



I. INTRODUCTION

A. BACKGROUND

The National Breast Cancer Coalition (NBCC) is dedicated to ending breast cancer through the power of grassroots action and advocacy. In 2010, NBCC launched **Breast Cancer Deadline 2020**[®] to focus resources and efforts to the areas that will lead to the knowledge needed to end breast cancer. The research component of **Breast Cancer Deadline 2020**[®] includes the Artemis Project[®], an advocate led mission driven approach of strategic summits, catalytic workshops, research action plans and collaborative efforts among various stakeholders. The Artemis Project[®] focuses on two areas:

- **Primary Prevention:** How do we stop people from getting breast cancer?
- **Prevention of Metastasis:** How do we stop people from dying of breast cancer?

Artemis Project[®]: Preventive Vaccine

The 2011 report from the first annual meeting provides an overview of the project and a description of the focus areas. After the first annual meeting in March, 2011, NBCC contracted with Science Application International Corporation (SAIC) to help prepare a detailed strategic plan for the Artemis Project[®], based on the outcomes of the annual meeting and follow-up interviews with attendees. This Project Plan was completed in December, 2011. Subsequently, NBCC issued a call for proposals to address initial steps in antigen identification. The report from the second annual meeting outlines the refined strategies developed for the early stages of the project, particularly around antigen identification and evaluation. Discussion during the third annual meeting focused on the specific tasks required within the next two years to remain on track for a vaccine product ready for clinical trials. The third annual meeting report provides updates on research progress within areas outlined in the Project Plan, including reports from groups awarded the initial grants.

Seed Grants Awarded

Through the generous support of the National Philanthropic Trust (NPT), seed grants have been awarded for the following research projects:

- **August 2012** to Dr. Paul Spellman and Dr. Joe Gray, of Oregon Health and Science University, to identify possible vaccine targets using existing and developing human genomic data within different breast cancer subtypes.
- **January 2013** to Dr. Paul Ewald, University of Louisville, and Dr. Vladimir Belyi, Robert Wood Johnson Medical School, to take a systematic look through two sets of breast cancer genomes for evidence of infectious agents, to supplement the search for appropriate vaccine targets.
- **October 2013** to Dr. Greg Hannon, Cold Spring Harbor Laboratory, and Dr. H. Kim Lyster, Duke Comprehensive Cancer Center, to conduct a thorough evaluation of the biological characteristics of human ductal carcinoma in situ (DCIS) in order to examine the molecular characteristics associated with disease progression into invasive carcinoma.
- **November 2013** to Dr. William Gillanders, Washington University, Dr. Keith Knutson, VGTI Florida, and Dr. Peter Lee, City of Hope, to advance the selection of final vaccine antigens and combinations through a focus on preclinical research on immunogenicity, safety, and efficacy.

B. FOURTH ANNUAL MEETING: GOALS

The purpose of this meeting was to further develop elements of the strategic plan leading to clinical trials for the development of a preventive breast cancer vaccine in the next three years, including a timeline and a budget. The meeting agenda focused on approaches to advance antigen identification and prioritization and development of a strategy to identify populations for vaccine clinical trials.

C. ATTENDEES

2014 Annual Meeting Participants

Leslie Bernstein, PhD, Professor and Director, Cancer Etiology, Dean for Faculty Affairs, City of Hope Beckman Research Institute

Amy Bonoff, MBA, Advocate

Frank Calzone, PhD, Biotech Consultant

Danny Douek, MD, Chief, Human Immunology Section, Vaccine Research Center, NIAID, NIH, DHHS

Yaniv Erlich, PhD, Fellow, Whitehead Institute for Biomedical Research

Paul Ewald, PhD, Professor of Biology and Director of the Program on Disease Evolution, University of Louisville

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

Silvia C. Formenti, MD, Professor of Medicine, Chair, Department of Radiation Oncology, New York University Medical Center

Suzanne Fuqua, PhD, Director of the Breast Center Microarray Core, Lester and Sue Smith Breast Center, Baylor College of Medicine

William Gillanders, MD, Professor of Surgery, Washington University School of Medicine

Gregory J. Hannon, PhD, Professor, Investigator, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory

Pat Haugen, Advocate

Simon Knott, PhD, Post Doctoral Fellow, Cold Spring Harbor Laboratory

Keith Knutson, PhD, Program Director in Oncology, Vaccine & Gene Therapy Institute of Florida, and Associate Professor, Department of Immunology, College of Medicine, Mayo Clinic

Debbie Laxague, RN, Advocate

Peter P. Lee, MD, Professor and Associate Chair, Department of Cancer Immunotherapeutics and Tumor Immunology, City of Hope

H. Kim Lyerly, MD, FACS, Professor, Duke University

Musa Mayer, MS, MFA, Advocate

Michele Rakoff, Advocate

Patricia Renzulli, Manager, Breast Cancer Grants, National Philanthropic Trust

Joy Simha, Advocate

Jill Slansky, PhD, Associate Professor, University of Colorado School of Medicine

Paul Spellman, PhD, Associate Professor, Molecular and Medical Genetics, Oregon Health & Science University

Asad Umar, DVM, PhD, Chief, Gastrointestinal and Other Cancers Research Group, National Cancer Institute, NIH

Fran Visco, JD, President, NBCC

Sara Whiting, Advocate

Meeting Support Staff

Annette Bar-Cohen, MA, MPH, Executive Director, Center for NBCC Advocacy Training

Giselle Hicks, MPH, Science Analyst

Isabel Hinstrosa, BA, Programs Assistant, NBCC

Kayla Kirsch, MS, President, Leapfrog Consulting

Marva Lewis, The Event Professionals

II. BACKGROUND PRESENTATIONS & UPDATES

The meeting began with updates and a review of relevant news since the last annual meeting, background presentations to enrich the context for discussion and reports from recipients of Artemis seed grants.

A. ADVOCATE UPDATES

Debbie Laxague, RN, NBCC advocate, presented an updated vaccine landscape, followed by Joy Simha, NBCC advocate and Board member, who reported on the various advocate projects conducted over the past year. NBCC advocates completed two research projects: a review of recent clinical trials in prevention, including breast cancer non-vaccine prevention trials and non-breast cancer vaccine prevention trials; and a report identifying priority biospecimens needed by Artemis® investigators, as well as existing resources where such samples might be obtained.

B. RESEARCHER UPDATES: NEW ISSUES IN ANTIGEN IDENTIFICATION

Direct Antigen Discovery Using Expanded TIN Epithelial Cells

H. Kim Lyerly, MD

Dr. Lyerly presented data on new methods for expanding and developing DCIS cell lines in order to more efficiently isolate antigens of interest. Working with Dr. Richard Schlegel's lab at Georgetown University, Dr. Lyerly used a Rho kinase inhibitor to successfully expand six cell lines and discovered the antigens identified by the Artemis Project® Data Mining Group within the peptide MHC of the expanded cell lines.

The Possibility of Mutant Estrogen Receptors as Antigens

Suzanne Fuqua, PhD

Dr. Fuqua posited that ESR1 mutations make attractive vaccine antigen targets because they occur at sites where there is good intracellular signal reception near large proteins, and the sites are immunogenic (at least in rabbits and mice). Such mutations may represent an early event in breast cancer development, since K303R is found in women with DCIS and those with high-risk familial breast cancer. ESR1 mutations also occur frequently in ER-positive breast cancer, a breast cancer subtype that has a simpler genomic background than other subtypes, and such mutations have measurable endpoints such as glomerular filtration rate (GFR) activation and signaling.

Phase 1 Clinical Trial of Mammaglobin-A DNA Vaccine

Will Gillanders, MD

Dr. Gillanders proposes that mammaglobin-A is a strong candidate antigen for a preventive vaccine because there is universal expression in all stages of breast cancer, and it has been shown to induce a CD8+ t-cell response.

Dr. Gillanders presented data on a Phase 1 open-label clinical trial evaluating the safety and immunogenicity of a mammaglobin-A DNA vaccine. To date, 15 patients with metastatic breast cancer were administered three escalating doses of 4mg plasmid DNA via intramuscular injection on Days 0, 28, and 56. No adverse events were observed. The vaccine was seen to induce mammaglobin-A-specific immune response in peripheral blood lymphocytes, and was associated with increased progression-free survival.

C. REPORTS FROM ARTEMIS SEED GRANTEES

Identification of Antigens for Preventative Vaccine Development

Paul Spellman, PhD

Using RNA sequencing (RNAseq) analyses of breast tumor and normal tissue samples obtained from The Cancer Genome Atlas (TCGA), Dr. Spellman described the use of an align-then-assemble genome-guided approach for greater sensitivity in detecting differential expression of the assembled transcripts. The final dataset included 329 normal tissue samples from TCGA and about 1,000 tumor samples. Of 86,000 native transcripts, 173 candidates met the criteria of a bimodal distribution with at least an 8-fold differential between the high and low expression clusters, at least 10% of the tumor samples in the high expression cluster, and tumor samples comprising 95% of the high expression cluster. One promising native candidate, MMP11, appeared to be more strongly enriched for luminal and HER2/neu-positive tumors than for basal-like tumors. Dr. Kami Chiotti, a colleague, assessed 116,000 predicted novel isoforms and identified 165 candidates that met the criteria. One promising novel isoform is GATA3, a binding protein that regulates t-cell development and is involved in luminal cell differentiation. Interestingly, when MUC1 was assessed as a known antigen comparison, it did not meet the criteria. However, it was noted that MUC1 is a glycosylate and so may be better assessed using a proteomic approach. It was also noted that although RNAseq analyses are relatively inexpensive and simple to perform, further validation is needed. Large statistical tests tend to overestimate effect sizes due to a lack of power; however, this effect could be mitigated by replicating the analyses using a different dataset. Dr. Lyerly's dataset was recommended as a potential resource.

Genomic Comparisons to Detect Candidate Viral Causes of Breast Cancer: Fundings and Implications for Artemis

Paul Ewald, PhD & Vladimir Belyi, PhD

Drs. Ewald and Belyi presented data updates from their work assessing breast cancer samples from TCGA for viral pathogens. They performed an RNAseq analysis of 310 breast cancer samples and a DNAseq analysis of 90 breast cancer samples to evaluate the presence of 4,000 viruses in the National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) database. Overall, neither DNA nor RNA was found to be present at the order of magnitude that oncogenic viruses are present in other cancers known to be of viral origin, such as cervical cancer and HeLa cells (HPV), and nasopharyngeal cancer and post-transplantation

proliferative disease (Epstein-Barr virus, EBV). There was, however, low EBV and anellovirus positivity in a small proportion of the samples.

Data Mining: Using the Human Genetic Data to Test the Pathogen Hypothesis

Yaniv Erlich, PhD

Dr. Erlich reported out on an aspect of the Artemis Project Data Mining Group's work on reviewing genome-wide association studies (GWAS) over the past year. His working hypothesis was that if pathogens contribute to the etiology of breast cancer, then genetic predisposition to breast cancer would be mediated through the HLA region. Natural polymorphisms can be used to see whether pathogens are associated with disease; if they congregate near the HLA region, the signal indicates a t-cell response. For example, Manhattan plots show a t-cell response for HPV in cervical cancer and Hepatitis B virus for hepatocellular cancer.

The Data Mining Group found no association between pathogens and HLA regions from the GWAS, reducing confidence that this will be a useful direction to pursue toward a preventive vaccine.

Characterizing DCIS

H. Kim Lyerly, MD & Greg Hannon, PhD

DCIS is treated as a precursor to breast cancer, but if it were eliminated, would breast cancer be eliminated as well? Drs. Lyerly and Hannon presented on their work to molecularly characterize DCIS and compare it to invasive disease, examine the development and evolution of non-invasive cancer, and identify the events that drive progression to invasive disease. Their aim is to determine the stage at which candidate antigens are expressed and when they become visible to the immune system.

The goal is to obtain samples from 30 patients. To date, samples from ten patients have been dissected, resulting in the accrual of 117 RNAseq libraries that passed quality control. Funding from the Department of Defense was received in March 2014 and will enable this pipeline to scale up.

D. FUTURE SEED GRANT PRESENTATIONS

Immunological Studies: Phase I

Peter Lee, MD, Keith Knutson, PhD & William Gillanders, MD

Dr. Lee presented a plan for addressing the key issues of targeting self-antigens versus novel/mutated antigens for a breast cancer preventive vaccine, and whether the immune system reacts differently to self-antigens and neo-antigens. Targeting self-antigens has the potential to induce an autoimmune response in healthy people, or conversely weaken immune response due to self-tolerance. The phase I immunological analyses and animal studies would assess safety and efficacy differences between self-antigens and neo-antigens in pilot studies, but would also establish a system to analyze possible antigens identified by the Artemis Project® antigen discovery team in order to move efficiently toward a product for the clinic within the time span of **Breast Cancer Deadline 2020®**.

E. BACKGROUND PRESENTATION

Identification of Women Being at Risk for Specific Breast Cancer Subtypes

Peter Fasching, MD

Dr. Fasching described the work of the Breast Cancer Association Consortium, a group identifying genetic risk factors for breast cancer and each of its molecular subtypes. The Consortium is an international working group with comprehensive epidemiological and germline DNA data from over 70,000 breast cancer patients and 90,000 healthy controls. With all this data available, Manhattan plots can be used to identify genetic mutations, the frequency of a mutation in the population, and validation of single-nucleotide polymorphisms (SNPs). The Consortium has identified 82 SNPs that need to be validated, but are thought to be true findings.

He concluded that prediction of molecular subtype might be clinically relevant, but that the approach should be a compromise between clinically relevant subtypes and on-time (on-demand) analysis of risk factors and molecular subtypes.

III. SMALL GROUP WORK & OUTCOMES

The meeting participants initially broke out into three groups to brainstorm strategies to answer the overall question of what to inject into whom. The three subgroups then came back together to report out and have a larger discussion. All three groups had similar themes and strategies, and it was decided to merge the plans with milestones around two focus areas of goal-driven research:

- Creation of a critical path, a series of intensive tasks that would use the current antigen target “best bets” to identify time-sensitive elements that would enable subsequent modifications to the antigen selection process while still moving forward with the urgency of the Deadline.
- Creation of an accelerator arm, consisting of tasks that could be rapidly completed separately from the time-consuming critical path steps, would identify opportunities to optimize antigen selection and facilitate a pipeline for testing candidates for the critical path.

Two groups were formed (critical path and accelerator) and asked to outline what specific steps were needed over the next 18-24 months to meet the milestone of a clinical trial with healthy people by 2017.

Artemis® participants were asked to put aside prior experience with FDA, and to just pose the question of what safety data would make them comfortable with injecting a vaccine into a healthy population and not be constrained by what the perceived barriers are.

Critical Path

Leslie Bernstein, Amy Bonoff, Frank Calzone, Danny Douek, Silvia Formenti, William Gillanders, Keith Knutson, Debbie Laxague, Peter Lee, Musa Mayer, Michele Rakoff, Joy Simha, Asad Umar

Milestone: Phase I clinical trial by 2017

There was agreement that a preventive vaccine would need multiple antigens as part of a scientific risk management approach to have a broader impact on all breast cancer subtypes. The number of antigens was decided upon as a balance of maximizing breast cancer subtype coverage and minimizing the complexity of the vaccine.

PATH 1: CLINICAL

For antigens that already have a toxicity profile, three well-characterized antigens should be selected and evaluated on their combined effect on tissue. These antigens will serve as a starting point to create an infrastructure and pipeline for antigens still to be identified and selected. The decision to focus on self-antigens was made primarily because there has been little evidence that there will be a strong driver neo-antigen, and to reach the milestone of a clinical trial by 2017. However, neo-antigens will not be excluded from future trials if they are prioritized and validated by the accelerator group.

HER2 and MUC1 have already been used in the clinic and in combination (Markovic and Sandler). There was debate about the third antigen choice. Although Mammaglobin-A has no murine ortholog, clinical safety data already exists from phase I trials. Other antigens were considered for this first phase, including IGF1R, IGFB2, MMP11, and Tert. However, the antigen with the least data will be the limiting factor.

There was a concern that a vaccine that would show efficacy among women with a history of disease may need to differ from one that would show efficacy among high-risk, healthy women with no disease history.

Women at high risk for recurrence could represent another trial sub-population, however, they are likely to have been previously treated with chemotherapy. Women treated only with hormonal therapy and not chemotherapy are likely to have a lower recurrence risk, illustrating the difficulty of identifying a trial sub-population.

PATH 2: PRE-CLINICAL

Antigens identified by the accelerator group will likely not be able to enter the clinic directly, and thus the determination of a different path is crucial to ensure progress. This path will focus on pre-clinical studies to examine the toxic and immunologic effects of combining multiple antigens.

Other elements of this pre-clinical critical path include vector optimization and adjuvant selection. Pre-clinical testing will take an “all-in” approach as opposed to testing each vaccine component separately. The different vector platforms - DNA, adenovirus and CMV - and any molecular adjuvants can be tested in combination with the antigens as part of the pre-clinical critical path. Mode of transmission (e.g., intramuscular, electroporation) will also need to be optimized in this phase.

Immunogenicity and safety will be the main endpoints of this path, since efficacy in mice does not necessarily predict clinical efficacy. Efficacy could serve as a secondary endpoint; it was noted that although positive efficacy results could boost publicity, negative efficacy results wouldn't stop trial progress. Immunogenicity could be assessed first with a short-term, two-week acute toxicity study followed by a two-month toxicity study with an immune response aspect in the therapeutic setting. A concern was raised that this may not be appropriate in the prevention setting.

Timeline: 1-2 months for antigen list, but will need capability to evaluate more than five antigens

Recommendations:

- Initial trial with subsequent staggered phased trials informed by accelerator group findings
- Form an alliance with the existing immune networks located at most institutions, and leverage the alliance for the pathway [Mac Cheever and Nora Disis]
- Meet with FDA soon to discuss requirements for starting a multiple antigen vaccine clinical trial

Accelerator Arm

Yaniv Erlich, Paul Ewald, Peter Fasching, Suzanne Fuqua, Greg Hannon, Pat Haugen, Simon Knott, Kim Lyerly, Jill Slansky, Paul Spellman, Sara Whiting

Milestone: Antigen selection by 2015

The focus of the accelerator group is to identify opportunities for better antigen selection through the utilization of genomics, proteomics, and GWAS to prioritize and validate candidates for the critical path. It was agreed upon that the technology exists to identify the optimal antigens for each breast cancer subtype. The focus is on a multiple antigen vaccine, and not a single “silver bullet” antigen for all breast cancer. A pipeline is needed to speed discovery, prioritization, and validation of candidate antigens.

DISCOVERY

Two sources of candidate antigens were identified for RNAseq analysis: TCGA and DCIS.

Dr. Spellman's analysis of TCGA data can be performed with subtype-specific criteria to help identify proteins that cross subtypes, and also to prioritize the existing candidate antigen list. The subtype criteria could include overexpression in at least 50% of the tumor samples in a given subtype and expression as RNA early in disease progression.

In parallel, RNAseq analysis of Dr. Lyerly's DCIS data could include a spectrum of breast samples ranging from atypia to DCIS to invasive breast cancer. Although atypia is a low risk factor for breast cancer and not an obligate progressive precursor, it is a criterion for early expression. The group recommended prioritization of DCIS samples with adjacent invasive breast cancer to overcome the criticism that DCIS may be unrelated to invasive breast cancer. Atypia samples could also be used as criteria.

It was also postulated that candidate antigens identified from the DCIS data be used to prioritize the

existing antigen list. It was suggested that Dr. Spellman's group could perform the same analysis on the DCIS data, identifying antigens that are present in both the TCGA and DCIS data.

Several hundred samples could be analyzed within a nine-month timeframe. However, the limiting factor of this analysis will be the breast pathologist dissecting and processing the tissue samples. The next steps will be to establish the pipeline, prepare the RNAseq libraries, and run the analyses as fast as the samples can be fed into it.

PRIORITIZATION

Peptide MHC pulldowns can be used to filter and prioritize the findings from the RNAseq analyses. Of the candidate antigens identified in discovery, only about 10% will be seen in an MHC pulldown. Of those, only half will stimulate a t-cell response in a peptide test. Synthesized peptides from mass spectrometry data can be used to filter out false positives by identifying which bind appropriately, and there can be high confidence for t-cell recognition for those that do bind. Of these antigens that exhibit appropriate binding, it is expected that only about one-third will have relevant proteomic data, such as protein functions linked to tumor driver genes. For instance, 500 candidate antigens from discovery could result in anywhere from zero to ten candidate antigens with relevant proteomic data prioritized for validation. The analysis of the peptide MHC pulldown could be conducted within twelve months on antigens identified from Dr. Spellman's TCGA analysis. Dr. Lyerly estimated that the RNAseq analysis of the DCIS data would take nine months, and the subsequent peptide tests an additional three months.

VALIDATION

Validation of the RNAseq analysis findings will require evidence of protein expression in tissue samples, including both normal breast tissue and tumor samples. Confirmation is needed because up to one-third of the RNA candidates identified from RNAseq will not be expressed the same way at the protein level. The first step will be to examine protein expression in silico, via computer simulation. A literature review may indicate whether an RNA candidate is not expressed or expressed at low levels in normal tissue. It will also provide useful information for discussions with FDA on the likelihood of an immune response given that a gene was identified with the presence of a peptide MHC. The third validation step will involve analysis through immunohistochemistry (IHC). MHC can be used to prioritize which antigen candidates should continue onto IHC validation. IHC will indicate a strong antibody presence, demonstrating expression in tissue and enabling validation of expression at the protein level. There is a human proteome atlas from Sweden with over 8,000 antibodies which could serve as a useful potential resource for this analysis. Microarrays have been conducted for different tissues, and although this data is publicly available, it might be difficult to check its reliability.

Recommendations:

- Reanalyze TCGA data by subtype; use DCIS data and existing MHC data to prioritize candidates for validation
- Analyze 100 DCIS samples within the next nine months
- Obtain funding for in silico work (computational salary), peptide validation testing (salary, materials), tissue array and antibody materials
- Keep in mind that the limiting factor will be the time needed for pathology micro-dissection of DCIS samples
- Build infrastructure among critical path participants
- Gain access to GWAS data to test hypotheses of patient population
- Capture samples from ongoing breast cancer vaccine trials, such as Mammaglobin-A and HER2, and information from immune-modulatory studies on antigen targets, and what the immune system is recognizing

During discussion, the creation of a scorecard with points awarded for characteristics such as level of overexpression, coverage of tumor subtypes, atypia, driver genes, and mouse ortholog, was briefly considered. Discussion timeframe limited further development of this concept. It was noted that using the availability of a mouse model as a selection criteria needed further discussion.