THE JOHN fettingfund FOR BREAST CANCER PREVENTION

PROJECT UPDATES 2023

BACKGROUND

The John Fetting Fund is an endowment that supports basic and translational breast cancer prevention research performed by or under the direct supervision of the faculty of the Johns Hopkins Breast Cancer Program. The priority of the Fund is genomic and epigenomic research, which distinguishes women at risk for breast cancer (and its consequences) from women not at risk. By refining our understanding of who is at risk, we can focus expensive and involved health care on those at risk and reassure those who are not. The Fund supports preliminary studies which position investigators to secure larger grants. The Fund will also provide stopgap or interim funding for promising work that has run out of funding while the investigator seeks more substantial funding.

EMILY AMBINDER, M.D. and JESSICA TAO, M.D.

Pilot Study to Evaluate the Role of Circulating Tumor DNA in Breast Cancer Detection

While breast cancer remains the second leading cause of cancer-related death for women, the mortality associated with breast cancer has decreased over the past three decades, in part because of implementation of mammography screening, which enables diagnosis at an earlier, more treatable stage. However, mammogram screening is not perfect. Between 60 to 80% of women who have abnormal findings on mammogram and need to undergo additional imaging or biopsy will ultimately be found to not have cancer. These false-positive screening results and subsequent unnecessary procedures cause significant physical, emotional, and financial burden. Additionally, not all cancers are detectable by screening mammogram. Cancers are more likely to be missed in certain groups of women, particularly those with dense breasts. Thus, despite the benefits that have been achieved with mammography, there remains an urgent need to improve breast cancer screening to both reduce over-diagnosis and overtreatment of false-positive, ultimately benign lesions, and to decrease missed diagnoses.

Jessica Tao Emily Ambinder

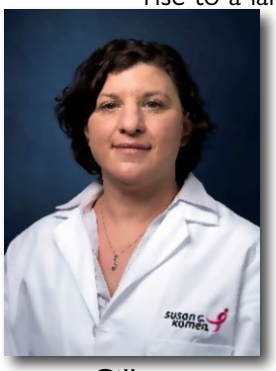
One potential tool to address these issues is liquid biopsy, an emerging technology in which pieces of free-floating DNA, called cell-free DNA (cfDNA), can be analyzed from a blood sample. In people without cancer, most cfDNA is derived from normal tissue turnover, predominantly from white blood cells. For patients with cancer, a portion of the cfDNA is derived from the tumor, and contains evidence of the genomic changes found in the tumor. For patients with early stage disease, the proportion of cfDNA that is tumor-derived can be very small, making detection very difficult. Fragmentomics is a newly developed approach that utilizes the fact that cfDNA pieces are not randomly broken. Instead, the pattern of fragmentation reflects the underlying biology of the cells from which the cfDNA is derived, such as the location of chromatin or the binding of transcription factors, and which can be different in cancerous versus normal cells. Because this technique looks broadly across the entire genome at thousands of potential differences, fragmentomics can enable the detection of very small amounts of tumor-derived cfDNA.

The team initiated EARLY, a pilot study of individuals with a breast abnormality to determine the potential role of ctDNA breast cancer detection and prevention. This study opened to enrollment in December 2022, and to date, nearly 60 participants have enrolled. Women and men receiving a breast biopsy will provide a blood sample before biopsy to evaluate the accuracy of ctDNA for breast cancer detection. Findings from cfDNA tests will be compared to and evaluated with imaging and pathology, and initial analyses from participants will begin this fall. Initiation of the study enabled a successful small grant awarded by Earlier.org. Additionally, team members have submitted a grant application for a multi-year Breakthrough Award Level 3 grant through the Department of Defense Breast Cancer Research Program. The Fetting Fund kick-started the first step in a multiyear project where Johns Hopkins will become a leader in the use of cfDNA in breast cancer detection and prevention. By developing a novel noninvasive, relatively low cost method to improve the accuracy of breast cancer screening, we hope to decrease mortality and ultimately accelerate progress towards ending breast cancer.

DANIELE GILKES, MS, Ph.D.

Understanding the role of breast density in preventing breast cancer

Breasts are made up of fat, fibrous tissue, and glandular tissue. Glandular and fibrous tissue cause breasts to appear dense on a mammogram and contribute to mammographic density. Mammographic density has been associated with an increased risk of developing breast cancer. Low breast density is independently and inversely associated with breast cancer risk. In fact, the risk associated with density is more significant than breast cancer associated with a family history of the disease or menstrual and reproductive risk factors.



Gilkes

Due to the increased risk of developing breast cancer, women with dense breasts may be offered additional screening to improve the sensitivity of detecting cancer. But this also increases false-positive results, leading to unnecessary invasive procedures such as biopsies. Interventions aimed at reducing mammographic density, such as prophylactic treatment with tamoxifen or raloxifene, have also been used as a preventive treatment for breast cancer. But such interventions have undesirable side effects and are only warranted for women who will go on to develop breast cancer. How do we identify these women? The Gilkes lab aims to determine why some women with dense breasts go on to develop breast cancer while others do not.

Dr. Gilkes and her team have demonstrated that fibrous tissue can act as a sponge holding growth factors that feed adjacent mammary epithelial cells, just as sea sponges contain nutrients for nearby fish. We have shown that breast cells placed in a fibrous environment can develop cancer stem-cell (CSC) like properties. The team has further determined that breast cells with high levels of a protein called fibroblast growth factor receptor (FGFR) are more likely to develop into CSCs than cells with lower levels. CSCs are believed to be responsible for initiating tumor formation and growth. Even a small number of CSCs can give rise to a large mass of cancer cells. The results of these studies are currently being assembled for publication.

With this new knowledge, Dr. Gilkes is now working to write a grant proposal aimed at (1) determining whether FGFR could be a biomarker used to identify the subset of women with high breast density that are most at risk of developing breast cancer (2) using preclinical models to determine whether FGFR inhibitors can be used as preventative therapy. FGFR inhibitors are a topic of active research and clinical trials and can be small molecules taken orally or antibodies administered intravenously.

DIPALI SHARMA, Ph.D.

Honokiol as a Chemopreventive for Breast Cancer

Honokiol is a bioactive compound purified from the bark of magnolia tree. The lab group showed that Honokiol can inhibit the growth of breast cancer cells, and identified the pathway how this compound may work. After defining the biomarkers of efficacy, the group examined the effectiveness of this compound in mice. Indeed, it inhibited breast cancer growth in mice but it did not abolish it completely. The group found that Honokiol induces a parallel mechanism known as cytoprotective autophagy, which may protect cancer cells to some extent. To overcome this, they propose using Honokiol in combination with Chloroquine to improve its anticancer potential. The team has also tested intra-dermal (through skin) delivery of this compound. The research team is continuing with the development of this compound, and is seeking grant support from the NCI-PREVENT program for further development.


Sharma

Bacteria and breast cancer

Breast cancer can develop in women and men with no apparent risk factors, and women with similar clinical profiles respond differently to prevention and treatment strategies. The team is working to uncover the changes in gut and/or breast bacteria (microbiome) which may modulate breast cancer initiation and response to therapy. The team is collecting stool samples from breast cancer patients to analyze their gut bacteria. These studies will uncover important bugs and establish us in the microbiome-breast cancer field. The team recently showed that bacteria not only change the breast cells but also change the immune cells in the breast. Another interesting aspect is that some pathogenic harmful bacteria secrete toxins which may help with cancer initiation. These studies are aiming to identify harmful bugs in breast as well as develop strategies to inhibit them. These studies acquired additional support from the Department of Defense.

Obesity and breast cancer

Obesity is an important risk factor for breast cancer—a 5-unit increase in BMI is associated with a 12 percent increase in breast cancer risk. In the United States, about 36 percent of adults are obese, and the prevalence is increasing globally. The group is uncovering new pathways as well as developing strategies to abrogate obesity-breast cancer axis and improving therapeutic response in obese women. Obesity is also associated with microbial dysbiosis hence the group is collecting stool and

serum samples from women undergoing weight loss intervention in a clinical trial setting. The study aims to identify any tangible ways to inhibit the harmful pro-cancer effects of obesity on breast cancer. Also, the group uncovered how obese state interferes with tamoxifen efficacy, and is now working towards developing some interventions. This is especially important as tamoxifen is used as a preventive measure.

SARASWATI SUKUMAR, Ph.D.

DNA methylation in suspicious-looking breast tissue for early detection

It is estimated that one hundred and ninety-nine times out of 200 times, a mammographic abnormality or a palpable mass turns out to be a benign lesion. This is because mammography is very sensitive and makes abnormalities in the breast visible. However, the anxiety accompanying these findings is intense until biopsy is performed and the pathology results confirm that the lesion is harmless. In a test that is currently undergoing rigorous testing in South Africa where routine breast cancer screening is not available, the Sukumar laboratory has developed an automated molecular test that examines cells from the lesions, drawn out from the breast with a fine needle. The test looks for cancer-specific alterations called DNA methylation in a panel of 6 genes in the cells. The test, run on a GeneXpert system, is completed within 2 hours, and can be done at the clinic with minimal technical expertise. The test could eventually reduce the number of biopsies, and cut time from finding a mammographic abnormality to diagnosis to a few hours. Pilot funding from the Fetting Fund has allowed the Sukumar laboratory to garner an NIH- ROI for an academic-industrial partnership to test and improve the cartridge, moving it closer to commercialization.


Sukumar

Similar tests are being developed along with Drs. Kala Visvanathan, Vered Stearns and Antonio Wolff for detecting cancer specific circulating methylated DNA in the blood- a liquid biopsy test that can be repeated over time to ascertain tumor load status of the patient during and after treatment. This study has also been recently funded by the DOD to conduct further investigation in the earlier stages of the disease.

KALA VISVANATHAN, M.D., M.H.S.

Evaluate the relationship between obesity and tamoxifen resistance

Mechanistic studies generated by Dr. Sharma's team strongly suggest that the upregulation of the Leptin receptor-Med1 axis in obese women may contribute to tamoxifen resistance. Acquired tamoxifen resistance could in turn impact disease progression and the effectiveness of tamoxifen to prevent breast cancer. To establish whether obesity modifies the relationship between tamoxifen and breast cancer progression we examined mortality in 8429 women with Stage I-III estrogen receptor positive breast cancer who were initiated on endocrine therapy. These women were part of the National Comprehensive Cancer Network Breast Cancer Outcomes Database (N = 25, 865).


Visvanathan

Consistent with their hypothesis, the team observed a statistically significant interaction between body mass index (BMI) and type of endocrine treatment with respect to breast cancer mortality. Mortality from breast cancer was higher among women taking adjuvant tamoxifen who were overweight or obese compared to women with a normal BMI taking tamoxifen and women taking aromatase inhibitors irrespective of their BMI. To our knowledge this is the first study to demonstrate an interaction between tamoxifen and obesity with respect to breast cancer mortality. A previous study by Sparano et al demonstrated a relationship between hormone therapy and obesity but was unable to look at differences by treatment. The team is currently putting together this manuscript. Given their findings, the team plans to validate preclinical work that demonstrated an association between upregulation of Leptin and Med 1and tamoxifen resistance in obese women. Moving into the preventive setting we would like to test whether the relationship between tamoxifen and BMI persists in women with DCIS treated with tamoxifen.

Dr. Visvanathan applied to NRG oncology for access to data and samples from the NSABP prevention trials but were unsuccessful despite a strong application. They are now looking for other DCIS trial samples.

CYNTHIA ZAHNOW, PhD.

Identification of Alcohol-Induced Alterations and Increased Risk of Breast Cancer

Breast cancer is the most commonly diagnosed and the second leading cause of cancer related death amongst women in the US. Lifestyle choices and diet are associated with increased risk of breast cancer and over 80 epidemiological studies have demonstrated that alcohol consumption consistently predicts for a 5%-45% (1-4 drinks/day respectively) increase in the risk of developing breast cancer. Although 4% of new breast cancers each year in the US are

attributable to alcohol consumption, the underlying genetic and epigenetic mechanisms remain unclear.


Zahnow

Mutations play a significant role in the development of breast cancer and 10-15% of breast tumors are associated with defects in DNA repair, including mutant BRCA1 and BRCA2 genes. Alcohol is a well-established mutagen that causes several types of DNA damage. We characterized the mutational burden in human mammary epithelial cells (HMECs) exposed to alcohol and correlated the alcohol-induced DNA mutations with established mutational signatures in the Catalogue of Somatic Mutations in Cancer (Cosmic). Several single base substitution signatures (SBS) were identified in alcohol treated HMECs, and the most significant signature was SBS3, which is observed in 357/915 breast cancers and strongly associated with BRCA1 and BRCA2 mutations and BRCA1 promoter methylation. The SBS3 mutational signature may serve as a predictor of defective DNA repair accompanying loss of BRCA1/2 and may thus help to predict response to cisplatin and PARP inhibitors. Although alcohol consumption has not been shown to increase the risk of breast cancer in women with BRCA1/2 mutations, we hypothesize that alcohol may create a BRCA1/2 "mutant-like" phenotype in breast cells via the inactivation of genes involved in DNA repair.

In addition to the SBS3 mutational signature, numerous other mutated genes were identified in our alcohol treated HMECs. The most frequently mutated gene in 100% of samples was TAF1. TAF1 is mutated in 2.51% of breast carcinoma patients, is known to be associated with alcohol consumption, and has been identified as a hub gene for numerous other genes that co-methylate across the genome of normal breast and breast cancer samples to regulate gene expression.These co-methylation data will provide important insight between the relationships and associations between different genes in normal breast tissue and breast cancer. Taken together these data support the hypothesis that the methylation and mutational status of TAF1 can be altered by alcohol and may be linked with an increased risk of breast cancer.

Drs. Zahnow, Visvanathan, and other colleagues are submitting the following paper for publication soon: Alcohol consumption does not modify the polygenic risk score-based genetic risk of breast cancer in postmenopausal women: the Atherosclerosis Risk in Communities (ARIC) study.

FETTING SCHOLARS & PUBLISHED STUDIES SUPPORTED BY THE FETTING FUND

Dr. Kala Visvanathan was the inaugural Fetting Fund Scholar in 2022. She is an expert in breast cancer prevention. As a Scholar, she contributed to extensive research related to risk and prevention of breast cacner. She is also engaged in many national and intenational activities setting guidelines.

Dr. Dipali Sharma was the second Fetting Fund Scholar in 2023. In her own words Dr. Sharma states: "If not for the support from Fetting Fund for Breast Cancer Prevention we would not be able to push through some of our innovative cancer prevention ideas. It is important to note that support for 'cancer prevention research' from NIH, DOD and pharma is very limited hence it is very hard to conduct cancer prevention research. Fetting Fund provided support to conduct the studies related to the development of Honokiol as a potential preventative as well as develop a better understanding of the risk factors including obesity and harmful bugs in gut and breast. We are currently conducting various translational and clinical studies related to breast cancer prevention from identifying the fine points of some risk factors to developing prevention strategies."

We are pleased to announce that Dr. Cynthia Zahnow is the 2024 Fetting Fund Scholar. As Fetting Scholars, each of the researchers has also engaged in additional activities to promote research of breast cancer prevention. The work has led to presentations in national academic meetings and to numerous publications in scientific journals.

DR. SHARMA'S PUBLICATIONS:

Title: Gut Colonization with an Obesity-Associated Enteropathogenic Microbe Modulates the Premetastatic Niches to Promote Breast Cancer Lung and Liver Metastasis.
Authors: Parida S, Siddharth S, Gatla HR, Wu S, Wang G, Gabrielson K, Sears CL, Ladle BH, Sharma D.
Journal and Year: Fronters in Immunology. 2023. PMID: 37503343.

Title: The Gut Microbiota in Breast Cancer Development and Treatment: The Good, the Bad, and the Useful!
Authors: Nandi D, Parida S, Sharma D.
Journal and Year: Gut Microbes. 2023. PMID: 37305949.

Title: Autophagy and Breast Cancer: Connected in Growth, Progression, and Therapy.
Authors: Wu Q, Sharma D.
Journal and Year: Cells. 2023. PMID: 37190065.

Title: Concomitant Analyses of Intratumoral Microbiota and Genomic Features Reveal Distinct Racial Differences in Breast Cancer.
Authors: Parida S, Siddharth S, Xia Y, Sharma D.
Journal and Year: NPJ Breast Cancer. 2023.PMID: 36702853.

Title: Label-Free Vibrational and Quantitative Phase Microscopy Reveals Remarkable Pathogen-Induced Morphomolecular Divergence in Tumor-Derived Cells.
Authors: Liu Z, Parida S, Wu S, Sears CL, Sharma D, Barman I.
Journal and Year: ACS Sens. 2022. PMID: 35583030.

Title: Induction of STK11-Dependent Cytoprotective Autophagy in Breast Cancer Cells Upon Honokiol Treatment.
Authors: Muniraj N, Siddharth S, Shriver M, Nagalingam A, Parida S, Woo J, Elsey J, Gabrielson K, Gabrielson E, Arbiser JL, Saxena NK, Sharma D.
Journal and Year: Cell Death Discov. 2020 .PMID: 32963809

Note: Dr Sharma is submitting a proposal to NCI-PREVENT program to further develop this compound and simultaneously work to develop it ourselves.

Title: A Procarcinogenic Colon Microbe Promotes Breast Tumorigenesis and Metastatic Progression and Concomitantly Activates Notch and β-Catenin Axes.
Authors: Parida S, Wu S, Siddharth S, Wang G, Muniraj N, Nagalingam A, Hum C, Mistriotis P, Hao H, Talbot CC Jr, Konstantopoulos K, Gabrielson KL, Sears CL, Sharma D.
Journal and Year: Cancer Discovery. 2021. PMID: 33408241.

Title: The Microbiome and Cancer: Creating Friendly Neighborhoods and Removing the Foes Within.
Authors: Parida S, Sharma D.
Journal and Year: Cancer Research. 2021. PMID: 33148661.

Title: Towards Taming the Bugs to Improve the Drugs for Breast Cancer.
Sharma D.
Journal and Year: Cancer Research. 2021. PMID: 34003771.

Title: Hyperleptinemia in Obese State Renders Luminal Breast Cancers Refractory to Tamoxifen by Coordinating a Crosstalk Between Med1, miR205 and ErbB.
Authors: Nagalingam A, Siddharth S, Parida S, Muniraj N, Avtanski D, Kuppusamy P, Elsey J, Arbiser JL, Győrffy B, Sharma D.
Journal and Year: NPJ Breast Cancer. 2021.PMID: 34389732.

DR. SUKUMAR'S AND VISVANATHAN'S PUBLICATIONS

Title: Intraductal Administration of Transferrin Receptor-Targeted Immunotoxin Clears Ductal Carcinoma In Situ in Mouse Models of Breast Cancer-a Preclinical Study.
Authors: Wang G, Kumar A, Ding W, Korangath P, Bera T, Wei J, Pai P, Gabrielson K, Pastan I, Sukumar S.
Journal and Year: Proc Natl Acad Sci U S A. 2022. PMID: 35675429.

Title: Evaluation of a Liquid Biopsy-Breast Cancer Methylation (LBx-BCM) Cartridge Assay for Predicting Early Disease Progression and Survival: TBCRC 005 Prospective Trial.
Authors: Visvanathan K, Cope L, Fackler MJ, Considine M, Sokoll L, Carey LA, Forero-Torres A, Ingle JN, Lin NU, Nanda R, Storniolo AM, Tulac S, Venkatesan N, Wu NC, Marla S, Campbell S, Bates M, Umbricht CB, Wolff AC, Sukumar S.
Journal and Year: Clin Cancer Res. 2023. PMID: 36534524.

Title: Liquid Biopsy Assay for Methylated Markers in Advanced Breast Cancer.
Authors: Fackler MJ, Tulac S, Venkatesan N, Aslam AJ, de Guzman TN, Mercado-Rodriguez C, Cope LM, Downs BM, Vali AH, Ding W, Lehman J, Denbow R, Reynolds J, Buckley ME, Visvanathan K, Umbricht CB, Wolff AC, Stearns V, Bates M, Lai EW, Sukumar S. Development of an automated
Journal and Year: Cancer Res Commun. 2022. PMID: 36046124.

Title: Functional Antagonism of Junctional Adhesion Molecule-A (JAM-A), Overexpressed in Breast Ductal Carcinoma In Situ (DCIS), Reduces HER2-Positive Tumor Progression.
Authors: Smith YE, Wang G, Flynn CL, Madden SF, MacEneaney O, Cruz RGB, Richards CE, Jahns H, Brennan M, Cremona M, Hennessy BT, Sheehan K, Casucci A, Sani FA, Hudson L, Fay J, Vellanki SH, O'Flaherty S, Devocelle M, Hill ADK, Brennan K, Sukumar S, Hopkins AM.
Journal and Year: Cancers (Basel). 2022. PMID: 35267611.

Title: Automated and Rapid Detection of Cancer in Suspicious Axillary Lymph Nodes in Patients with Breast Cancer.
Authors: Li J, Downs BM, Cope LM, Fackler MJ, Zhang X, Song CG, VandenBussche C, Zhang K, Han Y, Liu Y, Tulac S, Venkatesan N, de Guzman T, Chen C, Lai EW, Yuan J, Sukumar S.
Journal and Year: NPJ Breast Cancer. 2021. PMID: 34234148.

Title: DNA Methylation Markers for Breast Cancer Detection in the Developing World.
Authors: Downs BM, Mercado-Rodriguez C, Cimino-Mathews A, Chen C, Yuan JP, Van Den Berg E, Cope LM, Schmitt F, Tse GM, Ali SZ, Meir-Levi D, Sood R, Li J, Richardson AL, Mosunjac MB, Rizzo M, Tulac S, Kocmond KJ, de Guzman T, Lai EW, Rhees B, Bates M, Wolff AC, Gabrielson E, Harvey SC, Umbricht CB, Visvanathan K, Fackler MJ, Sukumar S.
Journal and Year: Clin Cancer Res. 2019. PMID: 31300453.

NEWS ARTICLES:

https://www.oncozine.com/researchers-find-breast-tumors-of-asian-black-and-white-women-to-have-different-cellular-features/

https://www.news-medical.net/news/20230327/Breast-tumors-of-women-from-different-racial-groups-have-diverse-cellular-microbial-and-genomic-features.aspx

https://www.hopkinsmedicine.org/news/newsroom/news-releases/study-finds-diverse-differences-in-microbes-in-breast-tumors-from-women-of-different-races

https://www.newswise.com/articles/study-finds-diverse-differences-in-microbes-in-breast-tumors-from-women-of-different-races?channel=

https://medicalxpress.com/news/2023-03-diverse-differences-microbes-breast-tumors.html

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