunization on adolescent girls behavior and risk of HPV subtypes.
Role: PI on Subcontract

[redacted] (PI) [redacted]
[redacted] 55 years with regards to the HPV vaccine.
Role: PI

[redacted] (PI) [redacted]
[redacted] and theories related to the effects of persuasive messages (i.e., inoculation theory), to systematically test the effects of brief persuasive message interventions on receipt of the first dose of HPV vaccine; and evaluate the effects of the interventions on follow-up with subsequent doses of vaccine(using reminder notices with persuasive message content).
Role: Co-Investigator

OMB Number: [redacted]
Expiration Date: 06/30/2011

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2010 *** End Date:** 06/30/2011 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		[redacted]		[redacted]		PD/PI	111,430.00	9.00			83,572.00	23,818.00	107,390.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	Total Funds requested for all Senior Key Persons in the attached file												

Total Senior/Key Person 107,390.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

	* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
		Post Doctoral Associates						
		Graduate Students						
		Undergraduate Students						
		Secretarial/Clerical						
		Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 107,390.00

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2010 *** End Date:** 06/30/2011 **Budget Period 1**

C. Equipment Description

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

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

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

E. Participant/Trainee Support Costs

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

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

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

Next Period

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2010 *** End Date:** 06/30/2011 **Budget Period 1**

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Other Costs	25,000.00
9.		
10.		
	Total Other Direct Costs	25,000.00

G. Direct Costs		Funds Requested ($)
	Total Direct Costs (A thru F)	132,390.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	8.00	132,391.00	10,591.00
2.				
3.				
4.				
	Total Indirect Costs			10,591.00

Cognizant Federal Agency [redacted]

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

I. Total Direct and Indirect Costs		Funds Requested ($)
	Total Direct and Indirect Institutional Costs (G + H)	142,981.00

J. Fee	Funds Requested ($)

K. * Budget Justification budget_justification_per1.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

OMB Number: [redacted]
Expiration Date: 06/30/2011

Previous Period

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2011 *** End Date:** 06/30/2012 **Budget Period** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		[redacted]		[redacted]		PD/PI	111,430.00	9.00			83,572.00	23,818.00	107,390.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	Total Funds requested for all Senior Key Persons in the attached file												

Total Senior/Key Person 107,390.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 107,390.00

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2011 *** End Date:** 06/30/2012 **Budget Period 2**

C. Equipment Description

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

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

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

E. Participant/Trainee Support Costs

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

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

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

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry * Start Date: 07/01/2011 * End Date: 06/30/2012 Budget Period 2

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Other Costs	25,750.00
9.		
10.		
	Total Other Direct Costs	25,750.00

G. Direct Costs		Funds Requested ($)
	Total Direct Costs (A thru F)	133,140.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	8.00	133,141.00	10,651.00
2.				
3.				
4.				
	Total Indirect Costs			10,651.00

Cognizant Federal Agency [redacted]

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

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	143,791.00

J. Fee	Funds Requested ($)

K. * Budget Justification budget_justification_per1.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2012 *** End Date:** 06/30/2013 **Budget Period** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		[redacted]		[redacted]		PD/PI	111,430.00	9.00			83,572.00	23,818.00	107,390.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 107,390.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 107,390.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2012 *** End Date:** 06/30/2013 **Budget Period 3**

C. Equipment Description

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

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

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

E. Participant/Trainee Support Costs

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

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

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3

Next Period

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2012 *** End Date:** 06/30/2013 **Budget Period 3**

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Other Costs	26,522.00
9.		
10.		
	Total Other Direct Costs	26,522.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	133,912.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	8.00	133,913.00	10,713.00
2.				
3.				
4.				
	Total Indirect Costs			10,713.00

Cognizant Federal Agency [redacted]

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

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	144,625.00

J. Fee	Funds Requested ($)

K. * Budget Justification budget_justification_per1.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2013 *** End Date:** 06/30/2014 **Budget Period** 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		[redacted]		[redacted]		PD/PI	111,430.00	9.00			83,572.00	23,818.00	107,390.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 107,390.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 107,390.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2013 *** End Date:** 06/30/2014 **Budget Period 4**

C. Equipment Description

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

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

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

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

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

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

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

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4

Next Period

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2013 *** End Date:** 06/30/2014 **Budget Period 4**

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Other Costs	27,318.00
9.		
10.		
	Total Other Direct Costs	27,318.00

G. Direct Costs		Funds Requested ($)
	Total Direct Costs (A thru F)	134,708.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	MTDC	8.00	134,709.00	10,777.00
2.				
3.				
4.				
	Total Indirect Costs			10,777.00

Cognizant Federal Agency [redacted]

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

I. Total Direct and Indirect Costs		Funds Requested ($)
	Total Direct and Indirect Institutional Costs (G + H)	145,485.00

J. Fee	Funds Requested ($)

K. * Budget Justification budget_justification_per1.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

OMB Number: [redacted]
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2014 *** End Date:** 06/30/2015 **Budget Period** 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		[redacted]		[redacted]		PD/PI	111,430.00	9.00			83,572.00	23,818.00	107,390.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	Total Funds requested for all Senior Key Persons in the attached file												

Total Senior/Key Person 107,390.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 107,390.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2014 *** End Date:** 06/30/2015 **Budget Period 5**

C. Equipment Description

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

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

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

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

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

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

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

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** 07/01/2014 *** End Date:** 06/30/2015 **Budget Period 5**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	28,138.00
9.	
10.	
Total Other Direct Costs	28,138.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	135,528.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. MTDC	8.00	135,528.00	10,842.00
2.			
3.			
4.			
Total Indirect Costs			10,842.00

Cognizant Federal Agency [redacted]

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

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	146,370.00

J. Fee	Funds Requested ($)

K. * Budget Justification budget_justification_per1.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

$25,000.00 yearly
Annual Budget:
Salaries with fringe
Funding will support a portion of our research technician's salary ($8,500)

Supplies + Reagents
PCR for genotyping, molecular biology reagents including those for protein purification ($5,000)
Bacterial culture, mammalian cell culture (including primary cells) ($4,000)
Processing and analysis of vaginal wash specimens from enrolled patients ($3,000)
Western Blot and ELISA assay reagents ($1000)

Travel
PI to travel to one major conference per year ($2,000)

Publications
Costs associated with publishing findings of the study including either page charges or open access fees ($1,500)

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		536,950.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		536,950.00
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		132,728.00
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	132,728.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		669,678.00
Section H, Indirect Costs		53,574.00
Section I, Total Direct and Indirect Costs (G + H)		723,252.00
Section J, Fee		

PHS 398 Cover Page Supplement

[redacted]

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

Prefix:
* First Name: [redacted]
Middle Name:
* Last Name: [redacted]
Suffix:

* New Investigator? ☐ No ☒ Yes

Degrees: [redacted]

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

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

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: [redacted]
Middle Name:
* Last Name: [redacted]
Suffix:

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

* Title: [redacted]

* Street1: [redacted]
Street2: [redacted]
* City: [redacted]
County: [redacted]
* State: [redacted]
Province:
* Country: [redacted] * Zip / Postal Code: [redacted]

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

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

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

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

PHS 398 Checklist

OMB Number: [redacted]
Expiration Date: 9/30/2007

1. Application Type:

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

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier: [redacted]

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

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

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

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

* Previously Reported: Yes ☐ No ☐

OMB Number. [redacted]
Expiration Date: 9/30/2007

4. * Program Income

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

☐ Yes ☒ No

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

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

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance , where applicable, provide an explanation and attach below.

Explanation: Add Attachment | Delete Attachment | View Attachment

PHS 398 Career Development Award Supplemental Form

[redacted]

1. Application Type:

From SF424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated here for your reference, as you attach the sections that are appropriate for this Career Development Award.

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Career Development Award Attachments:

Please attach applicable sections, below.

Introduction (if applicable)				
1. Introduction to Application *(for RESUBMISSION applications only)*		Add Attachment	Delete Attachment	View Attachment
Candidate Information				
2. Candidate's Background	rplan_cb.pdf	Add Attachment	Delete Attachment	View Attachment
3. Career Goals and Objectives	rplan_cg.pdf	Add Attachment	Delete Attachment	View Attachment
4. Career Development/Training Activities During Award Period	rplan_cd.pdf	Add Attachment	Delete Attachment	View Attachment
5. Training in the Responsible Conduct of Research	rplan_tr.pdf	Add Attachment	Delete Attachment	View Attachment
6. Mentoring Plan *(when applicable)*		Add Attachment	Delete Attachment	View Attachment
Statements of Support				
7. Statements by Mentor, Co-Mentors, Consultants, Contributors *(as appropriate)*	rplan_con.pdf	Add Attachment	Delete Attachment	View Attachment
Environment and Institutional Commitment to Candidate				
8. Description of Institutional Environment	rplan_env.pdf	Add Attachment	Delete Attachment	View Attachment
9. Insitutional Commitment to Candidate's Research Career Development	rplan_com.pdf	Add Attachment	Delete Attachment	View Attachment
Research Plan				
10. Specific Aims	rplan_nar.pdf	Add Attachment	Delete Attachment	View Attachment
11. Background and Significance	rplan_bas.pdf	Add Attachment	Delete Attachment	View Attachment
12. Preliminary Studies/Progress Report	rplan_prs.pdf	Add Attachment	Delete Attachment	View Attachment
13. Research Design and Methods	rplan_rdm.pdf	Add Attachment	Delete Attachment	View Attachment
14. Inclusion Enrollment Report *(for RENEWAL applications only)*		Add Attachment	Delete Attachment	View Attachment
15. Progress Report Publication List *(for RENEWAL applications only)*		Add Attachment	Delete Attachment	View Attachment
Human Subject Sections				
16. Protection of Human Subjects	rplan_hs.pdf	Add Attachment	Delete Attachment	View Attachment
17. Inclusion of Women and Minorities	Inclusion_Women_Upload.pdf	Add Attachment	Delete Attachment	View Attachment
18. Targeted/Planned Enrollment	Targetted_Enroll_Upload.pdf	Add Attachment	Delete Attachment	View Attachment
19. Inclusion of Children	Inclusion_Children_Upload.pdf	Add Attachment	Delete Attachment	View Attachment

PHS 398 Career Development Award Supplemental Form

2. Career Development Award Attachments (continued):

Other Research Plan Sections

20. Vertebrate Animals		Add Attachment	Delete Attachment	View Attachment
21. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
22. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
23. Resource Sharing Plan(s)		Add Attachment	Delete Attachment	View Attachment

Appendix (if applicable)

24. Appendix	Add Attachments	Delete Attachments	View Attachments

*3. Citizenship:

☐ U.S. Citizen or noncitizen national

☒ Permanent Resident of U.S.
(If a permanent resident of the U.S., a notarized statement must be provided by the time of award)

☐ Non-U.S. Citizen with temporary U.S. visa

CANDIDATE'S BACKGROUND

Preterm birth occurs in 12-13 percent of live born infants and is a leading cause of neonatal morbidity and mortality in the United States. Alarmingly, the incidence of preterm birth has risen by more than 30 percent over the last 20 years (1). Although tremendous advancements in the field of Neonatology have led to increased survival of preterm infants, little progress has been made with respect to reducing the significant associated morbidities. Subsequently, many of these surviving children are burdened with life-long impairments. A substantial and sustained reduction in the incidence is of preterm birth is therefore, of paramount importance. Critical to accomplishing this goal is an improved understanding of the risk factors and pathologic mechanisms contributing to the onset of preterm parturition, and this is my primary research focus.

[redacted]

[redacted]

Currently, our investigative efforts are directed towards elucidating the host's innate immune response to *G. vaginalis*, and in particular, the protective role of antimicrobial peptides. Recently published data linking vitamin D deficiency with development of BV in pregnant women [redacted] especially intriguing, as vitamin D is a major regulator of the innate immune response and induces the expression of these critical antimicrobial [redacted] The role of vitamin D in the pathogenesis of BV may be of particular relevance in explaining the striking racial disparities in the prevalence of BV. African-American populations, which are at highest risk for vitamin D-deficiency, also have the highest prevalence of BV and BV-associated preterm birth [redacted] **These observations have allowed us to formulate primary hypothesis of this research proposal: that vitamin D deficiency is a significant risk factor for the development of BV in non-pregnant, healthy adult women and that this effect is mediated by a deficiency of Vitamin D-responsive antimicrobial peptides**.

CAREER GOALS AND OBJECTIVES

The preliminary data obtained from our in vitro experiments provide convincing evidence that Vitamin D deficiency may play a substantial role in the development of BV. Exploration of this potentially modifiable risk factor in human subjects represents a unique opportunity to reduce the burden of this exceedingly prevalent disease and its primary associated morbidity, preterm birth. The proposed research represents a critical step in attaining my overall goal: to transition to an independent research career focused on mechanistic and translational studies of preterm birth.

The continued support of my department, the continued guidance of my mentors, the Master's program in Patient-Oriented Research that I have already embarked upon, and access to the abundant resources and rich intellectual environment across departments and schools at Columbia leave me well-positioned to embark upon a career in patient-oriented research. This Career Development Award will serve as a driving force in achieving this goal, providing the protected time and additional training required to become a successful independent investigator.

CAREER DEVELOPMENT/TRAINING ACTIVITIES

In an effort to expand my research training and move forward towards a career in patient-oriented research, I applied to the [redacted] program offered through [redacted] and was subsequently awarded a full scholarship to participate in this unique training opportunity. This program, funded by a Clinical and Translational Science Award from the [redacted], includes enrollment in the [redacted] for the pursuit of an MS degree in Biostatistics. In addition to the traditional statistical courses required for this degree, the program includes didactic training and colloquia designed to prepare young investigators for independent careers as clinical and translational scientists. Past participants in this program have enjoyed much success transitioning to positions as independently funded investigators. The [redacted] curriculum consists of 30 credits in total over a 2 year period. I began this program of study in [redacted] with the full support of my department, division, and mentors, and I will complete the degree in [redacted].

Highlights of this intensive training program that are particularly relevant to my stated career goals include the following courses:
Colloquium on [redacted] This seminar is designed to explore the methodological and practical issues related to establishing a satisfying academic career. The curriculum includes instruction on the development of scientific writing and presentation skills, attainment of funding, expansion of competencies in interdisciplinary research, and a successful transition to independence from your mentor.
Basic Laboratory [redacted] This course serves as an overview of widely used laboratory research methods, which may be employed to support clinical-translational research (including imaging modalities, molecular genetics, cell culture methods, use of animal models, high-throughput genetic and functional screening approaches, various methods for protein- and DNA-based analysis.) There is particular emphasis on the core facilities available at [redacted] including microarray and protein chemistry cores, the [redacted], and the [redacted].

Additional required courses include *Introduction to Biostatistical Methods, Principles of Epidemiology, Analysis of Categorical Data, Funding for Research Activities,* and *Basic Issues in Obtaining Support.* I will take elective courses in *Molecular & Cellular Mechanisms in Human Disease, Statistical Computing with SAS,* and *Applied Regression*, and I anticipate that these will be of tremendous benefit as I move forward with the proposed research project.

In addition to this didactic coursework, I will continue to attend and present at weekly seminars in the [redacted] provide opportunities for me to present and discuss preliminary data, as well as the chance to exchange ideas with other members of the [redacted], including Ph.D.s, M.D.s, and M.D./Ph.D.s at various levels of training. I anticipate that I will attend at least one domestic scientific meeting per year [redacted] in order to present my work to a broader audience and to facilitate collaborations with colleagues from other institutions.

I will serve as the Attending Physician at [redacted] in the neonatal intensive care unit for 6 weeks per year (12% overall effort). I anticipate that teaching responsibilities (resident/fellow lectures, journal clubs, etc…) will account for no more than 3% of overall effort. Therefore, 85% of my time will be devoted exclusively to laboratory research and didactic studies as outlined below. This plan has been approved by my department and

clinical division, and they understand and are supportive of this intensive research focus as I begin my career.

	Allocation of Work Effort During Proposed Award Period.				
Activity	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Career development and training activities (Didactic sessions, coursework, conferences)	20% MS/POR program	10%	5%	5%	5%
Proposed research project (Data collection, analysis and manuscript preparation)	60%	70%	70%	60%	45%
Future research development (transition to independence, preparation of R01)	5%	5%	10%	20%	35%
Total research-related activity	85%	85%	85%	85%	85%

Superb mentorship is an integral component of my career development plan and will be the driving force in my success. In addition to [redacted] a nationally recognized investigator in field of sexually transmitted infections, serves as my co-mentor for this proposed project. [redacted] is an accomplished clinical and translational researcher in the area of reproductive and neonatal infections, and [redacted] has served as the primary investigator for numerous large clinical trials designed to explore potential strategies for the prevention of herpes simplex virus infections. In addition, [redacted] is the [redacted] at [redacted]. Importantly, [redacted] has an exceptional track record of success in training independent investigators involved in patient-oriented research.

[redacted] and [redacted] are both committed to my development as an independent researcher. For this reason, they have helped me assemble a mentorship committee comprised of individuals who will not only provide added expertise relevant to the specific aims of this proposal, but also will function as outstanding resources for my academic development over the next 5 years. In addition to [redacted] and [redacted], the committee will include:

N[redacted] *Associate Dean of Clinical Research Operations at the* [redacted] *and* [redacted] served the Medical Director of the national [redacted] where [redacted]e oversaw the scientific priorities, policies and practices and the educational efforts of the Foundation, with emphasis on adverse birth outcomes including preterm/low birthweight and birth defects, and perinatal epidemiology.

[redacted] *Vice Chair of Pediatrics for Faculty Development* [redacted] has extensive expertise in clinical research and reproductive health. In addition to an active research career, [redacted] current role as Vice Chair focuses on faculty development, with a special interest in helping young faculty transition from a mentored position to independence.

This committee will meet formally at least four times per year, and I anticipate meeting with individual members on a monthly basis. Interactions with [redacted] will continue to occur daily in the laboratory, with scheduled meetings several times per week.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

[redacted] mandates that all research faculty receive training in the following areas: the responsible conduct of research, the use of human subjects, animal care, and HIPPA. I have completed these training requirements and will maintain my certification throughout the proposed award period.

The [redacted] program requires that all students participate in a seminar entitled *The Responsible Conduct of Research & Related Policy Issues* [redacted]. This course explores the ethical and policy issues that may arise during the conduct of basic, translational, and clinical biomedical scientific research. Topics addressed include research misconduct, human research participants, responsible use of animal subjects authorship practices in scientific publications, conflicts of interest arising from scientists acting as policy consultants and experts, and data sharing. Course sessions will include lectures, discussion periods, and analyses of case studies. This class meets weekly for a one hour period throughout the semester

[redacted]

Statements by Mentor

[redacted]

[redacted]

[redacted]

[redacted]

September 30, [redacted]

Dear [redacted]

I am delighted to write in support of your K23 application and to serve as a member of your mentorship committee.

It has been a pleasure to work with you as your course director in the Laboratory Methods course as part of the Patient-Oriented Research Master's program organized by the [redacted] for Clinical and Translational Research. As we have discussed, there is considerable overlap in our research interests. As the former [redacted], I am quite familiar with the challenges and the importance of research designed to understand and target the causes of preterm birth. Bacterial vaginosis is an area of particular importance and one that is only rarely addressed. Likewise, the importance of vitamin D to innate immune responses and to predisposition to diseases is only beginning to be teased out, thereby providing opportunities for excellent translational research. I have no doubt that your project - rare in that it crosses both clinical and basic research while addressing serious health and public health problems - will provide meaningful and exciting data in each of these areas.

I look forward to providing scientific expertise, clinical research experience, and mentorship throughout the K23 period and beyond. Your carefully chosen committee will provide the mentoring and the support needed for you to develop into an independent patient-oriented researcher, and I am delighted to help in any way that I can.

Sincerely,

[redacted]

[redacted]

Dear [redacted]

I am delighted to serve as a member of your mentorship committee and to continue to assist you with both your research project and your career development during this critical period. As you know, I have a long-standing interest in clinical and translational research, especially as it applies to reproductive health. I have carried out single- and multi-center studies of adolescent vaccination, herpes simplex virus infections, and microbicides. As a member of your committee, I will bring these relevant experiences as well as a wide network of potential collaborators in the field of reproductive health.

Importantly, consistent with my role as the [redacted] in the [redacted], I have a particular interest in the early career trajectory of young investigators such as you. While each member of your committee will be concerned about your scientific and career development, I will ensure that issues such as ongoing mentorship, appropriate collaboration within and outside the institution, proper forums for presentation of your work, and your transition to independence are brought up and addressed at our regular meetings.

In summary, I am excited to join your committee, fully support your application for support, and look forward to continuing to work together!

Sincerely,

[redacted]

[redacted]

[redacted]

[redacted]

Dear [redacted],

It is a pleasure for me to write this letter of collaboration for [redacted] K23 application. As you know, our group has extensive expertise in area of antimicrobial peptides and immune defense. Our groups have worked together to conduct binding and functional studies demonstrating a role for human alpha-defensins in inhibition of the cholesterol-dependent cytolysin family of bacterial toxins [redacted] We are happy to continue to provide expertise, purified defensins and analogues, and to continue to carry out collaborative experiments in support of [redacted] application.

Sincerely,

[redacted]

[redacted]

To the Committee:

The rich academic environment at [redacted] is particularly well suited for the multidisciplinary nature of [redacted]' research proposal as well as for [redacted] continued development as an investigator in the field of patient-oriented research.

The numerous core facilities and abundant shared equipment available at [redacted] will no doubt be of tremendous value to [redacted] as [redacted] works towards transitioning into an independent investigator. Both the [redacted] Research Center, sponsored by [redacted] and [redacted], are particularly relevant to the specific aims outlined in her proposal. In addition, this Institute continues to provide statistical guidance, as well as planning, regulatory and execution support for translational research here at [redacted]

The [redacted] OR) was created as a joint endeavor between the schools of public health, medicine, and sciences, with the overall goal of enabling its participants to compete more effectively for peer-reviewed research funding. The POR track delivers rigorous didactic training that prepares young investigators for independent careers as clinical scientists. Traditional coursework in Epidemiology, Biostatisics, and Molecular Epidemiology is complimented by practical instruction in the responsible conduct of research, the procurement of funding, and scientific writing. [redacted] participation in this program is an integral component of her career development plan and will certainly be a driving force in her success.

[redacted] carefully assembled mentorship committee is comprised of individuals who will not only provide added expertise relevant to the specific aims of this proposal, but also will function as outstanding resources for [redacted] academic development over the next [redacted] years. [redacted] dual appointment in the Departments of Pediatrics and Microbiology & Immunology facilitates collaboration with senior investigators in each of these disciplines. [redacted] participation in the weekly research seminars, journal clubs and data presentations both departments continue to serve as a source of tremendous academic enrichment.

In summary, as a full time faculty member of [redacted] will have continued access to all of the facilities, equipment and intellectual resources necessary for the achievement of her stated research goals, career development plan, and ultimate transition to independence in the discipline of patient-oriented research.

Sincerely,

[redacted]

[redacted]

To the Committee:

It is a pleasure to write this letter confirming the ongoing commitment of the Department of Pediatrics at [redacted] to the research and career development of [redacted] arrived at our institution in [redacted] to pursue a fellowship in Neonatal-Perinatal Medicine following outstanding clinical training in pediatrics as a resident and chief resident at [redacted]. During [redacted] training, [redacted] quickly distinguished [redacted] as a promising young investigator, well suited for a career in academic medicine. Upon completion of [redacted] fellowship, [redacted] was offered a full-time faculty position as Assistant Professor of Pediatrics in the Division of Neonatology, an appointment that **guarantees 80% dedicated time** for research.

[redacted] Chief of Neonatology, has been instrumental in ensuring [redacted] development as a physician-scientist. [redacted] has restricted [redacted] clinical responsibilities, teaching load and administrative tasks in order to make certain there is adequate protected time for [redacted] achieve [redacted] stated research goals. In addition, [redacted] has made guaranteed the flexibility in [redacted] clinical service time and call responsibilities required for [redacted] to excel in the MS in Biostatistics/Patient Oriented Research Track offered through [redacted]. Furthermore, the Division has provided [redacted] with an office and dedicated additional laboratory space needed to pursue the planned investigations as outlined in this proposal.

As a full time faculty member, [redacted] enjoys unlimited access to the abundant core facilities and shared equipment here at [redacted]. The continued involvement of senior faculty members from both within and outside the Department of Pediatrics will be of tremendous value as [redacted] moves forward with the proposed investigations and well as for the establishment of future collaborations.

In summary, Department of Pediatrics and the Division of Neonatology at [redacted] have both dedicated substantial financial, physical and intellectual resources to [redacted] since [redacted] appointment to our faculty. **Importantly, the continuation of this support is NOT contingent upon the receipt of this career development award**. We remain firmly committed to assisting [redacted] in the achievement of [redacted] goal to become a successful independent investigator in the field of patient-oriented research.

[redacted]

HYPOTHESIS AND SPECIFIC AIMS

Vitamin D is an important regulator of innate immune responses, and deficiencies of this critical vitamin have been associated with an increased susceptibility to infectious diseases. Recently, maternal vitamin D deficiency has been linked to the development of bacterial vaginosis (BV) during the first trimester of pregnancy. BV, the most common vaginal infection worldwide, is associated with significant adverse sequelae, including preterm birth and acquisition of sexually transmitted diseases, particularly human immunodeficiency virus. Identification of potentially modifiable risk factors for BV represents a unique opportunity to reduce the burden of this exceedingly prevalent disease and its associated morbidities.

We hypothesize that vitamin D deficiency is a significant risk factor for the development of BV in non-pregnant, healthy adult women and that this effect is mediated by a deficiency of Vitamin D-responsive antimicrobial peptides.

Aim 1. Investigate the role of vitamin D status in the development of BV in a cohort of non-pregnant, healthy adult women.

1a. Determine the association between serum 25-hydroxyvitamin D levels and the prevalence of BV.

1b. Determine whether specific known polymorphisms in the genes encoding the vitamin D receptor affect the risk of developing BV.

Aim 2. Investigate specific molecular mechanisms by which vitamin D signaling controls vaginal innate immune responses relevant to BV.

2a. Determine whether exposure to 25-hydroxyvitamin D results in increased production and secretion of antimicrobial peptides by vaginal epithelial cells.

2b. Determine the effects of vitamin D-responsive antimicrobial peptides on epithelial defense against *Gardnerella vaginalis,* the primary etiologic agent in BV.

2c. Detect and quantify specific vitamin D-responsive antimicrobial peptides in vaginal lavage specimens from the cohort of women studied in Aim 1.

BACKGROUND AND SIGNIFICANCE

Bacterial Vaginosis and Preterm Birth: The Scope of the Problem

Bacterial vaginosis (BV) is the most common vaginal infection worldwide and is associated with significant adverse consequences including preterm labor and delivery (8, 10), post-partum endometritis (16), and an increased risk of HIV acquisition (17-19). Reported prevalence rates for BV range from 10-40% depending upon the population studied (9). However, suboptimal methods of diagnosis and a high percentage of asymptomatic patients make the true prevalence difficult to ascertain.

BV is defined as a pathological state characterized by the loss of normal vaginal flora, particularly *Lactobacillus* species, and overgrowth of other microbes including *Gardnerella vaginalis*, *Bacteroides* species, *Mobiluncus* species, and *Mycoplasma hominis*. The hypothesis of a polymicrobial etiology of BV has garnered much support over the past several decades. However, more recent data suggest a primary role for *G. vaginalis* as the specific and sexually transmitted etiological agent in BV, as was initially postulated by Gardner and Dukes in 1955 (20-22).

The association of BV with the onset of preterm labor is of major public health importance as BV has been estimated to cause 90,000 excess preterm births per year (at an overall cost in excess of $1 billion) and to account for at least 30% of the racial difference in preterm birth rates (9). Epidemiologic studies reveal that African American women have not only a higher prevalence rate of BV, but the association between BV and preterm birth in these women is stronger (14). It is hypothesized that BV-associated preterm birth is the result of a pro-inflammatory response in the host, stimulating the release of mediators such as IL-1β and IL-8 that contribute to the onset of preterm labor (23-25). While successful treatment of BV in pregnant women is possible utilizing appropriate antimicrobial therapy, several large clinical trials have demonstrated that the use of antibiotics in these women has not been associated with a reduction in preterm birth (26-28). The failure of antimicrobial therapy to reduce BV-associated preterm labor may be attributable to a variety of factors including: a lack of uniformity in the diagnosis and treatment of BV, recurrent infections despite appropriate therapy, and an inability to mitigate the resultant inflammatory cascade already underway. Until understanding of the bacterial-host interaction is improved, further interventions directed towards the reduction of BV-associated preterm birth are unlikely to succeed (24).

Recently, Bodnar and colleagues identified vitamin D deficiency as a risk factor for the development of BV in pregnant women (12). This cross-sectional analysis revealed a significant, dose-dependent association between serum concentrations of 25-hydroxyvitamin D (25D), the major circulating vitamin D metabolite, and the prevalence of BV. These findings have enormous implications in light of the sheer prevalence of vitamin D deficiency in the United States. Utilizing data collected as part of the National Health and Nutrition Examination Survey 2001-2004, Ginde et al. found that a staggering 77% of individuals are vitamin D deficient. This represents a 22% increase in the prevalence of vitamin D deficiency since 1994 (29). Furthermore, serum 25D levels were **the absolute lowest for non-Hispanic black females** aged 12-59 years, a critical finding that may explain the profound racial disparities observed in epidemiologic studies of BV and BV-associated adverse sequelae. **Aim 1** of this proposal is specifically designed to investigate the role of vitamin D status in the development of BV in non-pregnant, healthy adult women.

Vitamin D Signaling and The Innate Immune System

The observed association between decreased serum concentrations of 25D and the development of BV in pregnant women was a novel but not surprising finding, as vitamin D is now understood to be a critical regulator of the innate immune system. Deficiencies in this important hormone have been associated with an increased susceptibility to numerous other infectious diseases including tuberculosis, influenza, bronchiolitis and HIV (30-32).
Vitamin D, obtained through the photochemical conversion of cholesterol precursors following sun exposure, and to a lesser extent through dietary intake, is hydroxylated in the liver to produce 25D. This stable metabolite, generally accepted as the most reliable indicator of an individual's overall vitamin D status, undergoes a second hydroxylation reaction, to produce 1,25 di-hydroxyvitamin D (1,25D), the hormonally active vitamin D metabolite. Intracellular 1,25D interacts with the nuclear vitamin D receptor (VDR), a ligand-activated transcription factor that binds to vitamin D response elements (VDREs) located in the regulatory region of vitamin D target genes (33). Microarray analyses have revealed hundreds of 1,25D target genes, **several of which are integral components of the innate immune system** (34). Many of these target genes encode antimicrobial peptides, most notably cathlicidin and human β defensin-2 (HBD-2) (35). Substantial direct evidence links vitamin D and upregulation of antimicrobial peptide expression in a variety of tissues (36-38).

Antimicrobial Proteins Link Vitamin D and BV

Antimicrobial peptides (AMPs) produced by epithelial cells in the lower genital tract serve as the first line of defense against numerous vaginal infections. Mounting evidence suggests that AMPs play a pivotal role in the development of BV as well. A study by Valore et al. revealed that BV is a pathological state associated with a local, reversible deficiency in several AMPs, including both alpha- and beta-defensins (39). Importantly, successful treatment and eradication of BV in these women was associated with restoration of normal concentrations of these peptides. These findings have been confirmed by other groups. For example, Fan et al. documented a similar deficit in vaginal AMP concentrations in women with BV (40).
Deficiencies in vitamin D-responsive antimicrobial peptides therefore provides a potential mechanistic explanation for the observed association between vitamin D deficiency and the development of BV. In **Aim 2** of this proposal, we will approach this hypothesis rigorously by undertaking a mechanistic investigation of the link between vitamin D, AMP deficiency, and BV.

PRELIMINARY STUDIES

Vaginolysin: Critical to the Pathogenesis of BV and BV-associated Preterm Birth. Recently, our laboratory identified and characterized vaginolysin (VLY), a novel pore-forming toxin produced by *Gardnerella vaginalis* [redacted]. VLY is a member of the cholesterol dependent cytolysin (CDC) family and is hypothesized to be a major factor in the pathogenesis of bacterial vaginosis and its associated morbidities. We have demonstrated that the pore-forming activity of this toxin is human specific, utilizing the GPI anchored protein CD59 as its receptor. Human red blood cells (hRBC) treated with VLY exhibit hemolysis in a dose dependent manner. However, sheep RBC (sRBC) are resistant to VLY-mediated lysis (**Figure 1A**, [redacted] More strikingly, the species-specific effect of VLY even discriminates among primate species. Human cervical epithelial cells (HeLa) are more sensitive to VLY-mediated cytolysis than the COS-7 cells, originating from *Chlorocebus aethiops*, the African green monkey (**Figure 1B**).

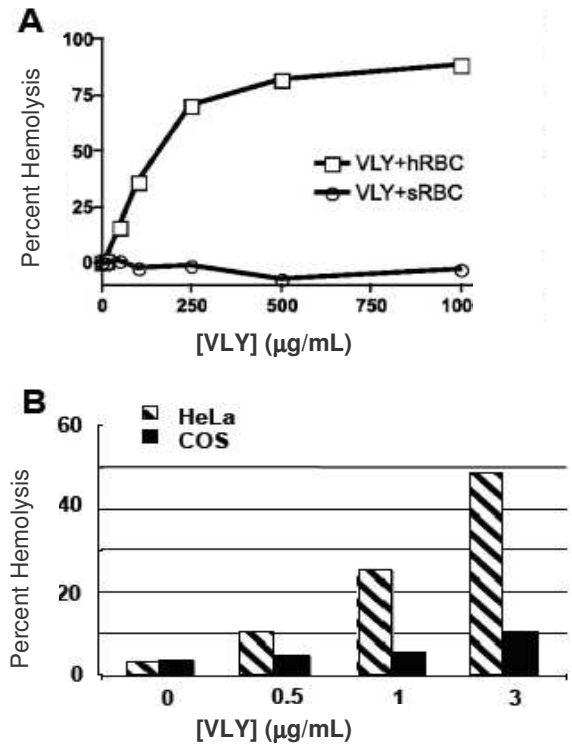


Figure 1. Species-specific activity of VLY. (A) VLY lyses erythrocytes from humans (hRBC) but not sheep (sRBC). (B) Lysis of human (HeLa) and African Green monkey (COS-7) cells by VLY. Lysis was measured by LDH release assay following 30 min of incubation with toxin. Values were normalized to 100% lysis for each cell line (Triton X-100) treatment.

The host response to vaginal colonization with *G. vaginalis* is postulated to play a critical role in the subsequent development of BV-associated preterm labor. Both IL-1β and IL-8 have been found in increased concentrations in the vaginal wash specimens from women with BV (24) Furthermore, mucosal IL-1β levels have been used to predict preterm birth in pregnant women with BV (42). We have demonstrated that VLY is capable of eliciting pro-inflammatory signaling in host epithelial cells. Preliminary studies performed in our laboratory reveal that the epithelial cell response to sub-lytic quantities of VLY includes phosphorylation of p38 mitogen-activated protein kinase (MAPK), processing and subsequent upregulation of pro-interleukin (IL)-1β, and induction of IL-8 transcripts **(Figure 2).**

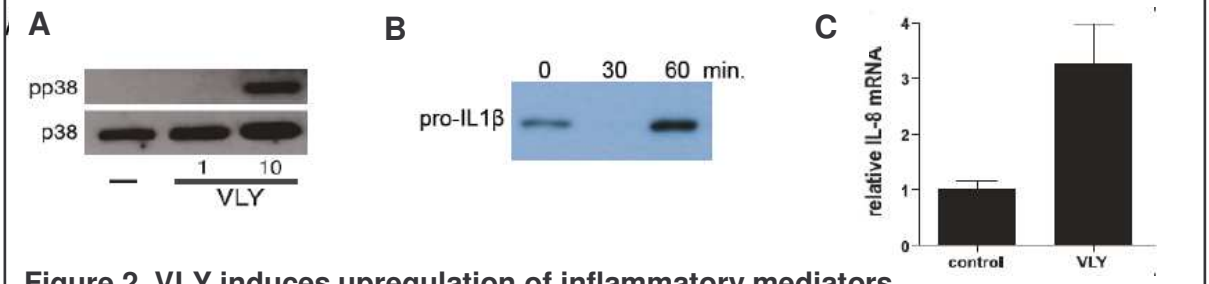


Figure 2. VLY induces upregulation of inflammatory mediators. Western blotting demonstrates (A) Activation of p38 MAPK in HeLa cells following treatment with VLY (1-10 µg/ml, 30 min) and (B) processing of pro-IL1β and subsequent translational upregulation in HeLa cells treated with purified VLY (5 µg/ml). (C) Upregulation of IL-8 mRNA in HeLa cells following VLY (10 µg/ml, 2 hr) as determined by real-time PCR.

Defensins Exhibit Antimicrobial Activity against *G. vaginalis*

Defensins are a large family of antimicrobial peptides (α-defensins, β-defensins, and θ-defensins) that play important role in the innate immune response to a variety of bacterial, fungal, and viral pathogens. Although multiple human θ-defensin genes exist, they do not undergo translation because of a premature termination codon. Recently however, a renewed interest in retrocyclin, a member of the θ-defensin subgroup, has emerged. Venkataraman et. al. have demonstrated that endogenous expression of biologically active retrocyclin can be restored following aminoglycoside-induced "read through" of the termination codon of the retrocyclin gene (43). Simliar to other members of the defensin family, Retrocyclin is capable of bacterial killing, toxin inactivation, and inhibition of viral entry into cells[redacted] Our preliminary data demonstrate that retrocyclin inhibits the growth of *G. vaginalis* in liquid culture as well as VLY-mediated hemolysis **(Figure 3).** In Aim 2b, we will determine whether other vitamin D-responsive AMPs (cathlicidin and HBD-2) similar antimicrobial activity against *G. vaginalis*.

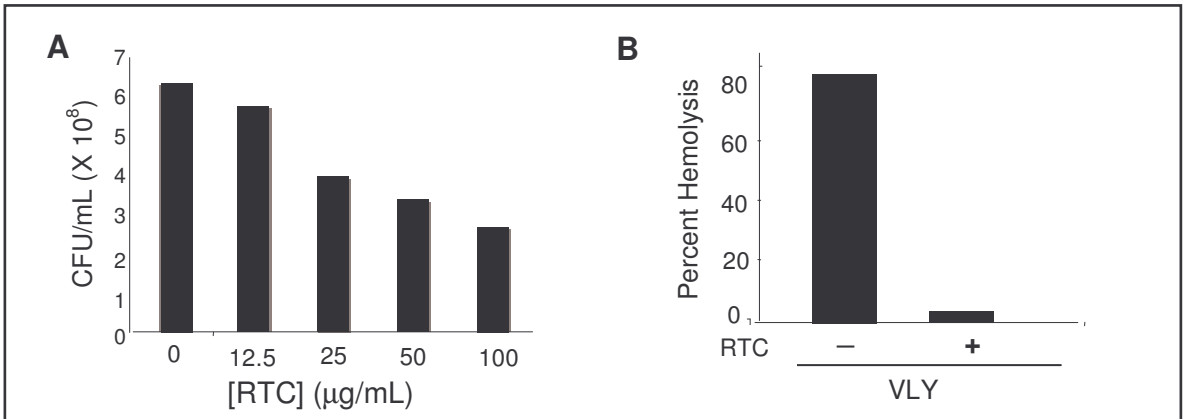


Figure 3. Antimicrobial activities of retrocyclin. (A) Retrocyclin (RTC) inhibits growth of G*. vaginalis* in liquid culture. Varying concentrations of RTC were added to liquid cultures of *G. vaginalis* (early to mid-exponential phase) for 1 hr with subsequent colony count determination. (B) RTC-mediated protection from VLY-induced hemolysis. VLY (500 ng/mL) was pre-incubated with RTC (25 µg/mL) or vehicle control X 30 min prior to exposure to human erythrocytes.

Vitamin D-Responsive Pathways Inhibit VLY-mediated Cytotoxicity

The interaction of 1,25D with the VDR culminates in increased transcription and translation of critical immunological mediators, including several AMPs. Multiple investigators have demonstrated that various epithelial cells types possess the enzymatic machinery necessary to convert circulating 25D to this active metabolite (38, 45, 46), thereby enabling these cells to regulate production of these immune mediators on a local level. We hypothesized that exposure of HeLa cells to exogenous 25D would similarly result in increased signaling via the VDR, culminating in the production of protective AMPs. Our preliminary data demonstrate a significant reduction in VLY-mediated cytotoxicity following pre-treatment of these cells with 25D **(Figure 4).** Aim 2a of this proposal focuses on the continued exploration of the vitamin D-responsive pathways in human vaginal epithelial cells.

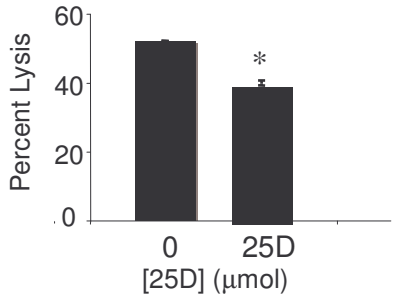
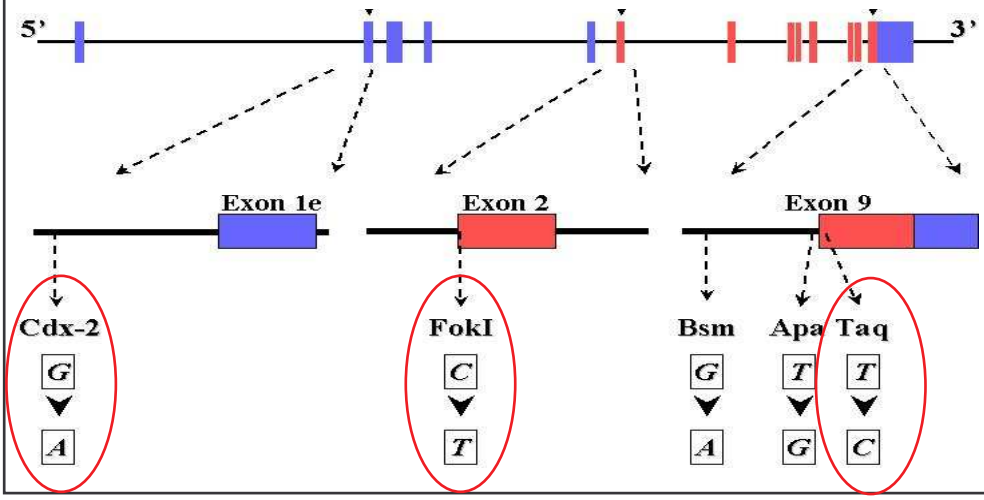


Figure 4. 25D confers protection from VLY cytotoxicity. HeLa cells were pre-treated with 25D or vehicle control x 24h. Lysis was measured by LDH release assay following 30 min of incubation with VLY (1 µg/mL). Values were normalized to 100% lysis (Triton X-100). Error bars represent standard deviations. *P*= 0.001

Genetic Variations in Vitamin D signaling: VDR polymorphisms

Multiple studies demonstrating an association between polymorphisms in the VDR gene and an increased susceptibility to infectious diseases provide further support for the critical role of vitamin D signaling in the innate immune system. Physical mapping studies reveal that this relatively large gene (> 100kb) is located on chromosome 12q12–q14. Restriction fragment length polymorphism (RFLP) analysis and regional sequencing of the VDR gene have identified several clinically relevant single-nucleotide polymorphisms (SNPs) with relevance to infectious diseases **(Figure 5).**

Figure 5. Structure of the VDR Gene. (Figure adapted from Genomos, Erasmus MC)



The *Fok I* polymorphism results from a cytosine to thymine transition that occurs in the first of the two potential initiating sites at exon II of the VDR gene [redacted]. The F allele is associated with the production of a "short" VDR variant (424-amino-acid) while the f allele results in transcription of the long VDR variant (427-amino-acid) [redacted] The shorter VDR variant has been found to be more transcriptionally potent than its longer counterpart [redacted]). *Taq*I is a synonomous single-nucleotide polymorphism resulting from a thymine to cytosine transition occurring in exon 10. The *Taq1* and *Fok1* polymorphisms in particular, have been shown to influence disease susceptibility [redacted]) and so we will explore the potential associated of these SNPs with the development of BV in Aim 1a of this proposal. In addition, we will similarly investigate the Cdx-2 polymorphisms (a guanine to adenine transition in the promoter region of Exon1e [redacted] because of its high prevalence in African Americans **(Table 1),** the group most commonly affected by BV.

Table 1. VDR Allele Frequencies across Major Ethnic Groups. Adapted from (49). Polymorphisms of interest highlighted in grey.

VDR Polymorphism	Minor Allele[a]	Ethnic Group (%)		
		Caucasian	Asian	African
Cdx2 (rs11574010)	A	19	43	74
Fok1 (rs2228570)	f	34	51	24
Bsm1 (rs1544410)	B	42	7	36
*Apa1 (*rs7975232)	A	44	74	31
Taq1 (rs731236)	T	43	8	31

[a] Minor allele in Caucasians

RESEARCH DESIGN AND METHODS

Our laboratory has recently received funding to conduct a clinical study designed to evaluate novel diagnostic strategies for BV and to determine whether specific polymorphisms in the VLY receptor gene affect an individual's risk of developing BV. This study is called Bacterial Vaginosis: Improved Diagnosis by ELISA and Sequencing (BV-IDEAS). **A major advantage of this research proposal is that the study design involves the same sample population and specimen collection procedures required for the *BV-IDEAS* study**. **Therefore, the vast majority of the methodologies proposed to address Aim 1 of this proposal will incur no additional costs.**

Aim 1. Investigate the role of vitamin D status in the development of BV in a cohort of non-pregnant, healthy adult women.

Aim 1a: Determine the association between serum 25-hydroxyvitamin D levels and the prevalence of BV.

Rationale

Recent epidemiologic work has revealed a strong relationship between maternal serum 25D levels and the prevalence of BV in the first trimester of pregnancy (12). However, the underlying mechanisms of this association and its relevance to non-pregnant women (the majority of those with BV) remain unclear. Hormonal changes occurring during pregnancy have profound effects on maternal vitamin D metabolism, with serum levels of 1,25D rising early in the first trimester of and remaining elevated until delivery (52). The effect of these changes on BV risk is unknown. In addition, no prior studies have evaluated the relationship between vitamin D status and the presence of BV in non-pregnant healthy women.

Experimental Design

Cohort

A cohort of non-pregnant, healthy adult women (ages 21-64) presenting for primary care at the [redacted] gynecology clinics will be recruited for this study. The extent of contact with the subject will be a single visit for the specimen collection. Exclusion criteria will include history of immunodeficiency, recent (<1 month) history of antibiotic or immunomodulatory therapy, current vaginal bleeding, and known reproductive tract abnormalities. These potentially eligible subjects will be approached for informed consent. Because a component of these studies will involve testing for genetic association, careful attention will be given to patient de-identification procedures. Participants will be able to opt out at any time with subsequent destruction of their specimens. Contact information for questions, rights and inquiries will be provided at the time of informed consent. Patients not requiring a speculum examination as part of their routine visit, as determined by the primary clinician, will not be included in this study due to the nature of required specimens.

Historical data

Members of the cohort will fill out a one-time questionnaire of current and historical data. This will include details of medical and obstetric history, current and past medication use, gynecologic history, and known history of BV or other reproductive tract infections. Known risk factors for BV, including self-reported race, smoking, oral contraceptive use, and number of sexual partners will be assessed in a checklist format.

Sample Collection and Processing

With the assistance of the primary care physician, the following samples will be collected:

1.) *A single 2 ml blood sample* will be obtained via venipuncture and centrifuged. The serum will

be immediately frozen and stored for later determination of 25D levels. The cellular component will be utilized for isolation of host DNA (required for vitamin D receptor genotyping, see methods for **Aim 1b**).
2.) A swab of the lateral vaginal walls will be used to prepare a glass slide for gram stain (Nugent scoring, see below).
3.) A vaginal lavage specimen will be obtained by instilling 1 ml of sterile PBS into the vagina with a flexible catheter-tipped 5 ml syringe and then immediately removing the fluid with the same syringe. This specimen will be immediately frozen and stored for quantification of antimicrobial peptide concentrations (see methods for **Aim 2c**).

Nugent Scoring of Gram Stained Slide

The Nugent scoring system for interpretation of Gram-stained vaginal smears was put forth in an attempt to standardize diagnosis of BV and increase inter-rater reliability (53). Scores are assigned to Gram-stained vaginal smears according to the number of specific bacterial morphotypes seen per microscopic 1000X visual field. The Nugent scoring system exhibits superior sensitivity and specificity compared to the more commonly utilized Amsel criteria, and is currently regarded as the gold standard for BV diagnosis (54). Slides will be collected as above, gram stained, and then scored by 2 blinded, independent reviewers using a 0-10 scale with specific criteria for each. Inter-rater reliability (κ) will be calculated. A score of 7-10 is considered diagnostic of BV.

Quantification of 25D Concentration

The serum concentration of 25D will be quantified using a commercially available ELISA assay kit (ALPCO). This assay is extremely sensitive and is capable of detecting concentrations as low as 2 nmol/l. Samples will be repeated in triplicate using at least 2 dilutions in order to obtain the most accurate estimate of 25D concentration.

Statistical Analysis

The presence of BV will be treated as a dichotomous variable. Subjects will be graded as positive (SCORE 7-10) or negative (SCORE <7) on the basis of Nugent scoring. Median serum 25D concentrations will be calculated for both groups and a Wilcoxon Mann-Whitney Test will be performed. In order to determine if an association between vitamin D status and the presence or absence of BV exists, women will be classified as either vitamin D sufficient ($\geq$75 nmol/l) or deficient (<75 nmol/l) as per recent published recommendations (55). Prevalence ratio estimates and respective 95% confidence intervals will be calculated. We will similarly analyze these data for an association between vitamin D status and black race, to confirm previously published data that this group is at highest risk for vitamin D deficiency (29).

Sample Size and Power Calculations (see methods for Aim 1b)

Anticipated Results

We anticipate that women with BV will have lower median serum 25D concentrations than those without BV. Furthermore, we predict that women classified as vitamin D deficient are more likely to be diagnosed with BV by the objective Nugent criteria than those women determined to be vitamin D sufficient.

Potential Pitfalls

We may not observe an association between serum 25D levels and BV, as there are several other critical components in the vitamin D cascade that may ultimately influence VDR-mediated production of AMPs. Individual polymorphisms in the VDR have previously been implicated in altered expression of AMPs and we will explore this further in Aim 1b. While 25D levels are generally considered the most accurate measurement of an individual's overall vitamin D status, there are anecdotal reports of a disassociation between clinically apparent vitamin D deficiency

and serum 25D concentrations (56). These observed discrepancies may be secondary to both quantitative and functional variations in vitamin D binding protein (DBP), another important mediator in the vitamin D signaling cascade. If no association between 25D concentrations and BV status is observed in this cohort, further exploration of individual variations in DBP may be warranted.

Aim 1b. Determine whether specific known polymorphisms in the gene encoding the vitamin D receptor (VDR) affect the risk of developing BV.

Rationale

The critical role of vitamin D signaling in the innate immune system is further supported by genetic studies implicating polymorphisms in VDR gene and an increased susceptibility to infectious diseases, the most notable of these being the *Fok1* and *Taq1* VDR SNPs (30, 31, 49). The Cdx-2 SNP is also of great interest as it is highly prevalent in African Americans, the group most commonly affected by BV. In this aim, we will explore the contribution of these three VDR polymorphisms to an increased susceptibility to BV.

Experimental Design

Cohort, Sample Collection, and Processing (as described for Aim 1a)

Host DNA isolation and Genotype Determination

DNA will be isolated from the cellular component of blood specimens using a commercially available kit (Qiagen). The DNA quality of each sample will be confirmed using control PCR of the housekeeping gene, GAPDH. PCR amplification of VDR sequences containing the target SNPs will be performed as previously described (57-59) using commercially available TaqMan real-time PCR reagents (Applied Biosystems). The amplified DNA products will undergo enzymatic digestion using *Fok1* and *Taq1* restriction endonucleases. Electrophoresis will be performed to identify resultant restriction fragments. SNP-containing regions will be sequenced to confirm RFLP findings.

Statistical Analysis

The departure of frequencies of *VDR FokI, TaqI,* and *Cdx-2* polymorphisms from expectation under Hardy–Weinberg equilibrium will be assessed. Subjects will be classified as follows: homozygous for the major allele, homozygous for the minor allele, or heterozygous for each polymorphisms. The presence of BV again will be treated as a dichotomous variable. Pearson's χ^2 test of independence will be used to test for an association between an observed genotype and the presence of BV. One-way ANOVA will be performed to assess whether there are significant differences among mean serum 25D concentrations for each observed genotype. Post hoc analysis will be performed using the Bonferroni method to correct for multiple comparisons.

Sample Size and Power Calculations (applies to cohort for Aims 1 and 2)

Relevant estimates include the prevalence of bacterial vaginosis (30% of the population, the majority of which are asymptomatic and not seeking treatment and our candidate SNPs (20% of the population, a conservative estimate based upon previously published data (49)). If we desire 90% power to detect a susceptibility allele that occurs in 20% of controls (in either the heterozygous or homozygous state) and two-sided α=0.001, our calculated number of cases needed is 229 (38). This gives a total sample size (based on prevalence of BV) of 764. We will target enrollment at 900 women over a two year period in order to account for possible withdrawals and missing data.

Anticipated results

We anticipate that homozygosity for any of the minor alleles of interest (f,t, and A) will be associated with an increased risk for BV, with the likely mechanism being decreased efficiency

of VDR-mediated signaling. This would provide further evidence in support of a critical role for vitamin D signaling in the local immunity at the vaginal mucosal surface and would be consistent with previously published data implicating these genotypes as a risk factor for other infectious diseases.

Potential Pitfalls

We have chosen to focus on the *Fok1* and *Taq1* VDR polymorphisms based upon previous data suggesting these polymorphisms are associated with altered susceptibility to other important infectious diseases and the Cdx2 polymorphism because of its high prevalence in African-American populations. However, there are now more than 60 SNPs identified for the VDR and it is possible that one of those may be more relevant to BV susceptibility. PCR amplification and subsequent sequencing of these new areas of interest in the VDR gene will be possible with appropriate storage of host DNA specimens. It is possible to haplotype the entire VDR allele by using a small number of "tag SNPs" using methods developed by the international HapMap program. If we focus on additional alleles, we will determine an appropriate number of tag SNPs using the Tagger algorithm (www.hapmap.org) and will type these using sequencing or real-time PCR. Statistical analysis for any of these will be as described above for the Fok1 and Taq1 polymorphisms, but careful correction for multiple comparisons will be required, especially with an increasing number of SNPs examined.

Aim 2. Investigate specific molecular mechanisms by which vitamin D signaling controls vaginal innate immune responses relevant to BV.

Aim 2a: Determine whether exposure to 25-hydroxyvitamin D results in increased production and secretion of antimicrobial peptides by vaginal epithelial cells.

Rationale

While it is clear that vitamin D deficiency and polymorphisms in the VDR are associated with increased risk for infectious diseases, the specific mechanisms mediating such predispositions remain unclear. However, it is has recently been established that epithelial cells are capable of converting 25D to 1,25D, a process that was once thought to occur exclusively in the kidneys. This hormonally active metabolite binds to the VDR, initiating a cascade of events culminating in the increased production of antimicrobial peptides (AMPs). This finding is consistent with our preliminary data, which show a protective effect of vitamin D on genital tract epithelial cells challenged with vaginolysin, the *Gardnerella vaginalis* cytolysin. In Aim 2a, we will test the hypothesis that vaginal epithelial cells are capable of producing both cathlicidin and human HBD-2, AMPs that are crucial for mucosal defense, in a 25D-dependent manner. These AMPs are hypothesized to play a pivotal role in host defense against BV, and their dependence on vitamin D metabolism provides a putative mechanistic link between serum vitamin D levels, VDR polymorphisms, and risk for BV.

Experimental Design

Human vaginal endothelial cells (VK2, ATCC CRL-2616) will be grown in serum free keratinocyte growth media (Invitrogen) as previously described [redacted]. Three-dimensional, highly differentiated primary vaginal epithelial layers (EpiVaginal; MatTek) will be grown in Dulbecco's Modified Eagle's Medium (DMEM) with supplementation as specified by the manufacturer. These primary vaginal tissue are cultured on specially prepared cell culture inserts and closely parallel native human tissues, thus providing a useful in vitro means to assess toxicity, response to infectious agents and production of antimicrobial products (39, 61). Cells or EpiVaginal layers will be treated with varying concentrations of purified 25D or vehicle control as discussed in

Preliminary Studies section. Following treatment, supernatants will be collected and immediately frozen prior to analysis. A subset of the cells will be collected for RNA extraction using a commercially available kit (Ambion). The remaining cells will be lysed for cellular protein analysis. We will assess vaginal epithelial cell production of the following proteins: 1) 1-α-hydroxylase and 24−hydroxylase, the inducible enzymes catalyzing the formation and degradation of 1,25D 2.) 1,25D 2) cathelicidin, and 3) HBD-2. Production of these proteins will be quantified at both the mRNA and protein level utilizing quantitative real-time PCR (qRT-PCR) and western blot analysis. Concentrations of 1,25D, cathelicidin and HBD-2 in cell supernatants will be determined by commercially available ELISA assay kits. (Immunodiagnostic Systems, Hycult Biotechnology and Phoenix Pharmaceuticals),

Statistical analysis

mRNA concentrations in both the treated and untreated groups will be will be determined using the comparative C_t method with normalization to the GAPDH housekeeping gene. Relative quantity of gene expression will be compared between 25D treated and untreated groups using an unpaired t-test. Median concentrations of each protein as determined by ELISA will be similarly compared in the treated and untreated groups.

Anticipated results

Based on our preliminary findings, we hypothesize that both immortalized and primary vaginal epithelial cells produce 1-α-hydroxylase and are therefore, capable of generating 1,25D from the 25D precursor. We anticipate that exposure to exogenously administered 25D will result in increased production and subsequent secretion of cathelicidin and HBD-2, consistent with our hypothesis that local vitamin D levels influence mucosal defense via the production of AMPs.

Potential pitfalls

Acute exposure of these cells to 25D may not be sufficient to induce measurable changes in AMP production and a variety of dosages and treatment time courses will need to be explored. In addition, we choose to investigate these particular AMPs based upon previously published data and our own preliminary experimental data. It is possible that other AMPs, regulated through 25D-dependent signaling mechanisms, have an important role in vitamin D mediated susceptibility to BV (such as α-defensins, secretory leukoprotease inhibitor). These peptides these could be similarly investigated.

Aim 2b. Determine the effects of vitamin D-responsive antimicrobial peptides on epithelial defense against *Gardernella vaginalis,* the primary etiologic agent in BV.

Rationale

AMPs provide protection from microbial pathogens through a variety of mechanisms including disruption of bacterial membranes, toxin binding/neutralization, and immunomodulatory signaling. Our preliminary data indicate that AMPs mitigate the lytic activity of VLY, the major pore-forming toxin produced by *G. vaginalis.* Because vitamin D signaling pathways regulate the expression of several AMPs, we hypothesize that this is the primary mechanism by which vitamin D deficiency mediates an increased susceptibility to BV.

Experimental Design

Continuing our group's longstanding collaboration with [redacted], an expert in the area of antimicrobial peptides and immune defense, we will utilize surface plasmon resonance technology to investigate binding of cathelicidin and HBD-2 to VLY. In addition, we will determine binding affinities over a range of pH values, as BV is characterized by dramatic alterations in vaginal pH. To determine whether these AMPs abrogate VLY-mediated

cytotoxicity, we will treat recombinant VLY with purified cathelicidin and HBD-2 (synthesized in the laboratory of [redacted]) as well as a negative control (scrambled peptide) for 30 minutes. VLY-mediated lysis well be assessed using an LDH cytotoxicity assay as previously described(11) . In separate experiments, the microbicidal activity of these peptides will be evaluated by adding various concentrations of each peptide to known concentrations of live *G. vaginalis* (in early to mid-exponential phase). Colony-forming units (CFU) will be determined over a range of time points and change in *G. vaginalis* concentration (log_{10} CFU/ml) will be calculated.

Statistical Analysis
Student's t test will be performed to compare the percent lysis following treatment of VLY with each AMP versus negative controls. ANOVA with appropriate post-tests will be used where multiple comparisons are involved. Dose-response and IC_{50} values will be computed for each peptide and similarly compared.

Anticipated Results
We predict that both cathelicidin and HBD-2 will bind VLY and subsequently inhibit VLY-mediated cytolysis, as has been observed with other AMPs (62) and as described in our preliminary studies. Furthermore, we anticipate that exposure to either cathelicidin or HBD-2 at physiologic concentrations will potently inhibit growth of *G vaginalis* in culture.

Potential Pitfalls
Cathelicidin and HBD-2 may not functionally bind VLY as they do other CDC toxins.
If this is the case, one potential mechanism may be to variations in the undecapaptide region of VLY. If so, other potential antimicrobial activities of these AMPs, such as disruption of bacterial membranes and immunomodulatory signaling, may require further exploration.

Aim 2c. Detect and quantify vitamin D-responsive antimicrobial peptides in vaginal lavage specimens from non-pregnant, healthy adult women.

Rationale
AMPs are known to play an important role in the maintenance of normal vaginal flora. Decreased concentrations of these critical peptides have been documented in the vaginal secretions of women with BV (39). We hypothesize that reduced AMP production may be associated with decreased serum concentrations of 25D, thereby providing a mechanistic link between vitamin D deficiency and BV susceptibility. Based on our findings in the prior subaims, we will examine the concentration of relevant AMPs in vaginal lavage specimens from women with and without BV and with varying levels of serum vitamin D in order to determine the relevance of this pathway to BV in vivo.

Experimental Design
Vaginal lavage specimens will be obtained from healthy adult women as described in Aim 1a. Detection and quantification of cathelicidin and HBD-2 proteins will be performed ELISA based techniques as described in Aim 2a. The microbicidal activity of these vaginal washes will be evaluated as delineated Aim 2b.

Statistical Analysis
Student's t-test will be used to compare the mean concentrations of both cathelicidin and HBD-2 in vaginal wash specimens of women 1) with and without BV, 2) women with and without vitamin D deficiency. In addition, we will utilize regression analysis to assess for correlation between AMP concentrations and serum 25D levels as determined in Aim 1a. The microbicidal

activity of vaginal wash specimens (expressed as log-change in *G.vaginalis* CFUs) obtained from women with and without BV will be similarly analyzed. In all cases, if the data are not normally distributed, non-parametric testing (Wilcoxon Mann-Whitney Test) will be substituted.

Anticipated Results

We anticipate that that the concentrations of these vitamin D-responsive AMPs will be significantly decreased in women diagnosed with BV and in those women classified as vitamin D deficient. Furthermore, we predict that there will be a positive linear correlation between the serum 25D level and the concentration of AMPs in the corresponding vaginal lavage specimen. Finally, we predict that that the microbicidal ability of vaginal wash specimens obtained from women with normal vaginal flora will exceed that of the specimens from those with BV. These data would confirm our hypothesis that the increased susceptibility to BV in women with inadequate vitamin D is mediated by a deficiency of Vitamin D-responsive antimicrobial peptides

Potential Pitfalls

Vitamin D-mediated protection from BV may be due to other VDR-induced alterations in mucosal defenses. Expanded investigation into additional vitamin-D regulated immune mediators, specifically IL-8 and IL-1β, may be warranted.

FINAL REMARKS

These two aims will allow us to rigorously assess the contribution of vitamin D deficiency, a reversible and exceedingly prevalent condition, to bacterial vaginosis. Using a combination of epidemiologic and laboratory-based techniques, we will address the hypothesis that vitamin D-responsive antimicrobial peptides are crucial to defense of the vaginal mucosa and that reduced levels of these peptides in the setting of vitamin D deficiency predispose women to developing BV. These investigations have important implications for our understanding of BV and for future therapeutic and preventative strategies and will set the stage for my continued development as an independent investigator. We thank you for your consideration.

Protection of Human Subjects

Risks to Human Subjects

Human Subjects Involvement and Characteristics

Human subjects are proposed for involvement in these studies. Over the 2 year period of the study, we will recruit 200 non-pregnant, healthy adult (ages 21-64) female volunteers presenting for primary care at [redacted] gynecology clinics. The extent of contact with the subject will be a single visit for the specimen collection. Exclusion criteria will include history of immunodeficiency, recent (<1 month) history of antibiotic or immunomodulatory therapy, current vaginal bleeding, and known reproductive tract abnormalities. These potentially eligible subjects will be approached for informed consent

Source of Materials

With the assistance of the clinician providing the patient's care, a single 2ml blood culture will be obtained for quantification of serum 25D and genomic analysis of the vitamin D receptor protein. The following samples will be collected during the speculum examination. Two swabs (done concurrently with a dual swab kit) of the lateral vaginal walls will be collected. These will provide material for rapid pH determination, wet mount and KOH prep, gram stain (for Nugent scoring). Following collection of the swabs, a 1 ml vaginal wash (obtained by instilling 1ml of sterile PBS into the vagina with a flexible catheter-tipped 5 ml syringe and then immediately removing the fluid with the same syringe) will be collected for detection and quantification of antimicrobial peptides. All other aspects of the visit will be at the discretion of the provider. There will be no collection or recording of any individually identifiable data from any of the subjects.

Potential Risks

A single blood specimen obtained via venipuncture may cause minor discomfort, but entails minimal risk to the patient. The speculum examination is part of a normal gynecologic examination and is something that most women will have experienced previously. The examination and lavage procedure will be explained in detail as part of the informed consent process, and volunteers will be given multiple explicit opportunities to withdraw from the study. The examination and lavage come with minimal physical risk -- minor discomfort during the procedure would be the most common. The entire procedure will be less than ten minutes in duration. A female chaperone will be present during the procedure at all times. Because no identifying data will be collected, no legal or financial risks to volunteers are anticipated.

Adequacy of Protection Against Risks

Recruitment and Informed Consent

Subjects will be recruited from the [redacted], an ethnically diverse area in which the [redacted] is located. Detailed informed consent will be obtained prior to enrollment. It will be made explicit that the samples being collected will only be used for in vitro studies and that no identifying data will be obtained. The purpose of the study will be explained to the subjects by the P.I. or co-investigators who will document consent, and opportunities to ask questions will be provided.

Protections Against Risk

Risks to subjects will be minimized. No identifying data will be collected. Data will only be reported in aggregate, without any individually identifying data. Vulnerable populations as listed in section 4.1.2b are not included in this research.

Potential Benefits of the Proposed Research to Human Subjects and Others
There are no potential direct benefits to the research participants. However, there are minimal risks of the study and the importance of the knowledge to be gained is great.

Importance of the Knowledge to be Gained
This proposal is designed to determine if vitamin D deficiency is associated with bacterial vaginosis, an incredibly common disease with serious sequelae. Identification of a potentially modifiable risk factor, such as inadequate vitamin D stores, would not only contribute to a better understanding of this disease process, but may represent a novel strategy for its prevention.

Inclusion of Women and Minorities

Only healthy adult women will be included in this research, as vaginal wash/swab specimens will be required. In addition, the research question of the proposal is relevant only to women. The targeted/planned enrollment table is attached. We plan to include Hispanic and non-Hispanic women in equal numbers and anticipate enrolling smaller numbers of African-American and Asian participants than white participants. We do not anticipate enrolling either American Indian/Alaska Natives or Native Hawaiian/Other Pacific Islanders given the demographics of our population. Recruitment will occur in the ethnically diverse neighborhood of [redacted].

Targeted/Planned Enrollment Table

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

Study Title: *Bacterial Vaginosis: Vitamin D Links Mucosal Immunity and Patient Risk*

Total Planned Enrollment: 200

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	100		100
Not Hispanic or Latino	100		100
Ethnic Category: Total of All Subjects *	200		200
Racial Categories			
American Indian/Alaska Native	0		0
Asian	20		20
Native Hawaiian or Other Pacific Islander	0		0
Black or African American	30		30
White	50		50
Racial Categories: Total of All Subjects *	100		100

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

Inclusion of Children

Children will not be included in this research as the question under study (the role of vitamin D status in the development of BV) is not directly relevant to children.