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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 standard of care for treating breast diseases and breast cancer. This multidisciplinary model integrates prevention, screening, diagnosis, treatment and continuing care, but the project is further unique in the incorporation of advances 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

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

I. 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 Mark Critz (formerly John P. Murtha) and Walter Reed Army Medical Center, with funding management by the Henry M. Jackson Foundation for the Advancement of Military Medicine.

We believe that there are three broad areas where the BC-COE (Breast Cancer – Center of Excellence) stands poised to make major contributions to breast cancer research and its translation into clinical practice. These areas include the identification of molecular profiles of disease with high clinical relevance, deepening our understanding of the genetic risk of breast disease and the enhancement of our understanding of breast tumor biology. These three themes are supported by the five pillars of the BC-COE. There is no doubt that our understanding of the biology of Breast Cancer in all of its various forms and manifestations remains incomplete. We believe that our high-value repository of biospecimens, our strong biomedical informatics infrastructure and our research base with strong internal and external collaborations puts us in an excellent position to make contributions to the understanding of breast disease that will have impact on the quality of life for breast cancer patients and their families.

Clinically Relevant Molecular Profiling: This is cross-cutting theme with clinical, risk assessment and basic research components. The primary focus of this theme is to evaluate the utility of existing molecular profiles that have relevance to risk assessment, diagnosis, prognosis and therapy in a clinical setting and to discover new profiles that can be evaluated in the clinic. Projects within this theme have well defined translational goals. The development of comprehensive and highly informative molecular profiles will be a foundation for the development and delivery of personalized/individualized medicine. A variety of research modalities will be used to identify these profiles including immunohistochemistry, gene and protein expression analysis and genetic profiling including Next Generation DNA Sequencing. Two major new initiatives are outlined below one involving the development and testing of clinically relevant immunohistochemical profiles for disease stratification and therapeutic guidance and the other using complete genomics sequencing of tumor and matched normal DNA to develop clinically relevant profiles that could aid in disease diagnosis, prognosis and therapy selection.

Genetic Risk: The rapid developments of high throughput genotyping and genomic sequencing of individuals has reminded the research community of the power of family

studies in the assessment of genetic risk. Evaluating family risk and translating that into individual risk is the primary goal of this theme. There is both clear clinical relevance and a strong basic research component to this theme. Understanding the underlying biology of observed racial disparities in disease prevalence, presentation and outcome will also be a major part of this effort. The interaction of the theme with the Risk Reduction pillar of the BC-COE and the number of projects outlined below that deal with research into the basis of the observed racial disparities in breast cancer morbidity and mortality point out the relevance of this theme to the overall goals of the BC-COE.

Tumor Biology: A unique combination of resources and expertise put the BC-COE in a strong position to further our understanding of the basic biology of breast disease including breast cancer. Many of the projects outlined in the Focused Research pillar address basic problems associated with tumor heterogeneity. The tumor microenvironment and stromal interactions, metastasis and recurrence, as well as the role of cancer stem cells and tumor evolution affecting the efficacy of treatment are emphasized. We firmly believe that a robust understanding of breast tumor biology is a key to the successful translation of the research performed at the BC-COE to the clinic.

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.

II. BODY

Pillar Specific Goals and Objectives:

1. Breast Cancer 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.
- Incorporation of newly identified markers of breast cancer risk into the assessment of breast cancer risk.
- Identify patients from families that might harbor mutations in the BRCA1 or

BRCA2 genes and offer testing to identify these mutations

- Identifying families with unexplained high frequencies of breast cancer as potential research subjects

2. Biorepository:

- Collect and store a broad spectrum of biospecimens from every patient undergoing a breast biopsy and/or breast surgery at WRAMC, WMC, AAMC, and our affiliated hospitals, who consent to participate in BC-COE IRB-approved protocols.
- Collect and store biospecimens (blood) from women who are free of breast disease who consent to participate in BC-COE IRB-approved protocols to act as controls.
- Utilize the power of this extensive biorepository as a major resource for breast disease research.
- Leverage the BC-COE biorepository to maximize the utilization of the repository, with BC-COE leadership approval, for the overall benefit of breast cancer patients and research, as able and appropriate.
- Participate in national/international projects that can benefit from resources of the BC-COE biorepository.

3. Focused Research (including: Genomics and Proteomics Research):

Genomics: Utilize high-throughput and translational research in a unique Discovery Science environment to include but not be limited to:

- DNA analysis with genotyping studies, Copy Number Variation (CNV), gene sequencing and whole genome sequencing
- RNA/cDNA micro arrays, to identify expression level differentials across the entire spectrum of breast disease and from cancer specimens of all stages and types, as well as the accompanying lymph nodes and metastatic deposits, and blood.
- Measure differences in microRNA levels in normal and diseased tissues
- Measure epigenetic changes associated with disease

Proteomics: Utilize high-throughput and translational research in a unique Discovery Science environment to include but not be limited to:

- Mass spectrometer pattern analysis and protein identification,
- Identify protein expression level differentials across the entire spectrum of breast disease and cancer specimens of all stages and types, as well as the accompanying lymph nodes and metastatic deposits, serum and blood
- Use Accurate Mass Tag (AMT) technology to assess protein expression changes in tumor tissues
- Search for novel protein biomarkers, individual or pattern.
- Store all this expression data in a data warehouse where it can then be utilized for biologic pathway development and in-silico biology research for hypothesis-driven research.

Collaborative Research:

- Perform affiliated translational laboratory research in support of the main expression profiling and biomarker discovery goals of the BC-COE research laboratories.
- Develop alliances with other research organizations and entities and carry out project-supported research in support of same. Currently we have established collaborations with the Pacific Northwest National Laboratory, Vanderbilt University, The Institute for Systems Biology, the Thomas Jefferson University, the MGR Global, etc.
- Collaborate with the NCI/NHGRI TCGA (The Cancer Genome Atlas) project to study the genomics of breast cancer.

4. Biomedical Informatics:

- Develop a comprehensive QA program and aid in SOP development for data collection and generation to ensure acquisition of high quality of data.
- Develop and support a robust laboratory information management system to ensure proper tracking of data acquisition.
- Develop and implement a clinically relevant and laboratory research-linked prospective, longitudinal computerized data warehouse to support translational research and ultimately support physician decision making
- Develop an analytical system including developing specific algorithms for integrative data analysis and mining, and deploying existing applications and algorithms to ensure execution of data analysis, mining, and modeling.
- Develop a breast knowledgebase to support clinical and research activities in BC-COE.
- Develop other needed infrastructure to support the activities in all other BC-COE pillars.

5. Clinical Care:

- Decrease the negative psychological impact on the patient evaluated or treated for breast disease.
- Create and maintain an environment (medical, physical, psychological) conducive 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. Serve as the portal for breast disease research by acquiring tissue, serum and clinically relevant data

III. PILLAR SPECIFIC SCIENTIFIC PLANS AND METHODS

Task 1: Identify and counsel no less than 100 patients at high risk for of breast cancer, and employ risk reduction strategies.

Task 2: Accrue over 500 patients annually to the “core” BC-COE protocols through consenting patients in the main BC-COE clinical sites.

Task 3: Acquire through consented protocol acquisitions, over 5,000 specimens annually (neoplastic and non-neoplastic breast tissues and tumors, lymph nodes, metastatic deposits, blood and its components, bone marrow) on patients with all types of breast diseases and cancer.

Task 4: Bank these biospecimens in the BC-COE Biorepository as the substrate for all molecular analyses carried out in BC-COE labs, as outlined in the BC-COE Core Protocols. Utilize this repository as the basis for intramural and extramural collaborations for secondary usage research.

Task 5: Perform focused research as outlined below on the biospecimens and clinical data collected under the BC-COE Core protocols including global expression analysis of the DNA, RNA, and Protein features and including targeted research into genomic analysis of Stages I, II, and III breast cancer, DCIS, LCIS, and pre-malignant neoplasia. Present findings in peer-reviewed national meetings and publications.

Task 6: Perform whole genome DNA sequencing on DNA from 40 cases of breast cancer.

Task 7: Develop and support a robust laboratory information management system to ensure proper tracking of data acquisition and a clinically relevant and laboratory research-linked prospective, longitudinal computerized data warehouse to support translational research and ultimately support physician decision making.

Task 8: Develop an analytical system for integrative data analysis and mining, and develop a breast knowledgebase to support clinical and research activities in BC-COE.

BC-COE PILLAR #1 BREAST CANCER RISK REDUCTION:**OBJECTIVES:**

- a. To collect data on all female patients 18 and older who present to the General Surgery Clinic at Walter Reed Army Medical Center and are found to be at an increased or elevated risk for developing breast cancer.
- b. To utilize this database to analyze the diagnosis, treatment, and treatment outcomes for patients found to be at an increased risk for developing breast cancer. Analysis will include but not be limited to: risk factors for developing breast cancer, effectiveness of various modalities of treatment, and actual risk of developing cancer.

BACKGROUND:

Each year, approximately 200,000 women in the United States are diagnosed with breast cancer, and one in nine American women will develop breast cancer in her lifetime. But hereditary breast cancer - caused by a mutant gene passed from parents to their children - is rare. Estimates of the incidence of hereditary breast cancer range from between 5 to 10 percent to as many as 27 percent of all breast cancers.

In 1994, the first gene associated with breast cancer — BRCA1 (for BREast CAncer1) was identified on chromosome 17. A year later, a second gene associated with breast cancer — BRCA2 — was discovered on chromosome 13. When individuals carry a mutated form of either BRCA1 or BRCA2, they have an increased risk of developing breast or ovarian cancer at some point in their lives. Children of parents with a BRCA1 or BRCA2 mutation have a 50 percent chance of inheriting the gene mutation.

In 1995 and 1996, studies of DNA samples revealed that Ashkenazi (Eastern European) Jews are 10 times more likely to have mutations in BRCA1 and BRCA 2 genes than the general population. Approximately 2.65 percent of the Ashkenazi Jewish population has a mutation in these genes, while only 0.2 percent of the general population carry these mutations.

MEDICAL APPLICATION:

Not all hereditary breast cancers are caused by BRCA1 and BRCA2. In fact, researchers now believe that at least half of hereditary breast cancers are not linked to these genes. Scientists also now think that these remaining cases of hereditary breast cancer are not caused by another single, unidentified gene, but rather by many genes, each accounting for a small fraction of breast cancers.

Hereditary breast cancer is suspected when there is a strong family history of breast cancer: occurrences of the disease in at least three first or second-degree relatives (sisters, mothers,

aunts). Currently the only tests available are DNA tests to determine whether an individual in such a high-risk family has a genetic mutation in the BRCA1 or BRCA2. Genetic counselors can help individuals and families make decisions regarding testing. For those who do test positive for the BRCA1 or BRCA2 gene, surveillance (mammography and clinical breast exams) can help detect the disease at an early stage. A woman who tests positive can also consider taking the drug tamoxifen, which has been found to reduce the risk of developing breast cancer by almost 50 percent in women at high risk. Clinical trials are now under way to determine whether another drug, raloxifene, is also effective in preventing breast cancer.

The field of oncology/surgical oncology is an ever-changing one with new developments in both diagnosis and treatment. We propose to collect data from all patients in the WRAMC Comprehensive Breast Center determined to be at an elevated risk for developing breast cancer in order to assess risk factors in this population for developing the disease and track outcomes of preventive and therapeutic interventions. Analysis of outcome will include comparison of various treatment modalities/regimens with regard to efficacy, risks for failure, complications, and overall morbidity/mortality/survival. The patient population of WRAMC can provide a significant number of patients to compare/contrast our findings with those of our civilian counterparts, specifically the Joyce Murtha Breast Care Center. The database will also allow us to analyze breast cancer risk data to provide scientific-based evidence that will guide the general surgeon and medical oncologist in optimal care of the patient at an elevated risk for developing breast cancer.

PLAN:

The risk Reduction Clinic at WRAMC CBCP and at Joyce Murtha Breast Care Center (JMBCC) is a multi-disciplinary program designed to identify, counsel and manage women at high risk for breast cancer. Patients receive an in-depth personal and family health history by a world renowned medical oncologist.

Current research shows there are risk factors that may influence the development of breast cancer. Identifying people with these risk factors and implementing closer surveillance and risk reduction techniques may detect cancer earlier. Earlier detection of breast cancer leads to better prognosis and outcomes. Calculations of risk are based on computer models extensively validated as accurate in identifying women at high risk.

Subjects: All female patients age 18 and older, seen in the WRAMC Comprehensive Breast Center at a high risk for developing breast cancer. Patients will have consented to WU # 01-20006, Tissue and Blood Library Establishment for Molecular, Biochemical, and Histologic Study of Breast Disease or WU # 01-20007, Creation of Blood Library for the Analysis of Blood for Molecular Changes Associated with Breast Disease and Breast Cancer Development. The core questionnaire completed by the patients contains the NCI Breast Cancer Risk Assessment Tool used to determine a patient's risk factor. Please see Addendum #1.

Study Design and Methodology: Patients being seen in the WRAMC Comprehensive

Breast Center at WRAMC or at the JMBCC in Windber, PA will be assessed for their risk of developing breast cancer by their history of LCIS or ADH or by applying the NCI Breast Cancer Risk Assessment Tool. Identified high-risk patients will be referred to the CBCP Risk Reduction Clinic. Patients are confirmed to meet the inclusion criteria and consented to one of two core protocols. Information collected will include the data contained on the enclosed database forms and may include data from previous clinic visits. All applicable patients will be followed indefinitely according to the applicable protocol.

If patients are referred for genetic testing, as per the American Society of Clinical Oncology, counseling involves the following eleven points:

1. Information on the specific test being performed
2. Implications of positive and negative results
3. Options for estimation without genetic testing
4. Risk of passing a mutation to a child
5. Technical accuracy of the test
6. Possibility that the test will not be informative
7. Fees involved in testing
8. Risk of psychological distress
9. Risk of insurance or employer discrimination
10. Confidentiality issues
11. Options for medical surveillance and screening following testing

It has been observed that healthy female relatives of individuals with ovarian or breast cancer tend to exaggerate their risk of incurring either form of cancer and, thus, accurate risk assessment is essential to quality genetic counseling for breast cancer. Breast cancer genetic counseling serves the goal of helping women to analyze their own and their relatives' risk of developing breast cancer.

In BRCA screening, genetic counselors offer services in compiling family histories, personalizing epidemiology, and, more recently, conducting genetic testing to empower healthy women of families stricken by breast cancer to alter their lifestyles and healthcare to ensure avoidance or early detection of breast cancer. In addition, breast cancer victims and healthy members of a single family can enable accurate screening of female family members by obtaining a sequence of their BRCA genes. Detection of a familial BRCA mutation in individuals outside of the Ashkenazi Jewish population requires time-consuming genetic analysis of a large number of affected and unaffected family members in order to identify the specific BRCA mutation for a particular family.

Testing positive or negative for a BRCA mutation is simply a risk assessment, not a certainty of experiencing or avoiding, respectively, breast cancer. Individuals with a BRCA mutation have an 80% risk of developing breast cancer by age 80. Therefore, 20% of BRCA mutation carriers never develop breast cancer. A first-degree relative of a carrier who tests negative for the mutation has the same breast cancer risk as women of the general population, namely 11%.

BC-COE PILLAR #2 BIOREPOSITORY

BACKGROUND

Although there have been remarkable improvements in breast cancer diagnosis and management, most of the complex molecular mechanisms associated with the onset, progression and/or severity of breast cancer are still not well understood. As part of the BC-COE we carry out molecular, biochemical and histological analysis of breast tissue and/or blood and blood components from breast cancer patients to provide insights into the molecular mechanisms that may be relevant in the development of breast cancer and breast diseases. To achieve this aim, a large supply and a wide variety of good quality tissue samples are needed. Unfortunately, good quality donor breast tissue is extremely scarce and when available is often not backed by a comprehensive medical history and/or is not a good representation of the target population or study area. The non-availability of a steady and consistent supply of good quality tissue limits the systematic analysis of tissues and negatively impacts the generation of biologically useful information in research laboratories and by extension negatively impacts new findings that benefit clinical practice. The objective of this project is therefore the acquisition and banking of breast tissue, lymph nodes, serum/plasma and other blood derivatives from informed and consenting donors.

Since the inception of the Clinical Breast Care Project the Biorepository Pillar has been critical to the success of the project. As we move forward into the establishment of the BC-COE it is important to look at the success of the biorepository and to understand the firm foundation that it has laid for building the Center of Excellence.

Figure BB-1 shows the cumulative patient accrual into the CBCP protocols since 2002. These patients, who have been recruited and consented into the CBCP protocols at Walter Reed Army Medical Center (WRAMC) and other participating CBCP clinical intake sites

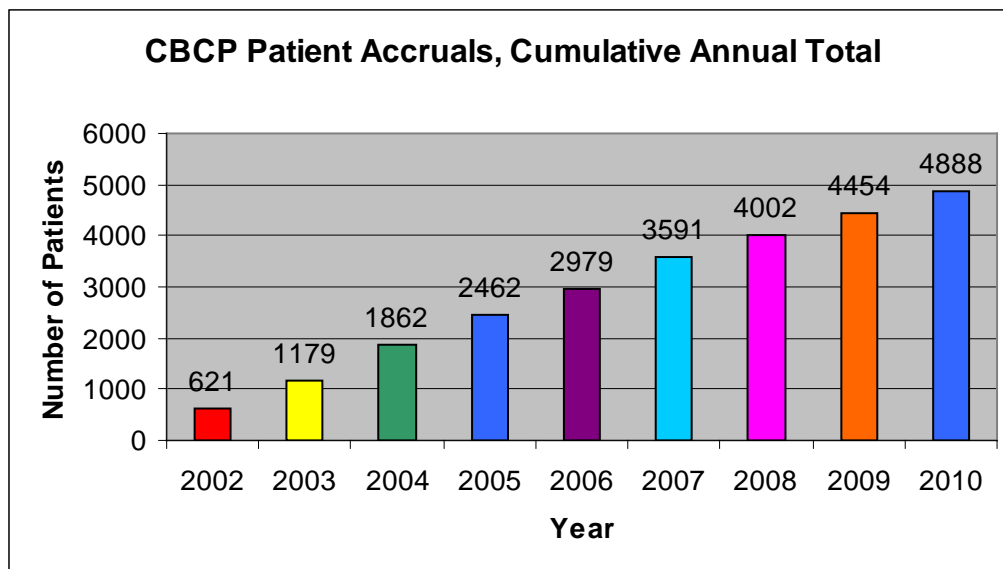


Figure BB -1. Cumulative patient accruals into CBCP protocols since 2002.

are the foundations of the translational research that has occurred within the CBCP and which will continue in the BC-COE. From these patients we have collected and stored in our biorepository over 42,000 biospecimens (Figure BB-2). These specimens represent a broad spectrum of tissues, blood and blood products (Figure BB-3) that are not only a

unique and valuable resource for the BC-COE but are also the substrates for our translational research program. Along with the biospecimens that have been collected from CBCP participants, each consented patient also provides nearly 800 field of demographic,

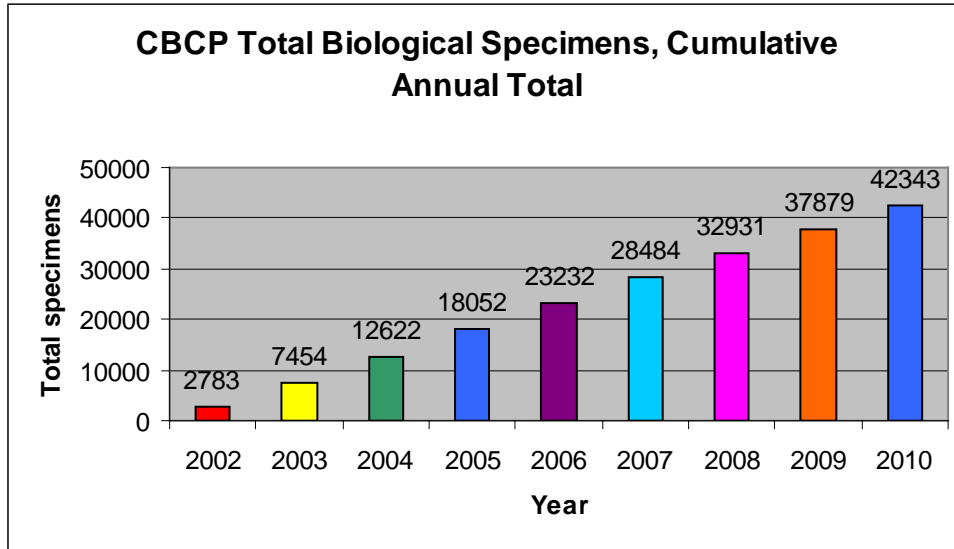


Figure BB-2. Total biospecimens collected and banked by the biorepository.

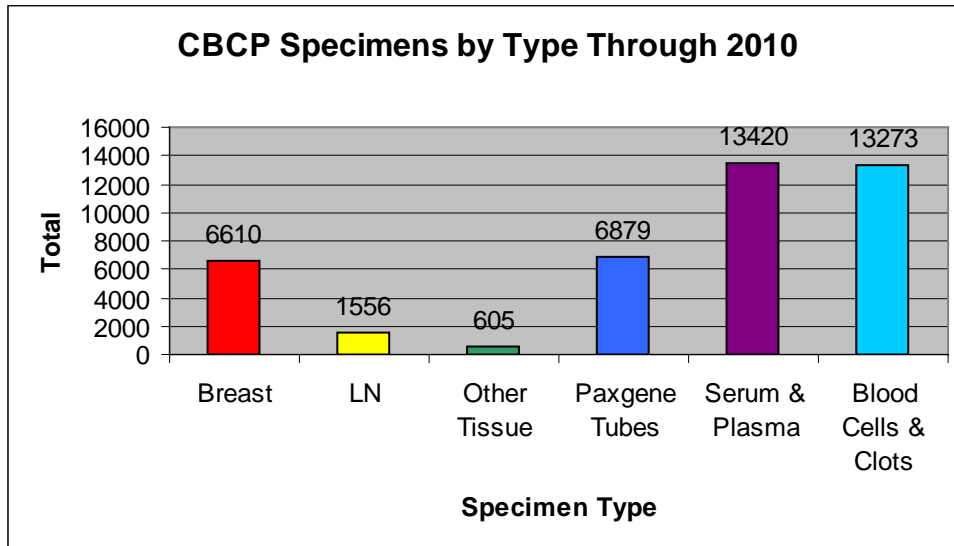


Figure BB-3. The numbers and types of biospecimens collected by the CBCP

medical, life and family history data as well as complete pathology data on donated tissues. The collection of tissues that the BC-COE inherits from the CBCP is even more valuable because of this rich annotation. Patients have been recruited from a number of

partnering clinical intake sites over the history of the CBCP (Figure BB-4). At the start of the BC-COE the active partners are WRAMC, the Joyce Murtha Breast Care Center in Windber, PA, and the Anne Arundel Medical Center in Annapolis, MD.

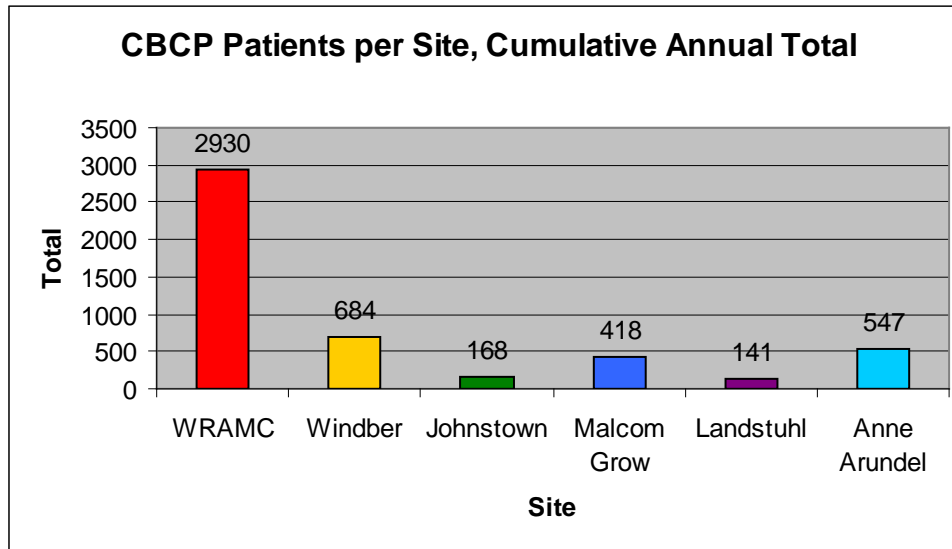


Figure BB-4. Numbers of patient recruited to CBCP protocols at various partner sites.

BC-COE PILLAR # 3 Focused Research including Genomics and Proteomics

The research pillar of the BC-COE focuses on the translational research program involving the clinical programs at Walter Reed Army Medical Center's Breast Center and the Joyce Murtha Breast Care Center and the genomic and proteomic analysis carried out at the Windber Research Institute.

The following is a list of the projects that make up the Focused Research Pillar of the BC-COE. Major new initiatives include a major project that will generate complete genomic DNA sequence from up to 40 breast cancer cases in FY2010. Another new initiative will utilize immunohistochemical to generate clinically relevant profiles of breast tumors to better stratify the disease in terms of prognosis and treatment options.

Focused Research

Estimating the Prognostic Value of Gene Expression Data from Primary Tumors in Predicting Lymph Node Status

Despite tremendous advances in early detection and treatment, breast cancer remains the second leading cause of cancer-related death in women. While a number of clinical parameters have been identified, axillary lymph node status remains the most important prognostic factor in predicting disease outcome. Previous studies in our laboratory using

allelic imbalance (AI) to detect chromosomal changes have shown that there are high levels of discordance of AI between primary breast tumors and corresponding axillary lymph node tumors (Ellsworth et al. 2005). In 2009, we published a RNA-based signature of metastasis of 51 genes that differ significantly between primary breast and matched metastatic lymph node tumors.

We have recently completed a study evaluating gene expression patterns in 74 primary breast tumors with and without metastasis and have submitted for publication in a special issue of International Journal of Breast Cancer. While an effective signature was not identified, the reasons why a global signature was not detected are intriguing, including genetic predisposition to metastasis. We have generated 100K SNP data from white women with invasive breast cancer and without breast cancer to look for genetic differences that may suggest host susceptibility, not to development of invasive tumors but for propensity to successfully metastasize. In addition, in collaboration with the Mass Spectrometry Research Center at Vanderbilt University, we are identifying protein expression patterns in primary tumors from patients with and without metastasis, protein differences in primary vs. metastatic lymph node tumors and between disease-free lymph nodes from patients with and without lymph node metastases.

Allelic Imbalance Studies in Breast Cancer

The allelic imbalance panel we developed in 2002 has been used for many use to evaluate genetic changes in all stages of breast tissue (disease-free through metastatic). We have completed all studies using this panel. In 2010, we published the results of our AI evaluation in non-neoplastic diseases, concluding that ADH and CCH are in fact, genomically naive and that the multitude of studies that have evaluated pre-neoplastic lesions from breasts with invasive breast tumor cannot represent the status of pure early lesions.

Our last study will be to evaluate patterns of allelic imbalance in the context of outcome. We have identified a number of chromosomal regions either present at significantly higher (suggestive of metastasis) or lower (protective effect) in tumors from patients who have died of disease.

Genomic differences in African and Caucasian American Women with Breast Cancer

Breast cancer incidence in women in the United States varies among different ethnic groups. The highest incidence occurs in Caucasian women; however, mortality is highest among African American women. Furthermore, African American women are more likely to be diagnosed at a younger age and with more advanced stages of the disease. These differences in outcome between Caucasian and African American women are frequently attributed to disparities in health care access and socioeconomic status. However, molecular factors may also be involved as indicated by the differences in tumor biology between African American and Caucasian women. Tumors in African American women tend to be larger and more aggressive with high-grade nuclear atypia and

lymphocytic infiltrate. Tumors from African American women are also more likely to be triple negative (ER-, PR-, and HER2-) making them refractory to treatment with current targeted therapies such as tamoxifen and herceptin. Initial profiling of the African American women enrolled in the CBCP was published in 2007, identifying both demographic and molecular differences between CCW and AW. Recently we generated gene expression data from both tumors and non-malignant tissues from AAW and CW. 18 genes were differentially expressed in tumors and 13 in non-malignant breast specimens, including PSPHL, SOS1 and CRYBB2, which are differentially expressed in both tumors and non-malignant tissues.

Breast Tumor Microenvironment

Alterations in cell signaling, angiogenesis, cell migration, motility, and proliferation establish the tumor microenvironment as a dynamic entity contributing to breast tumorigenesis. These alterations can be contributed to epigenetic, morphological, and genetic changes. Research in the past decade has increased our understanding of how the changing tumor microenvironment influences tumor development, and this is proving to be of significant importance in the progression of breast cancer research. An improved understanding of the molecular changes occurring in the breast tumor microenvironment may have significant clinical ramifications as well as provide an increased understanding of breast tumor etiology. Continued investigation into this research could provide insights into tumor development and progression, leading to meaningful advances in breast cancer. In 2009, we published an extensive review paper in *Expert Review of Molecular Diagnostics* weighing in on the debate as to whether morphologically normal breast stroma adjacent and distant from the tumor has chromosomal changes.

Copy number evaluation is being performed on fresh frozen genomic DNA samples from women with breast cancer, and a variety of pathological classifications using Affymetrix 500K SNP chips. Copy number and LOH analysis is being performed using Affymetrix genotyping console. Preliminary data were presented at the AACR meeting in April. Recently, we received a grant from CDMRP to evaluate gene expression differences in adipose close to and distant from breast tumors as well as adipose from non-malignant breasts to improve our understanding of how this long ignored component of the breast stroma contributes to tumorigenesis.

Combined Genomics and Proteomic Analysis of Metastatic Potential in Breast Cancers

We are studying the metastatic potential of breast cancers with collaborators at the Pacific Northwest National Laboratory (PNNL). A total of 76 estrogen receptor positive (ER+) tumor samples (20 LN-PM; 20 LN+PM; 19LN-PRM; 13 LN+PRM) have been collected by laser capture microdissection from histological tissue preparations for comparing the proteomic and genomic profiles from these four categories or groups. These samples underwent genomic analysis at WRI and are undergoing proteomic analysis at PNNL

WRI and PNNL have begun the exchange of data on the differentially expressed genes discovered by each group in their independent analysis (genomics- WRI, proteomics- PNNL). With this data exchange we are developing our strategies to integrate these data sets.

A proteomics methods paper has been drafted from the initial LC/MS proteomics methods optimization research on OCT embedded tissues and the manuscript has been submitted to the Journal of Proteome Research.

Proteomics (CBCP, WRI) report Y2010

Kumar Kolli

Body

Exploration/Identification of biomarkers through differential proteomics on retrospectively collected clinical breast cancer samples has been the major focus of proteomics research core facility at WRI and we have continued our efforts in Y 2010 to adapt the emerging protein microarray technology in the proteomics lab for high throughput profiling of serum samples. In this report, we summarize preliminary results on profiling of a panel of cytokines from breast cancer serum samples using antibody microarrays along with the serum profiling using GeLC/MS approach to evaluate the degradation protein products in the serum as markers to determine the invasiveness of breast cancer.

Profiling of breast cancer sera by cytokine antibody array to identify early disease markers

Introduction

Breast cancer is the most common cancer and the second leading cause of cancer death in women in the U.S with more than 40,000 mortalities each year. Early detection remains the most promising approach to improve the long-term survival of cancer patients. One area of research interest to explore the early markers for cancer is profiling serum cytokines. These proteins play an important role in innate immunity, apoptosis, angiogenesis and differentiation. The expressions of individual cytokines are altered in cancers and the interplay between immune and tumor cells further alters their expressions and measuring cytokine levels in serum might be useful in determining the specific stage and grade of tumors. With the emerging protein microarray based technologies, the discovery-driven translational research on cancer related clinical samples has drawn a considerable attention to rapidly profile the protein expressions using chip based protein microarrays for biomarker discovery in the area of disease diagnostics and prognostics. In the present study, we are exploring breast cancer serum samples to identify the early markers for cancer detection using cytokine antibody arrays.

Methods

Serum samples from 24 postmenopausal subjects which were evenly divided between benign and invasive cases were probed with glass slide based antibody array (AAH-CYT-

G2000-8; RayBiotech, Inc) to profile 174 cytokines simultaneously from each serum sample (30uL). The serum samples were prepared according to the protocol provided by vendor and profiled based on the principles of sandwich type immunoassay and the cytokine signals were read on a GenePix 4000B fluorescent scanner using Cy3 channel. Spot alignment was performed with GenePix Pro 4.0 where signal and background detection levels for each spot were defined as median intensity. Background subtraction and positive control normalization were done with RayBio Analysis Tool while average ratio and independent two-tailed t-test analyses were performed in Microsoft Excel to find the abundance difference of each cytokine between invasive and benign groups.

Results and Conclusion

The differential analysis of cytokine profiles from benign and invasive cases has shown only moderate expression changes for several cytokines. The two cytokines found to be elevated in the invasive group were MMP-9 and lowered expressions for MIP-1-delta, MIF, leptin R, SDF-1beta, TRAIL R4, prolactin, and LIF were observed in invasive group. Profiling of additional samples is under way, while extending the study to include other stages and node positive serum samples has also been planned. As the node positive and node negative BC tissue proteomics/genomics data were available, the cytokine profiling for the matched serum samples has been planned and this analysis will expand the scope of understanding the molecular mechanisms that drive breast cancer progression and invasion. Prediction models will be built from these microarray datasets to correctly identify the breast cancer cases.

Evaluating protein degradation products as serum biomarkers to determine the invasiveness of breast carcinoma

Introduction

The process by which cancer spreads from its site of origin to other tissues is a complex series of steps. The first of these involves the cancer cells freeing themselves from the primary tumor by degrading the proteins that make up the surrounding extracellular matrix (ECM), which separates the tumor from adjoining tissues. Degradation of the ECM is accomplished by the tumor cells secreting a variety of proteases, including the matrix metalloproteases (MMPs) and Plasminogen activators (PAs). Our hypothesis is that these secreted enzymes leak into the blood stream where they degrade serum proteins.

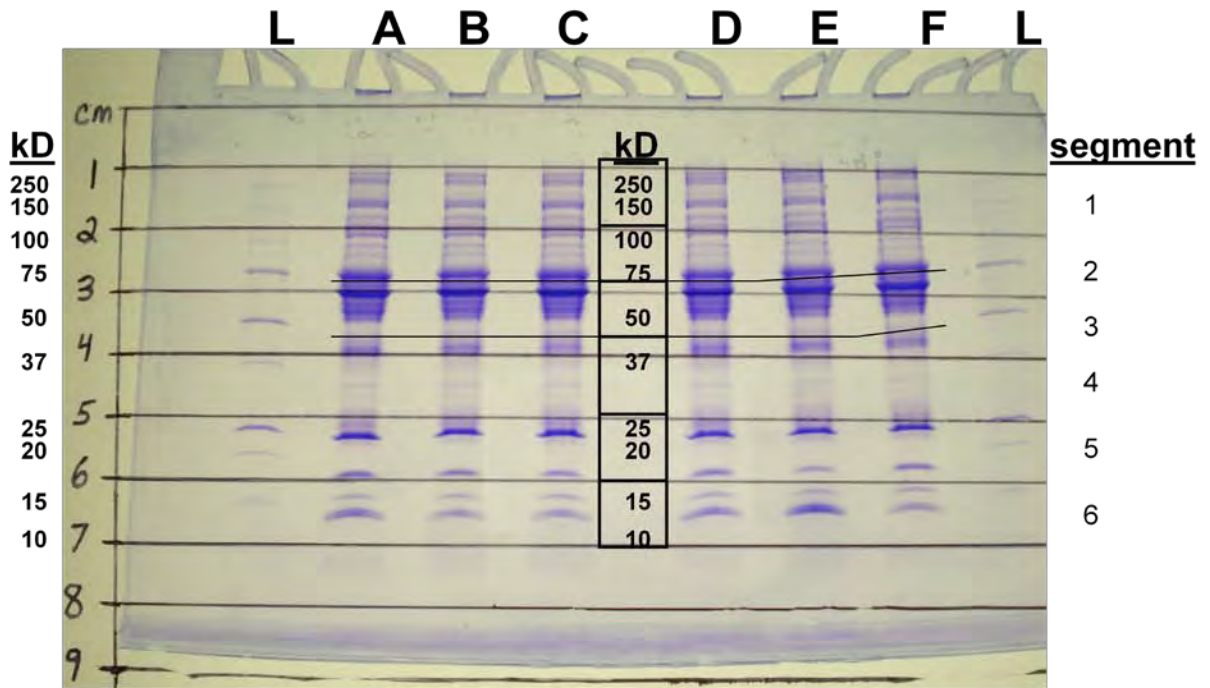
Methods

The breast cancer serum (20 µL) samples (20 benign and 20 invasive, stage I) were processed with Enchant multi affinity protein separation kit to remove the high abundant proteins. The depleted flow through fractions of serum samples from the respective groups were pooled to prepare three pooled samples for each group and the samples were separated on 1D gel (fig1). The gel lanes were cut into segments (fig1) and proteolytically digested, and the extracted peptides were run in duplicates on a LTQ-FT MS. MS/MS were searched against a database of human proteins with Sequest, and identifications were statistically validated at a 1% protein false discovery rate using the Provalt algorithm in ProteoIQ (BioInquire, Athens, GA). The proteoIQ data mining tools

were used to identify the protein degradation in the samples by assembling the data from each segment.

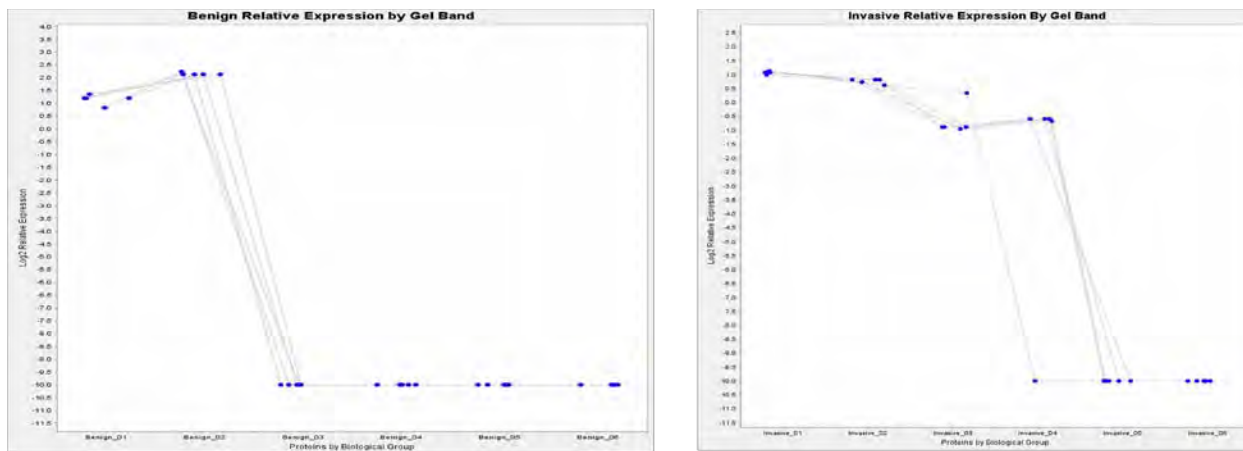
Results and conclusion

Based on our enzyme degradation hypothesis, we predict that the level of these fragments and their extent of degradation will be indicative of tumor invasiveness and thus capable of serving as serum biomarkers for this condition. To this end, we have applied a GeLC-MS approach for this study where intact proteins are separated via SDS gel electrophoresis prior to trypsin digestion and LC-MS/MS analysis. This sample-flow allows for the identification of proteins whose experimental MW (determined by SDS gel) differs significantly from their calculated MWs. This process has allowed us to identify several protein degradation products and other wise-abnormally modified proteins which may be related indirectly to tumor invasiveness. The presence of degraded inter-a-trypsin inhibitors can be clearly seen (fig2) as the presence of peptides from these proteins being detected across 4 gel slices in the invasive samples. In the benign samples, peptides are only detected in 2 of the gel slices (fig2). The preliminary results from this pooled sample study are encouraging and we are planning to apply this workflow on breast cancer samples individually to correlate the invasive characteristics of primary tumors to the extent of protein degradation products in the cancer serum samples.



ABC = Benign pooled samples
 DEF = Invasive pooled samples
 Each lane contains 30ug of protein.

Figure1. Separation of pooled serum samples on 1D gel and image shows the gel segments used for LC/MS/MS analysis.



Benign

Invasive

Figure 2. peptides of inter- α -trypsin inhibitor identified from different gel segments in invasive sample.

Collaborative Research

The proteomics group at WRI established collaborative research relationships with Scientists at University of Pittsburgh and Wistar Institute to develop the predictive models on the proteomics datasets generated from serum samples and to develop novel concepts of sub-categorizing the breast cancers (triple negative) through isoform level gene expression and predicting each sub-category by the status of isoform specific gene regulatory networks and this will be addressed by combination of innovative computational modeling approaches and use of NextGen sequencing technologies. These collaborative research partnerships resulted in developing proposals to apply for NIH and DOD grants in Y 2010 and the abstracts for the proposals were given below.

Bayesian Rule Learning for Disease Prediction and Biomarker Discovery (Biomedical Informatics, University of Pittsburgh; Dr. Vanathi Gopalakrishnan)

The problem: High-throughput biomedical data from biomarker profiling studies aimed at early detection of diseases like breast cancer and lung cancer are accumulating rapidly. Although many popular machine learning methods have been utilized for analysis of such high-dimensional datasets, no single method has consistently outperformed others. Moreover, scientists have the need to simultaneously address two related tasks: disease prediction and biomarker discovery, using the same sets of data and tools. One way, as undertaken in this project, to address this need is to find the most accurate classifier for the disease from a given set of profiles and present the discriminative markers used in that model to the scientist for further verification. The large space of possible models coupled with the small sample size of the data make it hard to accurately estimate predictive accuracy.

The solution: This project will develop, evaluate and refine novel Bayesian Rule Learning (BRL) methods that are algorithmically efficient, result in parsimonious models and accurately estimate predictive uncertainty from sparse biomedical datasets. BRL methods utilize a Bayesian score to evaluate rule models, thereby quantifying the uncertainty in the validity of the rule itself. This novel technique that combines the mathematical rigor of Bayesian network learning with rule-based modeling opens up a hitherto underexplored area of fundamental research in informatics involving such hybrid methodologies. Rules enable modular representation of knowledge and collaboration with scientists, as it is easier to present the model and extract markers both visually and computationally. Rule-based inference is also simpler and more tractable. The Bayesian approach enables prior knowledge to be incorporated and evaluated in a continual fashion with a human in the loop. The latter is very important for refinement of both tools and models.

The specific aims: This project will test the hypothesis that the BRL methods developed and extended herein produce more accurate and parsimonious models for disease state prediction than other state-of-the-art machine learning methods. This project evaluates BRL methods and models using existing proteomic datasets for three diverse diseases – rare, neurodegenerative Amyotrophic Lateral Sclerosis (ALS), and the two most common cancers in the world, lung and breast cancers. Experimental verification will be performed using a new set of retrospectively collected breast cancer sera samples to evaluate model generalizability.

The significance: This project will produce: (1) a novel biomedical data mining tool for analyzing data from biomarker profiling studies of any disease, (2) methodological insights into the applicability of this tool and current machine learning methods for such tasks, and (3) new data for research on the early detection of breast cancer. It has potential to help develop new diagnostic tests for early detection of ALS, lung and breast cancers and lays a firm foundation for building modeling frameworks that can incorporate both prior knowledge and data to provide the technological capability for combining evidence from multiple, heterogeneous sources.

Isoform specific miRNA:mRNA regulatory networks in breast cancers (Molecular and Cellular Oncogenesis, Wistar Institute; Dr. Ramana Davuluri)

Triple-negative breast cancer (TNBC), lacking estrogen and progesterone receptor expression and *HER2* amplification, accounts for approximately 15-20% of breast cancer diagnoses. TNBCs comprise a heterogeneous group of very aggressive tumors with poor prognosis. Some TNBC tumors are similar to basal-like breast cancers (BLBC), which are characterized by elevated expression of basal cytokeratins (CK5/6, CK14), myoepithelial marker (p63) and HER1. However, the similarity between BLBCs and TNBCs is only partial as defined by high-throughput gene expression analyses. The lack in complete similarity may in part be explained by both BLBC and TNBC comprising entities that in themselves are heterogeneous in their molecular profile.

Recent mRNA-seq data suggest that more than 90% of the human genes, including many tissue specific and developmentally regulated genes, produce multiple transcript

isoforms, through alternative splicing and alternative usage of transcription initiation and/or termination. Notably, there is growing evidence linking aberrant use of alternative mRNA isoforms and cancer formation: several oncogenes and tumor-suppressor genes (e.g. LEF1, TP53, TP63, TP73, HNF4A, RASSF1, and BCL2L1) are already known to have multiple promoters and alternative splice-forms, and moreover, it is known that the aberrant use of one isoform over another in some of these genes is directly linked to cancerous cell growth. However, nearly after a decade from the completion of the human genome draft sequence, we still assume “gene” as the basic physical and functional unit in the genome (Hayden Nature 2010). We argue that “gene/transcript isoform” should be the basic functional unit and biologists should look at the differential regulation of gene isoforms rather than that of genes between normal and disease conditions.

Similarly, non-coding RNAs (ncRNAs) have emerged as key regulators at both transcriptional and post-transcriptional levels. The discovery of small ncRNAs of size 20-31 nucleotides has revolutionized thinking about the control of gene expression. Although in many cases the target of small RNAs and their mechanism is still not clear, it is certain that many of these small RNAs play important role in several biological processes including development timing, cell differentiation, cell proliferation, cell death, metabolic control, transposon silencing and antiviral defense. Several classes of small ncRNAs have been discovered such as small interfering RNAs (siRNA), micro RNAs (miRNAs) and Piwi-interacting RNAs (piRNAs). The best understood among the small ncRNA classes is miRNAs. MiRNA modulates post-transcriptional gene regulation by base pairing to either full or partial complementary sequences primarily in the 3' untranslated region (UTR), but also inside the coding sequence (CDS) and the 5' UTR, of the corresponding messenger RNAs (mRNAs). The miRNA:mRNA interactions affect mRNA stability and translation. In normal cells the miRNA:mRNA interactions need to be very precise in order to regulate specific gene isoforms post-transcriptionally. A miRNA can target a specific mRNA isoform but not the other isoforms of the same gene (due to inclusion/skipping of exons/exonic regions), leading to balanced expression of mRNA isoforms of same gene in a given cell type. Therefore, it is critical to address the problem of miRNA:mRNA interactions at isoform level rather than at gene level. We propose a novel idea that miRNA:mRNA interactions are disrupted more at isoform level than at gene level leading to imbalanced expression of gene isoforms in cancer cells. We, therefore, hypothesize that the heterogeneity in TNBCs can be characterized by genome-wide combined signatures of mRNA expression at isoform level and expression of miRNAs. And, consequently different sub-categories of TNBCs can be predicted by the status of isoform-specific miRNA-mRNA interactions involving tumor suppressor or oncogenic proteins, such as p53 family members.

We will use integrative biology approach by performing mRNA-Seq and small RNA-Seq experiments, using Illumina GA massive parallel sequencing, and efficiently predict the miRNA:mRNA interactions by data-mining of the mRNA-seq and small-RNA seq data to define homogeneous sub-categories of TNBCs.

This challenging problem will be addressed by combination of innovative computational modeling approaches and use of NextGen sequencing technologies. We will:

- (i) develop a knowledgebase of mammalian gene isoforms, expression patterns, and miRNA:mRNA interactions at isoform level by integrating cross-species genomic

- data and high-throughput transcriptome data, including recent massive parallel sequencing data from the cancer genome atlas (TCGA) data portal;
- (ii) conduct mRNA-seq to identify and quantify full-length poly-A transcript isoforms and small RNA-seq to quantify miRNAs and other small RNAs (e.g. miRNAs, piRNAs and siRNAs) in 40 TNBC primary tissue samples and 20 ER positive breast cancer tissue samples;
 - (iii) perform bioinformatics and statistical analysis of mRNA-seq and small RNA-seq data to identify major homogenous sub-categories of TNBCs;
 - (iv) perform integrative analysis of mRNA-seq and small RNA-seq data, and develop novel computational methods to model the combinatorial interaction of miRNAs in targeting the protein coding genes at isoform level, and predict different sub-categories of breast cancer based on the status of the miRNA:mRNA networks that are specific to each sub-category.

The proposed work will not only pave a new way to think about identification of novel cancer subtypes, but will also lead the research effort to understand the molecular mechanisms involved in the pathogenesis of different subtypes of TNBCs. Understanding the isoform-specific miRNA:mRNA networks is clearly central if we are to develop rational and mechanistic therapies for complex genetic diseases, such as breast cancer, that arise from the accumulated contributions of many gene-gene interactions and environmental factors. It is anticipated that this research will lead to most effective treatment, matched to their cancer profile, eventually improving the breast cancer survival rates.

Reportable outcomes

(Conference abstracts)

Ron Orlando, Craig Shriver, Bernard Seth, David Kirchner, Richard Mural, James Atwood, Kyle Jones and **V.S. Kumar Kolli**. Evaluating protein degradation products as serum biomarkers to determine the invasiveness of breast carcinoma. American Society for Mass Spectrometry (ASMS), Salt Lake City, UT, May 23-27, 2010.

David Kirchner, Richard Katenhusen, Tapan Maiti, Craig Shriver, Richard Mural, **V.S. Kumar Kolli**. Shotgun proteomics of breast cancer sera to identify the markers for early cancer detection. American Society for Mass Spectrometry (ASMS), Salt Lake City, UT, May 23-27, 2010.

BC-COE PILLAR # 4 - Biomedical Informatics

The Data Warehouse for Translational Research

We have developed additional workflow utilities for improving the quality and use of the data in the Data Warehouse for Translational Research (DW4TR).

- i) A new utility, the Data Correction Utility using the InforSense analytical workflow to correct data errors in the DW4TR, has been developed and

- deployed. This application was completely developed by the Biomedical Informatics staff. Currently it is in full use and continue to be improved based on user feedback.
- ii) Several IHC data fields have been revised to reflect the new needs including the final status of HER2 based on IHC and FISH, and loading of the newly generated data with improved granularity of Ki67 and p53 for invasive breast cancer samples is in progress.
 - iii) The sample selection utility using temporal information is in use and improvements have been made based on user feedbacks.
 - iv) We have established regular procedure and routine for data loading to the DW4TR: data are extracted from CLWS, applying the data processed by DCU, and then loaded into the DW.
 - v) After the acquisition of InforSense by IDBS there are substantial personnel changes. IDBS is committed to continue to focus on translational research. Several teleconferences have been held. In July, Dr. Hai Hu visited InforSense—now part of IDBS, in Burlington, MA, to review the development of a microarray data analysis workflow utility and to start the strategic planning of the future relationship between the two organizations. In November, several IDBS leaders visited WRI. We are planning on an additional strategic meeting late January.

LIMS development of the clinical component

- i) A cosmetic revision was done on the CLWS. The pre-printing rule was changed: additional questions in the questionnaire can now be pre-printed out in the CLWS for follow-up patients per request from the clinical staff.
- ii) The development of the new modules for clinical/tissue components in collaboration with GenoLogics was discontinued due to the directional problem of GenoLogics. The company apologized, and we successfully negotiated for a full refund of the pre-payment for the development. As a result, we plan to develop a system to replace the CLWS in-house.
- iii) Our plan is to develop the LIMS in modules, aiming to replace the CLWS that is currently in use but already obsolete.
- iv) With ProLogic serving as a development subcontractor, we have developed 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. This Pathology Checklist tablet data capturing system development with is now done with the first phase development. Additional revision will be needed before this can be integrated with the new LIMS
- v) The Komen Promise Grant won by a 5-member consortium led by Dr. Hallgeir Rui of Thomas Jefferson, has been in execution since January 2010. It is a 5-year grant aiming to screen the expression of 250 drug targets across 5000 breast cancer tissues. Dr. Craig Shriver of WRAMC, Mr. Albert Kovatich of MDR Global, Dr. John Eberhardt III of DecisionQ, and Dr. Hai

Hu of WRI are the other 4 leaders of the consortium. Execution of the Komen Grant has a positive impact on CBCP. The WRI Biomedical Informatics team is responsible for the biomedical informatics infrastructure including the development of a LIMS and expansion of the DW4TR to support the project, and we will also support data analysis and research project design. The development of the LIMS is underway, which is coordinated with the development of the LIMS for CBCP CLWS replacement. One important component of the LIMS, the data entry form for the clinicopathologic data of the breast cancer cases, has been developed and is currently in use, which is useful for the development of the BC-COE outcome data form.

- vi) One experienced developer was hired on December 20, to focus on LIMS development for both this project and the Komen Promise Grant.

IT Infrastructure and Support

Historically, WRI IT support was outsourced, and from its inception the WRI IT infrastructure grew organically. We have reached a point where the whole system needs to be completely redesigned to lay the foundation for future research and support activities of the next phase of the research at WRI. With the help of professional consultants and a professional IT infrastructure company, we have completely redesigned our IT infrastructure. The new system applies virtual machine technology, with 4 servers hosting all the applications that were acquired through the 10 years of WRI's history. The servers have 32 GB memory each that are shareable, with a total of 36 TB shareable storage that can be further expanded as needed. The new network is supported by Level 3 with a speed of 1 Gbps

Effective January of 2011, Biomedical Informatics will provide WRI IT support as a newly added responsibility. For this purpose, a new IT Manager and an IT Support Specialist have been hired on December 20 and November 15, respectively. The original person in charge of the infrastructure re-structuring is no longer with WRI effective November 11. This change of personnel suspended the virtualization of the applications which has now been resumed. The status of the main tasks are shown below:

- i) Procurement of all hardware complete
- ii) Hardware installation complete, which includes network hardware, servers, and virtual storage
- iii) Network hardware configuration complete
- iv) Cut-off of WRI from the MANVT network complete and now Level 3 providing internet connectivity for WRI
- v) Virtualization of current physical servers – on going

SOP for clinical data entry (core questionnaire) have been reviewed and updated

- i) In collaboration with WRAMC, WRI team combined two SOPs (one for nurses and one for data entry) into single document. During this process, a number of discrepancies between these documents has been corrected and all changes to SOP have been highlighted
- ii) WRI team visited WRAMC and presented new SOP document to the clinical data staff. During the presentation, additional clarifications and corrections were made.

- iii) The WRI team also reviewed, clarified, and updated the SOP with the JMBCC nurses.
- iv) The SOP is now in a final version—but we are maintaining it as a ‘live document’ so new issues coming out from the data collection/QA/data entry process will be incorporated.

Research Project

Three research projects formed the bases of three conference poster presentations, see “Presentations”.

In addition, the analysis of peripheral blood gene expression of invasive cancer patients and normal subjects is in the final stage and manuscript preparation has been started.

Manuscripts

- i) Hu H, Correll M, Kvecher L, Osmond M, Clark J, Bekhash A, Schwab G, Gao D, Gao J, Kubatin V, Shriver CD, Hooke JA, Maxwell LG, Sheldon JG, Liebman MN, and Mural RJ. DW4TR: A data warehouse for translational research (submitted).
- ii) Bekhash A, Maskery SM, Kvecher L, Correll M, Zhang Y, Hooke JA, Liebman MN, Shriver CD, Mural RJ, and Hu H. Clinical Breast Care Project data warehouse as a research environment for breast cancer risk factor studies (submitted).
- iii) Zhang Y, Kvecher L, Sun W, Gutchell EM, Mural RJ, Shriver CD, Liebman MN, and Hu H. A quality assurance issue tracking tool to facilitate the enhancement of clinical data quality (in revision).

Presentations

- i) Li X, Rapuri P, Melley J, Brillhart G, Wu W, Kvecher L, Deyarmin B, Progar C, Hooke JA, Shriver CD, Mural RJ, and Hu H. Comparative analysis of gene expression profiles in human breast cancer from microarray data using breast tissues and peripheral blood samples. 18th Annual International Conference on Intelligent Systems for Molecular Biology. July 10-13, 2010. Boston, MA. (A poster presentation at the world’s largest bioinformatics conference. The lead author Dr. Xing Li won another travel award with an award rate of 30%.)
- ii) Bekhash A, Saini J, Li X, Rapuri P, Hooke JA, Kovatich AJ, Mural RJ, Shriver CD, and Hu H. Ethnicity Difference of Benign Breast Diseases in Breast Cancer and Non-Cancer Patients. Accepted by *The 33rd San Antonio Breast Cancer Symposium*. December 8-12, 2010. San Antonio, TX.
- iii) Saini J, Li X, Kvecher L, Larson C, Croft D, Yang YC, Hooke JA, Shriver CD, Mural RJ, and Hu H. Differential Gene Expression Analysis among Postmenopausal Caucasian Invasive Breast Cancer, Benign and Normal Subjects. Accepted by *The 33rd San Antonio Breast Cancer Symposium*. December 8-12, 2010. San Antonio, TX.
- iv) With the support of the consortium that won the Komen Promise Grant, Dr. Hai Hu organized a panel and led the development of a panel proposal entitled

- “Therapy-relevant stratification of breast cancer: integrating pathology and quantitative biomarker analyses”, which was accepted by the 2011 AMIA Summit on Translational Bioinformatics for presentation next March in San Francisco, CA.
- v) A speakers proposal entitled “A data warehouse for translational research”, submitted to BIO-IT World Conference 2011, was accepted for presentation in April at Boston, MA.

Other projects

- i) A document highlighting Biomedical Informatics activities has been created, which will be suitable for use on the BC-COE website after cosmetic improvement.
- ii) Re-organized the storage of hard-copy questionnaires for easier access. We are discussing the possibility of scanning in all the hard-copies and archiving them.

BC-COE PILLAR # 5 Clinical Care:

The objectives of the Clinical Care Pillar are to:

- a) Decrease the negative psychological impact on the patient of having an evaluation or treatment intervention for breast disease by utilizing objective measurement instruments to longitudinally assess the patient’s psychological response to evaluation and intervention, and base modifications of these procedures on those results.
- b) Create and maintain an environment (medical, physical, psychological) conducive to the multiple needs of the patient undergoing breast disease evaluation / treatment.
- c) Recruit patients into the various BC-COE protocols to obtain the clinical data and biospecimens needed to meet the BC-COE’s translational research goals.

This pillar of the BC-COE is the foundation upon which all the success of and project rests. Without patients enrolled in our biospecimen repository protocols, there would be no translational research center of excellence. These patients come from the clinical care environment. Since its inception in 2000, the CBCP (now the BC-COE) has had as a priority, the development and staffing of the core clinical centers at Walter Reed Army Medical Center, the Joyce Murtha Breast Care Center in Windber, PA and at our newest site, the Pat and Lesly Sajack Breast Center at Anne Arundel Medical Center in Annapolis, Maryland, that, under the direction of Lorraine Tafra, MD sees more than 500 newly diagnosed cases of breast cancer each year.

At each center the Staff is dually trained as clinical/research Providers, to seamlessly integrate the need for a strong research focus in the clinical center with the requirement to provide state-of-the-art clinical care to the patients.

The reputation of the BC-COE is that of an exceptional translational research project with very possibly the world's most pristine collection of breast tissue. This has resulted in a number of well respected medical centers expressing interest in joining us as research partners. Most recently, The Altoona Regional Health System has expressed interest in implementing our Core Protocols and becoming a partner as well.

The care of our patients is provided by Physicians and Advance Practice Nurses (Nurse Practitioners) and with all personnel having as their prime job description, the research aspects of the BC-COE.

Both the Walter Reed and Windber sites have built state-of-the-art comprehensive breast centers and our partners at Anne Arundel are about to open a brand new cutting-edge breast center. They have added 4 additional exam rooms, a bariatric exam room with a two procedure rooms, a counselor's office, conference room, patient library, multiple consult rooms to meet with patients & families, and a clinical trials room. At the Walter Reed site, the space for the new breast center was designated and approved by the command and in-depth architectural design and development plans were made in conjunction with the Patient Advocacy Group, which consisted of military and civilian breast cancer survivors.

The Comprehensive Breast Center at Walter Reed Army Medical Center is comprised of a Reception Area, Waiting Room/Patient Education Area and Quiet Room, Patient Examination Rooms, A Surgical Suite, Doctors and Nurses Work Stations, A Conference Room and Pathology Lab. Go to "[Tour the Breast Center](http://www.cbcp.info)" at our newly updated website www.cbcp.info to see the facility.

The TEMPLATE Day

At the Walter Reed Comprehensive Breast Center, we see between 5,000 and 6,000 patients per year and diagnose approximately 120 new breast cancers per year.

The TEMPLATE Day is a particularly significant accomplishment for the Walter Reed Comprehensive Breast Center. The purpose of this day is to: Provide an opportunity for the newly diagnosed breast cancer patient to meet all the providers that comprise the interdisciplinary breast care team. Providers include a breast surgeon, a medical oncologist, a radiation oncologist, a psychologist, nurse navigators/case managers, a physical therapist, and a plastic surgeon. Each specialty has individual private appointments to assess and evaluate each patient who, with significant others of their choice, is given a private room for the day. The benefit of the TEMPLATE day is a one day visit to see all the various providers instead of having individual appointments spread over several days or weeks.

This allows us to educate, facilitate and coordinate a comprehensive breast treatment plan for the patient that maximizes treatment options and streamlines patient care in a patient-focused environment.

It also allows us to discuss the various research protocols with patients and, if they agree, obtain informed written consent and complete with the assistance of a research nurse an extensive questionnaire that captures more than 500 fields of clinical data.

The breast care team assembles and conducts an interdisciplinary conference to discuss each patient's case, resulting in a comprehensive treatment plan built on a team consensus. The results of the conference are then reviewed with the patient/family and time is provided to clarify and ask questions.

In an article entitled "*Hidden in Plain View – Integration of Effective Patient Partnerships with Evidence Based Medicine in the Military Health System –The Walter Reed Army Medical Center Clinical Breast Care Project*" the TEMPLATE day is described by the following quote "This method of physician communication and consensus avoids conflicting messages to the patient and allows for the best evidence-based approach. The literature supports the notion that a group decision is superior to sequential individual ones. Staff and patient satisfaction, stability of staff retention, and continuous improvement attitude, creates optimal outcomes in a safe, high quality, and supportive, attractive physical environment. As Residency Director, clearly COL Shriver leads by example and has an impact on physicians during their graduate medical education by experiencing how idealized care can be operationalized in a military setting. This model of care should certainly be considered as the National Capital Area moves forward with the merger of Walter Reed Army Medical Center with the National Naval Medical Center and becomes the Walter Reed National Military Medical Center (WRNMMC) in 2011."

IV. KEY RESEARCH ACCOMPLISHMENTS

- Further enhancements have been made to 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. 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 and the underlying patient centric data model is being modified to support other types of disease.
- Gene expression difference have been found between African American women and Caucasian American women that may lead to insights into the differences in breast cancer severity seen between these populations. Recently we generated gene expression data from both tumors and non-malignant tissues from AAW and CW. 18 genes were differentially expressed in tumors and 13 genes in non-malignant breast specimens, including PSPHL, SOS1 and CRYBB2, which are differentially expressed in both tumors and non-malignant tissues.
- The Biomedical informatics core has continued development of the patient centric data model, enhanced tools for microarray data QA, and further analysis of breast disease co-occurrence.
- In 2010, we published the results of our Allelic Imbalance evaluation in non-neoplastic diseases, concluding that ADH and CCH are in fact, genomically naive and that the multitude of studies that have evaluated pre-neoplastic lesions from

breasts with invasive breast tumor cannot represent the status of pure early lesions.

- Copy number evaluation is being performed on fresh frozen genomic DNA samples from women with breast cancer, and a variety of pathological classifications using Affymetrix 500K SNP chips. Copy number and LOH analysis is being performed using Affymetrix genotyping console. Preliminary data were presented at the AACR meeting in April.
- In collaboration with Pacific Northwest Laboratory (PNNL) using the previously developed Accurate Mass Tag data base for proteins expressed in breast tissue we have examined breast tumors for the proteins that are markers of metastasis to lymph nodes.
- Work continues on a 6.7 million dollar Komen Promise grant awarded to Hallgier Rui of Thomas Jefferson University in 2009. 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”** As for the progress, Aim 1 of the grant involves the block and clinical data collection efforts and most of our work has been directed at this activity. The CBCP planned to submit approx. 500 cases. To date, we have collected blocks on 263 cases and have completed the clinical data forms on 196 cases. Of these 196 forms, we have performed a Quality Control check on 61 of them and sent them to the bioinformatics team for data entry testing. From the CBCP perspective, the next few months will also be directed at collecting blocks on the remaining 237 cases (approx.) and completing the completion and QC of all data forms.
- **The Cancer Genome Atlas project for NCI:**
The Material Transfer Agreement and Data Use Agreement have been completed for the TCGA Biospecimen Core Resource and IRB approval to participate in TCGA has been granted. In October, twelve eligible cases were shipped to TCGA and we were notified in November that all 12 qualified. We are in the process of collecting clinical data (enrollment, pharmaceutical therapy and radiation therapy data) on these 12 cases and will have entered the information in OpenClinica by 1/10/11. We are also collecting data on 5 additional cases which, based on new TCGA criteria, were qualified in December. During the past quarter we have also identified nineteen new patient samples that could be screened for eligibility in the TCGA project. These cases have been screened for prior malignancy and other clinical history that might compromise their utility in the program. Eligible cases were then reviewed for pathology inclusion metrics including diagnosis, tumor nuclei and necrosis. Shipment of those deemed eligible is scheduled for late January. We are approaching 50% completion as CBCP was scheduled to send approximately 156 cases and 74 have been sent.

V. REPORTABLE OUTCOMES

The CBCP Research Protocols and number of subjects recruited to each for the period January 1, 2010 to December 31, 2010 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 - **09**
- Tissue and Blood Library Establishment for Molecular, Biochemical and Histologic Study of Breast Disease – **175**
- 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 - **45**
- Tissue and Blood Library Establishment for Molecular, Biochemical and Histologic Study of Breast Disease – **65**

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 – **212**

Psycho-Social Oncology Services:

A dedicated psychologist assessed 88 new Friday Template breast cancer patients and counseled an additional 18 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.

Colonel Craig Shriver, M.D., MC, director of the Clinical Breast Care Project (CBCP), received the Impact Award bestowed by the National Consortium of Breast Centers at its 20th annual National Interdisciplinary Breast Center Conference, which took place in Las Vegas in March. The award recognizes individuals who have had a significant impact in the areas of general breast health, breast health care administration or breast center development.

Dr. Shriver was named the Chairperson of the Integration Team of the Comprehensive Cancer Center charged with ensuring that the new WRNMMC Cancer Center has attributes clearly recognized as World Class and lead to National recognition and designation.

VI. CONCLUSIONS

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.

VII. REFERENCES

N/A

VIII. 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 January 1, 2010 – December 31, 2010

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%
Chestang	Allan	Data Manager	100%
Chou	Carolyn	Budget Analyst	50%
Hamsher	Walter	Budget Analyst	50%
Courville	Faith	Research Nurse	33%
Cronin	Kerri	Administrative Assistant/ Office Manager	50%
Del	Ismail	Data Manager	100%
Eckhauser	Peggy	Research Nurse	100%
Ellsworth	Rachael	Genetist	100%
Enowold	Lindsey	Biostatitician	8%
Fantacone	Jamie Leigh	Pathology Assistant	100%
Gutchell	Veronica	Head Nurse, CBCP/ Nurse Practitioner	100%
Hamsher	Walter	Budget Analyst	25%
Hilton	Karrie	Research Nurse	100%
Hodgson	Carol	Nurse Practitioner	100%
Hooke	Jeffrey	Head of Pathology	100%
Kelley	Kay	Research Protocol Coordinator	100%
Leuth	Sunita	Administrative Assistant/ Office Manager	25%
Means	Marilyn	Lab Technician	100%

Miller	Donald	Data Manager	100%
Patterson	Carol	Medical Assistant	100%
Progar	Tina	Biomedical Informatics Coordinator	100%
Reece	Heike	Data Manager	100%
Rojas	Winifred	Lab Tech/Phlebotomist	100%
Rosenquist	Monica	Budget Analyst	100%
Smith	Stephanie	Research Nurse	100%
Stojadinovic	Alexander	Breast Surgeon	25%
Tracey	Dianne	Administrative Assistant/Office Manager	25%
Vilakasi	Patricia	Research Nurse	100%
Wareham	Janet	Pathologist's Assistant	100%
Williamson	Eric	Clinic Administrator	100%
Weiss	Raymond	Surgeon, Staff Researcher	100%
Yambaka	Baredu	Receptionist	100%
Zhao	Xinyan	Histology Technician	100%
Zhu	Kangmin	Epidemiologist	8%
Zhuo	Jing	Biostatistician	8%
Zingman	Rebecca	Histology Technician	100%

ATTACHMENT 2

PUBLICATIONS – 2010

Stojadinovic A, Eberhardt C, Henry L, Eberhardt J, Elster E, Peoples G, Nissan A, Shriver C. **“Development of a Bayesian Classifier for Breast Cancer Risk Stratification: A Feasibility Study”** *Eplasty*, vol 10, pp 203-216, 29 Mar 10, ISSN: 1937-5719. Open Science Co. LLC, Springfield, IL.

Bekhash A, Maskery S, Kvecher L, Correll M, Zhang Y, Hooke JA, Liebman MN, Shriver CD, Mural RJ, Hu H. **“Clinical Breast Care Project Data Warehouse as a Research Environment for Breast Cancer Risk Factors Studies”** *J Biomed Info*

Ellsworth DL, Ellsworth RE, Hooke JA, Shriver CD. **“Human Genetic and Structural Genomic Variation: Would Genome Wide Association Studies be The Solution of Cancer Complexity Like Alexander The Great for The ‘Gordian Knot?’ ”** *AnnSurgOncol* (2009) 16:776-777. DOI: 10.1245/s10434-008-0255-x

Ellsworth RE, Hooke JA, Love B, Ellsworth DL, Shriver CD. **“Molecular Changes in Primary Tumors and the Nottingham Histologic Score”** *PatholOncolRes*, 2009 Dec; 15(4): 541-7. DOI: 10.1007/s12253-009-9151-3. Aranyi Lajos Foundation 2009. PIMD: 19194786.

Buckenmaier III CC, Kwon KH, Howard RS, McKnight GM, Shriver CD, Fritz WT, Garguilo GA, Joltes KH, Stojadinovic A. **“Double-Blinded, Placebo Controlled Prospective Randomized Trial Evaluating the Efficacy of Paravertebral Block With and Without Continuous Paravertebral Block Analgesia in Outpatient Breast Cancer Surgery”** *Pain Medicine* 2010; 11:790-799. Wiley Periodicals, Inc.

Ellsworth RE, Ellsworth DL, Weyandt JD, Fantacone-Campbell JL, Deyarmin B, Hooke JA, Shriver CD. **“Chromosomal Alterations in Pure Breast Lesions: Implications for Breast Cancer Progression”** *AnnSurgOncol*, 27 Jan 2010. PMID: 20107913 [PubMed]

ABSTRACTS – 2010

“Chromosomal alterations associated with poor outcome in breast cancer patients”
Ellsworth RE, Fantacone-Campbell JL, Valente A, Shriver CD. SABCS, San Antonio, TX 8-12 Dec 2010

“Genetic Discrimination of Aggressive from Indolent DCIS”
Ellsworth RE, Deyarmin B, Patney H, Shriver CD, Ellison K, Thronton D, Tafra L, Cheng Z, Rosman M. SABCS, San Antonio, TX 8-12 Dec 2010

“Genetic signature discriminating metastatic from non-metastatic small tumors”

Ellsworth RE, Valente AL, Field LA, Kane JL, Love B, Tafra L, Shriver CD. SABCS, San Antonio, TX 8-12 Dec 2010

“Role of chromosome 16q in breast tumorigenesis”

Ellsworth RE, Patney HL, Kane JA, Hooke JA, Shriver CD. SABCS, San Antonio, TX 8-12 Dec 2010

“Comparative analysis of gene expression profiles in human breast cancer from microarray data using breast tissues and peripheral blood samples”

Li X, Rapuri P, Melley J, Brillhart G, Wu W, Kvecher L, Deyarmin B, Progar C, Hooke J, Shriver C, Mural R, Hu H. Molecular Biology Conference 11-13 Jul 2010, Boston, MA

“Breast Cancer Metastasis Gene Signature: The Search for the Holy Grail”

Ellsworth RE, Field LA, Love B, Kane JL, Hooke JA, Shriver CD. American Society of Clinical Oncology (ASCO), Jun 2010, Chicago, IL

“Genomic Instability of Breast Stroma”

Rummel S, Ellsworth RE, Kane J, Deyarmin B, Hooke JA, Ellsworth DL, Shriver CD. American Association for Cancer Research (AACR) 17-21 Apr 2010, Washington, DC

“Copy Number Variation in Low-and High-Grade Breast Tumor”

Ellsworth RE, Patney HL, Kane JL, Hooke JA, Shriver CD. American Association for Cancer Research (AACR) 17-21 Apr 2010, Washington, DC

“Chromosomal Alterations in Pure Breast Lesions: Implications for Breast Cancer Progression”

Ellsworth RE, Ellsworth DL, Weyandt JD, Fantacone-Campbell JL, Deyarmin B, Hooke JA, Shriver CD. Society of Surgical Oncology (SSO), 3-7 Mar 2010, St Louis, MI

“Molecular Characterization of Breast Cancer Progression: Early Lesions are not Genetically Advanced”

Ellsworth RE, Weyandt JM, Fantacone-Campbell JL, Deyarmin B, Ellsworth DL, Hooke JA, Shriver CD. Society of Surgical Oncology (SSO) 3-7 Mar 2010, St Louis, MO

“Is a Diagnosis of Invasive Breast Cancer an Effective Motivational Factor for Lifestyle Change?”

Ellsworth RE, Sann NJ, Kvecher L, Ellsworth DL, Shriver CD. National Consortium of Breast Centers (NCBC), 20-24 Mar 2010, Las Vegas, NV