K23

PI:	Title: Predictors of ovarian failure after chemotherapy in young breast cancer patients	
Received: 03/12/2009	FOA: PA09-043	Council: 10/2009
Competition ID:	FOA Title: MENTORED PATIENT-ORIENTED RESEARCH CAREER DEVELOPMENT AWARD (K23)	
1 K23	Dual: CA	Accession Number
IPF:	Organization:	
Former Number:	Department: Obstetrics and Gynecology	
IRG/SRG:	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 121,900 Year 2: 121,900 Year 3: 121,900 Year 4: 121,900 Year 5: 121,900	Animals: N Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N	New Investigator: N Early Stage Investigator: N

Senior/Key Personnel:	*Organization:*	*Role Category:*
		Other Professional-Consultant
		PD/PI
		Other Professional-Mentor
		Other Professional-Mentor
		Other Professional-Mentor
		Other Professional-Collaborator
		Other Professional-Collaborator
		Other Professional-Collaborator

Reference Letters

03/12/2009

03/12/2009

03/12/2009

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
03/12/2009	
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION *** Organizational DUNS:**

* Legal Name:

Department: Division:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: * ZIP / Postal Code:

Person to be contacted on matters involving this application

Prefix: * First Name: Middle Name:

* Last Name: Suffix:

* Phone Number: Fax Number:

Email:

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

7. * TYPE OF APPLICANT: O: Private Institution of Higher Education

Other (Specify):

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

8. * TYPE OF APPLICATION:

☐ New ☒ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

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

9. * NAME OF FEDERAL AGENCY:

National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Predictors of ovarian failure after chemotherapy in young breast cancer patients

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

N/A

13. PROPOSED PROJECT:

* Start Date: 12/01/2009 * Ending Date: 11/30/2014

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant b. * Project

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: * First Name: Middle Name:

* Last Name: Suffix:

Position/Title:

* Organization Name:

Department: Division:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: * ZIP / Postal Code:

* Phone Number: Fax Number:

* Email:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Tracking Number: Funding Opportunity Number: Received Date:2009-03-12T00:31:34-04:00

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	655,173.00
b. * Total Federal & Non-Federal Funds	655,173.00
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: Middle Name:

* Last Name: Suffix:

* Position/Title:

* Organization:

Department: Division:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: * ZIP / Postal Code:

* Phone Number: Fax Number:

* Email:

*** Signature of Authorized Representative** *** Date Signed** 03/12/2009

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: 4040-0001
Expiration Date: 04/30/2008

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

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RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name:
* Street1:
Street2:
* City: County:
* State: Province:
* Country: * ZIP / Postal Code:

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: 4040-0001
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: 08/06/2008

Exemption Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

Human Subject Assurance Number:

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

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 1234-IS_K23 Project Summary.pdf Add Attachment Delete Attachment View Attachment

7. * Project Narrative 1237-Projective Narrative.pdf Add Attachment Delete Attachment View Attachment

8. Bibliography & References Cited 1236-IS_References 0309.pdf Add Attachment Delete Attachment View Attachment

9. Facilities & Other Resources 1235-IS_K23 Resources 0309.pdf Add Attachment Delete Attachment View Attachment

10. Equipment Add Attachment Delete Attachment View Attachment

11. Other Attachments Add Attachments Delete Attachments View Attachments ☒

OMB Number: 4040-0001
Expiration Date: 04/30/2008

PROJECT SUMMARY/ABSTRACT

The candidate recently completed a reproductive endocrinology fellowship and the Master's of Science in Clinical Epidemiology program at the This mentored research award will prepare to become an independent investigator in reproductive epidemiology. Specifically, this proposal will enable the principal investigator to: 1) conduct a prospective cohort study; 2) learn methods in genetics epidemiology and predictive modeling; 3) collaborate with researchers multiple disciplines; and 4) gather data for an R01 submission.

The candidate's environment is optimal for both career development and the proposed research plan. The candidate is mentored by a team of three senior faculty members in the fields of reproductive epidemiology, reproductive aging and breast oncology. Through secondary appointment to has significant support for continued formal and informal training in epidemiologic and statistical methods. Finally, the proposed research project is feasible and well-supported in the candidate's environment. Through strong collaborations with breast oncology, general obstetrics and gynecology, radiology, and biostatistics and epidemiology, the investigator has direct access to study subjects, imaging and genotyping expertise, and biostatistical support.

More than two million American women are breast cancer survivors. The large number of reproductive aged women with breast cancer provides a unique opportunity to study the impact of this devastating disease and treatment on ovarian aging and reproduction. Accurately characterizing and predicting ovarian function in young breast cancer patients is an important issue to survivors because of implications on reproductive issues such as fertility and menopause and cancer survival. Current predictors of ovarian failure after chemotherapy are limited to age and chemotherapy regimen.

The goal of this research proposal is to identify significant hormonal, genetic and ovarian morphometric markers associated with ovarian failure after adjuvant chemotherapy. A 4-year prospective cohort study of pre- menopausal breast cancer patients, followed from diagnosis to post-chemotherapy, is proposed. We will test the hypotheses that: 1). Breast cancer patients with lower pre-chemotherapy anti-Mullerian hormone and inhibin B levels and higher pre-chemotherapy Follicle Stimulating Hormone levels will be more likely to develop ovarian failure after adjuvant chemotherapy; 2). Breast cancer patients with drug metabolizing enzyme polymorphisms resulting in higher cyclophosphamide exposure are more likely to develop ovarian failure after cyclophosphamide-based adjuvant chemotherapy; 3). Breast cancer patients with smaller pre-chemotherapy ovarian volume, diameter and antral follicle count, and larger changes in these measurements pre- and post-chemotherapy are more likely to develop ovarian failure; 4). A predictive index using variables from these aims can be derived to identify patients at high risk for ovarian failure after therapy.

Relevance: This study will identify hormonal, genetic and ovarian imaging markers to predict ovarian failure after chemotherapy. These tools will assist young breast cancer patients and their physicians in making treatment decisions that would impact cancer survival and reproduction.

BIBLIOGRAPHY

LABORATORY

Laboratory

The is located in the . The has certification from CLIA, State and Federal regulatory agencies and is supported in part by an NIH center grant (. The lab provides full clinical laboratory testing and immunoassays. The immunoassays for the current project will be conducted in the

Laboratory

The Laboratory comprises approximately 250 sq ft of space within the . on the within the laboratory of This space is occupied by one full-time technician and contains 2 freezers (-80 and –20), cold storage (4 degrees), refrigerated and non-refrigerated centrifuge. Genotyping studies are done in collaboration with and has dedicated laboratory space in the at the laboratory occupies approximately 750 square feet and has fully separated rooms for DNA extraction, PCR set up, and genotyping. Available equipment in laboratory includes 4 sets of agarose gel apparatus, seven 96-well PCR thermo cyclers ( ), high voltage power supplies, high-speed centrifuges, water baths and shakers, 2 refrigerators (4°C), 2 freezers (-20°C and -80°C), table-top and refrigerated high-speed centrifuges, Nanodrop for DNA quantitation, BioRad GelDoc Imager, and other miscellaneous equipment required for molecular biological analysis.

shares PCR-based genotyping systems with other investigators in the directed by in the at the laboratory occupies approximately 1100 square feet to support various genotyping platforms. These platforms include a Pyrosequencing PSQ M96 System with dedicated SNP Software and SNP Reagent Kits, the Applied Biosystem's 3130 xl Genetic Analyzer using capillary sequencing technology, SNPlex System kit for 48-plex genotyping per single sample, and Snapshot v 2.0 kit designed for multiplex single-base extension reactions, and the MJ Chromo4 Real Time PCR system including ABI Taqman genotyping assays and reagents. The LME also has a Tecan Genios FL microplate reader for DNA quantitation and ABI 377 and ABI 3100 automated sequencers for both sequence and fragment analysis.

CLINICAL

The is both a local hospital and national referral center, admitting approximately 20,000 patients per year to its 722-bed facility. is the largest of three major hospitals in the , which also includes and This network of hospitals provides access to a wide array of patients and health care settings that can be readily accessed for clinical studies. The central location of surrounded by over 50 primary care practices and located in the center of the encourages collaboration among physicians, epidemiologists, and basic scientists involved in clinical research.

The Department of Obstetrics and Gynecology

The Department of Obstetrics and Gynecology has dedicated clinical units for outpatient gynecology and obstetrics, family planning, high risk obstetrics and antenatal testing, gynecological oncology, infertility and endocrinology, assisted reproductive technologies and the premenstrual syndrome. Six residents each year are accepted into an approved 4-year residency training program in obstetrics and gynecology. The Department has sub-specialist physicians in maternal-fetal medicine, genetics, endocrinology and infertility, and gynecological oncology. Each of these sub-specialty programs has an approved clinical fellowship training program. There are over 40 faculty members within the Department.

The Division of Reproductive Endocrinology and Infertility

The care of women with reproductive/endocrine disorders is centered in the Department's Center for Reproductive Medicine and Surgery marketed under the brand name of which and are members. In the spring of 2003 moved to newly constructed 14,000 sq ft facilities on the campus of the This clinical space is complemented and fully integrated with an additional 8,000 sq ft of clinical and laboratory research space. This combined space includes 8 examination rooms, seven transvaginal ultrasound machines used for the purposes of clinical research and patient care, and a clinical procedure room equipped with an ultrasound machine, video monitoring and ability to capture electronic imaging for colposcopy and hysteroscopy. The Division is one of eight members of the Additionally, the Department's is an

The at the centralizes clinical research in the and is directed by The main unit occupies dedicated space (4000 sq ft) in a building adjacent to the inpatient facilities and operating rooms of the Hospital of the The unit also has satellite office for clinical coordination and research personnel in each division OB/GYN.

In particular, the satellite office adjacent to the and occupies dedicated space (8000sq ft). This satellite has access to seven transvaginal ultrasound machines used for the purposes of clinical research and patient care at In particular, a 5 MHZ real time ultrasound (Sonoline G50, Siemens, Berlin, Germany) is dedicated specifically to clinical research. The staff in the REI satellite is composed of 5 research coordinators, a dedicated administrative assistant, and up to an additional 4 part-time workers. This dedicated clinical research staff is available to perform aspects of the project including preparation of Institutional Review Board applications, recruitment, screening, clinical examinations, follow-up examinations and provide care outside of scheduled visits, thus allowing continuity of patient care. This staff is also experienced in data management with nine networked, dedicated, PC-compatible computers. All computers have internet access.

Since its inception, research in the has increased dramatically, and more than 1200 women have been recruited into clinical trials. The is experienced in the collection of clinical specimens from patients to use for research. The has conducted more than 31 multi-center trials and has participated in two NICHD clinical trial networks as well 2 NIH sponsored multi-center trials.

The at the is the home of the Human Behavior and Reproduction Program, directed by This program is dedicated to clinical research of behavioral and emotional factors that affect women's health. Major research areas of the Program are long-term longitudinal studies of hormones and behaviors linked to ovarian aging, clinical trials of treatments to improve women's reproductive health care, and the PMS Program.

The Center

The at the was founded in 1973 to serve as the focus of cancer-related research, patient care, and educational activities at the It is one of 40 cancer centers in the country approved and designated by the National Cancer Institute as a r and has been continuously funded by the National Cancer Institute since its founding.

The Center has more than 330 physicians and scientists who are drawn from 41 University Departments and 8 Schools. Most Cancer Center members are from the School of Medicine, while others are from the Schools of Veterinary Medicine, Dental Medicine, Nursing, Social Work, Wharton Business, and Arts and Sciences. Center members have over $90 million of extramural funding. Collaborative working

relationships are well established among Center members from different Departments and Schools as is evidenced in all aspects of Cancer Center research, patient care and educational activities.

The Center provides an organizational framework that promotes and enhances interdisciplinary cancer research within the School of Medicine and throughout the University. Its mission is defined through seven primary objectives: develop and support cancer-focused Research Programs (basic, translational, clinical, and cancer control) that are interdisciplinary and interdepartmental in nature; develop and support interdepartmental educational and research training programs for physicians, scientists, students, nurses, and others; recruit and support new faculty with a commitment to cancer research; facilitate investigator collaboration within and among Research Programs; develop and manage Shared Resources; acquire and allocate laboratory space for active cancer researchers; and foster interdisciplinary patient care programs and activities.

The Center is a dedicated interdisciplinary breast center introduced in 2000. is of this program. Located in 11,000 sq. ft of space in the new for at the the Center provides evaluation and ongoing care of patients with breast cancer or those at high risk. Among the services available at this single location are: outpatient medical, radiation and surgical oncology visits, rehabilitation medicine consultations, chemotherapy and infusion services, patient education, nutrition counseling, and psychosocial support. The success of this care model is evidenced by high volume, patient satisfaction scores over 90%, and strong physician support. Additional evidence includes recognition from national and regional breast cancer organizations. The recruitment of two additional medical oncologists this year was needed to meet patient demand. The Center believes that this kind of model represents the next level of interdisciplinary cancer care and service excellence.

clinical research staff is housed in 200 sq ft of space within the Center for Advanced Medicine, adjacent to the Center, where the proposed study will be conducted. Two full time and 3 part-time staff members specialize in data management, clinical data coordination and data entry. The space contains three Dell PC computers that utilize the network and server of the Research Institute. All study databases are developed in Microsoft Access. A large database ( ) of breast cancer patients presenting to the was implemented in January, 2000. This database contains more than 250 data elements, and currently contains more than 1600 subjects.

The Department of Radiology

All imaging that is part of the proposed project will be performed at the ( ) in the gy at the currently accommodates two whole-body MR imagers (3.0T Siemens Trio and 1.5T Siemens Sonata) entirely dedicated to research. The Siemens Trio 3.0 T whole-body MRI scanner and the Siemens Sonata 1.5T whole-body MRI scanner are equipped with 40 mT/m, 200T/m/s slew rate, whole-body gradients. In addition, a 1.5T Siemens Avanto and a 3T Siemens Tim Trio available 50% for research have recently been installed in the The Siemens Avanto 1.5 T whole-body MRI scanner is equipped with 45 mT/m, 200 T/m/s slew rate gradients. There are two SUN workstations, a Silicone Graphics Octane System and a Dell PC (P4, 2.4GHz) currently available in the MR Imaging Center. In addition, there are at least twelve other SUN, SGI and PC workstations distributed between the various research groups that use the MR facility. The clinical MRI operation is run through a Service Center that has four research technologists entirely dedicated to patient-related research.

The

The is an interdisciplinary and interdepartmental center at the Its mission is to improve the health of the public by linking epidemiology, biostatistics, and clinical medicine, bringing epidemiologic methods to clinical research, clinical insight to epidemiologic research, and an understanding of research methodology to clinical medicine. activities focus on the performance of clinical epidemiologic research, biostatistical research and methodology development, the conduct of multicenter clinical trials, collaboration with biomedical and clinical researchers across diverse scientific disciplines, and curriculum development and teaching of clinical epidemiology and biostatistics at the undergraduate and post-graduate levels.

The occupies 140 offices plus 20 workstation/computing pods, located on seven floors in , totaling approximately 34,000 net square feet. Within this space, the maintains one seminar room with capacity for 60 people and three large 16-person conference rooms. Renovation of additional space in for the representing approximately 3,000 net square feet of space, is scheduled for 2007. is adjacent to the main Medical School buildings and a short distance from , uilding (which houses many of the HIV basic science laboratories as well as the offices of the for AIDS Research), and the main campus of the University. In addition, the occupies approximately 1200 net square feet of laboratory space in the Basic Science Research Building II/III building, located across the street from . Finally, the has additional offices within the complex located on the "Avenue of Technology," at the edge of the . The occupies approximately 3,200 square feet of space at one location, including a large conference room and offices for approximately 15 staff. An additional 8,700 square feet is provided at the other location, with office space for approximately 50 staff.

ANIMAL
Not applicable.

COMPUTER
The Department of OB/GYN has over 100 desktop computers on a departmental wide-area network. We have established wide-area network connections between our laboratories and clinical facilities using the Hospital and University broad-band backbones. The has multiple high-speed T3 lines accessible via the University's wide-area network The Department maintains ethernet connections to and provides the full suite of Internet services (e-mail, FTP, USENET, gopher and World Wide Web) for nodes on our LANs. The Department has a full-time Network Administrator who is responsible for coordination of the Department's computer efforts, e.g., customizing software as needed and maintenance of the database servers. Data analysis software programs including SAS version 9.1 and STATA version 8.0 are currently used for data analysis purposes within the research unit.

OFFICE
Division of
The candidate has a 100 square foot office within the in at There are extensive computer facilities, including state-of-the-art statistical computing software and hardware, and access to patient medical records. office is equipped with a state-of-the-art computer, printer, and high-speed internet access.

Referee list:

1.

2.

3.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: * First Name: Middle Name:

* Last Name: Suffix:

Position/Title: Department:

Organization Name: Division:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: * Zip / Postal Code:

* Phone Number: Fax Number:

* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:**

***Attach Biographical Sketch** Add Attachment | Delete Attachment | View Attachment

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

PROFILE - Senior/Key Person 1

Prefix: * First Name Middle Name:

* Last Name: Suffix:

Position/Title: Department:

Organization Name: Division:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: * Zip / Postal Code:

* Phone Number: Fax Number:

* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:** Mentor

***Attach Biographical Sketch** Add Attachment | Delete Attachment | View Attachment

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

Funding Opportunity Number:PA-09-043 Received Date:2009-03-12T00:31:34-04:00

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: * First Name: Middle Name:
* Last Name: Suffix:
Position/Title: Department:
Organization Name: Division:
* Street1:
Street2:
* City: County:
* State: Province:
* Country: * Zip / Postal Code:
* Phone Number: Fax Number:
* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category**

***Attach Biographical Sketch** Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 3

Prefix: * First Name: Middle Name:
* Last Name: Suffix:
Position/Title: Department:
Organization Name: Division:
* Street1:
Street2:
* City: County:
* State: Province:
* Country: * Zip / Postal Code:
* Phone Number: Fax Number:
* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:** Mentor

***Attach Biographical Sketch** Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

Funding Opportunity Number:PA-09-043 Received Date:2009-03-12T00:31:34-04:00

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4

Prefix: * First Name: Middle Name:

* Last Name: Suffix:

Position/Title: Department:

Organization Name Division:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: * Zip / Postal Code:

* Phone Number: Fax Number:

* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:**

***Attach Biographical Sketch** Add Attachment | Delete Attachment | View Attachment

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

PROFILE - Senior/Key Person 5

Prefix: * First Name: Middle Name:

* Last Name: Suffix:

Position/Title: Department:

Organization Name: Division:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: * Zip / Postal Code:

* Phone Number: Fax Number:

* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:** Collaborator

***Attach Biographical Sketch** Add Attachment | Delete Attachment | View Attachment

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 6

Prefix: * First Name: Middle Name:
* Last Name: Suffix:
Position/Title: Department:
Organization Name: Division:
* Street1:
Street2:
* City: County:
* State: Province:
* Country: * Zip / Postal Code:
* Phone Number Fax Number:
* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:**

***Attach Biographical Sketch** Add Attachment | Delete Attachment | View Attachment

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

PROFILE - Senior/Key Person 7

Prefix: * First Name: Middle Name:
* Last Name: Suffix:
Position/Title: Department:
Organization Name: Division:
* Street1:
Street2:
* City: County:
* State: Province:
* Country: * Zip / Postal Code:
* Phone Number: Fax Number:
* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category**

***Attach Biographical Sketch** Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support 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

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
eRA COMMONS USER NAME	

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
	BS, AB MD MSCE		Biology, East Asian Studies Medicine Clinical Epidemiology and Biostatistics

Employment

Honors

Professional Societies

Publications

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

Ongoing Research Support

K12- (PI:
NICHD

(PI:

Completed Research Support

(PI:)
NIH

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

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
eRA COMMONS USER NAME	

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
	B.S.		Biology
	M.D.		Medicine
	M.S.C.E.		Clinical Epidemiology and Biostatistics

A. Positions and Honors.

Professional Positions:

NIH and Peer Review Participation:

Specialty Certification:

Honors, Awards. Committees:

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

C. Research Support.
Ongoing Research Support:

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

T32 (. PI /I co-PI)

U10 NIH/NICHD

Completed Research Support:

U01
NIH/NIAID

NIH/NIAID

NIH/NICHD

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

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
eRA COMMONS USER NAME	

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
	B.A.		Biochemistry
	M.D.		Medicine
	N/A		Hematology
	Residency		Internal Medicine
	Fellowship		Hematology-Oncology
	M.S.C.E.		Clinical Epidemiology

A. Positions and Honors.

Positions and Employment

Other Experience and Professional Memberships

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

Honors

B. Selected peer-reviewed publications (in chronological order). (Publications selected from 39

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

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

C. Research Support

Ongoing Research Support

Completed Research Support

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

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
eRA COMMONS USER NAME	

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
	M.S.S.		Social Work
	Ph.D.		Social Research

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

A. Positions and Honors

B. Selected peer-reviewed publications. (Selected from over 100 peer-reviewed publications)

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

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

C. Research Support

Current Research Support

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

Completed Research Support (last 3 years)

Principal Investigator/Program Director *(Last, first, middle):*

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
:	
eRA COMMONS USER NAMF	

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
	B.A.		Medical Science
	M.D.		Medicine
	M.S.C.E.		Clinical Epidemiology

A. Positions and Honors.

Other Appointments:

Selected Awards and Honors:

Selected peer-reviewed publications (last 5 years).

Principal Investigator/Program Director *(Last, first, middle):*

Principal Investigator/Program Director *(Last, first, middle):*

Research Support.

Principal Investigator/Program Director *(Last, first, middle):*

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

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
	BA		Statistics
	MA		Applied Statistics
	Sc.D.		Biostatistics

A. Positions and Honors.

Positions and Employment

Other Experience and Professional Memberships

Honors

B. Selected peer-reviewed publications out of 106 (in chronological order).

C. Research Support.

Ongoing Research Support

K12-
NIH

R01-
NIH

R01-
NIH

U10-
NIH

R01-
NIH

R01
NIH

□ Principal Investigator/Program Director (Last, first, middle):

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE

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
	BA		Chemistry
	BA		Mathematics
	MA		Mathematics
	MD		Medicine

A. Positions and Honors.

Positions and Employment

Honors

B. Selected peer-reviewed publications (in chronological order selected from 61 in total).

1.

2.

3.

4.

Principal Investigator/Program Director (Last, first, middle):

□ Principal Investigator/Program Director (Last, first, middle):

□ Principal Investigator/Program Director (Last, first, middle):

C. Research Support.

Ongoing Research Support

NIH/NHLBI

Completed Research Support

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

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
eRA COMMONS USER NAME: GCOUTIFARIS	

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
	A.B.		Biophysics
	M.D.		Medicine
	Ph.D.		Anatomy / Repro.Neuroendocrinology
	Post-doc		Reproductive Endocrinology

A. Research and Professional Experience:

Board Certification:

Selected Awards and Honors:

National Scientific Review Committees:

B. Selected Publications:

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

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

C. Research Support (active only):

U10-
National Institutes of Health

T32-
National Institutes of Health

K12-
National Institutes of Health

RO1-

National Institutes of Health

RO1-

National Institutes of Health,

U54-Roadmap grant, National Institutes of Health,

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

OTHER SUPPORT

;

ACTIVE:

:
NIH

R01-
NIH

NIH

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

ACTIVE:

NIH

U10-
NIH

R21 /

OTHER SUPPORT

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

ACTIVE:

PENDING:

K24
NIH

R01
NIH

R21
NIH

Overlap:
If funding received is greater than 100%, effort will be reduced as appropriate on some of the projects listed above.

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

OTHER SUPPORT

ACTIVE:

R01
NIH

R01
NIH

P50
NIH/NCI

R01
NIH

The

P30
NIH

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

OTHER SUPPORT

ACTIVE:

P30
NIH

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

OTHER SUPPORT

ACTIVE:

P50-

PENDING:

R21-
NIH

OVERLAP:

Support will not be permitted to exceed 100%. In the unlikely event that over 100% support were attained, the work on and support from existing projects would be adjusted downwards, with other faculty or staff assuming additional duties on each project.

Principal Investigator/Program Director:_____
(Last, first, middle)

PHS 398/2590 OTHER SUPPORT

ACTIVE

OVERLAP:
None

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

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

*** ORGANIZATIONAL DUNS:**

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

Enter name of Organization:

Delete Entry *** Start Date:** **End Date:** **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.							123,600.00	9.00			75,000.00	21,900.00	96,900.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 96,900.00

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) 96,900.00

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

* ORGANIZATIONAL DUNS:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry * Start Date: End Date: Budget Period

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:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry | Start Date: | * End Date: | 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.	Research development support	25,000.00
9.		
10.		
	Total Other Direct Costs	25,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	121,900.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.		8.00	109,032.00	8,723.00
2.				
3.				
4.				
	Total Indirect Costs			8,723.00

Cognizant Federal Agency

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

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	130,623.00

J. Fee

	Funds Requested ($)

K. * Budget Justification 1240-K23 Budget Justification0209.pdf

Add Attachment | Delete Attachment | View Attachment

(Only attach one file.)

OMB Number:
Expiration Date: 06/30/2011

Previous Period

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

*** ORGANIZATIONAL DUNS:**

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

Enter name of Organization:

Delete Entry *** Start Date:** *** End Date:** **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.							127,308.00	9.00			75,000.00	21,900.00	96,900.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person 96,900.00

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) 96,900.00

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

* ORGANIZATIONAL DUNS:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry * Start Date: * End Date: 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:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry | Start Date: | * End Date: | 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. Research development support	25,000.00
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	121,900.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	8.00	109,032.00	8,723.00
2.			
3.			
4.			
Total Indirect Costs			8,723.00

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

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	130,623.00

J. Fee	Funds Requested ($)

K. * **Budget Justification** 1240-K23 Budget Justification0209.pdf (Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

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

Previous Period

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

*** ORGANIZATIONAL DUNS:**

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

Enter name of Organization:

Delete Entry *** Start Date:** *** End Date:** **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.							131,127.00	9.00			75,000.00	21,900.00	96,900.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 96,900.00

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) 96,900.00

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

* ORGANIZATIONAL DUNS:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry * Start Date: * End Date: 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:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry | Start Date: | * End Date: | 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. Research development support	25,000.00
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	121,900.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	8.00	109,032.00	8,723.00
2.			
3.			
4.			
Total Indirect Costs			8,723.00

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

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	130,623.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-K23 Budget Justification0209.pdf (Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

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

Previous Period

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

*** ORGANIZATIONAL DUNS:**

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

Enter name of Organization:

Delete Entry *** Start Date:** *** End Date:** **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.							135,061.00	9.00			75,000.00	21,900.00	96,900.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 96,900.00

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) 96,900.00

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

* ORGANIZATIONAL DUNS:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry * Start Date: * End Date: 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:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry | Start Date: | * End Date: | 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. Research development support	25,000.00
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	121,900.00

H. Indirect Costs Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	8.00	121,900.00	9,752.00
2.			
3.			
4.			
Total Indirect Costs			9,752.00

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

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	131,652.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-K23 Budget Justification0209.pdf
(Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

OMB Number:
Expiration Date: 06/30/2011

Previous Period

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

*** ORGANIZATIONAL DUNS:**

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

Enter name of Organization:

Delete Entry *** Start Date:** *** End Date:** **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.							139,113.00	9.00			75,000.00	21,900.00	96,900.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 96,900.00

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) 96,900.00

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

* ORGANIZATIONAL DUNS:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry * Start Date: * End Date: 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:

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization:

Delete Entry | Start Date: | nd Date: | 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. Research development support	25,000.00
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	121,900.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	8.00	121,900.00	9,752.00
2.			
3.			
4.			
Total Indirect Costs			9,752.00

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

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	131,652.00

J. Fee	Funds Requested ($)

K. * Budget Justification: 1240-K23 Budget Justification0209.pdf (Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

will devote 9 calendar months of time to the proposed project during the period of support. Fringe benefits are calculated at 29.2%.

Research Support:
Tuition: Years 1-3 are budgeted for tuition costs of graduate level epidemiology and biostatisics courses for the principal investigator that are relevant to the area of study. The principal investigator will take courses listed in the training grant.

Funds are requested to offset the costs of a study coordinator in all years. The study coordinator will be key in subject follow up, medical records abstraction, data entry and blood specimen processing. In years 4 and 5, funds will be used toward statistical support.

Travel: Funds are requested to offset the costs of attending annual meetings of the American Society of Clinical Oncology and the American Society of Reproductive Medicine. These costs will cover transportation, registration and lodging for each conference.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		484,500.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		484,500.00
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		125,000.00
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	125,000.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		609,500.00
Section H, Indirect Costs		45,673.00
Section I, Total Direct and Indirect Costs (G + H)		655,173.00
Section J, Fee		

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

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

* New Investigator? ☐ No ☒ Yes

Degrees:

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:
Middle Name:
* Last Name:
Suffix:

* Phone Number: Fax Number:
Email:

* Title:

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

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: 0925-0001
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:

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. 0925-0001
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

OMB Number: 0925-0001

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)*	1241-1 IS_Introduction 03.09.pdf	Add Attachment	Delete Attachment	View Attachment
Candidate Information				
2. Candidate's Background	1242-2 IS_Candidatebackgrd 0309.p	Add Attachment	Delete Attachment	View Attachment
3. Career Goals and Objectives	1243-3 IS_Careergoal 0309.pdf	Add Attachment	Delete Attachment	View Attachment
4. Career Development/Training Activities During Award Period	1244-4 IS_Careerdvlp 0309.pdf	Add Attachment	Delete Attachment	View Attachment
5. Training in the Responsible Conduct of Research	1245-5 IS_trainingresponsiblersch	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)*	1246-Statements.pdf	Add Attachment	Delete Attachment	View Attachment
Environment and Institutional Commitment to Candidate				
8. Description of Institutional Environment	1247-8 IS_Institution 0309.pdf	Add Attachment	Delete Attachment	View Attachment
9. Insitutional Commitment to Candidate's Research Career Development	1248-SuLetter.pdf	Add Attachment	Delete Attachment	View Attachment
Research Plan				
10. Specific Aims	1249-10 IS_Specific aims 0309.pdf	Add Attachment	Delete Attachment	View Attachment
11. Background and Significance	1250-11 IS_Backgrdsignificance 03	Add Attachment	Delete Attachment	View Attachment
12. Preliminary Studies/Progress Report	1251-12 IS_Prelimstudies 0309.pdf	Add Attachment	Delete Attachment	View Attachment
13. Research Design and Methods	1252-13 IS_Rschdesign 0309.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		Add Attachment	Delete Attachment	View Attachment
17. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
18. Targeted/Planned Enrollment		Add Attachment	Delete Attachment	View Attachment
19. Inclusion of Children		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

1. INTRODUCTION TO THE APPLICATION

We thank the reviewers for their support and insightful comments for our proposal, "' Our review generated "outstanding reviewer enthusiasm for its overall scientific and training merit." The significant strengths of the proposal included a "highly motivated and impressive physician-scientist" candidate with a clear "potential to become a leader in this field", "outstanding mentoring", and "strong institutional support". Comments on the proposed study included "highly relevant", "impressive integration of different approaches" and "supported by a strong rationale and preliminary data". We are grateful for this positive feedback and now seek to address remaining concerns in a point by point fashion below. We have also significantly revised the text to reflect these important changes. Changes in the Research Plan are marked by vertical bars along the right page margin.

Reviewer 1

1. Specific Aim 1 power calculations

"Power and sample size calculations done for the study...lacked any pre-chemotherapy measures"

Specific Aim 1 tests whether pre-chemotherapy AMH, FSH and inhibin B are associated with post-chemotherapy ovarian failure in these young breast cancer patients. In our original submission, we performed power calculations using AMH, FSH and inhibin B values extrapolated from the literature and our post-chemotherapy breast cancer patients because we did not yet have pre-chemotherapy measures. We agree with the reviewer's suggestion to perform power calculations using pre-chemotherapy hormone values. We are now able to present revised power calculations using pre-chemotherapy hormone values from our newly recruited pre-chemotherapy breast cancer subjects (Preliminary data). Based on a sample size of 150 and 40% overall risk of ovarian failure, the study is adequately powered to detect very small differences in pre-chemotherapy AMH (0.0019ng/mL), FSH (1.5 mIU/mL) and inhibin B (2.1 pg/mL) between subjects who go on to develop ovarian failure compared to subjects who do not develop ovarian failure (Tables 8, 9 in Research Design and Methods).

2. Ovarian Reserve Index (Plan Ahead commercial kits)

"At the beginning of this year, a new test for ovarian reserve is now commercially available called the Ovarian Reserve Index (PlanAhead) by Repromedix...Given the greater reported reliability for this test, this now puts into question whether the grant's hypothesis is sound and current and it is important to address this new testing available in the context of the study. This is of importance to prevent the possibility of having the data obsolete by the time of publication of any papers five years down the road."

We thank Reviewer 1 for citing Plan Ahead, a new commercial kit for ovarian reserve. As our study proceeds, we absolutely plan on incorporating current, and newly validated approaches to measuring ovarian reserve into our analyses. With respect to Plan Ahead, our search through the science and popular literature found very limited data on this kit. Reported in two abstracts in 2008, the Ovarian Reserve Index and Egg Retrieval Score use proprietary cut points in FSH, AMH and inhibin B to predict retrieving 6 oocytes or fewer in two general IVF populations [1, 2]. The kit is promising because it demonstrates reasonably high sensitivity and specificity. However, to our knowledge, this test has not been validated in other populations (including the cancer population), is not FDA cleared, and is currently off the market in the U.S. Moreover, we currently plan to evaluate all the components of this "new" kit individually. Thus, at this time we do not feel our aim should be modified to incorporate this kit. If, or when, this kit does become commercially available, we can reassess its utility based on the supporting evidence.

There are two reasons why we do not anticipate that our data and results will be obsolete by study completion in 5 years. First, we are committed to incorporating the most valid hormone measures of ovarian reserve (whether through study-specific hormone cut points or assessed through other ovarian reserve algorithms such as Plan Ahead) as part of the final overall predictive index for breast cancer patients. Second, our predictive index is specific to young breast cancer patients. As such, we incorporate important predictors, such as chemotherapy agent and metabolism, that will never be part of any general algorithm for evaluating ovarian reserve in the infertility population. Therefore, we are confident that this data and the predictive index that will be developed will have pertinent and important contribution to the care of the young breast cancer patient population.

3. MRI ovarian morphometry intra- and inter-observer reliability

"There are no studies to date using MRI to measure the ovarian reserve in any infertility population or cancer survivors and therefore no intra- and inter-observer variability data is available."

Specific Aim 3 tests whether pre-chemotherapy measurements of ovarian morphometry are associated with the risk of post-chemotherapy ovarian failure. The reviewer raised legitimate concern about the lack of reliability data on antral follicle count (AFC) and ovarian volume by MRI in the breast cancer population. We also did not find this data in the literature. Therefore, we assessed these parameters in using our own newly acquired data in young breast cancer subjects prior to chemotherapy. In the 7 MRIs that we have performed thus far, the inter- and intra-observer coefficients of variation for ovarian volume were 8% and 9%, respectively. For antral follicle count, the inter- and intra-observer coefficients of variation were 12% and 15%. These numbers support the reliability of using MRI to evaluate ovarian morphometry and the strength of our radiology collaborators. As cited in the Research Plan, was the principal investigator of one of two existing, large studies on measuring ovarian parameters through MRI in the literature.

4. Sample size for ovarian morphometric assessment

"The sample size continues to be a limitation to the study and once again, even though it is possibly a significant financial burden to incorporate this specific aim and to power the analysis to an appropriate sample size, I still believe that it is important to readdress this point."

Specific Aim 3 tests whether ovarian morphometry, as measured by MRI and ultrasound, is associated with post-chemotherapy ovarian failure. The reviewer has been concerned about the small MRI sample size, initially limited to 38 subjects because of cost. We agree wholeheartedly with the importance of being adequately powered to detect smaller differences AFC and ovarian volume by ovarian failure status. We have now garnered additional funding for the study and are able to increase the imaging sample size from 38 subjects to 100 subjects. By increasing the sample size and performing power calculations based on our preliminary imaging data, we are powered to detect small differences in both AFC (1.6) and ovarian volume (1.4 ml) between subjects who develop ovarian failure and those who do not (in Research Design and Methods, Specific Aim 3, Sample Size and Power). These small detectable differences represent a 0.5 SD difference between the two groups.

5. Age of study subjects

"The issue of limiting study patients to less than 40 years of age continues to be an important factor. And although the investigators make a compelling argument as to why non-fertility related issues such as adjuvant hormonal therapy, menopause related symptom management and disorders associated with them such as coronary artery disease are important, the study is primarily designed to the prediction of fertility and ovarian failure and therefore the argument of trying to recruit younger patients continues to be valid."

In our original proposal, we sought to include breast cancer patients who were 45 years and younger. We agree with limiting the study population to patients 40 years and younger. In our initial recruitment, we have found that the vast majority of subjects are in fact younger than 40 (21/24 subjects). Therefore, we anticipate that restricting this protocol to subjects younger than 40 will only minimally prolong our recruitment to achieve the sample size of 150. Accordingly, we have decreased the expected risk of ovarian failure in our power calculations. The sample size is powered to test our hypotheses even in the setting of decreasing prevalence of the ovarian failure outcome.

6. Inclusion of minority subjects

"Description of minority group or lack thereof in the study is not well addressed."

We strongly agree that representation of minority subjects in research studies is important. Our enrollment table represents the clinical population of the Center of the University (78% non-Hispanic Caucasian, 12% African American, 6% Asian, 4% Hispanic). Our recruitment strategy has involved electronically screening every new visit to the breast cancer and approaching every eligible subject in person. Such face-to-face contact is highly effective in recruitment.

In our initial 24 pre-chemotherapy breast cancer subjects, we recruited two Minority women, both African American. Of the 4 subjects who declined joining the study, 3 were non-Hispanic Caucasian, and one was African American. Therefore, we have devised further efforts to promote study recruitment of minority subjects. Specifically, we will expand recruitment to

both of which have higher proportions of African American patients. The candidate will meet with oncologists in both systems to discuss the study and place study brochures in breast clinic waiting areas. Our goal is to increase significantly the proportion of minority subjects from what we have listed in our current enrollment table.

Reviewer 2

1. **Inclusion of minority subjects**

"Description regarding how the investigator will promote study recruitment of minority subjects is not outlined."

We appreciate this point and have detailed our strategy to enhance minority recruitment in Response 6 to Reviewer 1 above.

Candidate update

I would like to update the committee on my scientific progress. Since the last submission of this proposal, I have received the Master's of Science in Clinical Epidemiology (MSCE) degree from the . I have successfully initiated the , the prospective cohort study detailed in this grant. Over 4 months, 24 subjects have been recruited (85% recruitment rate), and their preliminary data have been incorporated into this application. Alongside the , I have continued to make progress on two other projects on ovarian function in the setting of breast cancer and breast cancer treatment. I have collected further hormone and contraceptive practices data from my initial cohort of post-chemotherapy breast cancer subjects. These data are undergoing final analyses and will culminate in two further manuscripts. Finally, I am the co-investigator of a third study on aromatase inhibitor-associated hot flashes and arthralgias in postmenopausal breast cancer survivors on . We have completed recruitment and genotyping of 400 subjects, and we have demonstrated an association between aromatase gene polymorphisms, estradiol and estrone levels and hot flash symptoms in these survivors on AIs. These data are undergoing analyses prior to manuscript preparation. The increase in my research pace and productivity is a direct result of protected research time. Moreover, the progress in these projects reflects the synergy and collaborations that I have built in my environment.

I have had some early success in competing for grant funding. I have been named a) for the first two years of my appointment as an instructor. Attaining the K23 award would provide crucial support for protected research time and allow rotation off of the . I have successfully competed for funding from the to support the cost of the . Recently, I have been awarded the t for a population-based database study evaluating the risks and benefits of hormone replacement therapy in the Asian population.

I believe my progress to date demonstrates my deep commitment to research in reproduction and becoming an independent investigator. The progress and momentum in my research program would not be possible without significant protected research time and further training through the K grant mechanism. Despite this early success, there is no doubt that additional protected time and training provided by the K23 is critical to continuing on the path to becoming an independent reproductive epidemiologist and physician scientist.

2. CANDIDATE'S BACKGROUND

I have had a longstanding commitment to research. In high school and college, I studied the role of neuropeptides in the developing mouse brain in the lab of [redacted] Health. I received the [redacted] for this work. My laboratory experience continued in the lab of [redacted] studying *ras* pathway proteins in C. elegans. These experiences fostered a fundamental understanding of laboratory science and my interest in asking scientific questions.

In medical school at [redacted], I worked with [redacted] to study inheritance patterns in premature ovarian failure (POF). I formulated, validated, and subsequently applied a questionnaire to patients with POF. My interest in reproductive aging emerged from this first project in clinical research. During my obstetrics and gynecology residency at [redacted], I merged my technical knowledge of laboratory techniques and interest in clinical research into a translation research project. With [redacted], I studied the role of amino acids in unexplained infertility through gene expression profiles of follicular fluid. In this endeavor, the prospect of identifying biomarkers and potential therapeutic targets for disease was extremely stimulating.

I decided to pursue a fellowship in reproductive endocrinology and infertility for several reasons: my clinical interest in reproductive medicine, a drive to answer questions in reproduction, and the opportunity for formal training in clinical research. In practice, I am most drawn to taking care of women with medical illnesses that impact reproduction. Therefore, my search for a Master's research project resulted in a study evaluating ovarian function in women with breast cancer after adjuvant chemotherapy.

3. CAREER GOALS AND SCIENTIFIC OBJECTIVES

My long-term career goal is a career in academic medicine with a focus on clinical and translational research. My goal is to conduct research that will significantly impact clinical care in reproductive medicine. I am dedicated to becoming an independent investigator who, in time, will mentor future investigators.

I have received the I This clinical research pathway was a change in direction from my earlier laboratory experiences. Thus, obtaining formal training in epidemiologic methods has been essential to my path to independent investigation.

My research program focuses on ovarian function in the setting of breast cancer and breast cancer treatment. Specifically, I am interested in evaluating hormonal, genetic and ovarian morphometric predictors of ovarian function in breast cancer patients. To this end. I have three ongoing, relevant studies. The first is the Study, the prospective cohort study of pre-chemotherapy young breast cancer patients proposed in this grant. In addition, I continue to follow a second large cohort of post-chemotherapy breast cancer patients through menopause to elucidate late reproductive aging and the menopausal transition in this population. Finally, to gain further experience in genetic epidemiology studies, I am the reproductive endocrinology investigator in a collaborative study with breast oncology and family medicine to evaluate genetic determinants of aromatase inhibitor-associated hot flashes and arthralgias in postmenopausal breast cancer survivors on aromatase inhibitors (AI).

Guided by experience from my initial post-chemotherapy cohort, I successfully initiated the several months ago. With protected time as a and support from the breast cancer center, I have recruited 24 subjects over 4 months (85% recruitment rate). This study has received high acceptability and enthusiasm from eligible subjects. Recruitment is ongoing and will be completed in two years as projected. Preliminary pre-chemotherapy hormone and ovarian morphometry parameters are presented in this application. Furthermore, interest in this cohort has resulted in collaboration with another cancer center investigator to evaluate chemotherapy-related fatigue in subjects. Finally, I have successfully competed for additional funding support for this study from the

I am following a second cohort of post-chemotherapy breast cancer survivors to evaluate ovarian function after adjuvant chemotherapy. Enrolled in a cross-sectional study in 2004, the 132 subjects of this cohort were approached for continuation in the study. I have successfully followed over 90% of the cohort annually for the past two years, obtaining annual questionnaire data and banking serum for hormone evaluation. The data on post-chemotherapy reproductive hormones, drug-metabolizing enzyme polymorphisms and chemotherapy related ovarian failure have resulted in two first-authored manuscripts and are presented as preliminary data in this grant. I have accrued further data on contraceptive practices and longitudinal changes in hormones. These data are undergoing final analyses and will culminate in two further manuscripts. I plan to follow the entire cohort through menopause to study the process of late reproductive aging in this population.
Finally, I have had an ongoing collaborative project with oncology and family medicine investigators on aromatase inhibitors and hot flashes in postmenopausal breast cancer survivors. We have recently completed recruitment and genotyping of 400 subjects, and we have demonstrated an association between aromatase gene polymorphisms, estradiol and estrone levels and hot flash symptoms in these survivors on AIs. These data are undergoing analyses prior to manuscript preparation.

With the support of my mentors and environment, I have had some early success competing for grant funding. I have been named a) for the first two years of my appointment as an instructor. Attaining the K23 award would provide crucial support for protected research time and allow rotation off of the award. I have successfully competed for funding from the to support the cost of the For my study on cyclophosphamide metabolism and risk of ovarian failure, I received the in-training award in research from the More recently, I have been awarded the study evaluating the risks and benefits of hormone replacement therapy in the Asian population.

I have had tremendous support from my clinical division and the Foremost has been my team of mentors, comprised of Dr. I chose this team of mentors because of the interdisciplinary nature of the project. Frequent meetings with these seasoned mentors have allowed me to take advantage of each of their area-specific expertise to formulate sound hypotheses and carry out a meaningful study.

- Dr. is an Associate Professor of Obstetrics and Gynecology and Epidemiology at the University and a Senior Scholar at the . He is also the Director of the Women's Health for the Department of Obstetrics and Gynecology. He is an extremely productive reproductive epidemiologist and savvy reproductive endocrinologist. This expertise has guided my study designs for the Master's project and the K23 proposal. His insight has been vital to my learning to consolidate ideas from multiple disciplines and formulate study questions directed toward the reproductive health of cancer survivors.

- Dr. is a Research Professor of Obstetrics and Gynecology at the University As Director of the Human Behavior and Reproduction Program, Dr. .s the principal investigator of the Study, one of the largest long-term longitudinal studies of hormones and behaviors linked to ovarian aging. Her expertise in ovarian aging, study designs incorporating repeated hormonal measures, and menstrual history collection has been invaluable to my research training. Further, Dr. has involved me in regular research meetings o investigators and helped me to incorporate healthy controls from her cohort to my study on breast cancer patients.

- Dr. is an Associate Professor of Medicine and Epidemiology at the University and a Senior Scholar at the She is also co-Director of the Center of the Center of the University As my primary mentor for the Master's project, Dr. has enhanced my understanding of breast cancer, breast cancer treatment and issues facing this patient population. She has been key in helping me access breast cancer patients, other oncology providers and research staff. She has also actively involved me in cancer center presentations and meetings. Finally, her expertise in the genetic epidemiology of drug metabolizing enzymes has helped me to formulate and test the hypothesis on the relationship between polymorphisms in drug metabolizing enzymes and ovarian failure.

My clinical division has also supported more than 90% protected time over the past year to nurture my research career. With limited clinical responsibilities, I have been able to concentrate on completing the Master's program and making research progress.

I have learned through this study that the clinical question at hand, determining reproductive and ovarian function after adjuvant chemotherapy, is extremely important to breast cancer patients. The enthusiastic response from our breast cancer subjects clearly supports further studies. I have been excited and fueled by this research. I acknowledge that my publication track record has been limited. My publication track record has been limited due in part to a shift in my research focus, but I am encouraged by the increasing pace of my research productivity.

The next step in my career development is a mentored research award that will provide critical protected research time over the ensuing five years. The goals of this mentored research award are to prepare me to become an independent investigator in reproductive epidemiology. Specifically, this proposal will enable me to: 1) conduct a prospective cohort study; 2) learn methods in genetics epidemiology, repeated measures and predictive modeling; 3) collaborate with researchers in oncology, radiology and biostatistics; and 4) gather data for an R01 submission. Despite some early success, there is no doubt that additional protected time and training through the K23 mechanism is critical to continuing on this path to become a successful reproductive epidemiologist and physician scientist.

4. CAREER DEVELOPMENT/TRAINING ACTIVITIES DURING AWARD PERIOD

The program and research project have provided me with a solid first step in clinical research. However, I need to build upon this foundation and improve my current knowledge and skill set in order to transition into an independent investigator, akin to a basic scientist who needs to develop specific laboratory skills. I propose the following training and career development plans during the 5-year award to achieve scientific independence:

1. Improve and acquire new research skills in study conduct and epidemiology: During this training period, I will gain experience in conducting a prospective cohort study. This will include patient recruitment, patient retention, data collection, data management and study personnel management. I will acquire new epidemiologic and biostatistical skills in analyzing repeated hormonal measures, working with genetic data and genetic epidemiology concepts, and formulating complex statistical models. I will also acquire knowledge of structural and functional pelvic imaging by magnetic resonance imaging.

2. Regular mentor meetings: My current mentoring structure will serve the K23 project well. In his role as my mentor for reproductive epidemiology, I will meet with Dr. every other week to discuss issues that arise in study design and conduct. will serve as my content mentor for ovarian aging. In weekly meetings of investigators of the t, I will report study progress. Dr. expertise in hormonal changes over reproductive aging will be central to analysis of Specific Aim 1. Dr. is my breast cancer content and genetic epidemiology mentor. I will meet with Dr. every other week, initially to review breast cancer patient recruitment and follow up and later for analysis of Specific Aim 2. As the award period progresses, these mentor meetings may decrease in frequency but will not be less than monthly.

3. Graduate coursework in epidemiology and biostatistics: I will take several courses through the to build upon my understanding of cancer epidemiology, genetics epidemiology and biostatistical methods ( ). I will complete this didactic portion of my training during the first three years of the research award. The classes include:
   -
   -
   -
   -
   -
   -

4. Seminars in reproduction, breast cancer, and epidemiology research: Attending seminars in reproduction, breast cancer and epidemiology research will expose me to ongoing research developments in these fields. This will be important to formulating new research ideas and questions. The holds weekly research seminars through the academic year on different aspects of human reproduction
   The offers weekly clinical epidemiology seminars The holds monthly seminars on breast cancer research. I plan to attend these seminars throughout the year.

5. Advisory committee meetings: and Dr. will serve as my K23 advisory committee. Their role is primarily to direct my career development. In addition to informal interactions with will formally meet with each advisor on a twice yearly basis to review research progress and obtain scientific and career advice. In addition, they will be available to help review manuscripts and presentations.
   -

institutional principal investigator of a large cooperative NIH grant on This involvement in the oncofertility consortium and his basic science bent will be important in formulating my next clinical and translational studies on fertility in cancer survivors.

- Dr. is a Professor of Medicine and Epidemiology at the University . He is also the Director of the at the Dr. will help direct my epidemiology training and my transition through the from student to Faculty Fellow to Associate Scholar.

6. Career development seminar: FOCUS is a collaborative and multidisciplinary network within the University to support the advancement and leadership of women in academic medicine. I will attend the FOCUS Annual Leadership Mentoring Conference yearly. This conference features nationally recognized guest speakers and workshops focusing on the professional needs of women in academic medicine.

7. Presentation of my research to the University community: I will present my research twice yearly to the and annually to the I will participate and present my results at least yearly at the work in progress meeting. In addition, I will have yearly presentations at the and the Department of Obstetrics and Gynecology grand rounds. These presentations are designed with the duel function of enhancing my public presentation skills and receiving peer review of my research.

8. Presentation and attendance at national meetings: I plan to attend and present my work at annual meetings of the

9. Writing and publication: I will continue to gain experience in manuscript preparation. In addition, I will seek opportunities to analyze data from the present study for abstract presentations, manuscripts on baseline characteristics, and future grants. I will set a goal of submitting one to two original manuscripts and one to two review papers per year during the award period.

Clinical Activities

I have been appointed as an Instructor in the and My activities are divided between 90% protected research time and 10% clinical time. My clinical time has been spent at the in an adolescent reproductive endocrinology practice. This clinical practice enriches my exposure to endocrinology issues during adolescence. In addition, my clinical time is supplemented by weekly journal clubs and in vitro fertilization conferences in the Division of Reproductive Endocrinology and Infertility. Finally, I will continue as an Instructor in the This will involve clinical teaching of medical students, obstetrics and gynecology residents, and reproductive endocrinology fellows. I will also lead medical student sections during the Reproductive Medicine module and give didactic lectures to residents and fellows.

5. TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

I have completed the , mandated by the The Certification Program requires training on Clinical Trial regulations including in-depth coverage of the Food and Drug Administration (FDA) and Office of Human Research Protections (OHRP) regulations that apply to Clinical Trials. The program covers requirements for the use of human subjects in research including coverage of Good Clinical Practice (GCP) guidelines, Institutional Review Board (IRB) Review, Informed Consent, Conflict of Interest, and policies and procedures that apply to the conduct of Clinical Research. I was required to demonstrate an understanding of the regulations by passing a test developed from the materials covered during the training. In addition, I completed a clinical trials course in the This course covered the fundamentals of conducting a clinical trial, including conflict of interest, responsible authorship, scientific misconduct, and data management and sharing.

I have also completed the two requirements related to ethics of clinical research. The first is a university sponsored online Bioethics Training Certification program. The second is a didactic lecture run by , Senior Fellow of the Center of This lecture familiarized students further with issues related to ethical issues of clinical research. I will continue to take annual or biannual renewal sessions of the Patient Oriented Research Certification Program and the Bioethics Training Certification Program.

February 27, 2009

NIH Study Section
Bethesda, MD

Re:

Dear Study Section Members and Colleagues:

I am writing to give my highest support for 's candidacy for the NIH K23
I will be her primary mentor for this award.
background and progress demonstrate her great promise for an outstanding career in clinical and translational research.

I first met when she was a resident in Obstetrics and Gynecology at the
had graduated from where was inducted into *Alpha Omega Alpha.* was a highly successful resident with clear signs of potential promise as a clinician and investigator. In work with one of my reproductive endocrinology colleagues, combined her prior laboratory experience and her interest in clinical and translational research to carry out a case-control study of the role of amino acids in unexplained infertility.

We were very pleased to recruit to our fellowship program in Reproductive Endocrinology and Infertility. I have served both as her mentor for her fellowship in REI, and a mentor for her Master's degree in clinical epidemiology and biostatistics (August, 2008). In addition to the rigors of a clinical fellowship has excelled in the Master's program, both in terms of her course work as well as in the inception and conduct of her research thesis on the effect of breast cancer and chemotherapy on reproductive hormones. She has carried out a number of additional research projects, further demonstrating her talents as a clinical researcher and her academic potential. We were delighted to recruit her to join our faculty as Instructor in the beginning in July, 2008. It has been my pleasure to mentor during these early years and I consider it a privilege to do so as part of this career development grant.

Despite her relatively short time conducting clinical research, has had great success in her Master's project on post-chemotherapy breast cancer survivors in terms of subject follow up, data collection, data management through creation of a working database, and management of a study coordinator. Over the past two years, she has accrued 90% annual follow up of this cohort. Through this project, she has developed strong collaborations with an extremely active clinical breast cancer researcher at , and

a senior researcher in ovarian aging. The positive preliminary results from this study, along with clinical interest in the intersection of medical illness with reproduction, naturally led to the development of this K23 proposal. This Master's research study has also lead to two first-author manuscripts on ovarian function after chemotherapy as well as new data for two further first-author manuscripts on contraceptive practices and longitudinal hormonal changes in this population.

research focus will be on the impact of cancer therapy on reproductive aging. The proposed study, after , is designed to assess biomarkers for predicting ovarian failure after chemotherapy. The study will be a cohort assessing novel genetic, serum hormone and magnetic resonance imaging ovarian morphometric parameters in breast cancer patients before and after adjuvant chemotherapy. Subjects from this cohort will be followed for up to four years. Data analyses and manuscript preparation will take place primarily in the four and fifth years of the K23. This study will allow her to develop an independent line of investigation from that of her mentors. The study design is well-conceived, and, more importantly, quite feasible. The strength of this application is not only in the clinical importance, the science, and the strong study design, but that it fosters cross-institutional collaboration among reproductive endocrinology, oncology, radiology and epidemiology and biostatistics. With protected time as a scholar, has successfully initiated the proposed study with high recruitment rates. Because has closely overseen every aspect of her study, has been able not only to learn from the process of study initiation, but also quickly troubleshoot and tailor the protocol to facilitate effective recruitment and data collection. Importantly, has successfully competed for additional funding to help carry out the study. There is no doubt that the early success of this study has been a clear result of her protected research time and environment.

The results of this study will help develop future studies in similar populations and younger cancer patients to directly address fertility after cancer therapy. will also use this experience to continue work on understanding the menopausal transition in healthy and cancer patients. s superbly qualified to successfully carry out this unique, important line of research. It clearly embodies her skills as a reproductive endocrinologist, a clinician, and a clinical epidemiologist.

is a pleasure to work with and is on the path to becoming highly productive. While publication record to date is limited, dedicated research time over this past year will yield multiple manuscripts from a number of projects. In addition to her primary project, is continuing to generate data from her initial post-chemotherapy breast cancer cohort, and she is actively involved in a collaborative study on hormone levels and hot flashes in breast cancer survivors. is performing the primary data analyses on the latter study and is beginning manuscript preparation. Lastly, has recently won the in-training research award, and she will receive the Research Grant for grant proposal on evaluating risks and benefits of hormone replacement therapy in postmenopausal Asian women using a population-based healthcare database. The sum of these studies is to hone skillset as a reproductive epidemiologist focused on ovarian function and aging.

current pace and recent accomplishments show both a deep commitment to research in reproduction and early signs of success. clearly demonstrates that has the potential to be a highly productive researcher given appropriate support in environment and for protected time. will greatly benefit from continued protected time through the K23 for enhancing her skills and gaining an in-depth understanding of her chosen area of expertise.

What makes me so confident in potential is that has a unique and advantageous training background. In addition to her prior laboratory training, is trained as an obstetrician gynecologist, a reproductive endocrinologist, and a reproductive epidemiologist. This training is very uncommon and will uniquely position her to continue her successful career in translational and clinical research.

Beyond training, has extremely strong mentors, research environment and institutional support. Among her collaborators is), a reproductive epidemiologist with tremendous experience in ovarian aging studies in her work with the cohort, and , a radiologist whose area of research is body MRI and functional MRI. I have assembled a career advisory committee of two senior faculty members to monitor progress and to provide both scientific and career development advice. advisory committee is comprised of and is the nd the Director for Reproductive Endocrinology at In addition to being a senior NIH-funded scientist and a nationally recognized leader in reproductive endocrinology, has a long track record of mentoring fellows and junior faculty. n is the of the at the and 3) at and a Professor of Medicine and Epidemiology. also has an outstanding track record of mentoring epidemiology students and faculty and will help guide transition from a Master's student to Faculty Fellow to Associate Scholar in the .

Mentoring Plan

has completed a Master's degree in Clinical Epidemiology and Biostatistics, but it is clear that for her to become a leader in reproductive function after cancer, her skill set needs to be increased and diversified. Therefore, we have put together a pragmatic yet ambitious mentoring plan which includes a number of didactic courses as well as participation in seminars and one-on-one mentoring.

course work will include enrolling in graduate level courses at the including Cancer Epidemiology, Biomarkers, and Prevention, Pharmacoepidemiology Research, Topics in Genetic and Molecular Epidemiology, Applied Regression Models for Categorical Data, and Advanced Methods for Analysis of Complex Genetic Traits. This course work will allow her to expand her knowledge base.

practical development will come in the form of interacting with colleagues in the numerous high quality seminars offered within our Division in the Center in Women's Health and the Center in Women's Health. Seminars which she will attend are designed to be diverse and include seminars within the Center for , the seminars for the Center for Women's Health, as well as Reproductive Endocrine journal clubs, clinical conferences, and research meetings. There will be a work-in-progress meeting where she will be able to critique others' developing research as well as formally present her own in front of a group of faculty, trainees, fellows, and students.

My specific mentoring with will include formal meetings to discuss research progress, course work, and overall plans. We anticipate that we will meet at least weekly. These meetings may taper off in frequency as long as continued progress is being made but will be no less than monthly. Additionally, I will formally meet with and review specialized mentoring forms used at the to document her progress. In this meeting, we will formally go over her curriculum vitae to assess the number and quality of publications, the

number and quality of research presentations, her teaching responsibilities, her clinical responsibilities, and assess whether she has attained the goals she has set in a biannual fashion. This formal mentorship is highly successful as the exercise in filling out these documents and submitting them to division heads, center directors and chairs forces the mentee to specifically document progress.

and will serve as additional mentors to because of their content area of expertise. is an expert in ovarian aging; one of r current projects is an ongoing cohort of healthy women over the who have been followed for ten years to date. will participate in weekly work-in-progress meetings with Study research group. is an expert in breast cancer care and genetic epidemiology. will help to access and recruit breast cancer patients from the Center at and help direct the analyses of the specific aim on drug metabolizing enzyme polymorphisms. will meet with e every two weeks initially. This frequency may decrease as the study progresses but will be no less than monthly.

Expectations

It is clearly understood by all mentors that 75% of time will be assigned to career development and her clinical research. In her first year as an instructor, 90% of time has been protected for research. Great care will be taken so that she will not exceed her allotment of clinical work or administrative responsibilities. I am confident that this transition will be very easy for as is already committed to a clinical research career and this will only enhance and focus her interests to increase her ability to be successful.

Transition towards Independence

The first two years of this training grant will be purposely devoted to course work and subject recruitment as described in this letter and elsewhere in the grant. After she has expanded her knowledge base with the courses described and is well along in conducting her research plan, future directions will clearly become evident. It is the inception of these ideas and implementing future projects that will assist her in her transition to independent investigation. She will clearly take the lead in designing these future projects, and her mentors will also shift their capacity to that of collaborators. As these grants are written and submitted for federal funding, it will allow her the opportunity of establishing her own independent lines of investigation. The definition of the true transition to independence will be the successful funding of these projects as stand-alone grants, with an RO-1 serving as a gold standard but recognizing that funding may also be attained by other R grants, program projects, federal contracts and/or other sources of foundation research support. I am truly confident that Irene possesses the skills, desire, and opportunity to make this successful transition.

Qualifications of Mentor

I am a tenured Associate Professor of Obstetrics and Gynecology and Epidemiology at the . I have a secondary appointment in the Department of Biostatistics and Epidemiology where I am a Senior Scholar for the Center for . Currently, I am the Director of the Woman's Center for Obstetrics and Gynecology, and the Associate Director

('). I have had continuous NIH support since 1998 in the areas of early pregnancy complications, family planning, and reproductive endocrinology. I am the co-PI for the

My mentoring in the past 5years has included eleven students pursuing Master's degrees in Clinical Epidemiology, six fellows in Reproductive Endocrinology, as well as mentoring four junior faculty members in the Department of Obstetrics and Gynecology. I am pleased to report that all fellows, residents or students who have finished their training at the under my mentorship now have academic appointments. Three have received faculty appointments at (two in Ob/Gyn and one in Family Practice), one at The , one a one as the University), one at , two at , and one at the University . The mentees I have been working with have conducted outstanding work. All have presented at national meetings in their specialties, including recognition with best presentations for a fellow in training at the 2004, 2005 , 2007 and 2008 meetings of the American Society for Reproductive Medicine.

Currently I am mentoring three junior faculty as part of the Program. All three have submitted grants for federal funding and have received (nonfederal) support. Federal support includes one awarded RO3 while an R21 and RO1 are currently under review. I am in the process of becoming PI for and I have been awarded the Berwick Award by the School of Medicine, which is given to the outstanding mentor and teacher in the field of translational research. Finally, I am a member of the Selection and Steering Committee of the inaugural NIH sponsored " Program " Enclosed with this letter is a table of five successful mentees.

Summary

Given the strength of this applicant, the excellent environment including the synergy of Ob/Gyn with other departments, and my strong past experience in mentoring, I believe that Irene has an outstanding chance of transitioning through this award to the desired independent research career. I am also quite confident that as she attains her independence, she will become a nationally and internationally renowned investigator who I will be privileged to work with in the future. I give my strongest support to Irene in this application.

Sincerely yours,

March 3, 2009

NIH Study Section
Bethesda, MD

Re:

Dear Study Section Members:

I am delighted to provide my unequivocal support for the K23 application of currently a first-year instructor in Reproductive Endocrinology and Infertility at the Having known for the past two years as primary mentor for the Masters of and ( ) program at , I can state with utmost confidence that she is one of the most talented, intelligent, hard working and ambitious trainees with whom I have had the privilege to work and that she is unreservedly committed to a career as a clinical and translational researcher in academic medicine.

came to me with a research background that was primarily laboratory-based. Because of her desire to pursue a clinical and translational research career, she sought formal epidemiology training through the program. expressed a strong clinical and research interest in studying the effect of medical illnesses on reproduction. As went through her core courses for the masters program, she formulated a study question and design under the guidance of her mentors (myself, and Dr). Matching breast cancer subjects from my Study with healthy women from Dr. 's , she has studied the effect of breast cancer and chemotherapy on reproductive hormones and examined whether drug metabolizing enzyme polymorphisms are associated with subsequent ovarian failure in breast cancer patients.

I am certain that will be a productive clinical researcher. For her Master's thesis, converted a cross-sectional study into a longitudinal cohort. She successfully designed a database for this cohort study, managed a study coordinator, and collected annual data on more than 90% of subjects for the past two years. These data have yielded two first-author manuscripts on after chemotherapy, two abstract presentations at national meetings and a research award from the . is in the midst of data analyses and two more first-author manuscripts from this cohort. Further, staff and patients alike have expressed excellent interactions with her.

K23 proposal on predictors of ovarian failure after chemotherapy in young breast cancer patients is an important and interesting project exploring biomarkers for ovarian failure in this population. The clinical question of how to determine ovarian function after adjuvant chemotherapy is extremely relevant and important to oncologists and patients alike. Further, this project will train in genetic epidemiology and predictive modeling methods, enable to present and disseminate her findings, and guide her in designing new studies and competing

for R01 funding. She has begun recruitment for this study and has made significant progress to allow her to present more preliminary data in this application.

As s mentor, I am completely committed to her professional development as an academic clinician with a focus on clinical and translational research. I have successfully mentored eight fellows and junior faculty within the the Department as well as numerous trainees within the Division of at all levels of training. I have been a previous recipient of a Young Investigator Award from the held my own K23 Mentored Patient-Oriented Research Career Development Award prior to being funded through an R-01 from the National Cancer Institute and various other sources of support.

As Co-Leader of the within the r, I will bring the full complement of the clinical research resources to s project. Furthermore, as director of clinical research within the . I will be intimately involved in the recruitment, data collection and follow-up of patients in the proposed study. My own background as a clinical breast oncologist, molecular epidemiologist and patient-oriented researcher has enabled me to develop a research infrastructure that will be available to Dr. in order to ensure the success of her proposed project. I will specifically provide space, informatics infrastructure and staff to assist in both clinical recruitment/data collection and laboratory-based specimen processing and assays. My clinical research staff is housed in three floors from the where the proposed study will be conducted. Two full time and three part-time staff members specialize in data management, clinical data coordination and data entry. I also have laboratory space within the on the campus, where my laboratory technician conducts genotyping assays. These resources will help recruit subjects and carry out the proposed study.

My role for the proposed study will involve mentoring on the genetics epidemiology portion of the project (Specific Aim 1), serving as the content advisor for breast cancer physiology, facilitating breast cancer patient recruitment, and providing career advice and guidance. I anticipate that we will continue to meet every two weeks initially, but no less than once per month over time. will also continue to participate in my laboratory meetings and breast cancer research meetings of the .

In summary, is the most promising patient-oriented research trainee that I have observed in my years as an independent investigator. This award will provide her the opportunity to launch what I have no doubt will be an extremely successful career in patient-oriented research and I wholeheartedly endorse her application without reservation. is a bright and highly motivated individual who will undoubtedly become a leader in reproductive health after cancer therapy.

Please let me know if I can provide any additional information.

Sincerely yours,

February 26, 2009

Center for Scientific Review
National Institutes of Health
Bethesda, MD

Re:

Dear Committee Members:

I am delighted to write this letter of enthusiastic support for for the Mentored Patient-Oriented Research Career Development Award. I am writing as co-mentor of this proposal. Having interacted with her in the research setting over the past year, I am confident that is both a superb candidate for this award and a clinical researcher certain to have a major impact in the field of reproductive health in cancer survivors.

training record to date is remarkable. received her undergraduate degrees from . continued in medical school at . Medicine, where she was inducted into . graduated in 2001. During medical school, worked on a project to determine inheritance patterns in premature ovarian failure with , a leading researcher on the menopausal transition. This exposure initiated her interest in clinical research. After completing , was recruited to the for residency in obstetrics and gynecology and subsequently a fellowship in reproductive endocrinology and infertility.

After the completion of the bulk of her clinical training in 2006, completed a Masters degree in clinical epidemiology at our institution in 2008. clinical and research interests are in reproductive aging and the impact of medical disease on the aging process. As designed her Masters project to study the effect of chemotherapy on ovarian function in breast cancer patients, we decided together that healthy women from my ongoing I g Study would serve as controls for her study. I have observed and am impressed by progress in conducting this study. first two manuscripts are under review, and she has begun data analyses on two more study questions using this cohort. has demonstrated her motivation, talent and scientific productivity over a short time.

As co-mentor in the career development award proposal " after chemotherapy in young breast cancer patients", I am fully committed to her continued research development. Given that her Master's project showed a positive association between post-chemotherapy reproductive hormone levels and subsequent ovarian failure, the next step is to conduct a prospective cohort study to evaluate hormonal and other biomarkers for breast cancer subjects from diagnosis to survivorship. As a scholar for the past 6 months, has begun this cohort study and has made quick progress initiating the study and beginning recruitment. The result of this project will impact not only care of breast cancer patients, but also have implications on the study of healthy ovarian aging. The conduct of this prospective cohort study will be an invaluable experience for this developing clinical researcher.

Over the past 25 years that I have been an independent investigator, I have mentored many talented researchers who are now pursuing careers as independent investigators across the country. Most recently, I have mentored three junior faculty as part of the Programs. It will be my pleasure to work with and mentor over the next several years. My role is primarily as the content mentor for ovarian aging. We will meet twice monthly initially and at least once monthly for the duration of the study. will also continue to collaborate on ongoing projects with the Study. We will continue to interact weekly at our study research meetings.

In summary, is an outstanding candidate who is ideally suited for the K23 Award, and I have no doubt that she will succeed.

Sincerely yours,

February 16, 2008

Dear ,

I would be delighted to serve on your Advisory Committee for your K23 application. Your proposal to examine genetic, hormonal and ovarian imaging biomarkers associated with ovarian failure after chemotherapy in young breast cancer patients is interesting and important. I look forward to continuing to meet with you on a twice yearly basis to review your progress and career development. In addition, I am available to advise on manuscripts and presentations.

As Director of the Clinical Epidemiology Unit at the I would like to express our strong support of your development as a clinical epidemiologist. Since your graduation from the Master's of Science in Clinical Epidemiology program in August, 2008, you have had an appointment in the as an I will help mentor your transition to Associate or over the next several years.

As you know, I believe the work you have proposed to be both important and methodologically rigorous. I look forward to working with you.

Sincerely yours,

February 23, 2009

Dear

I would be delighted to collaborate with you on your K23 proposal, an important study which seeks to examine genetic, hormonal and ovarian morphometric biomarkers associated with ovarian failure after chemotherapy in young breast cancer patients. As your biostatistics advisor for your Master's research project, I agree that this K23 study is the key next step in your research career. It will help you develop and refine skills in genetic epidemiology and complex predictive modeling. I am looking forward to helping you finalize study design and plan data analyses. I will also collaborate with you in data analyses and manuscripts preparation. I expect that we will meet on a monthly basis, with increased frequency around the time of data analysis. We will also have regular interactions through work-in-progress meetings of the Study with

Sincerely yours,

February 18, 2009

Dear

I would be delighted to continue to collaborate with you on your K23 career development award proposal on genetic, hormonal, and ovarian morphometric predictors of ovarian failure after chemotherapy in young breast cancer patients. I am very interested in using MRI to study chemotherapy-related structural changes in the ovary. This study could also give rise to pilot studies using MRI technology to assess ovarian function. I am happy to continue to oversee the MRI morphometric measurements and am eager to see the outcomes.

Sincerely yours,

8. DESCRIPTION OF INSTITUTIONAL ENVIRONMENT

The candidate has excellent facilities and resources for the conduct of research proposed in this application. The see Resources) has a large, multidisciplinary breast cancer program including investigators from diverse disciplines focused on the biology of breast cancer, genetic epidemiology, prevention, imaging and treatment. The candidate will have easy access to patients and will frequently interact with other investigators and clinicians involved in the care of breast cancer patients. Furthermore, the proposed project coincides with the opening of for Advanced Medicine, which provides a unique environment for the candidate to conduct the study. The Department of Obstetrics and Gynecology shares space with the hat includes 20 exam rooms, 4 consult rooms, touchdown space, blood drawing stations, and chemotherapy infusion rooms. Office space is provided for the project coordinator on this floor to facilitate subject recruitment and follow up. Imaging studies are conducted two floors down in the same building. Thus, this is an ideal facility for recruiting subjects and conducting the study.

The study's hormone assays and genotyping will also be performed onsite at the All hormone assays will be performed at the Clinical and Translational Research Center (CTRC) Core Laboratory (see Resources). The CTRC laboratory has extensive experience in reproductive hormone assays. This lab has provided data for both the candidate's preliminary study on postmenopausal breast cancer subjects and longitudinal study of the healthy menopausal transition (Study) for the past ten years. Genotyping studies are performed in the Laboratory (see Resources) in collaboration with Their labs have dedicated space, equipment, and personnel for DNA extraction, PCR set up and genotyping.

The offers a robust environment for the candidate's career development. has formal mentoring relationships with three strong, senior mentors representing the fields of reproductive endocrinology/epidemiology, ovarian aging and breast oncology/genetic epidemiology: and also has two seasoned advisors for the K23 project committee, and o review research progress and provide scientific and career advice.

Furthermore, the candidate frequently interacts with investigators not only in the Cancer Center, but also in the

These interactions occur through regular seminars in reproduction, breast cancer, and epidemiology research, and presentation of the candidate's research activities to the ommunity (see Career Development Activities). also offers a wide range of advanced courses in biostatistics and epidemiology, several of which have been planned into this K23 proposal. Finally, has several career development seminars for young investigators in which the candidate will continue to participate. In summary, the candidate's environment provides strong, well-established resources to support fully her career development and proposed research project.

March 3, 2009

Re:

I enthusiastically support 's application for a K23 Mentored Patient-oriented Research Career Development Award.

.., a graduate of and of Medicine, completed a residency in obstetrics and gynecology at the Hospital of the n 2005. was one of our most outstanding residents. During residency, under the mentorship of she completed a basic science research project for which she was received the . This is highly unusual for a resident in light of their clinical demands. She was also recognized for her teaching skills and selected by the medical students to receive the . Dr. completes her fellowship training in Reproductive Endocrinology and Infertility at the in June 2008. Dr. is committed to a career in academic medicine and intends to devote the majority of her effort to conducting patient-oriented research. Therefore, she accepted a position as a full-time Instructor in the Department of Obstetrics and Gynecology at the School of Medicine with the understanding that she will spend 90% of her effort devoted to her research project " " As an instructor, Dr. has all the rights and privileges of a full-time faculty member but this initial appointment will help to insure that she has protected time to conduct her research and transition to an independent investigator and an assistant professor. The remaining 10% of her time will be spent in one clinical session a week. Dr. has been named a for the first two years of her appointment. A K23 award will enable her to continue in a mentored relationship and develop the clinical research skills she needs to be well positioned to transition to a career as an independent investigator.

As a show of our commitment to , the Department provided her with a start-up package that includes supplemental salary support and support the remaining percentage of a research nurse for the duration of the grant, an office and computer. also has unlimited access to statistical support from our statistician, . We also provide administrative and grant support through the Center (, and the Department.

has a large, multidisciplinary breast cancer program including investigators from diverse disciplines focused on the biology of breast cancer, genetic epidemiology, prevention, imaging and treatment. Thus, has easy access to patients and will

interact with other investigators and clinicians involved in research and care of breast cancer patients. Furthermore, the proposed project coincides with the opening of . Center ( ), which provides a unique environment for Dr. to conduct the proposed studies. The Department's gynecologic oncology program shares space with the breast cancer program. We have 20 exam rooms, 4 consult rooms, touchdown space, blood drawing station and chemotherapy infusion rooms on the third floor. Imaging studies are performed on the first floor of the building. Imaging studies are two floors down on the first floor of This is an ideal facility for the conduct of the proposed project and recruitment of patients. In fact, Dr. has already begun the proposed cohort and has had early success with recruitment and data collection.

Furthermore, Dr. . is encouraged to participate in the faculty professional development programs at in addition to our departmental mentoring program. Each faculty member is assigned a mentoring team; I meet with each junior faculty member twice a year to review their progress and goals, and provide guidance and support. As chair I pledge my support for Dr. ; it is one of my goals to facilitate the development of young academicians and I appreciate the time, effort and resources needed to support the development of junior faculty.

It is with great pleasure and enthusiasm that I support Dr. s application for the K23 Career Development award. is an outstanding candidate with tremendous potential and commitment to a career in academic medicine. This award will allow her the protected time and resources she needs to develop into an independent investigator.

Sincerely,

10. HYPOTHESES AND SPECIFIC AIMS

More than two million American women are breast cancer survivors [3]. In 2008, almost 180,000 American women will be diagnosed with the disease [4]. One-third of these women are less than 54 years old, and 10 percent of the total are younger than 45 [4]. The large number of reproductive aged women with breast cancer provides a unique opportunity to study the impact of this devastating disease and treatment on ovarian aging and reproduction.

Ovarian aging refers to the complex process of oocyte attrition over a woman's reproductive life. Based on the principle that human ovarian reserve is finite, oocytes peak in number during fetal life, undergo attrition thereafter and do not regenerate [5]. Environmental factors such as chemotherapy accelerate this genetically determined time course, leading to shortened reproductive life and early menopause [6-8].

Breast cancer patients and their physicians have a deep interest in characterizing and predicting ovarian function after breast cancer therapy, because of implications on reproductive issues such as fertility potential and menopause [9, 10]. Ovarian function is also critically important to non-fertility-related issues such as breast cancer prognosis and choice of adjuvant therapy [11]. Therefore, the ability to accurately predict and identify ovarian function after cancer therapy would impact patient counseling, medical and surgical treatment choices and consideration of fertility preservation options.

The goal of this proposal is to identify significant biomarkers associated with ovarian failure both to provide a useful and necessary clinical tool for breast cancer patients and to further understanding of ovarian physiology. The investigator proposes a 5-year prospective cohort study of premenopausal breast cancer patients, followed from cancer diagnosis to post-chemotherapy. We will test the following hypotheses:

Specific Aim 1: To determine if pre-chemotherapy hormonal measures are associated with ovarian failure in breast cancer patients after adjuvant chemotherapy.
We hypothesize that breast cancer patients with lower pre-chemotherapy anti-Mullerian hormone (AMH) and inhibin B levels and higher pre-chemotherapy Follicle Stimulating Hormone (FSH) levels will be more likely to develop ovarian failure after adjuvant chemotherapy.

Specific Aim 2: To determine if cyclophosphamide drug metabolizing enzyme polymorphisms are associated with ovarian failure in breast cancer patients after cyclophosphamide-based adjuvant chemotherapy.
We hypothesize that breast cancer patients with drug metabolizing enzyme polymorphisms resulting in higher cyclophosphamide exposure are more likely to develop ovarian failure after cyclophosphamide-based adjuvant chemotherapy.

Specific Aim 3: To determine if morphometric measures of the ovary by magnetic resonance imaging (MRI) are associated with ovarian failure in breast cancer patients after adjuvant chemotherapy.
We hypothesize that breast cancer patients with smaller pre-chemotherapy ovarian volume, maximal diameter and antral follicle count (AFC), and larger change in pre- and post-chemotherapy ovarian volume, diameter and AFC are more likely to develop ovarian failure after adjuvant chemotherapy.

Specific Aim 4: To develop and validate a clinical predictive index to identify breast cancer patients at high risk for ovarian failure after adjuvant chemotherapy.
We hypothesize that variables collected in Aims 1, 2 and 3 can be used to develop and internally validate a clinical predictive index to identify breast cancer patients at high risk for developing ovarian failure after adjuvant chemotherapy.

To test these hypotheses, a prospective cohort of premenopausal breast cancer patients 40 years and younger will be enrolled and followed for three to five years. During follow-up, subjects will keep bleeding diaries to track menstrual pattern. Pre-chemotherapy bloods will be collected to isolate DNA and determine hormonal levels. Serial bloods will be collected during and after chemotherapy. In addition, two-thirds of subjects will undergo a pelvic MRI and ultrasound prior to and after chemotherapy.

11. BACKGROUND AND SIGNIFICANCE

I. Introduction

More than two million American women have a history of breast cancer [3]. Almost 180,000 new cases of breast cancer will occur in 2008 [4]. Of these, one-third arises in women less than 54 years old, and 10 to 20 percent will occur in women of childbearing age [4, 12]. While chemotherapy has decreased recurrences and improved survival, these medications also induce ovarian damage and aging, resulting in premature ovarian failure and early menopause in cancer survivors [6-8].

Ovarian function after breast cancer treatment is a clinically important survivorship issue. The degree of ovarian function affects breast cancer prognosis, choice of cancer adjuvant therapy, need for contraception, fertility potential and menopausal concerns [9-11]. Thus, tools to accurately predict and characterize ovarian function after cancer therapy would impact patient counseling, medical and surgical treatment decisions, and consideration of fertility preservation options.

II. Ovarian function after breast cancer adjuvant therapy

a. Adjuvant chemotherapy: Adjuvant chemotherapy is administered after primary breast canr surgery. Treatment is recommended for women with node-positive disease or tumors greater than 1 centimeter [13]. In the U.S., most breast cancer patients will receive adjuvant chemotherapy. Combination chemotherapy regimens commonly use cyclophosphamide, anthracyclines and taxanes [13]. Cyclophosphamide is likely the most gonadotoxic of these drugs. An alkylating agent, cyclophosphamide intercalates DNA in a non-cell cycle specific manner; this mechanism affects not only growing ovarian follicles but also quiescent primordial ones[14]. Ovarian histology after cyclophosphamide shows decreased or absent follicles and ovarian fibrosis [15, 16].

b. Adjuvant hormonal therapy: Tamoxifen and gonadotropin releasing hormone (GnRH) agonists can also be used as adjuvant therapy agents in premenopausal breast cancer patients with estrogen or progesterone receptor positive disease [13]. Tamoxifen competes for estrogen receptors, a mechanism that affects menstrual regularity and some hormonal measures (FSH and estradiol) [6, 17-19]. Therefore, tamoxifen use may modify interpretation of these common measures of ovarian function. Aromatase inhibitors are used as adjuvant hormonal therapy in postmenopausal breast cancer patients [20].

c. Current assessment of ovarian function after chemotherapy: Menstrual pattern and FSH are the most commonly used measures of ovarian function after chemotherapy.

1). Menstrual pattern: Studies of menstrual pattern after breast cancer treatment have used one of two outcomes: chemotherapy related amenorrhea (CRA) or menstrual regularity. CRA risk after cyclophosphamide/anthracycline-based regimens ranges from 15 to 80% [21]. This wide range results from heterogeneous CRA definitions, retrospective study designs, short follow up and heterogeneity in patient characteristics and treatments. First, the term CRA has been applied to amenorrhea of one missed period to two years [21]. Further, amenorrhea outcomes are often secondary endpoints and collected retrospectively [8]. This methodology is error-prone as menstrual history recall can be unreliable [22, 23]. Third, follow up is often short and unable to account for subjects who have eventual return of ovarian cyclicity [8, 21]. Similarly, short follow up is also unable to account for subjects who continue to menstruate but subsequently develop premature or early ovarian failure [7, 8, 21]. The proportion of women who resume menstrual function after significant amenorrhea is not well characterized [24]. In larger cohort studies, no return of menstrual function was observed after 18 to 24 months of amenorrhea [19, 25, 26].

Recently, a large, multi-center prospective cohort study reported incidence and time course of regular menstrual bleeding after breast cancer treatment. The majority of subjects experienced irregular bleeding or some amenorrhea in the first 12 months from start of chemotherapy [19]. As chemotherapy ended, the percentage of subjects recovering monthly menstrual bleeding increased to a high of 55% at 15 months[19]. This study systematically and prospectively collected menstrual data as a surrogate for ovarian function after breast cancer treatment. However, the outcome of menstrual regularity has limited clinical significance, including lack of association with fertility in cancer survivors. Ovarian failure or surrogate endpoints such as measures of ovarian reserve would be more clinically useful.

Therefore, we propose a prospective cohort design with 1. prospectively collected bleeding diaries, 2. reasonably long follow up (three to five years from chemotherapy dependent on recruitment time),

and 3. a conservative definition of ovarian failure (12 months of amenorrhea at least 18 months from start of chemotherapy).

2). Hormonal studies: FSH is the most commonly used hormonal measure of ovarian function after chemotherapy in breast cancer patients [21]. To a more limited extent, luteinizing hormone (LH), inhibin B, inhibin A and AMH have also been studied in this population [21]. AMH, inhibin A and inhibin B are produced by the ovary, while FSH and LH are secreted from the anterior pituitary. In healthy women, reproductive aging is associated with increasing FSH and LH, and decreasing inhibins and AMH [27-31]. Estradiol is useful for interpreting FSH, but is not a good measure of decreased ovarian reserve [31, 32]. FSH, LH, estradiol and inhibin levels fluctuate between and within menstrual cycles [33]. In contrast, AMH levels are gonadotropin-independent and relatively consistent within and between menstrual cycles [33]. Therefore, AMH levels are unlikely to be affected by tamoxifen use.

FSH and LH obtained concurrently with menstrual data are higher in amenorrheic versus menstruating women [34-37]. While higher FSH levels in amenorrheic survivors is known, lower AMH and inhibin B have only been reported by 3 smaller studies with short follow up and limited association with menstrual pattern [34-40]. One study associated lower pre-chemotherapy inhibin A to amenorrhea 6 months post chemotherapy [41]. These descriptive studies need longer follow up and a large sample size to yield clinically useful parameters for ovarian failure. However, these data assert a role for hormonal parameters in characterizing the extent of ovarian insult by chemotherapy.

The above hormonal studies have several limitations. First, it is unclear whether a single elevated FSH obtained in the setting of amenorrhea in this population is transient or indicative of permanent ovarian failure. Second, in the majority of these studies, blood was not obtained in the follicular phase even in cycling women, rendering interpretation of most hormonal levels difficult [35, 42]. Third, none of the studies in cancer survivors have attempted to determine cut points in hormonal levels associated with ovarian failure. Finally, only two small studies have used hormonal levels (inhibin A) to predict subsequent ovarian function [41] . **Therefore, the present study incorporates 1. serial bloods in the peri-chemotherapy period, 2. follicular phase sampling when applicable, 3. assessment of gonadotropin-dependent (FSH, estradiol, inhibin B) and gonadotropin-independent (AMH) hormones and 4. plans to analyze screening test characteristics (sensitivity, specificity, positive predictive value, negative predictive value) and receiver operating characteristic (ROC) curves to determine cut-points associated with subsequent ovarian failure.**

d. Current predictors of ovarian function after chemotherapy: Currently, only age and chemotherapy regimen are consistently associated with ovarian function after adjuvant chemotherapy. Risk of ovarian failure increases with age [8, 21]. Above the age of 40, a common cut point in available studies, the risk of ovarian failure or CRA is higher than age less than 40 [21]. The risk of ovarian failure ranges from 35% to more than 90% in women over age 40 [21]. Higher doses of cyclophosphamide have been associated with higher rates of amenorrhea, but this finding has not been consistent [43-47]. Age or chemotherapy dose alone do not accurately predict ovarian function or ovarian failure [21].

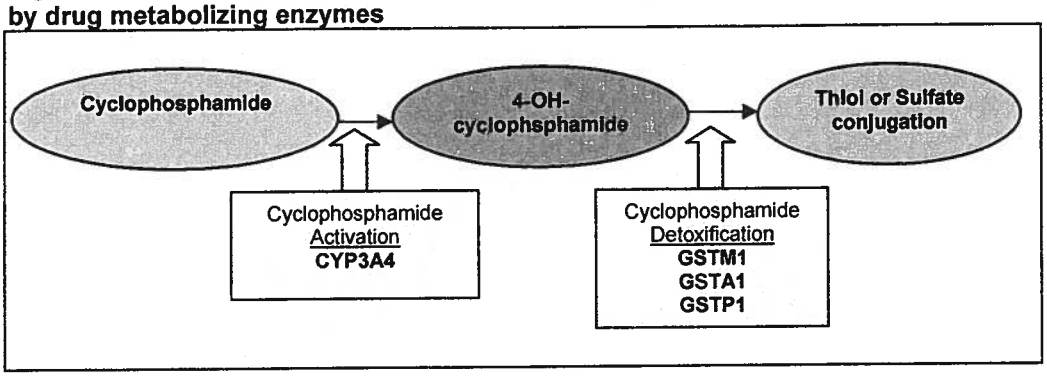
Given this background, there is a significant need for other biomarkers or combinations of biomarkers to accurately assess and predict ovarian function. **Therefore, this study proposes evaluating a number of genetic, hormonal and ovarian morphometric measures singly and in combination to determine risk for ovarian failure after adjuvant chemotherapy.**

III. Drug metabolizing enzyme polymorphisms

Functional single nucleotide polymorphisms (SNPs) in drug metabolizing enzymes (DME) can contribute to inter-individual differences in drug response. Enzymes involved in metabolizing chemotherapy drugs include members of the cytochrome P450 (CYP) and glutathione S-transferase (GST) families [48, 49]. Cyclophosphamide DME polymorphisms are targeted by our study for several reasons. First, cyclophosphamide is the most commonly used drug for breast cancer adjuvant therapy [13]. It is also used in a number of other cancers that affect reproductive age women, such as lymphomas and ovarian germ cell tumors [13]. DME polymorphisms in these genes that result in higher cyclophosphamide exposure have been associated with clinical outcomes such as improved breast cancer survival [50-54]. Finally, cyclophosphamide mechanism, exposure and dose have both been associated with ovarian damage [15, 16, 43-45]. Because cyclophosphamide behaves in a non-cell cycle specific manner, it can disrupt meiotically-arrested oocytes and/or the enveloping granulosa cells of primordial follicles.

In order to understand our selection of DME SNPs, it is important to summarize briefly cyclophosphamide metabolism. Figure 1 depicts cyclophosphamide activation and dextoxification by drug metabolizing enzymes CYP3A4, GSTM1, GSTA1, and GSTP1. Cyclophosphamide is inactive until metabolized by CYP enzymes such as CYP3A4 to its cytotoxic form 4-hydroxy - cyclophosphamide [49]. The **CYP3A4** germline mutation from CYP3A4*1A to CYP3A4*1B may alter transcription. The transcription regulation of this gene and functional significance of this polymorphism is controversial [55, 56]. In vitro assays suggest that CYP3A4*1B results in higher gene transcription, and hence, higher drug activation and increased exposure to activated cyclophosphamide [48, 57]. Clinically, however, two retrospective cohort studies have demonstrated that breast cancer patients who are CYP3A4*1B variants had worse disease-free survival compared to wildtype CYP3A4*1A/1A subjects [53, 54].

Figure 1: Simplified schematic of cyclophosphamide activation and detoxification by drug metabolizing enzymes



Inactivation of cytotoxic 4-hydroxycyclophosphamide occurs through conjugation with glutathione by several GST enzymes as shown in Figure 1 [48]. **GSTM1** Null genotype results in less drug detoxification and therefore higher exposure to active 4-hydroxycyclophosphamide. Breast cancer patients homozygous for the GSTM1 Null phenotype have improved disease-free and overall survival compared to individuals with non-null genotypes [54]. **GSTA1** has high detoxification activity; individuals with the GSTA1*B allele have reduced levels of GSTA1 and therefore higher exposure to cytotoxic 4-hydroxycyclophosphamide [58, 59]. In a cohort of 245 breast cancer subjects, those with the GSTA1*B/*B genotype had significantly increased 5-year survival compared to subjects with GSTA1*A alleles [51]. Similarly, a SNP in the **GSTP1** gene results in an amino acid change Ile105Val [52]. In vitro, enzymes containing Val have lower detoxification activity toward alkylating agents than those containing Ile [60]. Clinically, a cohort study of 240 breast cancer subjects demonstrated that women homozygous for the GSTP1 Val, who had higher exposure to cytotoxic 4-hydroxycyclophosphamide, had better overall survival than women with GSTP1 Ile variants (HR 0.3 [95% CI 0.1-1.0]) [52].

To date, there are no published studies examining the relationship between chemotherapy DME polymorphisms and ovarian failure after breast cancer adjuvant chemotherapy. In this proposal, we hypothesize that cyclophosphamide DME SNPs are associated with ovarian failure after cyclophosphamide exposure. That is, breast cancer patients with genotypes that increase exposure to activated 4-hydroxycyclophosphamide (by increased cyclophosphamide activation or decreased 4-hydroxycyclophosphamide detoxification) will be more likely to develop ovarian failure. **The present study will be one of the first to explore such a clinically relevant association.**

IV. Morphometric assessment of ovaries

Morphometric measurements of ovaries from antral follicle count (AFC) to ovarian volume are used in ovarian reserve testing [32, 61-65]. Currently, 2-dimensional ultrasound is the most widely used imaging modality to assess these measures [66]. The advantages of ultrasound ovarian imaging include lower cost, no contrast or radiation exposure and widespread use (can be performed in the office by gynecologists) [67, 68]. However, pelvic ultrasound is not well accepted by all patients, may be more operator-dependent, may be limited by increased body size, and has limited capacity to determine ovarian function [69-71].

MRI may offer several advantages over ultrasound. MRI is less operator-dependent, yields multiplanar tomographic images, and avoids a transvaginal study [68, 69]. MRI is a potentially useful tool in the evaluation of ovarian reserve. To our knowledge, there are no studies using MRI to measure ovarian reserve in cancer survivors (or infertility patients). As more breast cancer patients obtain breast MRIs for diagnosis and follow up, it will become more feasible to scan the pelvis during the same study [72, 73]. This would avoid a separate procedure and improve patient acceptability. In a small survey of 20 breast cancer subjects from our preliminary studies, 15 stated that they would prefer an MRI over a transvaginal ultrasound (data not shown). In addition, functional MRI technology is advancing rapidly. Functional MRI describes using noninvasive,

quantitative imaging techniques to monitor physiology, probe molecular processes and study metabolites [74, 75]. There is potential to use MRI technology such as Magnetic Resonance Spectroscopy to non-invasively assess ovarian function. **However, because even structural changes in ovaries exposed to chemotherapy have not been studied, we propose using MRI to measure ovarian size and AFC before and after chemotherapy as a first step.** This knowledge will facilitate pilot studies and future applications of functional MRI technology to ovarian physiology.

Only two MRI studies have examined age-related changes in ovarian morphometry. They demonstrated decreased ovarian size and AFC with age in healthy subjects [76]. . . performed a cross-sectional study of 100 healthy women divided by decade of life (age range 20 to 80). Ovaries were visualized in 97% of subjects; ovarian volume, requiring measurements in three orthogonal planes, could be calculated for 91% of the ovaries. Ovarian follicles could be visualized in 82% of women in their fourth decade. From these measurements, maximum ovarian volume, AFC and ovarian diameters were found between ages 30 and 40 and fell significantly thereafter. Of note, no differences in volume and AFC were found between 32 subjects scanned in the follicular phase and 39 subjects scanned in the luteal phase [77]. A second cross-sectional study performed by Dr. our radiology collaborator, examined 59 subjects between ages 21 to 60. At least one ovary was visualized in all subjects. Ovarian volume also declined after the fourth decade of life [76]. To our knowledge, there are no studies using MRI to assess ovarian parameters before and after chemotherapy in breast cancer patients.

V. Clinical predictive index

The goal of a clinical predictive index is to use a combination of pre-chemotherapy patient variables to accurately predict the risk of subsequent ovarian failure. These variables could include demographic, cancer, genetic, hormonal and ovarian imaging parameters. By optimizing the positive predictive value of the index, a patient who is identified by the index as high risk for ovarian failure will have a high likelihood of developing subsequent ovarian failure after chemotherapy. Therefore, a patient at high risk for ovarian failure may choose fertility preservation methods prior to adjuvant therapy. Conversely, a patient at low risk for ovarian failure may elect more aggressive chemotherapy or even surgical oophorectomy to decrease long-term estrogen exposure. Further, this tool could help future researchers design interventions for high risk groups. Currently, there is no such predictive index for breast cancer patient care.

VI. Significance

In summary, the goal of this proposal is to identify hormonal, genetic and ovarian morphometric parameters associated with ovarian failure in breast cancer subjects who receive adjuvant cyclophosphamide-based chemotherapy. Using endocrinologic evaluation, genetic epidemiology, and magnetic resonance imaging methods, the investigator will further current understanding of ovarian aging and formulate a useful and necessary clinical tool for care of young breast cancer patients. Future studies will be directed toward validating these predictors in other similar populations. In particular, the investigator is interested in testing these predictors in younger breast cancer patients to assess fertility potential.

12. PRELIMINARY STUDIES

The following studies are being conducted by the candidate. The results demonstrate:

- The candidate's ability to accrue young breast cancer subjects prior to chemotherapy
- The candidate's ability to accrue high subject follow up rates
- Reproductive hormones are affected by chemotherapy
- Post-chemotherapy levels of reproductive hormones are associated with ovarian failure
- Cyclophosphamide drug metabolizing enzyme polymorphisms may be associated with ovarian failure after cyclophosphamide-based adjuvant chemotherapy in younger women

Study

The candidate has initiated the Study, the cohort study detailed in this proposal. Over 4 months, 24 young breast cancer subjects have been recruited prior to chemotherapy (85% recruitment rate). Four eligible subjects declined enrollment because of the involved cancer treatment or distance from our cancer center (referred to only for a second opinion). We present descriptive statistics of the first 20 subjects in Table 1. These pre-chemotherapy values have been used for new power calculations for Specific Aims 1 and 3. The inter- and intra-observer coefficients of variation (cov) for ovarian volume were 8% and 9%, respectively. These cov for AFC were 12% and 15%, respectively.

Table 1: Pre-chemotherapy age, reproductive hormones and ovarian morphometry in the Study cohort (n=20)

	Mean	Range
Age at chemotherapy	36.5	26.4-40.8
AMH (ng/mL)*	1.4	0.05-10,9
FSH (mIU/mL)*	8.2	2.5-66.7
Inhibin (pg/mL)*	20.1	2.5-365.0
AFC (MRI)**	8.9	0-20
Ovarian volume (cm^3) (MRI)**	5.2	1.4-7.6
AFC (US)**	10.9	6-21
Ovarian volume (cm^3) (US)**	7.5	3.7-11.5

*Geometric mean; **n=7 subjects

Post-chemotherapy cohort study

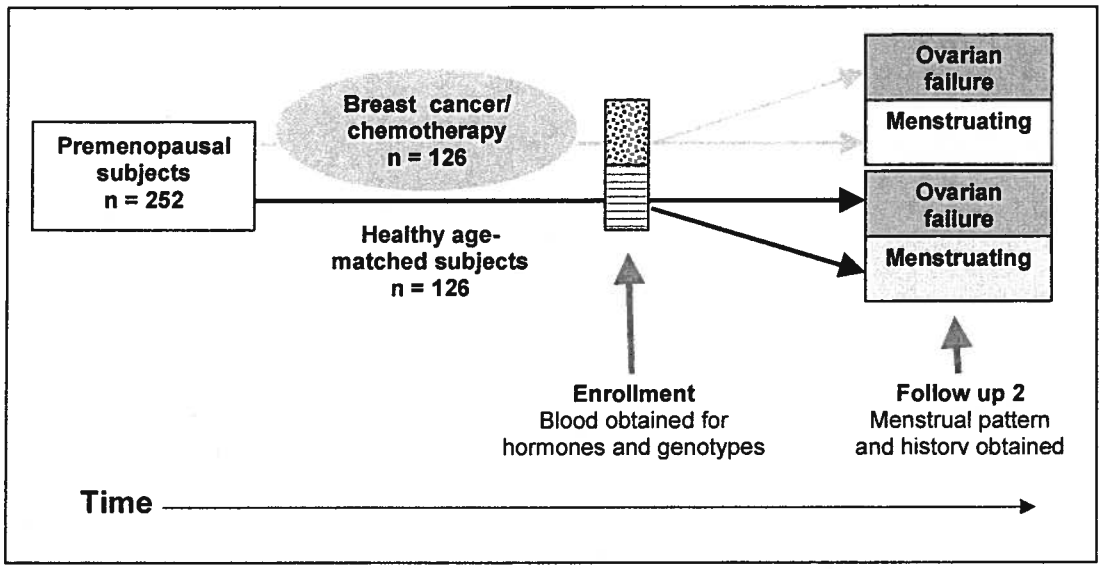
The candidate designed and conducted a cohort study in post-chemotherapy breast cancer patients to test three **hypotheses**: 1). AMH and inhibin B will be lower and FSH will be higher in women with breast cancer who receive adjuvant chemotherapy than in healthy age-matched women; 2). Lower post-chemotherapy AMH and inhibin B and higher post-chemotherapy FSH levels are associated with ovarian failure in breast cancer patients after adjuvant chemotherapy; and 3). Breast cancer patients who have drug metabolizing enzyme polymorphisms resulting in higher cyclophosphamide exposure are more likely to develop ovarian failure after cyclophosphamide-based adjuvant chemotherapy than patients who have DME polymorphisms associated with lower cyclophosphamide exposure. To our knowledge, these hypotheses have not been tested to date. The results from this work have allowed me to support and focus this K23 proposal.

Study population: To test these hypotheses, a cohort was formed using subjects from two existing studies at the , and Breast cancer subjects came from the study. These subjects were premenopausal at cancer diagnosis and recruited one to four years post-chemotherapy for a cross-sectional study on bone mineral density and chemotherapy related amenorrhea. Enrollment of 132 breast cancer patients occurred between 2004 and 2005. At enrollment, subjects had serum and DNA collected and stored.

Each subject (total n = 126) was age-matched to a healthy subject. is an ongoing population-based study of 436 healthy, late reproductive-age women from the area. At enrollment, all subjects were premenopausal. subjects have provided demographic information, medical history, exposures, menstrual history, BMI, and blood samples annually since 1995.

Study design and data collection: The study design is depicted in Figure 2. The entire cohort is comprised of 252 subjects. One-half, or 126 subjects, developed breast cancer and received adjuvant chemotherapy. Their age-matched controls (n = 126) are healthy women who do not develop breast cancer or have chemotherapy exposure. One to four years post-chemotherapy (Figure 2, Enrollment), breast cancer subjects had bloods collected (not specific to menstrual cycle day), from which serum and DNA are extracted and stored. Each healthy subject also had serum collected and stored in same time frame as the breast cancer to whom she is matched (Figure 2, Enrollment). All sera were assayed for AMH, FSH, inhibin B and

Figure 2: Study design



estradiol. DNA from breast cancer subjects were genotyped for single nucleotide polymorphisms (SNPs) in four cyclophosphamide metabolizing enzyme genes: GSTM1, GSTP1, GSTA1 and CYP3A4. As detailed in the **Research Design** portion of this grant application, these genotypes were used to classify breast cancer subjects as having higher or lower exposure to active 4-hydroxycyclophosphamide.

The primary outcome of ovarian failure was assessed via a menstrual history questionnaire 18 to 42 months after the initial enrollment visit (Figure 2, Follow up 2). Ovarian failure was defined as 12 months of amenorrhea occurring after start of chemotherapy. Over three months, the candidate was able to complete questionnaire follow up of 115 out of 132 breast cancer subjects (87% of the cohort). Six of 132 breast cancer subjects could not be paired to an age-matched subject and were excluded from paired analyses. Menstrual history from age-matched healthy subjects was abstracted from the closest matched annual follow up for the (Figure 2, Follow up 2).

Of note, the candidate is also obtaining pelvic ultrasounds and a second blood specimen on breast cancer subjects for Follow up 2. Over six months, the candidate completed ultrasound in 96 of 115 (83%) and blood collection in 109 of 115 (95%) subjects who completed the questionnaire portion of Follow up 2. Ten percent of subjects declined to undergo ultrasound evaluation, the most cited reason being pelvic discomfort. Based on the success of enrollment and data collection, we propose a similar study design for our K23 study.

Data Analysis: Initial data analysis included descriptive and bivariate analyses. In descriptive statistics, categorical variables were summarized by frequencies and proportions, while continuous variables were summarized by the mean, median, standard deviation and range. Categorical variables were characterized by proportions and compared by chi-square, exact methods, or McNemar's test (for paired data), as appropriate. Relative risks and 95% confidence intervals were calculated for dichotomous risk factors. Student's t test (for normally distributed data) or Wilcoxon rank-sum test (for ordinal or non-normally distributed variables) were used to compare independent continuous variables. Paired t-test was used for normally distributed paired data.

Table 2: Enrollment characteristics

Mean age at start of chemotherapy (range)	43.2 (26.7 – 57.8)
Mean age at study visit (range)	45.3 (28.9-60.6)
Mean years since start of chemotherapy (range)	2.1 (0.5-4.9)
Breast cancer stage (%)	
I	29 (22%)
II	83 (63%)
III	20 (15%)
Estrogen receptor +	97 (73%)
Progesterone receptor +	89 (67%)
Her-2/neu +	27 (20%)
Cyclophosphamide based chemotherapy	130 (98%)
Race	
Caucasian	117 (89%)
African American	6 (4.5%)
Other/Not reported	9 (7%)
Surgical menopause	10 (7%)

Results: Baseline characteristics of the initial breast cancer cohort (n = 132) are summarized in Table 2. Compared to subjects at enrollment, the 115 subjects at Follow up 2 were older (mean age 49.1, range 32-62.4, $p < 0.001$), and a greater proportion (19/115, 17%, $p = 0.04$) had undergone bilateral oophorectomies, resulting in surgical menopause. The cohort remained unchanged in other baseline characteristics (data not shown). Of the 17

women who were lost to follow up, 2 were deceased, 1 declined follow up and 13 could not be reached. At Follow up 2, ovarian failure status could be evaluated in 96 of 115 subjects (83%), because 19 subjects had surgical menopause. Ovarian failure at Follow up 2, defined as 12 months of amenorrhea, occurred in 63 of 96 subjects (66%), while 33 subjects continued to menstruate (34%).

Age and chemotherapy dose as risk factors for ovarian failure at Follow Up 2: Age was significantly associated with ovarian failure. Age at chemotherapy (mean ± SD) of breast cancer subjects who developed ovarian failure was 47.0 ± 4.3 years, compared to 38.1 ± 4.9 years in breast cancer subjects who continued to menstruate ($p < 0.01$). Because of the wide age range of our subjects, we also evaluated subjects in two age groups: less than and greater than 45 years. The risk of ovarian failure was significantly higher in women older than 45 years (95% versus 42%, RR 2.32 $p < 0.001$). Based on these data, we focused our present K23 proposal on the age group (≤ 45 years) where determination of ovarian failure risk has the most clinical impact.

We did not see an association between higher cumulative dose of cyclophosphamide and the development of ovarian failure ($p = 0.56$). This finding may have been overshadowed by other variables such as age. However, based on the data below, we hypothesize that risk of ovarian failure may be affected by chemotherapy metabolism rather than dose. We plan to pursue this hypothesis by looking at cyclophosphamide drug metabolizing enzyme polymorphisms that result in differential activation and detoxification of cyclophosphamide.

Breast cancer and adjuvant chemotherapy affect reproductive hormone levels: There were 126 breast cancer patients who were age-matched to healthy subjects. Age at blood draw (mean ± SD) was 45.8 ± 5.9 years for breast cancer subjects and 45.9 ± 5.3 years for healthy subjects ($p=0.11$). Geometric means of AMH, FSH and inhibin B for breast cancer subjects and healthy subjects are depicted in Table 3. Significant differences between inhibin B and FSH levels were observed (Table 3). While AMH did not differ between the 126 pairs, a sub-analysis of 57 subject pairs where both subjects had detectable AMH demonstrated a significant difference in AMH between breast cancer subjects (0.35 ng/mL, 95% CI 0.23-0.55) and healthy subjects (0.14 ng/mL, 95% CI 0.1-0.2) ($p = 0.001$).

Table 3: Reproductive hormones* in post-chemotherapy breast cancer subjects and age-matched healthy subjects

	Breast cancer subjects (n = 126)	Healthy subjects (n = 126)	p-value
AMH (ng/mL)	0.028 (0.2-0.64)	0.036 (0.18-0.44)	0.43
Inhibin B (pg/mL)	15.9 (12.4-20.2)	39.8 (32-49.4)	< 0.001
FSH (mIU/mL)	33.8 (28.8-39.8)	19.5 (14.7-25.8)	0.001
Estradiol (pg/mL)	39.0 (31.8-47.9)	42.3 (33.5-53.5)	0.57
*Geometric mean (95% CI)			

Additional analyses examined important cut-off values for these reproductive hormones. Subjects were dichotomized into undetectable and detectable AMH, undetectable and detectable inhibin B, and FSH < 11.4 versus FSH ≥ 11.4 mIU/mL. Breast cancer subjects were more likely to have undetectable inhibin B (OR 4.7, $p < 0.001$) and high FSH ≥ 11.4 (OR 6.7, $p < 0.001$) than their healthy counterparts. Odds of undetectable AMH was no different between paired breast cancer and healthy subjects ($p = 0.88$).

These data support the hypothesis that chemotherapy exposure adversely affects measures of ovarian reserve, in particular inhibin B and FSH. We then tested whether these surrogate markers were associated with the clinically important outcome of ovarian failure. In the present proposal, we plan to investigate these findings with greater power in a more focused age group.

Post-chemotherapy hormones are associated with ovarian failure in breast cancer subjects: Sixty-six percent of breast cancer subjects (63/96) were classified with ovarian failure at Follow up 2, 5.4 years (range 2.7-7.6) after initiation of chemotherapy. Post-chemotherapy AMH and inhibin B levels were significantly lower, and FSH levels were significantly higher, in breast cancer subjects who developed ovarian failure than in those who continued to menstruate ($p < 0.001$, $p = 0.03$, and $p < 0.001$, respectively). We also evaluated the association between reproductive hormones and ovarian failure by age ≤ 45 (n = 52) and age > 45 (n = 44) (Table 4). In breast cancer subjects less than 45 years old (Table 3, shaded rows), post-

Table 4: Post-chemotherapy reproductive hormones* by ovarian function status and age

	Age	Ovarian failure	No ovarian failure	p-value
AMH (ng/mL)	≤ 45	0.024 (0.007-0.084)	0.109 (0.045-0.262)	0.05
	> 45	0.009 (0.004-0.018)	0.067	NS
Inhibin B (pg/mL)	≤ 45	11.1 (5.4-22.8)	24.1 (13.8-42.1)	NS
	> 45	2	9.2 (5.3-16.3)	<0.001
FSH (mIU/mL)	≤ 45	49.1 (33.1-72.8)	19.2 (13.3-27.8)	< 0.001
	> 45	53.7 (44.2-65.2)	51.4	NS
Estradiol (pg/mL)	≤ 45	34.9 (20.0-60.9)	95.0 (64.2-140.7)	0.004
	> 45	19.6 (15.1-25.4)	36.1	NS

*Geometric mean (95% CI)

chemotherapy AMH was lower and post-chemotherapy FSH was higher in those who developed ovarian failure than in those who continued to menstruate. Inhibin B appeared lower in ovarian failure subjects ≤ 45, but was not statistically different.

Breast cancer subjects were also dichotomized into undetectable versus detectable AMH, undetectable versus detectable inhibin B, and FSH < 11.4 versus FSH ≥ 11.4. Subjects with undetectable post-chemotherapy AMH, undetectable inhibin B, or FSH ≥ 11.4 had higher risks of ovarian failure (AMH RR 1.5, p = 0.004; inhibin B RR 1.5, p = 0.009; FSH RR 3.2, p < 0.001). In subjects less than 45 years old, similar relative risks of ovarian failure were observed for undetectable versus detectable AMH (RR 1.6, p = 0.02), undetectable versus detectable inhibin B (RR 1.75, p = 0.09) and FSH ≥ 11.4 versus < 11.4 (RR 3.2, p < 0.001).

These data reveal several important findings. They support the association of hormonal measures of ovarian reserve with ovarian failure in this population. In addition, these data support the potential use of cut-points in hormonal levels to assess risk of ovarian failure. Through this proposal, we hope to extend these findings to pre-chemotherapy reproductive hormone levels as outlined in our first study aim. The ability to predict ovarian failure using hormone levels prior to chemotherapy would enable clinicians and patients to modify their care.

Cyclophosphamide drug metabolizing enzyme polymorphisms and ovarian failure in breast cancer subjects: Breast cancer subjects were genotyped for DME polymorphisms in 4 cyclophosphamide metabolizing enzyme genes: GSTM1, GSTA1, GSTP1 and CYP3A4. Genotype distributions were in Hardy-Weinberg equilibrium (data not shown) and were consistent with reported reference SNP frequencies.

The risk of ovarian failure was 73% in GSTM1 Null genotype (higher cyclophosphamide exposure) compared to 53% in GSTM1 non-Null genotype (lower cyclophosphamide exposure) (RR = 1.4, p = 0.07). The relative risk of ovarian failure in higher cyclophosphamide exposure compared to lower cyclophosphamide exposure genotypes was 0.8 for GSTA1 (p = 0.16), 0.9 for GSTP1 (p = 0.63) and 1.5 for CYP3A4 (p = 0.5).

Because the risk of ovarian failure differed by age (stratified into age ≤ 45 and > 45 as described above), we tested whether risk of ovarian failure by genotypes differed by age stratum. There were 52 subjects ≤ 45 and 44 subjects > 45 years old. In women less than 45, all four genotypes postulated to result in higher cyclophosphamide exposure were associated with higher risk of ovarian failure, though this was not statistically significant (Table 5). These preliminary results, the first to test the association between drug metabolizing enzymes and ovarian failure after chemotherapy, support the current design of a larger study on younger women.

Table 5: Risk of ovarian failure by GSTM1, GSTA1, GSTP1 genotypes and by age

Gene	Age	High Cyclophosphamide Genotypes*	Low Cyclophosphamide Genotypes**	Relative risk Ovarian failure (High vs. low)	p-value
GSTM1	≤ 45	46%	32%	1.5	0.3
	> 45	94%	100%	0.9	0.4
GSTA1	≤ 45	44%	38%	1.1	0.7
	> 45	83%	97%	0.9	0.2
GSTP1	≤ 45	50%	39%	1.3	0.6
	> 45	75%	97%	0.8	0.06
CYP3A4	≤ 45	60%	23%	2.6	0.1
	> 45	100%	89%	0.9	1.0

*GSTM1 Null, GSTA1*B/*B, GSTP1 Val/Val, CYP3A4*1B Variants
**GSTM1 non-Null, GSTA1*A Variants, GSTP1 Ile Variants, CYP3A4*1A/*1A

Temporary ovarian failure in breast cancer subjects: Of 115 subjects at Follow up 2, seven subjects who were classified as ovarian failure case at the enrollment visit (for more than twelve months of amenorrhea) had subsequent resumption of menstrual cyclicity. At enrollment, the mean follow up time from start of chemotherapy for these 7 women was only 1.2 years. The mean follow up time from chemotherapy to Follow

Up 2, when these 7 cases were identified, was 4.4 years (range 3.2 to 5.6 years). The duration of amenorrhea before resumption of menstrual cyclicity was not captured.

Table 6 depicts age and enrollment hormone levels. All seven cases had elevated FSH and appropriately low estradiol. However, detectable AMH and/or inhibin B was observed in 4/7 subjects. These results support the need for longer follow up time and further studies using AMH and inhibin B to assess ovarian function in amenorrheic subjects after chemotherapy.

Table 6: Breast cancer subjects who had > 12 months amenorrhea at enrollment and subsequently resumed menstruation by Follow up 2

Subject	Age at chemotherapy	AMH (ng/mL)	Inhibin B (pg/mL)	FSH (mIU/mL)	Estradiol (pg/mL)
1	38.4	Undetectable	Undetectable	57.2	24.7
2	40.3	0.087	19.2	38.8	19.1
3	41.4	0.01	Undetectable	52.8	8.7
4	42.1	Undetectable	23.7	153.6	27.2
5	42.5	Undetectable	Undetectable	36.3	27.9
6	43.2	Undetectable	Undetectable	42.4	18.7
7	44.8	0.17	Undetectable	51.8	39.0

Summary

The post-chemotherapy breast cancer cohort had a high follow up rate (87%), and subjects were very motivated to undergo additional blood work and ovarian imaging. Compared to healthy age-matched subjects, breast cancer subjects after adjuvant chemotherapy had higher FSH and lower inhibin B. This finding supports the adverse effect of chemotherapy on ovarian follicles, resulting in decreased measures of ovarian reserve. In examining risk factors for ovarian failure after adjuvant chemotherapy, increased age was strongly associated with increased risk of ovarian failure. Ovarian failure was also associated with lower post-chemotherapy AMH and inhibin B and higher post-chemotherapy FSH.

Statistically significant associations between cyclophosphamide DME polymorphisms and risk of ovarian failure were not found. However, several analyses suggested that a larger sample size using more restricted age groups may yield significant results.

Finally, the data raised the question of how ovarian failure should be defined in breast cancer patients after chemotherapy. Using 12 months of amenorrhea as the criteria for ovarian failure, 10% of breast cancer subjects who met this criteria for ovarian failure at enrollment (n = 7) had subsequent return of menstrual function. These women were young and had short follow up times at enrollment (less than 2 years). Clinically utilized FSH and estradiol from their enrollment visit bloods would not helped to identifying these 7 cases of prolonged but temporary amenorrhea.

The , proposed in this grant, has successfully started enrolling subjects and will address the limitations of this preliminary study by:

- Obtaining pre-chemotherapy bloods for hormonal studies. Determining the association of pre-chemotherapy reproductive hormones and subsequent ovarian failure could be used clinically to affect reproductive and chemotherapy choices.
- Obtaining follicular phase bloods for hormonal studies to ensure comparability of gonadotropin-dependent hormones such as FSH and estradiol.
- Restricting age to women less than 40.
- Obtaining menstrual history prospectively and frequently for accuracy and to capture the lengths of amenorrhea prior to resumption of menstrual cyclicity.
- Defining ovarian failure by both length of amenorrhea (≥ 12 months) AND length of follow up since start of chemotherapy (≥ 18 months).

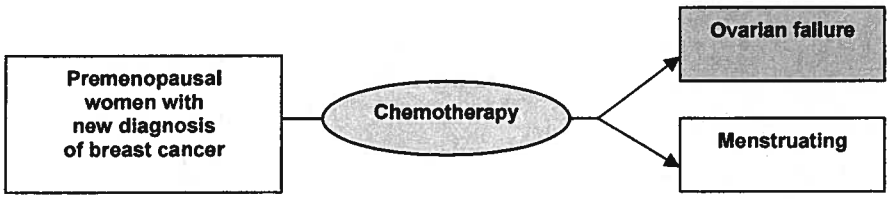
13. RESEARCH DESIGN AND METHODS

Overall research plan

A 5-year, prospective cohort study to determine genetic, hormonal and ovarian morphometric biomarkers associated with ovarian failure after adjuvant chemotherapy in premenopausal breast cancer patients is proposed. Eligible subjects will be women with newly diagnosed breast cancer 40 years old or younger who will undergo adjuvant chemotherapy. Subjects will be recruited from the at the University prior to chemotherapy. Baseline information will be collected by the Breast Care Questionnaire, an initial study questionnaire and medical chart abstraction. Patients who decline participation in the study will be asked for their permission to review their medical records. With this information, we will be able to compare demographic and disease characteristics between those who agreed or declined to participate. This will help to determine the generalizability of the study.

Subjects enrolled into the full study will have serial blood work and maintain a daily uterine bleeding diary for the duration of the study (Figure 3). At enrollment, subjects will have blood drawn for hormonal levels and DNA extraction. Blood for hormonal levels and bleeding diaries will be collected every 3 months during chemotherapy and every 6 months after chemotherapy. Two-thirds of subjects will be randomly selected to undergo a pelvic MRI and a pelvic ultrasound before and within six months after chemotherapy.

Figure 3: Study design



	Breast cancer diagnosis (Pre-chemotherapy)	Chemotherapy (Duration 3-6 months)	Post-chemotherapy to end of study
Enrollment into study	√		
Blood draw	√	√ every 3 months	√ every 6 months
MRI/ Ultrasound	√ 25% of cohort		√ 25% of cohort
Daily bleeding diary	√	√ collect every 3 months	√ collect every 6 months

Serum, collected during early follicular phase where applicable, will be assayed for AMH, FSH, inhibin B and estradiol. DNA will genotyped for cyclophosphamide metabolizing enzyme polymorphisms. By genotype, a subject will be categorized as "high risk" and "low risk" for developing ovarian failure. Ovarian volume, diameters and antral follicle count will be assessed by MRI and ultrasound studies.

The primary study outcome will be ovarian failure. In this population, ovarian failure will be defined by both 1. **length of amenorrhea** (≥ 12 months) and 2. **length of study follow up since start of chemotherapy** (≥ 18 months). Therefore, a subject will be considered to have ovarian failure if her last menstrual period was more than 12 months ago and she has been followed for more than 18 months since start of chemotherapy.

Data will be analyzed in several steps. First, descriptive statistics of ovarian failure, demographic information, cancer history, reproductive hormones, drug metabolizing enzyme polymorphisms, and ovarian dimensions will be calculated. Second, the investigator will test the association between ovarian failure and individual risk factors in demographics (such as age), cancer history (such as estrogen receptor positive disease), pre-chemotherapy reproductive hormone levels, drug metabolizing enzyme polymorphisms, and ovarian morphometric parameters (such as change in ovarian volume after chemotherapy). By examining screening test characteristics and using ROC curves, the investigator will then establish optimal cut-points for these continuous variables for predicting ovarian failure. Finally, clinically and statistically important risk factors will be used to develop a clinical predictive index to predict the outcome of ovarian failure in premenopausal

breast cancer patients who receive adjuvant chemotherapy. This predictive index will be internally validated using a bootstrapping approach.

Study population, recruitment and study visits

Study Site: Subjects will be recruited from the Breast Center at the University The Breast Center has 6 breast cancer oncologists. Each year, the center sees approximately 600 new patients. In 2005 and 2006, 20% of patients (n = 240) were ages 35 to 45 diagnosed with breast cancer. Twenty-five percent of patients were less than 45 years old.

Subject Eligibility and Recruitment:

Inclusion criteria

- Female
- Age 40 or younger
- Diagnosis of AJCC Stage I, II, or III breast cancer
- No prior chemotherapy
- Premenopausal (defined as menses in the past 12 months)
- Intact uterus and at least one ovary
- Treatment plan for adjuvant cyclophosphamide-based chemotherapy

Exclusion criteria

- Medical illnesses known to affect FSH, estradiol or inhibin B levels. Examples are anorexia nervosa, hypothalamic amenorrhea, undertreated overt hypothyroidism
- Pregnancy or lactation
- Use of psychotropic drugs

Rationale for eligibility criteria: The age range was selected to include very young women with breast cancer and exclude older women (age >40) in whom risk of ovarian failure after chemotherapy is very high. We will recruit at least 150 women who are 40 or younger at chemotherapy. We purposefully selected this age group because predicting ovarian failure after chemotherapy is clinically important to this population. Continued ovarian function in this age group has implications including, but not limited to future fertility. Ovarian function would result in estradiol production and impact cancer prognosis, choice of adjuvant chemotherapy, menopause-related symptom management and prevention of diseases associated with premature ovarian failure such as coronary artery disease. The sample size of 150 women who are 40 and younger will yield a reasonable proportion of outcomes (ovarian failure versus continued ovarian function) in the three to five-year initial follow up period. This will aid in the power of our study. The cohort size of 150 is seen below in sample and power calculations. The main exclusion criteria address women who have medical conditions or treatments that affect hormonal levels, confounding interpretation of results.

Recruitment: Subjects will be identified by screening new patients of the Patients will give informed consent to join the study cohort. Once enrolled, the subject will undergo pre-chemotherapy blood draw and teaching for completing bleeding diaries. A random subset will undergo a non-enhanced pelvic MRI and pelvic ultrasound. Subjects will be reimbursed for parking fees for study visits and will not receive other financial compensation for their time.

Rationale for recruitment process: Recruitment was chosen to take place at diagnosis, prior to chemotherapy, to ascertain the effect of adjuvant chemotherapy on ovarian function. Further, any counseling or individualization of treatment that may result in the future from this study would best be implemented prior to start of gonadotoxic therapy.

Study visits (Figure 3): After enrollment, subjects will undergo one pre-chemotherapy study visit, timed for the first 5 days of the menstrual cycle where possible. Subjects will be taught to record bleeding diaries and will have blood collected for DNA isolation and hormonal studies. Every 3 months during chemotherapy and every 6 months after chemotherapy, subjects will have a study visit timed with oncology follow up for blood draw, collection of bleeding diaries, and review of recent menstrual history. These visits will be timed, if possible, with the early follicular phase of the menstrual cycle. Two-thirds of subjects will undergo a pelvic MRI and ultrasound before and within 6 months after completing chemotherapy. This subset will be selected using a computer random number generator.

Data collection and storage

Baseline information: Demographics, breast cancer history, cancer treatment plan, reproductive history, family history, medical history and body mass index will be abstracted from the Breast Care Questionnaire, the study questionnaire and medical records by the study coordinator.

Hormonal studies: Serial AMH, inhibin B, FSH and estradiol will be evaluated. These hormones were chosen after a literature review of hormonal measures of ovarian reserve and the menopausal transition (see Background). There may be up to 10 blood samples per subject. Samples will be centrifuged and frozen in aliquots at -80 degrees C. Hormonal assays will be performed in batches and in duplicate by the University Center. Commercial kits were chosen to improve the generalizability of our results. AMH will be measured using commercial ELISA kits (Diagnostic Systems Laboratories, Webster, TX). Inhibin B will be assayed using commericial ELISA kits from AbD Serotec (Oxford, UK). FSH and estradiol will be measured by radioimmunoassay using Coat-A-Count commercial kits (Diagnostic Products, Los Angeles, CA). Means of duplicates will be used for analysis. Intra- and inter-assay coefficients of variation will be calculated.

Drug metabolizing enzyme polymorphisms: Four single nucleotide polymorphisms in cyclophosphamide metabolizing enzymes will be genotyped (Table 7). These polymorphisms were selected after a literature search for SNPs that result in functional change and are associated with breast cancer survival. DNA will be extracted from pre-chemotherapy blood samples, and a laboratory technologist will perform genotyping studies in year 3 after enrollment is complete. GSTM1 homozygous Null mutations and GSTA1 SNP will be identified by multiplex polymerase chain reaction, followed by visualization on 4% metaphor gel [53]. A multiplex pyrosequencing assay will be used for CYP3A4 and GSTP1 polymorphisms [54].

Table 7: Selected cyclophosphamide metabolizing enzyme polymorphisms

Drug metabolizing enzyme gene	Chromosome	Single nucleotide polymorphism	Genotypic comparison	Change in enzyme function	Exposure 4-hydroxy-cyclophosphamide	Assay method
GSTM1	1p13	Null mutation	Null vs. non-Null	↓ drug detoxification	Increased	PCR and gel electropheresis assay
GSTA1	6p12	69 C>T	*B/*B vs. *A/*A and *A/*B	↓ drug detoxification	Increased	PCR and gel electropheresis assay
GSTP1	11q13	313 A>G	Val/Val vs. Ile/Ile and Ile/Val	↓ drug detoxification	Increased	Pyrosequencing multiplex assay
CYP3A4	7q21.1	-392 A>G	1A/1B and 1B/1B vs. 1A/1A	↑ drug activation	Increased	Pyrosequencing multiplex assay

Pelvic imaging:

Pelvic MRI: Pelvic MRI will be performed at 1.5T with a pelvic phased-array torso coil using axial T1-weighted fast spin echo, axial, sagittal and fat-suppressed coronal T2-weighted fast spin echo [76]. No contrast agent will be administered. Radiology collaborators will ascertain the diameters (in three orthogonal dimensions), volumes and AFC of the ovaries. The outer contours of the ovaries will be manually traced as regions of interest (ROI) using picture archiving and communications system (PACS) workstation (Centricity; GE Health care, Milwaukee, WI) to provide area values. Area values will be summed and totals will be multiplied by slice thickness to obtain ovarian volumes. These measurements, along with change in these measures between pre- and post-chemotherapy MRI, will be used to test the association between ovarian imaging measures and ovarian failure.

Pelvic Ultrasonography: Ovarian volume, dimensions and AFC will be determined by transvaginal pelvic ultrasonography within the first four days of a menstrual cycle when possible. Pelvic ultrasonography will be performed by the principal investigator using a standard protocol. Both ovaries will measured in the maximum transverse, anterior-posterior and longitudinal diameters. Volume will be estimated as $\pi/6 \times 3$ diameters. AFC will represent the total number of antral follicles (2 and 10 millimeters in diameter) from the ovaries.

Outcomes (menstrual history, ovarian failure and time to ovarian failure):

Menstrual history: Bleeding diaries and menstrual history from study visits will be used to determine the primary outcome of ovarian failure and secondary outcomes such as time to ovarian failure. Bleeding diaries will be self-recorded on pre-printed calendars and collected at each visit. For each day of the month, participants will record whether they had bleeding and self-rate bleeding as none, spotting, mild, moderate, or heavy. In addition, dates for last menstrual period, prior menstrual period and number of periods in the interval between study visits will be elicited at each study follow up.

Ovarian failure:

Ovarian failure is the primary outcome; time to ovarian failure is a secondary outcome. In this population, ovarian failure will be defined by both 1. length of amenorrhea (12 months) and 2. time since start of chemotherapy (18 months). The prevalence of **ovarian failure** and **time to ovarian failure** will be examined in this study. In women who develop ovarian failure, time to ovarian failure will be defined as the number of months from start of chemotherapy to the final menstrual period. Women who continue to menstruate throughout the study will contribute the number of months that they remained in the study.

Surgical menopause:

A number of subjects will undergo prophylactic oophorectomies during this follow up period. This proportion is estimated to be 10 to 15% [19, preliminary data]. These subjects will be ineligible for analyses using ovarian failure as the outcome. For analyses using time to ovarian failure as the outcome, these subjects will be censored at surgery.

Data storage: All data will be stored in an Access Database created by the investigator. This database is functional and currently in use.

Data analysis

Specific Aim 1: To determine if pre-chemotherapy hormonal measures are associated with ovarian failure in breast cancer patients after adjuvant chemotherapy.

Pre-chemotherapy AMH, inhibin B, and FSH will be tested individually for their association with ovarian failure and time to ovarian failure. Each hormone will be analyzed as a continuous measure. In addition, FSH and AMH will also be dichotomized by a threshold value consistent with decreased ovarian reserve (and hence increased risk of ovarian failure) in the literature and by our assays. For example, an FSH value of 11.4 mIU/mL or greater from our institutional laboratory is associated with decreased ovarian reserve and exceeds 2 standard deviations above the mean level for normal women of peak reproductive age [29, 78]. Therefore, subjects will be dichotomized into FSH $\geq$ or < 11.4 mIU/mL. Similarly, AMH will be dichotomized into detectable versus undetectable [40, 79, preliminary data]. There are no well-established clinical thresholds for inhibin B in the literature and in our experience. Finally, new cutpoints that optimize the positive predictive value for ovarian failure will also be determined for each of these three hormones. We have chosen to optimize the positive predictive value because it is important to be certain that patients at high risk actually develop ovarian failure. Chemotherapy treatment decisions by physicians and patients are unlikely to change unless a positive test that identifies women at high risk of ovarian failure after chemotherapy is closely linked to subsequent development of ovarian failure.

Estradiol levels will be used to help interpret FSH. That is, FSH levels will be considered "true" and not falsely suppressed if estradiol is appropriately low. We will analyze the data using FSH values where concomitant estradiol is $\leq$ 60 pg/mL (2 standard deviations above the mean level for early follicular phase estradiol at our institution).

Statistical analyses: First, descriptive statistics of reproductive hormones will be calculated. Hormone measures will be transformed to natural log values to minimize the impact of their skewed distributions. If normality assumptions are met, Student's t-test will be employed to test whether pre-chemotherapy levels of each hormone differed by the outcome of ovarian failure. The Wilcoxon rank-sum test will be used for non-normally distributed variables. Poisson regression models will be used to model the shape of the association between hormone levels and risk of ovarian failure while adjusting for confounders and effect modifiers. Based on the study data, we will assess screening test characteristics of sensitivity, specificity, positive predictive value and negative predictive value across the range of cutpoints for FSH, AMH and inhibin B. Cutpoints that optimize the positive predictive value for ovarian failure will be selected for each of the three hormones. Chi-

square test and Poisson regression will be used to examine the association between dichotomized AMH, inhibin B and FSH levels and the outcome of ovarian failure. Survival analysis using time to ovarian failure as the outcome and dichotomized AMH, inhibin B and FSH levels as the exposure will also be performed. Cox regression analysis will be performed to adjust for confounders and effect modifiers of time to ovarian failure. Relationships between hormonal levels as a continuous variable and time to menopause will also be assessed using the Cox regression framework.

Sample size and power: Based on the literature on post-chemotherapy ovarian failure, the risk of ovarian failure in women who are 40 or younger after adjuvant chemotherapy is assumed to be 40%. Sample size was set at 150 for study feasibility. Accounting for dropout due to oophorectomy, loss to follow up or death, the analyzable sample size decreases by 25% to 120. Mean hormone levels and standard deviations were obtained from our preliminary data on 20 young, pre-chemotherapy breast cancer subjects (Preliminary data), Table 8 demonstrates the detectable differences in mean hormonal values by outcome (ovarian failure versus not). Power was assumed to 80% and Type I error was 0.05. This study is powered to detect a difference of 0.0019 ng/mL in AMH, 1.5 mIU/mL in FSH, and 2.1 pg/mL in inhibin B in pre-chemotherapy bloods between subjects who go on to develop ovarian failure compared to subjects who do not develop ovarian failure.

Table 8: Smallest detectable difference in means of pre-chemotherapy AMH, FSH and inhibin B by ovarian failure.

Hormone	Minimum detectable difference	σ (standard deviation)*
AMH	**0.0019 ng/mL**	1.2
FSH	**1.5 mIU/mL**	0.77
Inhibin B	**2.1 pg/mL**	1.4

* Standard deviations of logarithmically transformed hormone levels

Assumptions for the proportions of subjects with detectable AMH and FSH $\leq$ 11.4 mIU/ml were also derived from our 20 pre-chemotherapy breast cancer subjects. Dichotomizing subjects by varying thresholds for abnormal pre-chemotherapy hormone levels (10th to 40th percentiles), the study is powered to 1.5 to 2.2 relative risk of ovarian failure for those with an abnormal pre-chemotherapy AMH or FSH compared to those with normal hormone values (Table 9). Based on our preliminary data where 10% of our population had undetectable AMH, the detectable relative risk of ovarian failure for undetectable versus detectable AMH would be 2.2. Based on our preliminary data where 35% of our population had an FSH $\geq$ 11.4, the detectable relative risk for ovarian failure for high versus low FSH would be 1.55.

Table 9: Detectable relative risks of ovarian failure by cut points in pre-chemotherapy AMH and FSH

Threshold for abnormal hormone cut point	Detectable relative risk of ovarian failure (abnormal to normal values)
10%ile	2.2
20%ile	1.8
25%ile	1.7
30%ile	1.6
40%ile	1.5

* Overall risk of ovarian failure assumed to be 40%

Anticipated results: Subjects who develop ovarian failure will have lower AMH, higher FSH, and lower inhibin B than subjects who do not develop ovarian failure. High FSH ($\geq$11.4 mIU/ml) and undetectable AMH will be associated with increased risk of ovarian failure and shorter time to ovarian failure. Inhibin B will be negatively correlated with time to ovarian failure.

Limitations and alternative approaches: Follicular phase hormonal measurements are advantageous because of ease of obtaining the tests and the relative abundance of literature on these measures in cancer patients [8, 21]. Alternatively, dynamic testing such as GnRH agonist stimulation test, the clomiphene citrate challenge test, or the exogenous FSH ovarian reserve test was considered. However, the nature of this testing, which may involve injections, repeat blood draws, and stimulation of transient estradiol elevations, pose an undue burden on the patient and would be difficult in a large cohort. In addition, these dynamic tests may be difficult to apply by the primary oncologist or gynecologist as part of the clinical predictive index.

Specific Aim 2: To determine if cyclophosphamide drug metabolizing enzyme polymorphisms are associated with ovarian failure in breast cancer patients after cyclophosphamide-based adjuvant chemotherapy.

Four cyclophosphamide DME polymorphisms in GSTM1, GSTA1, GSTP1 and CYP3A4 genes will be tested individually and in groups for their association with ovarian failure and secondary outcomes. For each DME, a subject will be classified by genotype as having higher or lower exposure to active 4-

hydroxycyclophosphamide. Genotypes resulting in higher exposure to active cyclophosphamide are hypothesized to be associated with higher risk of ovarian failure. Conversely, genotypes resulting in lower exposure to active cyclophosphamide are hypothesized to be associated with lower risk of ovarian failure.

GSTM1 Null will be compared to GSTM1 non-Null genotypes. Previously reported breast cancer survival data demonstrated improved survival in subjects with GSTA1*B variant genotypes compared to wildtype genotype [51]. Similarly, GSTP1 Val/Val genotype will be compared to pooled Ile/Ile and Ile/Val variants [52]. CYP3A4*1A/*1A genotypes will be compared to pooled *1B/*1B and *1A/*1B genotypes [54].

Polymorphisms will also be examined in genotype groups based on the hypothesis that combinations of functional polymorphisms could impact circulating drug levels and the outcome of ovarian failure [53]. Specifically, subjects will be categorized into low, intermediate and high cyclophosphamide exposure groups. Subjects with all four genotypes associated with low cyclophosphamide exposure will be categorized as "low exposure". Subjects with all four genotypes associated with high cyclophosphamide exposure will be categorized as "high exposure". Subjects with combinations of low and high cyclophosphamide exposure genotypes will be categorized as "intermediate exposure".

Statistical analyses: First, descriptive statistics of drug metabolizing enzyme polymorphisms will be calculated. Next, bivariate associations between genotype or genotype combinations and ovarian failure will be estimated using Pearson chi-squared test, or Fishers exact test as required. Stratified contingency tables will be examined to identify potential effect modifiers and confounders of identified associations. Next, Poisson regression models will be utilized to adjust for these confounders and effect modifiers. Kaplan-Meier curves and log-rank statistics which use time to ovarian failure as the outcome and DME genotype as the exposure will also be performed. Cox regression models will also be fit to adjust for confounders and effect modifiers of time to ovarian failure. Because the four genetic polymorphisms are all part of the cyclophosphamide metabolism pathway and are therefore not truly independent, we have set the statistically-significant p-value to 0.025 to decrease potential for Type I errors.

Sample size and power: The following assumptions were made for sample size and power calculations. The risk of ovarian failure in women who are 40 or younger after adjuvant chemotherapy was 40%. Sample size was set at 150 for study feasibility. Accounting for dropout, the analyzable sample size was decreased by 25% to 120. Assuming power to 80% and Type I error of 0.025, Tables 10-13 demonstrate the differences in relative risk of ovarian failure that is detectable between genotypes for each enzyme polymorphism. Based on these assumptions, this study is powered to detect relative risks of 2.0, 2.0, 2.2 and 6.2, respectively, in development of ovarian failure for GSTM1 (Table 10), GSTA1 (Table 11), GSTP1 (Table 12) and CYP3A4 (Table 13) genotypes.

Table 10: GSTM1 detectable relative risks, assuming GSTM1 null genotype prevalence of 50%

Overall risk of ovarian failure	Detectable relative risk of ovarian failure (Non-null to Null)
50%	1.8
45%	1.9
40%	**2.0**
35%	2.2
30%	2.4

Table 11: GSTA1 detectable relative risks, assuming GSTA1*B/*B prevalence of 17%

Overall risk of ovarian failure	Detectable relative risk of ovarian failure (*B/*B to others)
50%	1.8
45%	1.9
40%	**2.0**
35%	2.2
30%	2.4

Anticipated results: We anticipate that ovarian failure and time to ovarian failure will be significantly associated with genotypes that increase a subject's exposure to activated 4-hydroxycyclophosphamide: GSTM1 Null genotype, GSTA1*B/*B genotype, GSTP1 Val/Val genotype and the CYP3A4*1B variants genotypes. In addition, subjects in the "low exposure" combined genotype group will have lower risk of ovarian failure than subjects in the "intermediate exposure" and "high exposure" groups.

Table 12: GSTP1 detectable relative risks, assuming GSTP1 Val/Val prevalence of 10%

Overall risk of ovarian failure	Detectable relative risk of ovarian failure (Val/Val to others)
50%	2.0
45%	2.1
40%	**2.2**
35%	2.4
30%	2.8

Limitations and alternative approaches

Sample size is the largest limitation to this genetic epidemiology aim, restricting both number of SNPs tested and the minimum detectable relative risk. However, the prospective cohort study design is integral to collecting valid menstrual history and bleeding patterns for the outcome of ovarian failure. Therefore, the sample size will be limited in this mentored project. Further, the four targeted SNPs were carefully chosen from known clinical data and biologic plausibility. If at least one SNP is associated with ovarian failure, then this data will be used to conduct larger future studies.

Table 13: CYP3A4 detectable relative risks, assuming CYP3A4*1A/1A prevalence of 90%

Overall risk of ovarian failure	Detectable relative risk of ovarian failure (*1A/*1A to others)
50%	4.5
45%	5.5
40%	**6.2**

Specific Aim 3: To determine if morphometric measures of the ovary by MRI are associated with ovarian failure in breast cancer patients after adjuvant chemotherapy.

Two-thirds of subjects (n = 100) will be randomly selected to undergo a pelvic MRI and pelvic ultrasound before and after chemotherapy. All MRIs will be read by our radiology collaborator. One-third of MRIs will also be read by a second radiologist to determine inter-observer reliability. One-third of the will also undergo a second reading by Dr. to determine intra-observer reliability. Pre-chemotherapy ovarian volume, AFC and maximal ovarian diameters will be individually tested for their association with ovarian failure and time to ovarian failure. Change in pre- and post-chemotherapy ovarian volume, AFC and maximal ovarian diameters will also be tested for their association with ovarian failure and time to ovarian failure. In addition, subjects will be dichotomized by pre-chemotherapy antral follicle count into $\geq$ 3 AFC and < 3 AFC, a threshold count consistent with decreased ovarian reserve in the literature [32]. The risk of ovarian failure by high or low AFC will be ascertained.

As a further control, measurements of ovarian volume, AFC and maximal ovarian diameters will be extracted from pelvic MRIs performed in women 40 years old or younger for clinical indications at the University of Pennsylvania during the same time period. MRIs of women with a history of pelvic malignancy, chemotherapy, radiation and ovarian cysts will be excluded.

Statistical analyses: First, descriptive data of ovarian volume, AFC, maximal ovarian diameters, change in pre- and post-chemotherapy ovarian volume, AFC and maximal ovarian diameter will be calculated from MRI and ultrasound studies. Intra- and inter-observer reliability for MRI measurements will also be calculated using Pearson or Spearman correlation, as appropriate. If normality assumptions are met, Student's t-test will test whether these ovarian morphometric parameters differed by imaging modality. Similarly, Student's t-test will test whether these pre-chemotherapy parameters in breast cancer subjects differed from parameters in the control (non-cancer) population. Next, Student's t-test will be employed to test whether pre-chemotherapy imaging parameters or pre- to post-chemotherapy changes in imaging parameters differed by the outcome of ovarian failure. The Wilcoxon rank-sum test will be used for non-normally distributed variables. Poisson regression models will be used to model the shape of the association between imaging parameters and development of ovarian failure while adjusting for confounders and effect modifiers. Similarly, Chi-square test and Poisson regression will be used to examine the association between dichotomized AFC and the outcome of ovarian failure. Survival analysis using time to ovarian failure as the outcome and dichotomized AFC as the exposure will also be performed. Cox regression analysis will be performed to adjust for confounders and effect modifiers of time to ovarian failure. Relationships between morphometric parameters as a continuous variable and time to menopause will also be assessed using the Cox regression framework.

Sample size and power: Sample size was set at 67% of the study population (100/150). From our preliminary data, mean AFC $\pm$ SD by MRI was 8.9 $\pm$ 7.1.Assuming 80% power and alpha of 0.05, this study is powered to detect a difference in AFC of 1.6 (0.56 SD) between women who go on to experience ovarian failure compared to menstruating subjects. By MRI, mean ovarian volume was 5.2 $\pm$ 2.1 ml. This study has good power to detect a small difference in ovarian volume of 1.4 ml (0.56 SD) between ovarian failure subjects and menstruating subjects. Finally, this study is powered to detect a 0.56 standard deviation difference in the pre- and post-chemotherapy measurements of volume, maximal diameter and AFC.

Anticipated results: Subjects who develop ovarian failure will have lower pre-chemotherapy ovarian volumes, AFC and maximal diameters than subjects who continue to menstruate. We also hypothesize that ovarian failure will be associated with larger changes in pre- and post-chemotherapy volume, AFC and

maximal ovarian diameters. Ovarian volume, AFC and maximal diameter will be positively correlated with time to ovarian failure. Finally, low pre-chemotherapy AFC will be associated with a higher risk of ovarian failure.

Limitations and alternative approaches: The primary limitation to ovarian imaging parameters is the sample size, which was selected based on feasibility and cost. While ultrasound is a less expensive and more established method of ovarian imaging, this study will provide MRI data on ovarian morphometry after chemotherapy as well as change in ovarian morphometry with chemotherapy. It is understood that this is the most exploratory of our aims. However, this aim will provide important descriptive data, and the investigator will gain experience in using MRI technology to evaluate ovarian change through chemotherapy. Then, in patients obtaining breast MRI, a pelvic MRI can be performed at the same time to evaluate the ovaries. Further, this aim will foster collaboration with MRI researchers to develop and perform pilot studies of functional imaging of the ovaries by MRI.

Specific Aim 4: To develop and validate a clinical predictive index to identify breast cancer patients at risk for ovarian failure after adjuvant chemotherapy.

The goal of a clinical predictive index is to use a combination of pre-chemotherapy patient variables to accurately predict the risk of subsequent ovarian failure. This index could include demographic, cancer, genetic, hormonal and ovarian imaging parameters. Unlike explanatory statistical models that determine which pre-chemotherapy risk factors are associated with subsequent ovarian failure, the predictive model will determine which combination of risk factors best predicts the outcome. The final clinical predictive index will be able to accurately predict the risk of subsequent ovarian failure such that clinicians and patients will be able to use the index with certainty.

Statistical analyses:

Optimize positive predictive value: The goal of this analysis is to optimize the positive predictive value of the predictive index. Positive predictive value is the proportion of subjects with a positive test that actually develops the outcome of ovarian failure. Chemotherapy treatment decisions by physicians and patients are unlikely to change unless a positive test that identifies women at high risk of ovarian failure after chemotherapy is closely linked to subsequent development of ovarian failure. In other words, it is more important to be certain that patients at high risk actually develop ovarian failure (optimized positive predictive value) than to determine all patients at risk for developing ovarian failure (optimized sensitivity).

Develop a multivariable predictive model: Multivariable Poisson and/or logistic regression will be performed with clinically and statistically important ($p < 0.2$) pre-chemotherapy variables associated with ovarian failure. Variables that will be considered in the model must be associated with at least 10 cases of ovarian failure to minimize Type I error. This model will be the basis for the clinical predictive index.

Model simplification: For clinical use, the predictive model must be simplified to contain the fewest number of risk factors needed to predict ovarian failure accurately. Simplified models and parent models will be compared by examining discrimination and calibration. *Discrimination* refers to the ability of the predictive index to distinguish between high-risk and low-risk patients and will be assessed by examining ROC curves. These will be evaluated graphically (sensitivity vs. 1-specificity) and numerically (c-statistic) [80, 81]. *Calibration* measures how closely predicted outcomes agree with actual observed outcomes by examining the correspondence between expected and observed cases of ovarian failure across strata defined by the prognostic score from the model. This will be tested with the Hosmer-Lemeshow statistic [82].

Clinical predictive index: Scoring for the predictive index will be determined using two approaches. First, the variables that comprise the predictive index will be scored according to their estimated regression coefficients from the final model (rounded to the nearest integer). Each subject will have a risk score by adding up the points associated with each observed risk factor. A second approach is to calculate the score as the sum of the relevant risk factors. Here, continuous measures will need to be dichotomized as part of the simplification process above. The predictive probability of ovarian failure will then be determined for each subject based upon her score. The threshold or cut-off for the clinical predictive index will optimize the positive predictive value of the index and minimize misclassification of patients.

Internal validation: Validation of the index involves demonstrating that its repeated application as part of the process of clinical care would lead to the same result [83, 84]. The bootstrap technique is useful for simulating this process as it evaluates a predictive model using a new data set that has been created by drawing a random sample of observations from the original data set with replacement. The model from the original

sample is then applied to the new re-sampled data set. The performance of each of this technique has been evaluated, and has been shown to provide stable estimates of performance (i.e., discriminative ability, calibration, and overall accuracy) with low variability [85]. As such, we will perform internal validation of the clinical predictive index using a bootstrapping approach [86].

Sample size and power: Sample size calculation was based on the confidence interval around the positive predictive value (PPV) estimate of the clinical predictive index. That is, the lower bound of the 95% CI around the PPV was set at 80%. We also assume a PPV point estimate of 90%. Assuming 80% power, Type I error of 0.05 and 50% incidence of ovarian failure, 70 total subjects would be required to determine a PPV with a lower bound of the 95% CI exceeding 80%. The expected number of subjects (n = 120) exceeds the number required by this sample size calculation.

Anticipated results: We will be able to develop a clinical predictive index which will have an estimated PPV with a lower bound of the 95% CI at 80% or higher.

Limitations and alternative approaches:

The internal validation of the predictive index can result in an over-estimate of its true value. An alternative to bootstrapping for validation of the prediction rule is an external validation using a separate sample from the same patient population, which is planned for future study.

Secondary analyses

There are several pre-specified secondary analyses that will be performed with this data. First, the investigator will explore the association between fertility post-chemotherapy and the proposed hormonal, genetic and ovarian morphometric parameters. Subjects will be asked whether they are attempting pregnancy, and data from pregnancies achieved post-chemotherapy will be collected. Second, other descriptive data that will be ascertained include the maximum length of amenorrhea after which there is no resumption of menses and the prevalence of hormonal values in the "normal" reproductive range during amenorrhea. Third, subjects will be categorized by STRAW criteria (menstrual pattern and FSH levels) at each visit. These data will be compared to that of healthy women in the Study staged by STRAW. Finally, morphometric measurements will be compared by imaging modality (ultrasound versus MRI).

Histologic evaluations of ovarian tissues from subjects who undergo prophylactic oophorectomies will be considered to provide histologic correlations with hormonal and imaging measures. Follicle count, AMH and inhibin B staining can be performed using archived surgical pathology slides on these subjects.

Time Line

Subject enrollment is expected to take place over the first two years of the study. Along with a study coordinator, the principal investigator will actively recruit and follow these subjects. During the first three years, the candidate will also continue advanced coursework on epidemiologic and biostatistical methods. Hormonal assays will be performed in batches annually. Ovarian imaging studies will occur over the first three years to capture pre- and post-chemotherapy measurements. Batch Genotyping of DME polymorphisms will occur in Year 3 (after enrollment completion). Genotyping will be performed by a laboratory technologist over two months. Data analysis and manuscript preparation for the primary aims will occur in Years 4-5. However, the Candidate will begin planned secondary analyses, manuscript preparation, and new protocol development in Year 3. One such secondary analysis is the comparison of MRI and ultrasound ovarian morphometry. These activities will allow the Candidate to start R01 submissions and transition to independence.

	Year 1	Year 2	Year 3	Year 4	Year 5
Study time line					
Subject enrollment	√	√			
Subject follow up	√	√	√	√	√
Hormonal assays	√	√	√	√	√
MRI reading	√	√	√		
Ultrasound reading	√	√	√		
Genotyping			√		
Data analysis			√	√	√
Manuscript preparation			√	√	√

Career development time line					
Course work	√	√	√		
New protocol development			√	√	√
R01 development and submission				√	√

General limitations

Selection bias: Selection bias may occur in the process of identifying the study population if those who consent to enrollment systematically differ from those who decline to participate. Our approach is to ask patients who decline participation for permission to review their medical records. With this information, we will be able to compare demographic and disease characteristics between those who agreed or declined to participate and determine whether a selection bias occurred. We will also have extensive data on the subset of subjects who undergo prophylactic oophorectomies during the course of the study and become ineligible. These analyses will help to determine the generalizability of the study population.

Information bias: Information bias may occur if measurement of the outcome ovarian failure is inaccurate. Thus, prospective bleeding diaries and close, regular follow up of menstrual history will minimize recall bias.

Confounders: Confounders such as age and chemotherapy dose will be selected on the basis of clinical knowledge and accounted for in our analyses through adjustment.

Missing data: Missing data from bleeding diaries will occur over the course of the study. Therefore, we will minimize loss of information by retraining participants on bleeding diaries and obtaining recalled menstrual history every three months during chemotherapy and every six months after chemotherapy. Short gaps in bleeding calendars will not likely differ between subjects who develop ovarian failure and those who continue to menstruate. This design takes advantage of the close follow up that patients have with their oncologists. Because subjects physically return to the oncologists at these intervals, follow up will likely be more complete.

Loss to follow up: Loss to follow up is one of the drawbacks of a cohort study design. We have liberally allowed for 25% loss to follow up to ensure adequate statistical power for the primary study aims.

Sample size: The sample size for the entire study, based on feasibility and power calculations, will yield the ability to test hormonal and genetic risk factors for clinically relevant differences in risk of ovarian failure. The smaller sample size for the MRI study is intended to yield preliminary data for a larger future study.

Generalizability: This study will be applicable to 10-20% of women diagnosed with breast cancer each year. Further studies to validate our findings in other cancers in reproductive aged women will be needed.

Summary and future directions

In summary, this study will identify important hormonal, genetic and ovarian morphometric biomarkers that predict ovarian failure in young women with breast cancer who require adjuvant chemotherapy. At the conclusion of the study, the candidate will have made significant contributions to current understanding and prediction of ovarian function in breast cancer survivors. Future directions will involve continuing to follow this well-characterized cohort over time. Continued observation of this cohort will yield further knowledge of the long term consequences of chemotherapy on the menopausal transition. In addition, the investigator is interested in applying these study findings to younger breast cancer patients who pursue fertility. Finally, further studies to validate these measures in other cancer populations will be a natural extension of this work.

This K23 award will prepare the candidate to become an independent investigator in reproductive epidemiology. At the end of this study, the candidate will have gained the ability to carry out a prospective cohort study, collaborate with multiple disciplines in medicine and clinical research, and use genetic epidemiology and complex predictive modeling methods.

16. PROTECTION OF HUMAN SUBJECTS

1. Risks to the subjects

a. *Human Subjects Involvement and Characteristics*

We anticipate recruiting and following a cohort of 150 adult women with newly diagnosed breast cancer. At enrollment, inclusion criteria are: female, age 21 - 40 years, American Joint Committee on Cancer Stage I-III breast cancer, premenopausal (menses within 12 months of recruitment), treatment plan for cyclophosphamide-based chemotherapy, and intact uterus and at least one ovary. Exclusion criteria include medical illnesses known to affect FSH, estradiol or inhibin B levels, hypothalamic amenorrhea, undertreated overt hypothyroidism, pregnancy or lactation, and use of psychotropic drugs.

These subjects will undergo a study questionnaire about medical and reproductive history at enrollment. They will have bloods drawn pre-chemotherapy, every three months during chemotherapy, and every 6 months post-chemotherapy until the end of the study (up to 10 blood draws per subject). Subjects will keep daily bleeding calendars which will be returned to the study at every blood draw. In addition, 67% of subjects will be randomly selected to undergo a non-contrast pelvic MRI and pelvic ultrasound.

Women who are pregnant at recruitment will be excluded from the study. However, during the course of follow up, women who become pregnant will be followed for their pregnancy outcomes. These women will not continue blood draws or bleeding diaries until after the pregnancy is completed. Pregnant women will be excluded from the imaging studies. Otherwise, this study does not involve other vulnerable populations.

b. *Sources of Materials*

Research materials obtained from our subjects will include:

- Demographics, breast cancer history, cancer treatment plan, reproductive history, family history, medical history, surgical history, body mass index and menstrual history. This information will be abstracted from the Breast Care Questionnaire, the study questionnaire, medical records and study follow up visits.
- Serum from each study visit. Up to 10 specimens may be collected from each subject through the course of the study. These sera will be used to assess reproductive hormones AMH, inhibin B, FSH and estradiol.
- DNA will be extracted from bloods obtained at enrollment. DNA will be genotyped for single nucleotide polymorphisms in four drug metabolizing enzymes: GSTM1, GSTA1, GSTP1 and CYP3A4.
- Ovarian volume, antral follicle count and diameters will be obtained in 25% of the study cohort.

All materials and data except for the Breast Care Questionnaire and patient medical records will be collected specifically for this proposed research study. Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). All collected data will be entered onto the study case report form (CRF) and stripped of patient identifiers. The paper CRF is the primary data collection instrument for the study. Data collected on CRF will be entered into the study database under each subject's study identifier. A separate paper and electronic database file linking a subject to her study identifier will be maintained in a locked research file and a password-protected computer file with no internet access. Only the principal investigator and the study coordinator will have access to files linking a specific subject to her study identifier.

c. *Potential Risks*

There is a small risk to subjects during blood draws, which may include bruising, bleeding, fainting, and/or local infection. Subjects may also experience minor discomfort from transvaginal ultrasonography or lying in the MRI machine. In addition, subjects may find it uncomfortable talking and answering questions about their cancer experience or their menstrual history.

2. Adequacy of protection against risks

a. *Recruitment and Informed Consent*

Approval by the Institutional Review Board at the will be obtained prior to subject recruitment. Subjects will be recruited from patients of the at the Eligible subjects will be identified by the PI or study coordinator through screening new outpatients of the . Specifically, the Breast Care Questionnaire completed by all patients will be screened for inclusion/exclusion criteria. Eligible patients will then be approached by the PI or study coordinator for possible study enrollment. The risks and benefits will be explained to the patient in detail by the PI or research coordinator in order for the patient to make an informed decision regarding participation. Once the patient understands the protocol, they will be asked to sign the study consent form and HIPAA consent form if they wish to participate. They will also be given a copy to keep in their records. In addition, the PI or study coordinator will contact the patient's oncologist to make sure that he/she is willing to have the patient participate.

b. *Protection Against Risk*

As described above, strict procedures will be instituted to maintain confidentiality. Each subject will be assigned a unique study identifier to be used on all CRF and in the study database. One paper file and one database file linking subject names and data codes will be locked, password-protected and maintained separately. This data will only be accessed by the PI or the study coordinator.

Possible medical risks involve finding abnormalities on ovarian imaging as a part of this study. If this occurs, the subject and her primary oncologist will be notified in a timely fashion in order to manage the patient appropriately.

3. Potential benefits of the proposed research to the subjects and others

Subjects will derive no direct benefit by participation in this study. However, as discussed above, abnormalities that are found from ovarian imaging will be made available to the subject and her physician for appropriate management. The risks of the study are minimal in relation to the information that will be provided to improve understanding of long-term risks associated with adjuvant chemotherapy for breast cancer.

4. Importance of the knowledge to be gained

The knowledge gained from this study will potentially significantly affect clinical care of young women with breast cancer. Further, this study may provide insights to biomarkers of reproductive aging in general.

17. INCLUSION OF WOMEN AND MINORITIES

The Targeted/Planned Enrollment Table is on the following page. Because ovarian failure is relevant only to females, all study subjects will be women. Minority subjects will be included in this study and will primarily reflect the minority composition of the patient population of the To increase recruitment of minority subjects, we will expand recruitment to and Hospital, both of which have higher proportions of African American patients. The candidate will meet with oncologists in both systems to discuss the study and place study brochures in breast clinic waiting areas.

Targeted/Planned Enrollment Table

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

Study Title: Predictors of ovarian failure after chemotherapy in young breast cancer patients

Total Planned Enrollment: 150

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	6		
Not Hispanic or Latino	144		
Ethnic Category: Total of All Subjects *	150		
Racial Categories			
American Indian/Alaska Native			
Asian	9		
Native Hawaiian or Other Pacific Islander			
Black or African American	18		
White	123		
Racial Categories: Total of All Subjects *	150		

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

19. INCLUSION OF CHILDREN

Children will not be included in this study. Breast cancer is very rare in females less than 21 years old. Ovarian failure as the outcome is also uncommon in this young population. A separate, childhood cancer survivor study would be more appropriate to study the effects of chemotherapy on ovarian function and outcomes such as fertility.