AWARD NUMBER: W81XWH-05-2-0053

TITLE: Comprehensive Reproductive System Care Program – Clinical Breast Care Project (CRSCP-CBCP)

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REPORT DATE: November 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
1 November 2009	Annual	1 Nov 2008 – 31 Oct 2009

4. TITLE AND SUBTITLE

Comprehensive Reproductive System Care Program – Clinical Breast Care Project (CRSCP-CBCP)

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-2-0053

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Henry M. Jackson Foundation for the Advancement of Military Medicine
Rockville, MD 20852

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The Windber/Walter Reed Clinical Breast Care Project will help lead the way in the 21st century in the crusade against breast disorders. The project will utilize a multidisciplinary approach as the stand of care for treating breast diseases and breast cancer. This multidisciplinary model integrated prevention, screening, diagnosis, treatment and continuing care, but the project is further unique in the incorporation of advanced in risk reduction, informatics, tissue banking and research. These efforts focus on decreasing the morbidity and mortality of breast cancer among American women.

15. SUBJECT TERMS
Tissue Banking, Biomedical Informatics, Focused Research, Translational Research, Genomics, Proteomics, Risk Reduction, Comprehensive Breast Care

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	23	USAMRMC

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

Comprehensive Reproductive System Care Program—Clinical Breast Care Project (CRSCP-CBCP)
Annual Report

1. INTRODUCTION:

The Clinical Breast Care Project (CBCP) is the outcome of the initial FY00 and subsequent Congressional appropriations, and consists of an extensive collaborative effort between Windber Medical Center (Windber, PA – 12th Congressional District of the Honorable John P. Murtha) and Walter Reed Army Medical Center, with funding management by the Henry M. Jackson Foundation for the Advancement of Military Medicine. "The Clinical Breast Care Project (CBCP)" moniker is modified to better reflect its expanded congressional mission, and in FY05 became officially entitled, "The Clinical Breast Care Project of the Comprehensive Reproductive System Care Program". In correspondence, conversation and general usage the shortened form is the "CBCP (of the CRSCP)".

Ultimate Goal of this project: Decrease morbidity and mortality of breast cancer among American Women. Through the interlacing of the five pillars, the CBCP will help lead the crusade against breast disorders.

- Develop a comprehensive breast care center/system that enables health care providers with a multidisciplinary team approach to work toward a common goal.
- Empower women with breast cancer and other breast disorders with the decision-making tools and environment to enhance quality of life and to meet psychosocial needs of the patients and their families.

The five pillars of the CBCP of Walter Reed/Windber are (1) Risk Reduction (2) Focused Research (Genomics and Proteomics); (3) Tissue banking; (4) Biomedical informatics; and (5) Clinical Care.

Pillar Specific Objectives:

1. ***Risk Reduction***:
    - Identify the population of patients at above average risk for the development of breast cancer.
    - Decrease this identified population's rate of breast cancer development.
    - Analyze potential cost differential in the prevention of breast cancer development.

2. ***Focused Research***:
    - Utilize our CBCP-developed panel of microsatellite chromosomal markers to genomically assess various stages of breast disease, malignant and benign, in our on-going effort to elucidate the biologic development timeline of breast cancer.
    - Analyze our in-depth serum and blood repository utilizing various proteomic identification and pattern technologies in our ongoing effort to identify new biomarkers that can be predictive of breast cancer risk and development.
    - Utilize our microarray gene expression profiling capabilities in our effort to analyze the gene expression changes of the continuum of breast disease and cancer development.
    - Analyze the relationship of certain breast cancer protein aspects, eg. ORP-150 protein, to prognosis and other known variables of breast cancer biology.
3. ***Tissue Banking***:
    - Collect and store specimens of breast tissues, lymph nodes, bone marrow aspirates, serum, blood cells (leukocytes), and plasma from every patient undergoing a breast biopsy and/or breast surgery at WRAMC, WMC, MGMC, and LRMC who consent to participate in this study. Use the power of this tissue bank to dramatically further breast disease research.
4. ***Bio-Informatics***:
    - Develop and implement a clinically-relevant prospective, longitudinal computerized database for use in patients with all types of breast care needs.
    - Link this database information through the Internet to data set at a rural primary breast care center with appropriate security and firewall protections.
    - Develop the database to allow for "on-the-fly," relational, clinically-relevant statistical analysis.
    - Develop an informatics companion to the prospective serum / breast tissue bank.
5. ***Clinical Care***:
    - Decrease the negative psychological impact on the patient of having an evaluation or treatment intervention for breast disease.
    - Create and maintain an environment (medical, physical, psychological) conductive to the multiple needs of the patient undergoing breast disease evaluation / treatment.
    - Utilize objective measurement instruments to longitudinally assess the patient's psychological response to evaluation and intervention, and base modifications on those results.

Summary of the methodology of the project.

The five pillars of the CBCP of Walter Reed/Windber are (1) Clinical Care; (2) Tissue banking; (3) Risk Reduction; (4) Biomedical informatics; and (5) Focused research (Genomics and Proteomics).

- The clinical care pillar was established by building state of the art breast care facilities at the Windber and Walter Reed sites. These sites were critical to the ability to implement all other pillars of this Project. The Walter Reed Comprehensive Breast Center opened in July 2001, and the Joyce Murtha Breast Center in Windber opened in February 2002.

- The tissue banking pillar was established at both sites in collaboration and entails acquisitions, storage, and movement amongst the sites for research purposes of tissue garnered from all breast surgeries being performed at both locations. The robust IRB-approved protocol that enables this pillar is unique in four critical aspects: It is a tissue usage protocol, not a tissue repository protocol. It is hypothesis-generating, not hypothesis-driven research; It allows for patients to pre-consent for secondary, future uses of the tissues in presently-unknown research; It contains a unique fail-safe mechanism to protect the complete diagnostic integrity of all samples.

- The biomedical informatics effort, is a collaborative development effort between Walter Reed, Windber and Inforsense, driving the development of a comprehensive data warehouse storing clinical and molecular data related to breast disease This is a resource for all CBCP investigators and is exportable for use by other investigators, programs, and organ sites. The data warehouse uniquely integrates clinical data with genomic and proteomic analysis of patient samples and is being extended to incorporate an image repository. This is being developed in close collaboration with industry leaders in the high-end database field, specifically Inforsense and Concentia Digital. Extensive efforts to model the pathways of breast cancer and its risk factors are underway at Windber with consultation from Walter Reed.

- The risk reduction pillar is a vital portion of this mutual project which has resulted in the capability of the project to establish a screening program to identify women who are at high risk for developing breast cancer, and to enter them into a very time- and resource-intensive risk reduction program which can only be appropriately resourced through an appropriation such as this, to decrease significantly these patients' chance of getting breast cancer in the future.

The research aspect is centered on functional genomic/microarrays/proteomics analysis of the tissues and biospecimens, which are acquired as described above. The collaborative research on the functional genomics is established through a high-end microarray, genomics, and proteomics facility at the CBCP Windber Research Institute and will be used as the prime research center for the tissue collaborations, which are developed through this project.

- An important outgrowth of this effort is and will be the bringing of more patients into the Windber and Walter Reed sites for breast cancer evaluations and treatment options; also, the economic development at Windber is being enhanced through job creation and establishment of the scientific research center.

2. BODY:

The CBCP established six primary tasks in its approved Statement of Work for the 2009 fiscal year. These six tasks consist of:

- Task 1. Enroll over 500 patients annually to the "Core" CBCP protocols through consenting in the main CBCP clinical sites.
    a. Core protocols of Tissue and Blood acquisition and molecular testing at the DNA, RNA and Protein level, allied with the clinical and demographic databases

- Task 2. Continuing molecular analysis in CBCP labs, as outlined in the CBCP Core Protocols allowing for global expression analysis of the DNA, RNA and Protein features.
    a. Utilize this repository as the basis for all molecular analysis in CBCP labs, as outlined in the CBCP Core Protocols allowing for global expression analysis of the DNA, RNA, and Protein features.
    b. Utilize this repository as the basis for intramural and extramural collaborations for secondary usage research.

- Task 3: Continuing software development of the CLWS (Clinical Laboratory Workflow System) and its further deployment into the clinical/research arms of the CRSCP-CBCP.

- Task 4. Identifying and counseling no less than 100 high risk patients for development of breast cancer and employ risk reduction strategies.
    a. Perform BRCA gene mutation testing on 10 patients annually in contract with MYRIAD Genetics.

- Task 5. Performing targeted research into genomic analysis of Stages I, II, and III breast cancer, DCIS, LCIS and pre-malignant neoplasia, and presenting findings at national meetings and in peer-reviewed publications.

- Task 6. Perform mass spectrometry fingerprinting of 200 sera samples from patients with diagnosis of breast diseases and analyze for distinct patterns based on disease state.

Task 1: Enrolling over 500 patients in the "core" CBCP protocols.
See section 5 Reportable Outcomes for the breakdown and number of subjects enrolled in "core" CBCP protocols.

Task 2: Continuing molecular analysis in CBCP labs, as outlined in the CBCP Core Protocols allowing for global expression analysis of the DNA, RNA and Protein features.
One of the Core activities of the CBCP is the collection and banking of human biological specimens for research. Tissue specimens are collected from a variety of donors under different conditions. For the period of October 1, 2008 – September 30, 2009 **417** donors contributed **4481** samples. The goal is to collect good quality specimens that will provide products such as DNA, RNA and proteins for research. To achieve this goal, the Tissue Banking arm of the CBCP has incorporated the science of Biospecimen Research into its activities. We have performed research studies to determine how temperature changes and time of collection to processing affect the quality of breast specimens collected for research. These findings have been presented at national conferences and are currently being prepared for publication in peer reviewed journals. Our results will contribute to the shaping of the NCI Office of Biorepositories & Biospecimen Research (OBBR)'s Best Practices and their proposed guide lines for specimen collection and processing for tissue banks.

Task 3: Biomedical Informatics Infrastructure development and research Data Warehouse

We have now named the WRI Data Warehouse the Data Warehouse for Translational Research (DW4TR). To represent sample information and molecular data, a sample-centric data model has been conceived in parallel to the patient-centric clinical data model. The model for sample information representation has been developed in the data warehouse with corresponding interface development. Currently, using the Aggregated Biomedical Information Browser (APBIBrowser, the literal name for OLAP we used before), we can analyze not only the number of participants but also number of samples associated with aggregated conditions.

A new level of security, "Virtual Private Database" (VPD), has been implemented. This is a feature of the Oracle system that allows for row level

access control within the DW. While one of the strengths of our system is the ability to perform cross-study analysis, in many instances it is necessary to restrict data visibility among different users of the system. In our security model, all users of the system are assigned roles which dictate the data they are permitted to access. Our VPD system is designed such that every data element in the DW is tagged with a study identifier that is associated with the different user roles, enabling data access control.

On top of the data warehouse, we developed a set of biomedical informatics tool for cohort selection and molecular data analysis. These tools have been published and are available through biomedical informatics portal. We also designed and implemented an algorithm that precisely describes each sample related to patient's clinical and pathological information. Using this algorithm, system searches "best fit" for samples related to patient's information and flags the relationship using set of pre-defined flags. Please refer to "An approach to correlate the temporal information to facilitate specimen selection in the breast cancer research project" in the research projects part for more information.

LIMS (Laboratory Information Management System):
We completed implementation of Geneus and Proteus (two commercially available modules developed by GenoLogic, now conceptually merged with a name of Synapeus). Implementation includes installation in-house version of the software, workflows configuration, and user training. Using this system, people in the lab have created more then 40 projects and tracked more then 4000 samples. The Biomedical Informatics group is responsible for managing this system including system updates, daily incremental backups, and monthly full backup of both servers

We continue working with GenoLogics in developing the BMI (tissue banking and clinical information tracking) part of the LIMS system. This should replace CLWS system (developed in collaboration with Cimarron) should improve clinical data collection as well as tissue banking operation. Five different modules would need to be integrated into the system to cover our needs. Each of the modules has different timeline of implementation per GenoLogics' plan. For efficient and accurate transitioning we will only substitute our existing (CLWS) system when all five modules are ready for implementation.

New Pathology Checklist form and data entry system: With ProLogic serving as a development subcontractor, we have started to develop a new tool for completing and tracking pathology checklist and sample attribute sheet using TAB PC technology. This was accompanied with a drastic revision of the current Pathology Checklist led by the CBCP Head Pathologist with close teamwork involving the biomedical informatics group here, the ProLogic team, and the MDR Global leader. The design part of the project is done and the development is on track. We expect the testing and implementation to start in the second quarter

of next year. This tool will eventually be integrated into the GenoLogics BMI solution.

Outcome data collection and entry form: We have worked closely with the clinical staff in WRAMC and spearhead the drafting of the Outcome Tool for compilation of the outcome and treatment information of CBCP cancer patients. While the form is still awaiting for finalization, we have developed a data entry tool to enable direct data entry by the clinical staff reviewing medical charts.

Recognition of the quality of our biomedical informatics infrastructure: The quality and capability of the CBCP Biomedical Informatics infrastructure is not only recognized by this program, but also gradually by other scientists in clinical and translational research. We have succeeded in a joint Komen Promise Grant application headed by Dr. Hallgeir Rui of Thomas Jefferson University, and the WRI will provide biomedical informatics support to the project with Dr. Hai Hu serving as one of the two Co-PIs. The grant will support the study of the expression of 250 drug targets in 5000 breast cancer tissues, and to conduct the clinical trial of a kinase inhibitor.

Other current active research and development projects

- **Microarray Data Analysis Using Peripheral Blood Samples of Breast Cancer Patients and Normal Subjects:** Statistical analysis including Gene Set Enrichment Analysis (GSEA) is done using the microarray GeneChip raw data. The GSEA is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states, in this case cancer and normal. We also find some crucial pathways enriched in each group. Most of the pathways downregulated in the blood of patients in the invasive group are related to the immune response. In contrast, most of pathways upregulated in the blood of patients in the invasive group are associated with metabolism. An abstract has been accepted to SABCS 2009, and a manuscript is in preparation.
- **Use of the CBCP clinical data warehouse for IHC-based breast cancer subclassifications:** Breast cancers have been classified into 5 subtypes based on gene expression studies. IHC-based technology uses ER, PR, and Her2 can currently classify 4 of them. We are performing a study to characterize the subclasses in the CBCP population. An abstract has been accepted by SABCS 2009, and a manuscript is in preparation.
- **A review of ANN application in breast cancer diagnosis and study:** To better understand the current state-of-art of artificial neural network work application to breast cancer diagnosis and study, we are performing a literature review and a manuscript is in preparation.
- **Use of the CBCP clinical data warehouse as a research environment for breast cancer risk factor assessment:** The data warehouse and the On-Line Analytical Processing tool as the interface have proven to be a powerful tool in supporting scientific research at WRI and WRAMC. We

have tested the idea that the CBCP clinical data warehouse can serve as a research environment for breast cancer risk factor assessment, and have presented the preliminary data at SABCS 2007. Current a manuscript is under review by the Journal of Biomedical Informatics.

- **An approach to correlate the temporal information to facilitate specimen selection in the breast cancer research project:** Temporal information management is very important in translational research. In the CBCP, subjects' information is collected at different temporal points and for different temporal periods. We have developed a temporal data model to represent such information but a transitional solution is needed to immediately satisfy the CBCP research. We have designed and implemented an algorithm to use a set of pre-defined flags to precisely describe each sample related to patient's clinical and pathology information in the temporal domain. The temporal criteria for sample selection are now represented by the relationships between these flags, and can be implemented through several filtering processes. The described algorithm drastically reduces the time needed for precise sample selection from several days for manual efforts to several hours. An abstract has been accepted by the SABCS 2009.
- **Differential Benign Breast Disease Co-occurrence with Cancer in Caucasian and African American Women:** Breast Cancers (BCs) in Caucasian (CA) and African American (AA) women have different characteristics. There are reports that Benign Breast Diseases (BBDs) may be risk factors for BCs. In the CBCP 66 BBDs are recorded for patients with a biopsy. We studied the data from a total of 1479 CA and 484 AA women for the association between BBDs and BCs (including in situ, invasive, and malignant NOS) in these two ethnic groups using the chi-square test. We found that, 1) in both populations 6 BBDs are positively associated with BCs, and on the contrary 3 BBDs are negatively associated with BCs; 2) 8 BBDs differentially co-occur with BCs between AA and CA. An abstract has been accepted by the SABCS 2009. More research is undergoing to understand the implications of this distinct co-occurrence pattern.
- **Src, phospho-Src, and breast cancer:** Src and phospho-Src has been implicated in and considered as a drug target in breast cancer patients expressing a moderate level of Src. In collaboration with Dr. Marius Sudol of Weis Center for Research, we have started a project to test whether a gene named DUSP5 can be used as surrogate marker for phosphor-Src as the current assay for the latter is expensive. A joint DoD grant application was submitted last April. A pilot study of two dozens of samples has been completed, and no correlations in human breast cancer tissues were identified, contrary to the results in model systems. Correlation between total Src and phospho-Src was detected, however. A new research plan is being developed pending other results such as those from Expression Pathology.
- **MTDH and breast cancer:** MTDH is a protein that is implicated in

breast cancer metastasis. In collaboration with Mr. Albert Kovatich of MDR Global we have developed an IHC-based research project to test the role of MTDH in breast tissues of different cancer and metastasis status. The project has been approved by the CBCP PI and the lab work is starting.

Task 4: Identifying and counseling high risk patients for development of breast cancer and employ risk reduction strategies. The risk reduction pillar continues to be a vital portion of this mutual project which has resulted in the capability of the project to establish a screening program to identify women who are at high risk of developing breast cancer, and to enter them into a very time-and- resource intensive risk reduction program which can only be appropriately resourced through an appropriation such as this, to decrease significantly these patients' chance of getting breast cancer in the future. A significant achievement this year has been the implementation of the risk reduction program at the Joyce Murtha Breast Cancer Center in Windber, PA. Dr. Raymond Weiss, medical oncologist saw his first patient at JMBCC in April of 2009.

A total of 493 patients were entered into the program this year. The extensive risk assessment, family history and pedigree generation, computerized modeling of individual risk, genetic mutation testing when appropriate (BRCA-1 and BRCA-2), implementation and follow-up of intervention strategies to include chemoprevention, novel diagnostic testing, and even surgical prophylaxis, resulted in a highly successful program where breast cancer truly is being prevented before it ever occurs in many women.

Task 5: Performing targeted research into genomic analysis of Stages I, II, and III breast cancer, DCIS, LCIS and pre-malignant neoplasia, and presenting findings at national meetings and in peer-reviewed publications: CBCP continues to have tremendous success in this regard. We have verified that important genetic changes occur across multiple chromosomal regions as breast epithelium transitions from non-neoplastic into neoplastic states. We have identified these changes and published the specific markers for chromosomal loci that we identified as having changed during this transition. Whether or not any of these changes are causal in the malignant transformation process remains to be determined. As can be seen from the meeting and publication list we are actively continuing the research direction. We are considering, as an organization, whether to seek patent protection for this panel of markers, which may be considered a diagnostic and prognostic marker panel for breast cancer and breast cancer development.
See ATTACHMENT 2 for the list of publications and presentations.

Task 6: Perform mass spectrometry fingerprinting of sera samples from patients with diagnosis of breast diseases and analyze for distinct patterns based on disease state: The activities of proteomics facility continue on the data

analysis of Maldi-TOF profiles generated from the acetone processed BC serum samples, new serum processing workflows are being implemented to couple them to Maldi-TOF and LC/MS/MS analyses. We are also optiming workflows for tissue proteomics in collaboration with the PNNL group.

We continue to apply a simple differential precipitation procedure using acetone to enrich the low molecular wt peptides/proteins from breast cancer serum samples for MALDI-TOF profiling and utilized the MS profile patterns to develop a model that discriminates the two disease groups (benign and invasive) involved in the study. Serum samples, evenly divided between benign and invasive cases, were processed by acetone in triplicates and each replicate sample was analyzed twice on MALDl-TOF instrument. The assessment of MS profiles and clinical covariate data yielded 31 benign and 30 invasive cancer cases for comparative analysis. To determine the discriminative features that distinguish benign and invasive cases, both univariate and multivariate analyses were performed on the features. The univariate analysis revealed several features were differentially expressed between benign and invasive BC cases. The unsupervised hierarchical clustering analyses indicate that there were some subgroups among the cancer and benign groups. The discriminative potential of peak clusters was analyzed using a Classification and Regression Tree (CART) algorithm implemented in Ciphergen ProteinChip^{TM} Biomarker Pattern software. CART modeling selects a set of predictors and their interactions that optimally predict the outcome of the dependent variables. Samples were split into 70% training sets and 30% test sets. The menopausal status (categorical variable) was balanced between the benign and invasive groups in the training sets. The average performance of all the predictive models that were built using CART was 50%. The distributions of menopausal status, age, and HRT history for the subjects examined in this study could be discriminated, with statistical significance, between case and control groups. Only the menopausal status was able to be accommodated in the forming of training sets for multivariate analysis. The inability to balance for clinical variables may contribute to inconsistency and high ACE in classification model performance.

3. ADDITIONAL ACCOMPLISHMENTS

a. Meetings were held on February 4 and 5 with Jennifer Brusstar from the Tug McGraw Foundation, Max Wallace from ABC2 (Accelerate Brain Cancer Cure),which is affiliated with the Steve and Jean Case Foundation, Dr.Sandberg from AFIP, Dr. Paul Mischel from UCLA, Dr. Renata Greenspan from USMCI and Dr. Craig Shriver from the Clinical Breast Care Project. Discussion centered around working with the military to advance a cure for brain cancer as the initial project. As a result of those meetings, The Partnership for Military Medicine Symposium and Gala is planned for November 6 and 7. The symposium will feature leaders from military and civilian medicine who will address the following topics: PTSD, TBl and Neuroscience Research; Humanitarian Aid and Disaster Response; and lnfectious Diseases. Registration includes breakfast, luncheon

with keynote speaker Faith Hill, and a networking reception. The date: Friday, November 6, 2009Time: 7:30 a.m. – 6:30 p.m. Location: Omni Shoreham Omni Hotel, 2500 Calvert Street NW, Washington DC 20008.

Additionally, HJF and the Tug McGraw Foundation (TMF) will host the Country United Gala—a black-tie charitable event to honor medical researchers and educators who advance warrior care on Saturday, November 7, 2009. The gala will also raise funds to support military medicine. Bob Costas, Emmy Award-winning NBC and HBO Sports broadcaster, will emcee the event, which will conclude with a performance by Grammy Award-winning country music stars Tim McGraw and Faith Hill.

b. LTC Jack Davis conducted a site visit on Monday, March 2, 2009 and met with the directors of the 5 Centers of Excellence. COL Shriver arranged for a video teleconference with Windber research Institute and staff from the Comprehensive Breast Center at WRAMC as well as staff from the WRI individually introduced themselves and told LTC Davis how the work they do contributes to a translational Research Center of Excellence. A result of that visit was a decision to fund the Breast Center of Excellence with RDT&E dollars in the future .

Much work has been done since congressional language in National Defense Authorization Act (NDAA)-FY09 apportioned $40 million for the 5 Centers of Excellence at WRAMC.
The NDAA states that the SECDEF is "directed to include funding for these centers beginning with the fiscal year 2010 budget submission."

- Directive did not clarify color of money FY10+ or funding level
- No funding stream was provided to support these programs beyond FY09
- HP&S briefed MEDCOM CoS March 2009 for decision and in preparation for TMA budget brief
- Outcomes: CoEs were doing research; should be funded with RDT&E; MRMC would provide oversight; and FY10 requirement was $43M
- CoE's status was briefed to TMA based on CoS decision
- According to MRMC, HA formally assigned oversight of the CoEs to the Joint Technology Coordinating Groups (JTCG) – 19 May 2009 email from Mr. Hofflinger
- 26 April 2009 – Received PCP tasking from OTSG PA&E
- 29 April 2009 – CoE's FY10 budget numbers communicated to PMs
- 6 May 2009 – MRMC PA&E contacted NARMC about PCPs for 4 of the 5 CoEs (indicated USU would submit CPDR)

On Monday, June 15, MAJ Blunt, Mr. Hofflinger and COL Janet Harris met with the CBCP and the agenda was as follows:
AGENDA

1. Review of Current Projects (FY07 and FY08)
   - Overview of the CoE noting various Cores/Projects.

- Please annotate Projects/Aims by FY.
- External Partners and contributions to the CoE

2. Review of FY09, FY10, and FY11 Core/Project plans
- RDT&E Budget Activity (6.1, 6.2, 6.3, etc.) determination for FY10 and FY11
- External Partners and contributions to the CoE

3. Fiscal review
- Status of Prior Year funds (FY07, FY08) – funds received,obligation,disbursement
- Status of FY09 funds - funds received, obligation, disbursement
- FY10 budget requirements
- FY11 program requirements
- Potential contract carryover to offset possible FY10/FY11 budget/program shortfalls
- Other non-DoD funding sources

c. The annual meeting of the National Consortium of Breast centers was held in Las Vegas from March 14 – 18 2009. COL Shriver gave two presentations and has accepted a position as a member of the Board of Trustees.

d. Numerous NNMC/WRAMC Breast Center Integration meetings have been held and COL Craig Shriver has been named the integrated chief of Breast Care for the new WRNMMC and the new facility at Fort Belvoir.

e. A 6.7 million dollar Komen Promise grant was awarded to Hallgier Rui of Thomas Jefferson University. On behalf of the Clinical Breast Care Project at Walter Reed Army Medical Center, the Henry M. Jackson Foundation is committed to participate as a consortium collaborator on this grant submission: **"Therapy-relevant Stratification of Breast Cancer Patients: Integrating Pathology and Biomarker Analyses"**

4. KEY RESEARCH ACCOMPLISHMENTS

- Gene expression difference have been found between African American women and European American women that may lead to insights into the differences in breast cancer severity seen between these populations.
- Further enhanced the use of the database and data warehouse system that CBCP has developed for last five years, to integrate the clinical, molecular, pathologic, and biorepository aspects of CBCP translational research. It's robust query capability and analysis tools have assisted in stratifying patients populations for our studies. The data warehouse and the On-Line Analytical Processing tool as the interface have proven to be a powerful tool in supporting scientific research at WRI and WRAMC.

- We completed implementation of Geneus and Proteus (two commercially available modules developed by GenoLogic.
- Other developments in the Biomedical informatics core have included development of a new patient centric data model, new tools for microarray data QA, MS data protein peak detection and alignment and an analysis of breast disease co-occurrence.
- Temporal information management is very important in translational research. In the CBCP, subjects' information is collected at different temporal points and for different temporal periods. We have developed a temporal data model to represent such information but a transitional solution is needed to immediately satisfy the CBCP research.
- Gene expression analysis of primary (node negative) versus primary (node positive) revealed a 70 gene signature distinguishing two types of tumors. These results are undergoing further analysis.
- Gene expression of blood RNA from 100 patients with invasive breast cancer compared to blood RNA from 100 disease free controls has identified several candidate markers of breast cancer detection. Statistical analysis including Gene Set Enrichment Analysis (GSEA) is done using the microarray GeneChip raw data. The GSEA is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states, in this case cancer and normal. We also find some crucial pathways enriched in each group. Most of the pathways down-regulated in the blood of patients in the invasive group are related to the immune response. In contrast, most of pathways upregulated in the blood of patients in the invasive group are associated with metabolism.
- A collaboration with Vanderbilt University continues to examine proteomics differences associated with lymph node metastasis continues and has resulted in the discovery of patterns of protein expression that may distinguish tumors that has lymph node metastasis from those that do not.
- We are currently performing targeted research aimed at identifying blood based biomarkers for the early detection of breast cancer. For this research activity, we are utilizing a multiplex assay platform (xMAP) to analyze a panel of biomarkers made up of matrix metalloproteinase 1, 2 and 9 (known mediators of the extracellular matrix modification) and growth factors. Such a combination will provide positive predictive values that approach 100% compared to the earlier studies that utilized only the matrix metalloproteinases with predictive values of 80%.
- A collaboration with Pacific Northwest Laboratory (PNNL) using proteomic analysis of normal and breast cancer tissue to identify protein biomarkers for metastasis and disease progression is progressing. Mass Spectrographic analysis of normal and breast cancer tissue has lead to the development of an Accurate Mass Tag data base for proteins expressed in breast tissue. This data base will be used to analyze mass spectra from breast cancer tissue samples from patients with and with lymph node metastases to look for biomarkers predictive of metastases.

5. REPORTABLE OUTCOMES

The CBCP Research Protocols and number of subjects recruited to each for the period October 1, 2008 to September 30 2009 are as follows:
Clinical Breast Care Project Walter Reed Army Medical Center

- Creation of a Blood Library for the Analysis of Blood for Molecular Changes Associated with Breast Disease and Breast Cancer Development - **22**
- Tissue and Blood Library Establishment for Molecular, Biochemical and Histologic Study of Breast Disease – **228**

- Molecular Phenotyping of Bone Marrow Aspirates and Peripheral Blood Collected As Part of The Walter Reed Army Medical Center Clinical Breast Care Project (CBCP) – **0**

The Windber Joyce Murtha Breast Care Center Research Protocols and subjects recruited to each is as follows:

- Creation of a Blood Library for the Analysis of Blood for Molecular Changes Associated with Breast Disease and Breast Cancer Development - **55**
- Tissue and Blood Library Establishment for Molecular, Biochemical and Histologic Study of Breast Disease – **86**

Anne Arundel Medical Center Research Protocols and subjects recruited to each is as follows:

- Tissue and Blood Library Establishment for Molecular, Biochemical and Histologic Study of Breast Disease – **169**

Psycho-Social Oncology Services:
A dedicated psychologist assessed 60 new Friday Template breast cancer patients and counseled an additional 22 patients on an ongoing or crisis basis. The services that continue to be provided by this resource are as follows:

- Psycho-social assessment and evaluation of newly diagnosed cancer patients
- For those patients who exhibit a high level of distress, a system has been established that allows close monitoring of patients to include one-on-one time during chemotherapy.
- Individual and family therapy are available for all breast cancer patients who are in need of support. For patients who live at a distance, telephone sessions are available.
- A Buddy System that provides support to newly diagnosed breast cancer patients from breast cancer patients who have completed treatment.
- On-going psycho-social consultation with patients' medical providers.

Three types of Support Groups are also available:

- A group for patients actively engaged in cancer treatment is provided. The group is a structured 8-week group that meets for 90-minutes once per week. The group format is concrete and offers practical support to patients.
- A group for patients who have completed all treatment. This cancer survivorship group also meets for 8 sessions, 90-minutes, once per week. The format is also concrete and practical.
- A group for parents who have cancer that provides guidance to them to help their children thrive as they overcome cancer. The same format is also used for this group.
- All groups can be conducted in person or via video-teleconferencing. Video teleconferencing allows patients who live a distance from Walter Reed, or are too ill to travel a distance, to participate in the support group process.

6. CONCLUSIONS

The CBCP proposal and budget submitted to USAMRAA on 21 August 2008 was approved and on 23-December Amy Metzger notified COL Shriver that HJF had just received the modification to the Clinical Breast Care Project Cooperative Agreement. This document has been signed by HJF and returned to USAMRAA for final execution (signature by the Grants Officer). We have requested that the period of performance be changed to reflect October 1 2008 through September 30 2009.

We are still waiting to hear about this request and as of this date have not received our 09 dollars.

As we stated in the previous annual report, the next great advances in breast cancer prevention and treatment will be based upon an increased understanding of the changes that occur in the cells of normal breast tissue, as they transition into cancer cells. The CBCP, through its unique and inter-connected 5 pillars, leverages the strengths of its clinical care arm focusing its research arm to study these cells as they change into cancer. To date, we have been the first to show that the way that breast cancer "behaves", is possibly pre-determined very early in the change of the cells as they are becoming cancerous, as opposed to the cancer cells getting "worse" as they grow and develop. In other words, our important findings are indicating that the behavior of the cancer cells is determined in the development of the cancer, not later. The implications of these findings are critical in our understanding of breast cancer biology, and are leading to new understanding in developing prevention strategies and treatment programs. Our tissue repository has grown into the world's largest and best characterized (annotated) biorepository of human breast tissues, receiving great acclaim from

research organizations around the world, and is being shared with other research organizations of great renown, in an effort to speed the pace of discoveries through sharing of this irreplaceable resource. We are finalizing our study into whether or not we can identify "the breast cancer blood test", through the use of serum repository, linked to one of the world's foremost organizations capable of identifying protein patterns in serum from various organ system cancers.

Breast cancer is the most common non-skin cancer in women. It is the single greatest cause of cancer deaths among women under 40, and is a significant cause of mortality for women in the United States Armed Forces. Breast cancer mortality among women <50 years accounts for >40% of years of life lost due to this disease. The economic, social and emotional cost to families is far greater when a young woman dies than when an older woman dies of breast cancer. The more aggressive nature of the disease in young patients along with the attendant costs underscores the importance of early detection of breast cancer in young women. Breast cancer is a curable disease if it is detected early; as such early detection is related to survivorship, cost of treatment and quality of life for the affected woman.

The majority (>90%) of women in active military service are < 40 years of age. The Department of Defense (DOD) with its high percentage of young women and its commitment to health care is particularly concerned about breast cancer. When discovered at a later stage, treatment of breast cancer is expensive, aggressive and results in considerable disruption to the woman's ability to contribute to society. Cost and disruption to life are considerably less when the carcinoma is discovered at an earlier stage. Furthermore, the DOD has a high percentage of African-American (~40%) and Hispanic (~10%) women. Death rates from breast cancer tend to be particularly high in these ethnic groups owing in part to later stage of detection and to the more aggressive nature of breast cancer in these groups. CBCP Breast Center is the Army-recognized specialty referral center for active duty personnel from around the globe with medical disorders related to all breast diseases and breast cancer. CBCP Breast Center routinely cares for women on active duty Army from places such as Iraq / OIF, Korea, Europe, and the Far East. CBCP annually cares for over 5,000 patients at its site at Walter Reed.
In summary the Clinical Breast Care Project, a collaborative effort between Walter Reed and Windber, has resulted in excellent working relationships and collaborations between the two sites on all five of the project's main pillars. The project continues to achieve its goals and looks forward to further continuance of this great vision and what will be a national resource, into the future.

As reported last quarter, The CBCP has been asked to become the donator of breast tissue for The Cancer Genome Atlas and has been described by Carolyn Compton of NCI as the platinum standard for tissue biospecimen repositories in the world. We have completed all paperwork and are ready to begin shipping the first group of specimens. This is indeed a phenomenal accomplishment for this project

7. REFERENCES

N/A

8. APPENDICIES

- ATTACHMENT 1 List of personnel receiving pay from the research effort in FY 2008
- ATTACHMENT 2 : List of publications and meeting abstracts for FY 2008

ATTACHMENT 1

CBCP PERSONNEL RECEIVING PAY FROM THE RESEARCH EFFORT
October 1, 2008 – September 30, 2009

Last Name	First Name	Role	Percent of Effort
Shriver	**Craig**	**Principal Investigator**	**25%**
Basham	Janice	Licensed Practical Nurse	100%
Boone	Jaime	Admin Mgr, CBCP Physician Staff	100%
Bronfman	Eileen	Administrative Director	100%
Campbell	Jamie Leigh	Pathology Assistant	100%
Chestang	Allan	Data Manager	100%
Chou	Carolyn	Budget Analyst	100%
Courville	Faith	Research Nurse	100%
Cronin	Kerri	Administrative Assistant/ Office Manager	100%
Del	Ismail	Data Manager	100%
Enowold	Lindsey	Biostatitician	25%
Gutchell	Veronica	Head Nurse, CBCP/ Nurse Practitioner	100%
Hilton	Karrie	Research Nurse	100%
Hodgson	Carol	Nurse Practitioner	100%
Hooke	Jeffrey	Head of Pathology	100%
Kelley	Kay	Research Protocol Coordinator	100%
Louallen	Rachelle	Receptionist	100%
Means	Marilyn	Lead Medical Clerk/Receptionist	100%
Miller	Donald	Data Manager	100%
Patterson	Carol	Medical Assistant	100%
Progar	Christina	Biomedical Informatics Coordinator	100%
Reece	Heike	Data Manager	100%
Rosenquist	Monica	Budget Analyst	100%
Stojadinovic	Alexander	Breast Surgeon	25%
Vilakasi	Patricia	Research Nurse	100%
Williamson	Eric	Clinic Administrator	100%
Zhao	Xinyan	Histology Technician	100%
Zhu	Kangmin	Epidemiologist	20%

ATTACHMENT 2

PUBLICATION, ABSTRACT AND PRESENTATION DATA

October 1, 2008 – September 30, 2009

PUBLICATIONS – 2009

Field LA, Love B, Kane J, Deyarmin B, Hooke JA, Ellsworth RE, Shriver CD. **"Gene Expression Differences in Normal Breast Tissue from Disease-Free African American and Caucasian Women".** Breast Cancer Research Journal, BioMed Central Ltd, Middlesex House, London, UK, Jan 09

ABSTRACTS – 2009

Weyandt J, Ellsworth RE, Hooke JA, Shriver CD, Ellsworth DL. **"Molecular Characteristics of Early Breast Disease".** AACR, 18-22 Apr 2009, Denver, CO

Ellsworth RE, Weyandt JM, Fantacone-Campbell JL, Deyarmin B, Ellsworth DL, Hooke JA, Shriver CD. **"Molecular Characterization of Breast Cancer Progression: Early Lesions are not Genetically Advanced".** AACR 18-22 Apr 09 Denver, CO

PUBLICATIONS – 2008

Field LA, Love B, Kane J, Deyarmin B, Hooke JA, Ellsworth RE, Shriver CD. **"Gene Expression Differences in Normal Breast Tissue from Disease-Free African American and Caucasian Women".** In press

Yang S, Guo X, and Hu H. MOF-an R Function to Detect Microarray Outlier Slides. **"Genomics, Proteomics and Bioinformatics".** (In press).

Maskery SM, Hu H, Hooke J, Shriver CD, Liebman MN. **"A Bayesian Derived Network of Breast Pathology Co-Occurrence".** Journal of Biomedical Informatics. In print 2008

Becker TE, Ellsworth RE, Deyarmin B, Patney HL, Jordan RM, Hooke JA, Shriver CD. Ellsworth DL. **"Genomic Heritage of Lymph Node Metastases: Implications for Clinical Management of Patients with Breast Cancer."** Clinical Biochemistry Journal, submitted.

Weyandt J, Ellsworth RE, Hooke JA, Shriver CD, Ellsworth DL. **"Environmental Chemicals and Breast Cancer Risk – A Structural Chemistry Perspective".** Current Medicinal Chemistry, 2008, 15, 2680-2701.

ABSTRACTS – 2008

Patney HL, Deyarmin B, Hooke JA, Ellsworth RE, Love B, Shriver CD. **"Relationship Between Alterations of Chromosome 8q24 and Poorly-Differentiated Breast Carcinoma".** SABCS 10-14 Dec 08

Ellsworth RE, Heckman C, Love B, Shriver CD. **"Identification of Breast Cancer Metastasis Initiation and Virulence Genes".** SABCS 10-14 Dec 08, San Antonio, TX

Field LA, Love BJ, Kane J, Deyarmin B, Hooke JA, Ellsworth RE, Shriver CD. **"Role of Phosphoserine Phosphatase-like in Breast Disease in African American Women".** SABCS 10-14 Dec 08, San Antonio, TX

Ellsworth DL, Ellsworth RL, Patney HL, Oviedo A, George A, Croft, DT, Jr., Love B, Jordan RM, Deyarmin B, Becker TE, Hooke JA, Shriver CD. **"Fingerprinting Genomic Heterogeneity in Primary Breast Carcinomas and Among Sentinel Lymph Node Metastases: Implications for Clinical Management of Breast Cancer Patients".** SABCS 10-14 Dec 08, San Antonio, TX

Ellsworth DL, Gillard D, Love B, Ellsworth RE, Deyarmin B, Hooke JA, Kostyniak PJ, Shriver CD. **"Abundance and Distribution of Polychlorinated Biphenyls (PCBs) in Breast Tissue".** SABCS 10-14 Dec 08, San Antonio, TX

Croft D, Mao X, Hooke JA, Shriver CD, Shriver M, Ellsworth RE. **"Admixture Mapping to Identify Breast Cancer Susceptibility Loci in African American Women".** SABCS 10-14 Dec 08, San Antonio, TX

Patney HL, Deyarmin B, Hooke JA, Ellsworth RE, Love B, Shriver CD. **"Relationship Between Alterations of Chromosome 8q24 and Poorly-Differentiated Breast Carcinoma."** SABCS 10-14 Dec 08, San Antonio, TX

Ellsworth RE, Heckman C, Love B, Shriver CD. **"Identification of Breast Cancer Metastasis Initiation and Virulence Genes".** SABCS 10-14 Dec 08, San Antonio, TX