I. INTRODUCTION

A. BACKGROUND

The Artemis Project® of the National Breast Cancer Coalition (NBCC) brings together a collaborative group of advocates and scientists to take a strategic, systematic, yet broad approach to developing a breast cancer preventive vaccine within five years. The Artemis Project® is innovative, advocate led, and mission driven to ensure a focus on the end result. The project was launched in 2011 and the 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. An initial seed grant was awarded in 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. A second grant was awarded in early 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.

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.

B. PURPOSE OF THIRD ANNUAL MEETING

The third annual Artemis Project® meeting took place on March 8–11, 2013, in Calistoga, California. The purpose of this meeting was to receive updates on research progress within areas outlined in the Project Plan, including reports from groups awarded the initial grants, and to refine the Plan. Discussion focused on the specific tasks required within the next two years to remain on track for a vaccine product ready for clinical trials.

C. ATTENDEES

2013 Annual Meeting Participants

Vladimir Belyi, PhD, Assistant Professor, The Cancer Institute of New Jersey

Amy Bonoff, MBA, Advocate

Frank Calzone, PhD, Biotechnology Consultant

Brian Czerniecki, MD, PhD, Surgical Director, Immunotherapy Program, Abramson Cancer Center, University of Pennsylvania

Danny Douek, MD, PhD, Chief, Human Immunology Section, National Institutes of Allergy and Infectious Diseases, National Institutes of Health (NIH)

Yaniv Erlich, PhD, Fellow, Whitehead Institute

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

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

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

Gabriel M. Gutierrez, PhD, Project Manager, Science Application International Corporation

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

Pat Haugen, Advocate

Jarrod Holmes, MD, Medical Oncologist, Redwood Regional Medical Group

Stephen Johnston, PhD, Director, Biodesign Institute, Arizona State University

Simon Knott, PhD, Postdoctoral Fellow, Cold Spring Harbor Laboratory

Keith Knutson, PhD, Program Director, Oncology, Vaccine & Gene Therapy Institute of Florida; 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

Susan Love, MD, MBA, President, Dr. Susan Love Research Foundation

Doug Lowy, MD, Deputy Director, National Cancer Institute, NIH

H. Kim Lyerly, MD, George Barth Gellar Professor of Cancer Research, Duke University School of Medicine

Elaine Mardis, PhD, Co-Director, The Genome Institute, Washington University School of Medicine

Laura Nikolaides, MS, Director of Research & Quality Care Programs, NBCC

George Peoples, MD, Chief of Surgical Oncology, Brooke Army Medical Center; Director and Principal Investigator, Cancer Vaccine Development Program, Uniformed Services University of the Health Sciences; Deputy Director, US Military Cancer Institute

Michele Rakoff, Advocate

David Raulet, PhD, Professor of Immunology and Pathogenesis, University of California, Berkeley

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

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

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

Fran Visco, JD, President, NBCC

Meeting Support Staff

Debby Berlyne, PhD, Medical Writer

Kayla Kirsch, MS, President, Leapfrog Consulting

Marva Lewis, President and Managing Director, The Event Professionals

II. ARTEMIS PROJECT® PROGRESS

The first full day of the meeting was spent with presentations and discussion around research and project progress.

A. RESEARCH RESULTS WITHIN PROJECT PLAN

Native and Neoantigens in Breast Cancer

Paul Spellman, PhD

Dr. Spellman and Dr. Joe Gray, through the generous support of the National Philanthropic Trust, received a grant from NBCC to identify native and neoantigen peptides from RNA sequencing (RNAseq) analyses of 708 breast tumor specimens and 329 normal tissues from The Cancer Genome Atlas (TCGA). The samples generated approximately 15,000 novel predicted transcripts. Of these, 730 have higher expression in tumors. Eighty-eight genes have novel transcripts that are more highly expressed in tumors and are predicted to have more than 12,000 distinct epitopes that bind to a specific human leukocyte antigen (HLA) allele.

The project, which should be completed by August 1, 2013, will produce a list of "entry-level" targets. Experts in epitope research can then identify suitable targets from this list and determine how to validate them.

Participants noted that the normal breast tissue specimens for this study, which come from adjacent tissue in TCGA, might not be truly normal. A better resource for normal specimens might be the normal tissue bank at Indiana University, which collects normal breast tissue and matched serum, plasma, and DNA. Approximately 30 of these samples might be sufficient for the RNAseq study. Another suggestion was to focus on potential targets that are more prevalent in early-stage breast cancer and will be important for developing a primary prevention vaccine.

Dr. Doug Lowy noted that the TCGA is populated with larger tumors that generally have not metastatized (therefore resected by surgery). A follow up note by Dr. Lowy produced the stages of the TCGA dataset:

Stage 0: 0

Stage I: 75

Stage IA: 71

Stage IB: 7

Stage 2: 9

Stage IIA: 306

Stage IIB: 204

Stage III: 2

IIIA: 129

IIIB: 36

IIIC: 43

IV: 15

Genomic Comparisons to Detect Candidate Viral Causes of Breast Cancer

Paul Ewald, PhD & Vladimir Belyi, PhD

Dr. Ewald and Dr. Belyi, through the generous support of the National Philanthropic Trust, received a grant from NBCC to examine breast tumor and normal breast samples using RNAseq for evidence of infectious agents. Candidate viruses in breast cancer include Epstein-Barr virus (EBV), mouse mammary tumor virus (MMTV), human papillomavirus (HPV), and bovine leukemia virus (BLV). The validation of detection tools and algorithms is currently underway. The investigators will follow the validation with an analysis of TCGA data for pathogens known to be associated with oncogenesis, such as HPV and EBV; any of the more than 4,000 annotated viruses; and unknown pathogens.

A preliminary analysis of 24 breast cancer and 6 control samples found scattered EBV reads in half the tumor and normal samples, which might represent latent infection of epithelial cells or B-cell infiltration. At least one tumor sample had trace amounts of HPV, but none of the reads in the samples mapped perfectly to MMTV or BLV. About 80% of the samples had at least one viral hit.

Considerations expressed by the group include:

- Searching for the presence of viral genetic information in metastatic tumor tissue is important in gaining acceptance for causal links
- Epidemiological data to corroborate findings will be important
- Important to look at long-term follow-up of women who have received HPV vaccination
- If infectious agents are responsible for a subset of cancer, this subset typically represents a distinct pathobiological entity
- Several studies suggested that women with untreated HIV infection have a reduced risk of breast cancer but the benefit disappears when they begin antiviral treatment. This evidence could indicate that viral infection suppresses cancer.
- Perhaps chronic inflammation, and not infection, causes breast cancer, or chronic inflammation interacts with infection to cause breast cancer.

Breast Cancer Native Antigens

Laura Nikolaides, MS

Laura Nikolaides, Debbie Laxague, and Maria Wetzel reviewed the published literature to identify the best, known breast cancer native antigens for consideration and further evaluation as preventive vaccine components. The advocates reviewed the prioritized list published by Dr. Mac Cheever et al in 2009 *(Clin Cancer Res. 2009;15:5323-37)* as well as native antigens identified since the Cheever list's publication. The team did not review HER2-associated antigens as there is already significant expertise and knowledge within the Artemis Project® group.

Of the 75 antigens in the Cheever list, the advocate team found 37 to be associated with breast cancer. After reviewing the literature for evidence, six of these antigens or classes of antigens were identified as present in the majority of breast tumors and associated with early breast tumorigenesis:

- **Wilms' tumor gene (WT1):** WT1 was ranked first on the Cheever list and is overexpressed in almost 90% of breast tumors but not in most normal breast tissue. The abnormal expression of WT1 may be one of the earliest steps in the development of breast tumors, and correlates with poor prognosis. A 2012 review of clinical vaccination trials with WT1 found no severe adverse events, including no reports of autoimmune reactions. Recently published work identified 41 previously unreported epitopes of WT1 that were able to induce T cell responses.

- **Cancer testis antigens:** Cancer testis antigens are attractive cancer vaccine targets because there is normal expression in the germ cells of the testis and ovaries and in various cancers but not in adult somatic tissues.

  - SPA9 is a more recently identified cancer testis antigen and was not on the Cheever list. It may be an attractive target as the expression is high in breast cancer and may appear early in tumorgenesis. A 2009 study found SPA9 expression in 87% of the breast tumor specimens, and in all DCIS samples, though the number of DCIS samples was small. The same researchers found SPA9 antibodies in 80% of the patients with SPA9 expressing tumors. The protein is involved in c-Jun N-terminal kinase (JNK)-signaling and functions as a scaffold protein for JNKs, and has been associated with cellular proliferation, migration and invasiveness of cancer cells.

  - NY-ESO-1 and MAGE A are cancer testis antigens that have been more widely studied. Expression is found in several tumors, including breast tumors and DCIS, though at lower rates than SPA9. These antigens are highly immunogenic, and received high therapeutic index and stem cell expression scores from the Cheever group. The proteins are important in cellular proliferation, stem cell function, and carcinogenesis. MAGE-A3 was ranked #8 and NY-ESO-1 was ranked #10 on the Cheever list. There is significant therapeutic vaccine research involving NY-ESO-1 and MAGE-A3, particularly through the Ludwig Institute for Cancer Research. GlaxoSmithKline (GSK) has licensed NY-ESO-1 and MAGE-A3 from LICR and is currently conducting phase III clinical trials of a MAGE-A3-based cancer vaccine in non-small cell lung cancer and melanoma.

- **hTERT:** hTERT is linked to tumor growth and development, and hTERT-specific T cells may contribute to tumor immunosurveillance. Expression is absent from most normal cells but present in more than 85% of human cancers and up to 99% of breast tumors. Significant work has been reported on multiple epitopes derived from hTERT and several formulations have been or are being evaluated in clinical trials as treatment vaccines.

- **Survivin:** Survivin plays a crucial role in tumor survival and is not present in normal cells. It is expressed highly in most cancers and up to 90% of human breast cancers. Survivin expression has been found in DCIS, particularly in samples with ER+ tumors and microinvasion. Antibodies to survivin have been found in cancer patients and are absent from healthy volunteers. Its increased expression is an unfavorable prognostic marker in breast cancer correlating with decreased overall survival. Survivin has been evaluated in clinical trials as part of a multi-peptide vaccine with hTERT. Survivin was ranked #21 on the Cheever list, with high scores for immunogenecity and oncogenecity. In a study of 420 breast cancer cases, survivin was detected in 378 (90%).

- **NY-BR-1:** NY-BR-1 is a transmembrane protein, present in the normal ductal epithelium of the breast and overexpressed in up to 80% of breast tumors. It is a differentiation antigen and a strong and early marker of early breast tumorigenesis. Some reports indicate that expression is lost as the tumors progress. NY-BR-1 was originally discovered using SEREX (serological analysis of recombinant tumor cDNA expression libraries) to identify breast cancer tumor antigens. Expression was found in breast and testis samples, but not in other normal tissues. NY-BR-1 was ranked #53 on the Cheever list. Work is currently underway to identify appropriate epitopes.

- **Tumor-associated carbohydrate antigens:** Overexpression of tumor-associated carbohydrate antigens (TACA) caused by malignant transformation may offer possible targets for a preventive vaccine, if the inherent low immunogenicity and tolerance of the immune system can be overcome. Despite the low immunogenicity, tumor-associated MUC1 was ranked second of 75 tumor-associated antigens, on the Cheever priority list. Subsequent research, including clinical trials with vaccines against MUC-1 with various adjuvants, have demonstrated safety and the ability to generate immune responses. The antigen is overexpressed in many tumor types, is found on cancer stem cells, and has a functional role in tumorigenesis. Of note, a smaller carbohydrate structure that is produced as MUC1 is glycosolated – Thomsen-Nouvelle - has received significant attention, and may be an attractive target for a preventive vaccine.

  - **Thomsen-Nouvelle (Tn):** Tn is present in 90% of human carcinoma tissue and has been proposed as a universal carcinoma marker. High expression (almost 90%), is found in both invasive breast cancer and DCIS. Tn may be a very early marker for breast tumorgenesis. Cancer vaccines containing the Tn antigen as a single tumor antigen or as a component of a polyvalent vaccine have progressed into phase I and II clinical trials (mainly prostate cancer). Tn was ranked #50 on the Cheever list.

Immunotherapy of DCIS

H. Kim Lyerly, MD & Danny Douek, MD, PhD

It is possible that strategies that prevent DCIS or its progression to invasive breast cancer could be effective for preventing primary breast cancer. Other cancers, such as colon cancer and cervical cancer, begin with a noninvasive phase, but whether all breast cancers begin with a noninvasive phase is not known. The limited data on DCIS indicate that it might express the same genes as invasive breast cancer, and be immunogenic; for example, one study showed that 28% of DCIS cases were erbB2-positive.

To prioritize antigens associated with DCIS for a vaccine, Dr. Lyerly is first identifying genes associated with a poor prognosis and copy number aberrations in frozen samples of invasive breast cancer, and in DCIS, and identifying accompanying genes that are co-amplified on the same chromosomal region. He will then confirm that the patterns of genes identified are present in archival specimens of early breast cancer and DCIS, in formaldehyde fixed and paraffin embedded specimens, and available cell lines. The next steps will be to use proteomics to identify/confirm processing and MHC presentation of T-cell epitopes encoded by these genes, confirm that they are presented by DCIS, test the T-cell antigens' immunogenicity, expand antigen-specific T-cells in vitro, and show that these T-cells can recognize and lyse DCIS cells. Dr. Lyerly will use a set of frozen breast samples collected by mammotome, consisting of almost 1,000 early breast tumor samples, including more than 200 pure DCIS samples with 10 years of follow up, for this analysis.

In addition, these archived specimens can be used to not only to identify antigens but, rather, to determine whether evidence exists of an adaptive immune response in DCIS. This can be accomplished by detecting the expansion of clonal or oligoclonal populations of T cells with DCIS samples.

Dr. Douek, followed up from the October 1 small group meeting on immune issues, by demonstrating in the lab that it is possible to amplify T cell receptor B transcripts from DCIS samples. Dr. Douek extracted RNA from the purified mRNA of five DCIS samples than used his technique to amplify T cell receptor beta subunit transcripts. From those amplicons he made libraries, clustered and sequenced them. This pilot project demonstrated the feasibility, and follow up studies to demonstrate the T cell response to DCIS can be performed on this sample set.

Preventive Vaccine Research Update

Stephen Johnston, PhD

Dr. Johnston's preventive vaccine research focuses on frameshift-mutated neoantigens that result from microsatellite instability. He has used bioinformatic and genetics analyses to identify aberrant transcripts that are associated with tumor cells encoding frameshift neo-peptides. Dr. Johnston is developing an array containing 330,000 random peptides based on hundreds of breast cancer and normal samples that will be

used with the frameshift peptide database to predict the presence of positive frameshift antigens in people with cancer.

Every frameshift peptide that is also present in mouse tumors is examined in a mouse model to determine whether that peptide provides any protection. The next step is testing in vitro T-cell activity of the candidate peptides in cancer patient specimens to determine whether these antibodies are part of the signature diagnosis of breast cancer. A list of candidate frameshift peptides will soon be available, and six peptides are expected to cover more than 90% of people with cancer.

Dr. Johnston plans to design a Phase 2 efficacy trial that will use a diagnostic based on a random peptide array that can detect cancer at a very early phase to exclude patients with very early tumors (before they present the relevant antigen). Patients will then be randomly assigned to receive a vaccine or a mock vaccine and will be monitored using the early detection diagnostic. Mouse studies have shown that the diagnostic can detect very aggressive tumors 2 weeks before these tumors are histologically evident based on their immuno-signatures.

B. BREAST CANCER VACCINE CLINICAL TRIALS RELEVANT TO ARTEMIS PROJECT® VACCINE DEVELOPMENT

Immunologically Targeting HER Family for Breast Cancer Prevention

Brian Czerniecki, MD, PhD

Dr. Czerniecki has conducted two clinical trials of a vaccine consisting of type 1 polarized dendritic cells (DCs) pulsed with six HER-2/neu promiscuous major histocompatibility complex (MHC) Class II-binding peptides and two HLA-A2.1 Class I-binding peptides administered directly into the groin lymph nodes of patients with HER2-positive DCIS four times prior to surgery. The investigators generated DCs from the patients' own blood through apheresis.

Most patients had HER2-reactive CD4 and CD8 T-cells, suggesting a complete immune response. Women with estrogen receptor-negative DCIS had no recurrence following vaccination and lumpectomy. In addition to eliminating HER2, the vaccine appeared to reduce proliferation in breast cancer stem cells in luminal phenotypes. None of the patients in either trial had long-term cardiac sequelae after 84 months of followup.

Multi-Epitope Folate Receptor Alpha and HER-2/neu Peptide Vaccines

Keith Knutson, PhD

Dr. Knutson's initial HER2/neu-based vaccines included an extracellular domain vaccine with three Class II peptides, an intracellular domain vaccine with three Class II peptides, and an HLA-A2 vaccine with three Class II peptides that encompassed HLA-A2 motifs. The vaccines were tested in Phase 1 clinical trials in patients with Stage III or IV breast cancer and a few patients with ovarian cancer. Patients had microscopic disease or no evidence of disease. The vaccines were administered monthly for 6 months with granulocyte-macrophage colony-stimulating factor (GM-CSF) as the adjuvant.

These initial trials were conducted before trastuzumab (Herceptin®) became available. At that time, approximately 30–40% of patients with Stage III breast cancer survived for 8 years without treatment, and survival rates were lower in those with Stage IV disease. After vaccination, approximately 75–80% of patients survived for 8 years. However, these were Phase 1 single-arm trials, and more advanced trials would be needed to draw any conclusions from these data.

Using improved bioinformatic approaches, Dr. Knutson subsequently identified four new HLA Class II epitopes HER2/neu based on evidence that these epitopes are naturally targeted by the immune system in breast cancer patients. These epitopes have been formulated into a new vaccine and are being tested in a Phase 1 clinical trial of this vaccine plus GM-CSF that began in September 2012 and will ultimately enroll 22 patients with HER2 2+ and 3+ breast cancer to test the vaccine's immunogenicity, safety, and feasibility.

In another trial, Dr. Knutson is also testing a folate receptor alpha based vaccine consisting of 5 epitopes mixed with GM-CSF. The folate receptor is expressed in up to 70% of breast cancer and is associated with poor outcome. No immune data are available for either trial at this time, but in the first five patients on each trial, the only adverse effects were injection site reactions and fatigue.

AE37, E75, and GP2 Vaccines to Prevent Breast Cancer Recurrence

George Peoples, MD

Dr. Peoples summarized his clinical trials of three HER2/neu peptide vaccines. The first of these, E75 (now known as NeuVax and licensed by Galena Biopharma), is derived from the protein's extracellular domain. In a Phase 1/2 trial 108 women with node-positive or high-risk node-negative breast cancer who had completed standard therapy received this vaccine and were compared to 79 women with node-positive or high-risk node-negative breast cancer who did not receive the vaccine. The 60-month disease-free survival (DFS) rate was 90% in vaccinated women and 80% in the control group. Injection site reactions were the most common adverse effect. Among patients with HER2 1+ and 2+ disease, the DFS rate was 89% in the vaccine group and 76% in the control group. DFS was also higher in patients who received the optimal dose and those who received booster vaccines.

Prevention of Recurrence in Early-Stage Node-Positive Breast Cancer with Low to Intermediate HER2 Expression with NeuVax Treatment (PRESENT), a prospective, randomized, double-blind Phase 3 trial of E75 with GM-CSF compared to GM-CSF alone, is now underway. It is enrolling 700 patients with HLA-A2+ or HLA-A3+ positive, node-positive, and HER2-negative breast cancer. All patients will receive the optimal dose and boosters, and the study's primary endpoint is DFS at 36 months.

A Phase 2 trial is evaluating whether a GP2 vaccine or AE37 vaccine combined with GM-CSF (or GM alone) reduces breast cancer recurrence rates in 400 patients with node-positive breast cancer or high-risk, node-negative invasive breast cancer. The 24-month interim analysis shows that DFS, at almost 90%, is similar in the GP2 and AE37 vaccine groups, compared to 80% in the control group. Both peptides appear to be safe and effective in increasing HER2 immunity.

A Phase 2, single-blinded trial led by Dr. Jarrod Holmes will evaluate a combination of trastuzumab and NeuVax followed by boosters in 300 patients with HLA-A2+ or HLA-A3+, low expressing, HER2 breast cancer who have completed standard therapy. The primary endpoint is 24-month DFS.

Personalized Breast Cancer Vaccines Based on DNA Sequencing

William Gillanders, MD

Many cancer vaccines developed to date have targeted shared tumor antigens, which are expressed in a limited subset of normal tissues and are overexpressed in several cancers, but these studies have often had disappointing results. Unique tumor antigens, which are typically expressed in a single cancer, have a low autoimmunity risk, induce high-affinity T-cell responses, and might limit antigen loss. All intrinsic subtypes of breast cancer appear to have many candidate unique tumor antigens, suggesting that a personalized vaccine approach could be used in all breast cancer patients, regardless of intrinsic subtype or HLA type.

Dr. Gillanders and colleagues established patient-derived breast cancer xenografts in NOD/SCID mice and used primary tumor and germline sequencing and HLA binding algorithms to identify candidate unique tumor antigens. Based on these data, 32 of 37 candidate unique tumor antigens were predicted to bind to at least one HLA allele. The team has generated human CD8 T-cell lines that are specific for four unique tumor antigens identified by genome sequencing. Three of these cell lines lysed WHIM2 cells (the patient-derived xenograft that was sequenced and analyzed) but not WHIM12 cells (an irrelevant patient-derived xenograft). This was the first demonstration that unique tumor antigens are processed and presented in breast cancer.

Dr. Gillanders plans to design and validate a personalized DNA breast cancer vaccine targeting unique tumor antigens identified by genome sequencing using an unbiased approach that integrates all of the

candidate unique tumor antigens into a polyepitope DNA vaccine. Several in vitro and in vivo studies in mice and humans have shown that vaccination with peptide, viral, or DNA polyepitope constructs can successfully elicit CD8 T-cell responses.

In a planned Phase 1 clinical trial, DNA from the primary tumor and peripheral blood of patients with locally advanced breast cancer will be sequenced; nonsynonymous mutations will be identified and validated; and the investigators will design, synthesize, and validate a polyepitope DNA vaccine that integrates all candidate unique tumor antigens. These vaccines will be administered by electroporation after patients complete their initial therapy, and the trial's endpoints will be safety and feasibility.

Immune Issues Relevant to Antigen Identification and Evaluation: Report from Artemis Project® Immune Issues Meeting, October 1, 2012

Peter P. Lee, MD

Dr. Lee summarized the discussions from the October 2012 meeting of the Artemis Project's immune issues team and outlined future action steps:

- Screen blood from DCIS patients to validate antigens
- Screen blood from healthy women for evidence of T-cell or antibody responses to novel antigens
- Sequence T-cell receptors from DCIS samples to understand the diversity of natural T-cell responses and identify major natural targets of dominant clones
- Broadly consider all mouse models of breast cancer, conduct proof-of-concept studies using transgenic inducible models, and develop large-animal breast cancer models
- Consider glycosylated antigens, such as MUC1
- Meet with FDA to obtain guidance on the agency's requirements, especially if the Artemis Project plans to test a self-antigen-based vaccine in healthy women
- Develop a clinical trials network and reference laboratory
- Begin planning human clinical studies now, including a Phase 1 trial of a single-antigen vaccine

III. UPDATED ARTEMIS PROJECT® RESEARCH PLANS

Artemis Project® team members formed breakout groups to outline the specific tasks required over the next two years within three areas: 1) Antigen Prioritization and Validation 2) Pre-clinical Studies and 3) Clinical Studies.

A. ANTIGEN PRIORITIZATION AND VALIDATION

Vladimir Belyi, Frank Calzone, Paul Ewald, Yaniv Erlich, Gregory J. Hannon, Stephen Johnston, Simon Knott, Debbie Laxague, Elaine Mardis, Laura Nikolaides, Patricia Renzulli, Jill Slansky, and Paul Spellman

The antigen prioritization group recommended that the Artemis Project® team develop a vaccine containing approximately five distinct antigens and give highest priority to developing a list of tumor neoantigens that frequently arise from the tumor genome. The criteria for selecting the neoantigens to pursue are:

- High priority: Microsatellite frameshift mutations and alternative mRNA transcripts
- Consider pursuing (but insufficient alone): Overexpressed native proteins

- Include in new searches: Mitochondrial genome mutations
- Do not pursue: Amino acid substitutions, translocations, and trans-splicing

Neoantigens will require vigorous validation but some of the validation has already been completed on crossover antigens. For this reason, a suitable crossover antigen could be moved forward more quickly than a neoantigen. One strategy may be to pursue crossover antigens now while waiting for more data on neoantigens, although the vaccine will ultimately combine crossover and neoantigens.

Pathogen antigens are not a high priority for the coming year because of timeframe constraints and the uncertain outcomes of the pathogen genomics mining. Similarly, because antigens identified by immunological methods are unlikely to be available within the next year, these antigens are not a high priority for the next year.

Table 1 lists the tasks involved in prioritizing and validating antigens, along with the person responsible and deadline for each task.

Task	Person(s) Responsible	Deadline
Identify initial candidate neoantigens	Neoantigen mining team: Yaniv Erlich, William Gillanders, Gregory Hannon, Simon Knott, Stephen Johnston, Elaine Mardis	August 1, 2013
Determine whether to pursue self-antigens that have been previously described, including cancer testis antigens and WT1	Crossover antigen team: Brian Czernicki, Keith Knutson, Laura Nikolaides, George Peoples, and Mark Schwartz	August 1, 2013
Complete pathogen genomics mining project on schedule	Pathogen antigens team: Vladimir Belyi and Paul Ewald	December 2013
Validate initial lists of antigens against RNAseq data	Paul Spellman	September 1, 2013
Meet to discuss RNAseq results and revise candidate antigens list as necessary	Neoantigen and crossover antigen teams	September 30, 2013
Complete RNAseq on Duke DCIS tumor set	Kim Lyerly/Greg Hannon/ PI?	October 31, 2013

Table 1. Antigen Prioritization and Validation Tasks

Task	Person(s) Responsible	Deadline
Obtain RNA samples	PI TBD	October 31, 2013
Prioritize candidate antigens based on frequency in tumors, number of breast cancer subtypes, and DCIS expression	Neoantigen and crossover antigen teams	November 30, 2013
Use RT-PCR on an RNA panel of at least 30 samples of each breast cancer subtype to independently confirm the high-priority antigens **Cost: $50,000**	PI TBD	December 31, 2013
Complete data analysis and report findings	TBD	N/A
Complete immunological validation of 5–10 antigens using the Arizona State University peptide profiling platform by determining whether • **Antibodies are present in breast cancer patient serum at frequencies predicted by tumor gene expression.** • **The antigen elicits a T-cell response *ex vivo* in cells from patients with BRCA1/2 mutations who express the antigen.** • **The antigen shows efficacy as a vaccine in a murine syngeneic mouse model (optional).**	Stephen Johnston, Peter Lee, and Susan Love	N/A
Validate translation of selected antigens using protein expression arrays to determine whether the antigen is detected in breast cancer cell lines and tumors by western blot and immunohistochemistry	TBD	N/A
Coordinate antigen prioritization and validation activities	NBCC advocate(s)	Ongoing

Table 1. Antigen Prioritization and Validation Tasks *(continued...)*

Suggestions from participants were to:

- Use mass spectrometry to identify peptide major histocompatibility complexes (MHCs) on the surfaces of breast cancer cells
- Instead of detecting antigens using RT-PCR, gather evidence that a sufficient amount of protein is made
- Determine whether T-cells from breast cancer patients react to tumor using different peptides and MHC proteins

Preclinical Studies

Amy Bonoff, Brian Czerniecki, Danny Douek, Gabriel Gutierrez, Keith Knutson, Peter Lee, David Raulet

The preclinical studies group outlined steps to evaluate candidate self-antigens for immunogenicity and to generate safety data for the investigational new drug (IND) application. Ideally, the vaccine will generate a response that involves several levels of the immune system, activating CD4 and CD8 T-cells, and B-cells. Identification of the appropriate adjuvant will be part of the pre-clinical studies.

Mouse studies will be needed to test the immunogenicity of self-antigens individually and in combination. FDA will require evidence of responses to these antigens and safety studies in rodents. The preclinical group estimates that clinical assay development would cost $3 million in an industry facility, where such assays are usually developed. However, an academic GMP facility could probably create these assays at a much lower cost. The final product at the end of the year will be a vaccine that can be tested in a clinical trial.

During the discussion, participants said that FDA is unlikely to allow the vaccine to be tested in healthy women in the initial clinical trial, especially if the vaccine uses self-antigens. However, some self-antigens (e.g., WT1, hTERT, and survivin) have already been tested in humans. The pre-IND discussions with FDA should address whether FDA would permit first-in-humans studies of the vaccine in healthy women and any special toxicology requirements because the product will ultimately be used in healthy women of childbearing age. The results of early pre-IND discussions can then be addressed in the preclinical animal studies.

The vaccine could be tested initially in healthy women who have finished childbearing or do not plan to have children, and the sample could be subsequently expanded to women of childbearing age and those who are lactating. Because the vaccine will ultimately be given to women of childbearing age, its potential teratogenic effects must be studied. Macaques might be an appropriate animal model to study the vaccine's effects on lactating mothers. Studies could also be done in prophylactic mastectomy or oophorectomy samples from BRCA1/2 carriers or using breast milk from Army of Women volunteers, to look for evidence of the vaccine antigens.

Table 2 provides the timeline and costs for preclinical studies to evaluate three self-antigens.

		June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Costs*
Design and make constructs	Mouse	Viral/DNA construction of three candidate antigens												$25,000
Pre-IND		Discussions with FDA to determine needed toxicology and safety data												

Table 2. Timeline and Costs for Preclinical Studies of Three Self-Antigens

*Direct costs only

		June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Costs*
Testing in Mice	Immuno-genicity**	T-cell assay (EliSpot) of single antigens and combination												$600,000
		B-cell assay (ELISA) of single antigens and combination												
	Safety**	Immunohistochemistry												
		Tissue pathology, blood counts, and enzymes***												
Manufacture Hu construct							Analytical assay development, release testing, stability (in an academic GMP facility or an industry facility)							$300,000 to $3 million
Non-clinical Hu construct											Safety and toxicology study (in rats or rabbits) in a GLP facility			$20,000 to $350,000

Table 2. Timeline and Costs for Preclinical Studies of Three Self-Antigens *(continued...)*

*Direct costs only
**Includes analysis of glandular tissue
***Conducted by a pathologist certified by the American College of Veterinary Pathologists

Clinical Studies

Silvia Formenti, William Gillanders, Pat Haugen, Jarrod Holmes, Susan Love, Doug Lowy, Kim Lyerly, George Peoples, Michele Rakoff

The clinical studies group outlined the action steps to undertake in planning for a Phase 1 clinical trial. The primary action steps are as follows:

- Consult with an experienced biostatistician with expertise in drug development/FDA marketing approval studies and vaccines to determine the sample size and timeline for each clinical trial in the development pathway and whether novel trial designs might be appropriate
- Present an overview of the preclinical and clinical trial plan to FDA in an pre-IND meeting
- Investigate surrogate endpoint(s) using existing or planned Phase 3 clinical trials to demonstrate the breast cancer vaccine's clinical efficacy
- Consult with the preclinical team to design and plan Phase 1 clinical trial(s) of the vaccine in healthy women or the population of women to be targeted for the first FDA marketing approval study

Additional action steps are:

- Take inventory of existing breast cancer vaccine and other cancer vaccine trial data that could be applicable to breast cancer prevention
- Determine whether data on any therapeutic or adjuvant breast cancer vaccines (such as primary endpoints used or biospecimens collected) can inform the clinical trial's design
- Consult with FDA and a biostatistician about strategies to advance secondary prevention trials to a primary prevention pathway

- Determine whether data (such as event rates) from ongoing breast cancer primary prevention trials can be used to identify high-risk populations
- Determine whether data (such as risk of recurrence, metastasis, or secondary cancer) from other breast cancer trials can be used to identify high-risk populations

Deliverables and associated costs are listed in Table 3.

Deliverable	Cost
Report of biostatistician regarding power, sample size, and other clinical trial design issues	$25,000
Report on pre-IND meeting with FDA	$25,000
Finalized design of Phase 1 clinical trial(s)	$25,000
Inventory of breast cancer and cancer vaccine trials	$0
Report on collaborative opportunities to validate endpoints or immune biomarkers	$0

Table 3. Clinical Trial Deliverables and Costs

Tamoxifen offers an example of the approval pathway that FDA might follow for a breast cancer prevention vaccine. FDA initially approved tamoxifen for use in women with metastatic breast cancer before granting approval for use in the adjuvant setting and then in healthy women. If the development of a primary prevention breast cancer vaccine needs to follow this path, the steps required (such as testing in high-risk postmenopausal women, then high-risk women who have completed their childbearing, then healthy young women) will need to be determined through discussions with FDA.

During the discussion, participants cautioned that a great deal must be learned about the vaccine before it is tested in young healthy women. However, a vaccine that prevents primary breast cancer might not be effective in women with metastatic breast cancer. Furthermore, participants should not assume that FDA will require the first clinical trial of the vaccine to include only women with metastatic breast cancer. Other vaccines have been tested in healthy people in initial clinical trials, and FDA has demonstrated a willingness to approve testing of a cancer vaccine in the adjuvant setting without proof of effectiveness in metastatic cancer. If, for example, the antigen used does not exist in normal breast and safety data are available, FDA might allow testing in healthy women.

IV. ARTEMIS PROJECT® FOR A PREVENTIVE VACCINE: NEXT STEPS

In addition to the steps outlined by the groups to prioritize and validate antigens, to formulate the vaccine, and to prepare for clinical trials, the group identified additional action steps which would complement the work outlined by the breakout groups. These action steps are outlined in Table 4.

Action	Person(s)/Group Responsible
Continue to mine data on pathogen antigens and expand this effort to non-viral pathogens**	Vladimir Belyi, Paul Ewald
Determine whether the Komen Tissue Bank can provide suitable normal breast specimens for Paul Spellman's RNAseq study	NBCC
Determine whether the RNAseq results identify the tumor-associated antigens on the Cheever list	Paul Spellman
Collect follow-up data on breast cancers in women who have received the HPV vaccine**	NBCC
Send names of genes to Dr. Spellman for inclusion in his RNAseq analyses	All Attendees
Determine which specimens and data to request from the Army of Women	NBCC
Consult a biostatistician about designs of Phase 1 and 2 clinical trials (might cost $50,000)**	TBD
Share information on a new early breast cancer detection biomarker platform	Gabe Gutierrez
Send names of experts on biomarkers of breast cancer incidence to NBCC	George Peoples, Elaine Mardis, Keith Knutson
Convene a small-group meeting to discuss breast cancer biomarkers	George Peoples, Elaine Mardis, Keith Knutson
Send names of genes to Dr. Spellman for inclusion in his RNAseq analyses	NBCC
Complete RNAseq in 200 DCIS samples (might cost approximately $250,000)**	Kim Lyerly, Greg Hannon, additional PI?

Table 4. Action Steps and People or Groups Responsible for Each Action

** High-priority activity that requires funding in the coming year