NATIONAL BREAST CANCER COALITION

ARTEMIS Project®

Artemis Project® Summary of Seed Grants

Identification of Breast Cancer-Specific Antigen Targets for Development of Anti-Cancer Vaccines

Paul Spellman, PhD

PURPOSE: The purpose of this grant was to advance the development of a preventive breast cancer vaccine through the identification of neo-antigens present in diverse breast cancers. This would be achieved by assessing the frequency of non-native antigens in genomic and RNA expression data within the context of breast cancer subtypes. A candidate neo-antigen is a non-native peptide sequence arising from genomic aberrations or other mechanisms that affect encoded or post-transcriptional alterations. This project sought to develop a thorough and comprehensive portfolio of native and non-native antigens across the major breast cancer subtypes. This project involved collaboration between Drs. Joe Gray and Paul Spellman at Oregon Health and Science University.

RESULTS TO DATE: Preliminary results presented at the fourth annual Artemis Meeting in March 2014 included the identification of 173 native transcripts and 165 predicted novel isoforms that exhibited bi- or tri-modal expression, had at least an 8-fold differential of expression between tumor and normal tissues, and were made up of >95% tumor samples and represented >10% of tumors in the dataset. The translation potential of the novel isoforms was assessed, and each was assigned a priority score depending on coding differences and translated novel sequence alignment. Candidates were prioritized using heuristic ranking scores, and a list of the top 26 novel breast cancer epitopes was determined.

Genomic Comparisons to Detect Candidate Viral Causes of Breast Cancer

Paul Ewald, PhD

PURPOSE: The primary focus of this grant was to advance the development of a breast cancer preventative vaccine through the investigation of candidate viral causes of breast cancer. Two sets of breast cancer genomes were assessed to ascertain the full spectrum of known infectious agents in each breast cancer specimen for a population of breast cancer patients. The presence and prevalence of known viruses and cellular pathogens in breast cancer genomes were determined using bioinformatic tools to identify pathogens with the biochemical characteristics to classify as essential causes of cancer. The presence of viral infections in breast cancer tumors were analyzed utilizing available tumor datasets such as a private dataset at the Cancer Institute of New Jersey, the Cancer Genome Atlas (TCGA) RNA dataset, and the Cancer Genome Atlas (TCGA) DNA dataset. This grant

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would allow for the identification of candidate pathogens with the potential to be utilized as vaccine targets through a thorough analysis of viruses and cellular pathogens associated with breast cancer.

RESULTS TO DATE:: Preliminary results presented at the fourth annual Artemis Meeting in March 2014 showed that neither DNA nor RNA was present at the order of magnitude that oncogenic viruses are present in cancers that are currently accepted as being caused by viruses, such as the presence of human papilloma virus (HPV) in cervical cancer cells. However, a low copy number of Epstein-Barr virus (EBV) and Anellovirus were found in a minority of samples.

Evaluating the Biology of Human DCIS through Transcriptome Sequencing (RNAseq)

H. Kim Lyerly, MD & Greg Hannon, PhD

PURPOSE: This grant sought to advance the development of a breast cancer preventive vaccine through a thorough evaluation of the biological characteristics of human ductal carcinoma in situ (DCIS). Transcriptome sequencing (RNAseq) of DCIS lesions would allow for the generation of gene expression profiles and an analysis of oncogenic signaling and adaptive immune response in these lesions. This grant additionally examined whether DCIS lesions distribute among the accepted classifications of breast cancer. A gene expression analysis of the stromal environment surrounding the lesions were performed to examine the characteristics associated with disease progression into invasive carcinoma.

RESULTS TO DATE: Preliminary results presented reported on the dissection of ten patient samples, resulting in the formation of 117 RNAseq libraries that passed quality control. An RNAseq analysis of these samples showed the clustering of DCIS samples when compared with invasive breast cancer (IBC) and normal samples. Gene expression data showed an overexpression of ERBB2 in DCIS samples compared to normal samples. It was also found that IBC samples overexpress progesterone receptor (PGR) when compared to DCIS samples.

Large Scale Resources for Studying Breast Cancer Recurrence with DNALand

Yaniv Erlich and Joe Pickrell, NY Genome Center & Peter Fasching, UCLA; Friedrich-Alexander University, Erlanger-Nuremberg, Germany

PURPOSE: This project sought to create a database of genomic and phenotypic information from thousands of individuals by crowdsourcing to facilitate studies of etiological factors of breast cancer recurrence. Genomic data was gathered by leveraging DNA information from direct to consumer (DTC) genomics companies such as 23andMe, Ancestry DNA, or FamilyTreeDNA. Participants uploaded their genomic information to the database and answered a series of clinical questions. The participants were also able to upload their family tree and share information about breast cancer of other family members. A clinical questionnaire was used to link a thorough set of clinical data with genomic data. The clinical questionnaire was developed by NBCC advocates, Daniel Speyer,

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New York Genome Center, and Peter Fasching, MD, Associate Professor of Gynecology and Obstetrics, Department of Gynecology and Obstetrics, Friedrich-Alexander University, Erlangen-Nuremberg, Germany, Visiting Researcher, Department of Medicine, Division of Hematology and Oncology, University of California at Los Angeles, CA.

RESULTS TO DATE:: The clinical questionnaire was launched in April 2017. As of September 2019, about 41,500 users started the NBCC survey, and nearly 33,000 (79.4%) users completed it. Of the nearly 33,000 users who completed the survey, nearly 5000 users agreed to share their emails with NBCC researchers. In terms of phenotypic data, 4341 (13%) users reported at least one family member (including themselves) with breast cancer. Approximately 65% (2865/4341) of them indicated that only their mother had breast cancer, while approximately 13% (569/4341) reported a personal history of breast cancer. Approximately 10% (454/4341) of the users with breast cancer history indicated that they have one sibling with breast cancer, and 1.7% (74/4341) indicated that their father had breast cancer. The remainder (~10%) reported that they have more than a single breast cancer incidence in their family, which could be of interest for future studies.

Overall, the participation rate was relatively constant, with some slowdown in the latter months of data collection. Nevertheless, we hit our target of 25,000 participants without any marketing efforts. At present, hard drives encrypted for security, with imputed VCF files of the genomic data from participants, along with clinical questionnaire data are stored in a secure safety deposit box, along with the SQL databases that document the results.

Preclinical research on the development of a preventive breast cancer vaccine, focus on immunogenicity, safety and efficacy under a Master Service Agreement

Keith Knutson, Mayo Clinic

PURPOSE: Develop preliminary clinical protocol and conduct Pre-IND discussions with the FDA to establish a preclinical safety plan. Develop questions, research plan and discussion items for review by the FDA. Conduct Pre-IND meeting and prepare and distribute Post Pre-IND meeting report.

RESULTS TO DATE: Pre-IND information package was submitted, and a call with the FDA was completed on March 6, 2018. In 2019, NBCC hired a trial project manager to streamline the transition to the Phase 1 trial of the vaccine. In 2020, NBCC and Mayo investigators were awarded a contract with the National Cancer Institute (NCI) as part of its competive PREVENT Cancer Preclinical Drug Development Program. The PREVENT program is a peer-reviewed agent development program designed to support preclinical development of innovative interventions and biomarkers for cancer prevention and interception towards clinical trials. As a result of this award, NBCC and the Artemis Preventive Vaccine project team are working with NCI who will manufacture and supply at no cost, the vaccine required for Phase 1 testing. Working in conjunction with NCI, Mayo is in the process of finalizing the Investigational New Drug application (IND) for formal submission to the FDA for review and approval. A work plan has been developed to track key milestones needed to launch the Phase 1 trial.

Pilot project to determine whether adaptive immune system can recognize and kill dormant disseminated breast tumor cells and, if not, determine which aspect of DTC biology should be targeted to enhance T Cell recognition

H. Kim Lyerly, Duke, Cyrus Ghajar, Fred Hutchinson & Josef Penninger, Institute of Molecular Biotechnology of the Austrian Academy of Science

PURPOSE: The purpose of this grant is to determine whether the adaptive immune system can recognize and kill dormant DTCs, and if not, which aspect of DTC biology should be targeted to enhance T cell recognition. The goal is to leverage the findings to develop approaches to eliminate dormant DTCs, prevent late recurrences, and reduce deaths from breast cancer. Specifically to determine: a) if expression of a given antigen in the presence of molecularly defined T cells specific to said antigen results in recognition and killing of dormant DTCs, b) the relative contribution of CD4-or CD8-positive T cells to this process, and c) if recognition and/or killing depends on the microenvironment of dormant DTCs. Determine how DTC growth status and microenvironment influence the efficacy of recognition and killing by class I and/or class II interactions mediated by cytotoxic (CD8+) and helper (CD4+) T cells. Further, to Determine how DTC growth status and microenvironment influence the efficacy of recognition and killing by class I and/or class II interactions mediated by cytotoxic (CD8+) and helper (CD4+) T cells.

RESULTS TO DATE: The investigators found that quiescent breast epithelial cells (normal or malignant) downregulate human leukocyte antigen I (HLA I), which is an MHC class I molecule essential for CD8+ T cell recognition and killing. They further observed that quiescent breast tumor cells in organotypic culture exhibit down-regulated HLA-A2 expression. They also found that CAR T cells were able to target proliferative and dormant cells presenting a model antigen with equal efficacy. In 2020, results were presented at the Artemis meeting demonstrating that this effect carried over in vivo, in a small cohort of mice. Namely, CAR T cells eliminated metastases and single cells in the bone marrow of mice harboring metastases prior to CAR T infusion. Next, they will expand these studies and also contrast them with T cell receptor-mediated approaches using model neoantigens.