NATIONAL BREAST CANCER COALITION

ARTEMIS PROJECT

National Breast Cancer Coalition
The Breast Cancer Deadline 2020

ANNUAL MEETINGS
MARCH 10-13, 2017

I. INTRODUCTION

The National Breast Cancer Coalition (NBCC) was formed in 1992 to end breast cancer through the power of grassroots action and advocacy. Since that time, NBCC has built a strong coalition of advocates and organizations that support its mission. In 2010, NBCC launched the **Breast Cancer Deadline 2020®** campaign a strategic plan of action, set out in a blueprint, that is designed to identify by 2020, the knowledge, approaches and tools, needed to end breast cancer.

This unprecedented campaign includes a research component, known as the **Artemis Project®**, a collaboration that involves researchers, advocates, and other key stakeholders who set priorities and design and implement research plans that focus on two areas:

- **Primary Prevention:** How do we stop women and men from getting breast cancer?
- **Prevention of Metastasis:** How do we stop them from dying of breast cancer?

The various reports from previous annual meetings, found at (http://www.breastcancerdeadline2020.org/about-the-deadline/artemis-project.html) lay out the history of the Artemis Project. This report is a summary of discussions and recommendations made at the 2017 annual Artemis meeting. This meeting included more than 30 participants including advocates and those with scientific expertise ranging from immunology, biophysics, genetics, to molecular biology, and clinical oncology.

2017 ANNUAL MEETING PARTICIPANTS

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

Frank Calzone, PhD, Biotechnology Consultant

Joe Camardo, Sr. Vice President, Global Medical Affairs, Celgene

Jayanta Debnath, MD, Professor and Vice Chair for Research, Department of Pathology, University of California, San Francisco, Member, Helen Diller Family Comprehensive Cancer Center

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

Stephen J. Elledge, PhD, Gregor Mendel Professor of Genetics and Medicine, Department of Genetics, Harvard Medical School, Division of Genetics, Brigham and Women's Hospital

Yaniv Erlich, PhD, Assistant Professor of Computer Science, Columbia University, Core Member, New York Genome Center

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

Silvia C. Formenti, MD, Chair, Department of Radiation Oncology, Weill Cornell Medical College, Radiation Oncologist-in-Chief, New York Presbyterian/Weill Cornell Medical Center

Cyrus Ghajar, PhD, Director, Laboratory for the Study of Metastatic Environment, PSH Program: Translational Research Program, Fred Hutchinson Cancer Research Center

Pat Haugen, BA, Advocate, Inflammatory Breast Cancer Research

Judi Hirshfield-Bartek, RN, MS, OCN, Advocate, Dr. Susan Love Research Foundation

Amreen Husain, MD, Global Development Team Leader, Atezolizumab Breast and Gynecologic Cancers Program, Genentech, Inc.

Stephen A. Johnston, PhD, Director, Center for Innovations in Medicine, Director of the Biological Design Graduate Program at the Biodesign Institute, Professor of Life Sciences, Arizona State University, CEO, Calviri, Inc.

Simon Knott, PhD, Assistant Professor, Associate Director, Center of Bioinformatics and Functional Genomics, Cedars-Sinai Medical Institute

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

Debbie Laxague, RN, Advocate, BCSSC

Mark Lee, MD, PhD, Head of Clinical Development and Medical Affairs, GRAIL, Inc.

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

Vivian Lee, Advocate, Breast Cancer Connections

Christopher Li, MD, PhD, Research Professor, Epidemiology Fred Hutchinson Cancer Research Center

Susan Love, MD, MBA, Chief Visionary Officer, Dr. Susan Love Research Foundation

H. Kim Lyerly, MD, FACS, George Barth Geller Professor for Research in Cancer and Professor of Surgery, Duke University Medical Center

Stuart S. Martin, PhD, Professor of Physiology, Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine

Josef Penninger, PhD, Scientific Director, Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Professor of Immunology and Medical Biophysics, University of Toronto, Professor of Genetics, University of Vienna, Austria, Honorary Professor, Chinese Academy of Sciences, Peking Union Medical College

Joseph Pickrell, PhD, Junior Investigator and Core Member, New York Genome Center, Adjunct Assistant Professor, Department of Biological Sciences, Columbia University

Michele Rakoff, Advocate, CABCO

Paul Spellman, PhD, Professor, Department of Molecular and Medical Genetics, Director, Quantitative Oncology Program, Knight Cancer Institute, Oregon Health & Science University

Sohail Tavazoie, MD, PhD, Senior Attending Physician, Leon Hess Associate Professor, Elizabeth and Vincent Meyer Laboratory of Systems Cancer Biology, The Rockefeller University

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

Fran Visco, JD, President, NBCC

Alana Welm, PhD, Associate Professor, Department of Oncological Sciences, University of Utah, Investigator, Huntsman Cancer Institute, Member, Cell Response and Regulation Program

MEETING SUPPORT

NOTE TAKERS:

Erika Crosby, PhD, Postdoctoral Researcher, Department of Surgery, Duke University Medical Center

Jaime Fornetti, PhD, Postdoctoral Researcher, Huntsman Cancer Institute, University of Utah

Nancy Gough, PhD, Director Research Collaboration, NBCC

Giselle Hicks, MPH, Advocate

FACILITATOR:

Kayla Kirsch, MS, President, Leapfrog Consulting

LOGISTICS:

Marva Lewis, The Event Professionals

II. BACKGROUND AND DISCUSSION

Friday evening, March 9, was set aside for introductions, background and general scientific presentations.

The session on Prevention of Metastasis began Saturday, March 11 to Noon, Sunday, March 12, followed by the session on Primary Prevention, Preventive Vaccine.

BACKGROUND PRESENTATIONS (EACH FOLLOWED BY DISCUSSION AMONG ALL PARTICIPANTS)

Review of Vaccine Landscape

Debbie Laxague, RN, Advocate, BCSSC

Debbie reported that there was one prevention vaccine report published in 2016 and that study included a treatment component. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4931621/) There were many articles published on breast cancer prevention through lifestyle factors and were also some medication trials using tamoxifen, aromatase inhibitors, or other non-breast cancer-specific medications like nonsteroidal anti-inflammatory drugs (NSAIDs, such as aspirin). An Australian trial used two medications, a selective androgen receptor modifier (SARM) and an aromatase inhibitor (AI); however, the endpoint was reduced mammographic density in peri-menopausal women rather than survival.

There were 25 new immunomodulation trials conducted in different settings, from advanced cancer to the neoadjuvant setting. Many are using PD-1 inhibitors in combination with standard treatment or after as a single agent. Most are small, phase I trials.

Among the five breast cancer vaccine trials, all were therapeutic (not preventive) and most were in the neoadjuvant setting. Three were small, phase I trials. The two phase II trials were both in TNBC patients with pathologic complete response as the endpoint. Both are folate receptor alpha vaccines with GM-CSF and oral cyclophosphamide. In one the randomization was with/without oral cyclophosphamide before vaccine administration, and in the other the randomization was to vaccine or not.

Ongoing trial results are difficult to find, and at least one study, the NEUVAX trial of patients with HER2-low breast cancer, has been terminated due to futility. A poster summarizing the vaccine landscape is attached as Addendum 1.

Biomarkers of Disseminated Tumor Cells (DTCs)/Metastasis: Overview of Published Data

Alana Welm, PhD, Associate Professor, Department of Oncological Sciences, University of Utah, Investigator, Huntsman Cancer Institute, Member, Cell Response and Regulation Program

Alana presented data from three articles published late in 2016 on breast cancer tumor cell dissemination and dormancy.

(1) Johnson et al. (Nat. Cell Biol., Oct 2016; https://www.ncbi.nlm.nih.gov/pubmed/27642788) used mouse breast cancer models and xenograft models with human breast cancer cells in mice to look at whether the bone microenvironment maintains breast cancer cell dormancy through activation of the LIF-LIFR pathway. LIF (leukemia inhibitory factor) is a secreted signal of the interleukin-6 (IL-6) family.

(2) Hosseini et al. (Nature, Dec 2016; https://www.ncbi.nlm.nih.gov/pubmed/27974799) compared primary tumor cells and metastatic tumor cells from the same mouse and found that 80% of lung metastases derived from early disseminated cells. These early cells had disseminated before the primary tumor had acquired 50% of its genetic mutations, illustrating the importance of taking into account the heterogeneity of tumor cells when targeting the seeds of metastasis. These results also suggest a model of both early disseminating cells, which mutate separately from the primary tumor resulting in metastases that have different mutations than the primary tumor, and of late disseminating cells, such that the mutations match between the primary and metastatic tumors derived from these late cells.

(3) Harper et al. (Nature, Dec 2016; https://www.ncbi.nlm.nih.gov/pubmed/27974798) also used mouse models to illustrate that early dissemination and metastasis in HER2+ mammary cancer may involve aberrant activation of a developmental program for mammary ductal branching, an event that occurs during mammary development.

Lymph Nodes: Soil/Seed

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

Peter provided an overview of how cancer affects the lymph nodes and how these lymph node changes relate to metastasis and the ability of cancer cells to evade immune detection. So, why is lymph node metastasis predictive of advanced disease? Van der Weyden et al. (Nature, 2017; https://www.ncbi.nlm.nih.gov/pubmed/28052056) showed that, in mice with an intact immune response, metastasis did not occur. The animals that did develop metastasis were found to have defects in the immune response. Thus, metastasis occurs once the immune system has been disrupted. Furthermore, the tumor cells that invade the lymph nodes may contribute to the disruption of the immune response.

Numerical (the proportion of different types of immune cells) and spatial relationships (such as, dendritic cell clustering) between immune cells are altered within tumor-draining lymph nodes (TDLNs). Changes in these two properties, the numerical and spatial relationships among immune cells, in particular dendritic cell clustering, are predictive of clinical outcome in breast cancer. Dendritic cell clustering is only seen in the lymph nodes, not with dendritic cells at peripheral sites or in the dendritic cells that invade tumors, and dendritic cell clustering in the lymph node is part of a normal immune response. Patients with intact T cells and clustered dendritic cells have higher survival rates, independent from the presence of histologically detectable tumor cells in the lymph node. The presence of lymph nodes with few CD33+ immune cells also correlates with survival. Tumor-invaded lymph nodes lack clustered dendritic cells. Thus, analysis of immune profiles and the numerical and spatial relationships in TDLNs provides prognostic information. The hypothesis is that breast cancer cells in TDLNs engage and then disarm the host immune system, thereby disrupting immune control of distant metastasis.

Immunotherapy in Breast Cancer

Amreen Husain, MD, Global Development Team Leader, Atezolizumab Breast and Gynecologic Cancers Program, Genentech, Inc.

Amreen presented an overview of how cancer cells can evade the immune system and described efforts to leverage immunotherapy in breast cancer.

III. ARTEMIS PROJECT ON PREVENTION OF METASTASIS

SEED GRANT UPDATES

Genetic Determinants of Metastasis: DNA.Land

Yaniv Erlich, PhD, Assistant Professor of Computer Science, Columbia University, Core Member, New York Genome Center and Joseph Pickrell, PhD, Junior Investigator and Core Member, New York Genome Center, Adjunct Assistant Professor, Department of Biological Sciences, Columbia University

Yaniv provided an overview on the progress on gathering genetic data through DNA.Land and reported on the status of the NBCC breast cancer questionnaire. DNA.Land is a website for consumers to upload their DNA test results. DNA.Land is partially funded by a seed grant from the Artemis Project. Such a website enables "case-control association mapping by proxy" using family history of disease. DNA.Land has partnered with NBCC to develop a short, initial questionnaire of family history with questions relevant to breast cancer. In addition to including a consent form, the questionnaire includes questions, such as type of breast cancer, stage, year of diagnosis, and whether it recurred or not for the responders' own history and that of family members.

The resulting database will be a resource for researchers asking questions about recurrence and progression.

Understanding Tumor Dormancy: Investigating Adaptive Immune Recognition Of Quiescent DTCs

Cyrus Ghajar, PhD, Director, Laboratory for the Study of Metastatic Environment, PSH Program: Translational Research Program, Fred Hutchinson Cancer Research Center and H. Kim Lyerly, MD, FACS, George Barth Geller Professor for Research in Cancer and Professor of Surgery, Duke University Medical Center

Cyrus presented the project that is designed to understand tumor dormancy and the immune response to DTCs.

Because the papers that Alana discussed indicate that late disseminating DTCs may have more in common with the primary tumor cells than they do with early disseminating DTCs, it is critical to explore DTCs in context and not rely on just analysis of the primary tumor. There are likely different populations: Some that are proliferating and some that are capable of leaving the perivascular niche and invading the bone (or other tissue) to form a metastatic site.

PREVENTING METASTASIS WORKING GROUPS

After discussion, participants identified four topics for further focus and broke out into small working groups to discuss what tools, technology, and knowledge are needed to prevent metastasis. Participants were encouraged to take a "blue sky" approach, rather than focusing on currently available resources. There were two rounds of discussions in the working groups, except for those focused on the topics "Who/What/Why" and "Act Now".

1. Disseminated Cells: Who Is Who? Who Does What? Why?

Jayanta Debnath, Daniel Douek, Silvia Formenti, Debbie Laxague, Peter Lee, Vivian Lee, Stuart Martin [Stephen Johnston joined after the break]

The discussion focused on questions related to the relationship between circulating DNA, circulating tumor cells (CTCs), DTCs, how any of these cells relate to the macro metastases that develop and how to identify those cells that are destined to form lethal metastasis.

The consensus was that identification methods for CTCs are far from perfect, and those for DTCs are even worse.

The group began to design a study to learn more about the tumor cells at each stage of metastasis. One important distinction is between clinical dormancy (tumor is too small to detect) and identification of dormant cells residing in the marrow. Another issue is the possible role for changes in the patient (host) that determine if or when dormant tumor cells awaken and develop into overt metastasis.

Initial study design:

- Human patients with node-positive, locally advanced breast cancer, all subtypes; if population needs to be narrower for faster outcomes, study could be limited to TNBC patients
- Collect samples from primary tumor, lymph nodes, CTCs (blood), DTCs (bone marrow biopsy), metastatic tumor (if it occurs)
- Analyze with RNA-Seq, metabolic profiling, immune profiling with plasma metabolites, immune cell surface markers, and transcriptomes of cells in the blood or plasma
- Examine host factors associated with increased metastatic occurrence: weight gain during chemotherapy, death of spouse, transplant or other immune suppression, surgery for other indications (e.g., hip replacement, dental implants)

Caveat: This study design will result in key information about whether CTCs or DTCs (or both) form macro metastases; however, this study may not address early DTCs which may be the most important.

2. Data Commons

Leslie Bernstein, Yaniv Erlich, Joe Camardo, Judi Hirshfield-Bartek, Stephen Johnston, Mark Lee, Christopher Li, Susan Love, Joseph Pickrell, Paul Spellman

The goals of DNA.Land are to create the dataset, organize the data, and facilitate creative uses of and discovery with the data. Only data of a certain size enables new hypotheses, and this effort is building the infrastructure to generate data on that scale.

The group discussed and identified the large shared datasets that already exist and discussed how they could all work together. The first hurdle is data access. Using the Cohort Consortium, as an example, data are accessible only to organizations with a cohort of at least 10,000 people. NBCC would meet this criterion with the DNA.Land cohort of over 40,000 people and could join the Cohort Consortium and work to increase data access.

The second challenge is finding a target for drug development or intervention to prevent or eliminate recurrence and to identify modifiable recurrence risk factors.

Two data sources will contribute to the "NBCC cloud data extravaganza" with the aim of identifying extreme phenotypes for recurrence predicted by genotype and clinical factors:

1) **Retrospective:** There are cohorts of patients from the past (CTS, WECARE), some of whom have already been genotyped (4,700) and others who could be genotyped (4,000). Jeff Trent at TGEN is already moving forward with this, however, it will take time to pull the samples from the freezers at UC Irvine and to obtain resources, such as a statistical geneticist, for data management. This is estimated to take about a year and cost $50,000. However, it will serve as a model for other cohorts.

2) **Prospective:** Data from DNA.Land could be used as a pilot study to test hypotheses arising from the existing cohort data.

While NBCC is recruiting contributions and participation in the breast cancer survey for DNA.Land to obtain at least 25,000 informative participants by the end of 2017 they must obtain cloud space for the data and establish a team to curate the data, to ensure data harmonization, assess if the data capture recurrence effectively, answer questions about the data, and assess the legitimacy of proposals for research using the data.

3. How To Make The DTC Microenvironment Hot

Cyrus Ghajar, Amreen Husain, Simon Knott, Michele Rakoff, Asad Umar, Alana Welm [Jayanta Debnath, Vivian Lee, and Daniel Douek joined after the break]

The main questions for discussion were how to eliminate the reservoir for residual disease, and are the DTCs the reservoir?

The group discussed which patient population should be studied to ensure a reasonable timeframe and number of patients. Ideally, the goal would be to analyze DTCs in the same patient to understand how dormant DTCs and their niche differ from proliferative DTCs and their niche near a metastatic site, and how these two differ from metastatic cells and their microenvironment. Another goal would be to understand how DTCs from stage I patients (less likely to develop into metastasis) differs from DTCs from stage III patients (more likely to develop into metastasis).

The two strategies to prevent metastasis are to keep cells dormant forever or to remove them. To remove dormant cells, we need to target the survival mechanisms or the mechanisms by which these cells resist previous treatments. It is important to analyze patient specimens, not only mouse models or cultured cells, to

ensure clinical translation of the information. It is also important to preserve the architecture of the DTCs and their neighbors, respectively. Such in situ analysis of gene expression can be performed with new technology.

Trial proposal:

- Accrue 50 patients at two sites (Fred Hutchinson Cancer Center and Utah)
- Test population: Luminal B, Stage III breast cancer (30% recurrence in bone within 3 years)
- Obtain blood samples and bone marrow core biopsies pre-treatment (surgery), post-treatment, and at recurrence
- Compare DTCs and metastatic cells and neighboring cells of each using multiplexed in situ analysis, correlate with recurrence
- Outcomes: Identify candidate vaccine targets to kill DTCs, markers for true dormancy versus activatable DTCs (quiescence versus deep quiescence), and signals that maintain dormancy

4. Understanding Immunotherapies: Success And Failures

Frank Calzone, Stephen Elledge, Paul Ewald, Pat Haugen, Keith Knutson, Kim Lyerly, Josef Penninger, Sohail Tavazoie

Why do checkpoint inhibitors work or fail, and why is it important? Understanding these mechanisms will allow us to increase the proportion of responders. The group discussed a prospective trial with TNBC patients, including pre-treatment biopsies, anti-PD1 or anti-PD-L1 treatment, serial blood draws, and post-response biopsies. Analysis would include RNA-Seq, tumor immune profiling, and blood immune profiling.

Clinical trials of checkpoint inhibitors in breast cancer have consistently shown only 10-15% of patients have a partial or complete response. Although partial or complete response may not be the best parameters for measuring "success," because stable disease could also indicate resistance to therapy, understanding the responders at the molecular level would identify mechanisms underlying the positive immune response.

To prove an adaptive T cell response against the tumor and identify an effector T cell population that targets non-private antigens, TIL and peripheral blood lymphocyte analysis are needed.

Main Question #1: Understanding why responders respond?

Action item #1: Analyze longitudinal tumor RNA-Seq and peripheral blood mononuclear cells (PBMC) for markers of response

Main Question #2: Of the responders, can we use tumor cells or TILs to inform our vaccine or T cell therapies?

Action item #2: Create longitudinal profile of PBMC and tumor T cells of checkpoint inhibitor responders

- RNA-Seq of metastasis to build cDNA library from tumors in responders to identify possible tumor vaccine antigen targets
- RNA-Seq of metastasis to build cDNA library from tumors in responders to pulse matched antigen-presenting cells and find broadly, tumor reactive T cells or T cell receptors
- Recreate the TIL T cell receptor repertoire by sequencing T cell receptors of individual cells or clones

Two-year plan: Approach sponsors for trials, identify patients with meaningful response on current immune checkpoint blockade trials, and then identify available samples.

5. New Work Group

For the second round of small group discussions, most of the participants from the "Disseminated Cells: Who Is Who? Who Does What? Why?" group created a new group, ACT NOW, focused on doing something with what we already have to accelerate progress.

Novel Interventions: ACT NOW!

Daniel Douek, Paul Ewald, Silvia Formenti, Stephen Johnston, Debbie Laxague, Peter Lee, Susan Love, Stuart Martin

The group discussed a number of possibilities:

- Anti cytomegalovirus (CMV) and checkpoint inhibitor therapy for patients whose primary tumor have CMV+ cells;
- Include anti-pain medication during surgery instead of just general anesthesia. General anesthesia prevents the brain from perceiving pain, however, the body still reacts to the pain. Blocking the pain may be associated with better outcomes.
- For patients with both a primary tumor and metastasis, the primary tumor could serve

as the source of epitopes for a vaccine that could mount an immune response against the primary tumor and treat the metastasis with the hope that the metastasis shares some of the same epitopes. Create a metastasis-preventing vaccine that is administered when a primary tumor is diagnosed, but there are no detectable metastases. Modulating myeloid cells were also suggested as a target because these cells modulate the metastatic niche and other components of the immune system. Using a myeloid suppressing drug may be safer and better than aspirin.

The group ultimately proposed the following plan:

Can radiation therapy reset the immunologic phenotypes of the tumor? Radiation therapy increases the homing of lymphocytes into the tumor, even in advanced disease. In addition, there is an unmet need because approximately 6-10% of new breast cancer cases are initially Stage IV or metastatic, and in 2012, the median survival was 2-3 years.

In situ "vaccination" of primary tumor in de novo Stage IV metastatic breast cancer patients was proposed with the following trial design to determine if a combination of radiation therapy and immune therapy could cause increased immune response to the tumor (use Simon Trial Design):

- Patient population: de novo Stage IV metastatic breast cancer patients (any subtype) with a minimum of one metastatic lesion (pilot of 14 women)
- Pre-treatment biopsy prior to study enrollment
- Multiple blood samples throughout treatment to confirm patients are really immunized
- Intervention:
  - Day 1 – Anti-CTLA-4 to eliminate regulatory T cells
  - Day 7 – Local radiation of tumor, intratumoral injection of a toll-like receptor agonist (CPG) to stimulate an immune response, anti-PD1 treatment; if chemotherapy is necessary, then perhaps give taxol because this drug enhances the immune response where as other chemotherapies suppress the immune system
- **Endpoints:** response of primary tumor and metastatic tumor (imaging), time to progression, and immune infiltrate into primary tumor

During discussion, participants commented that the trial design was similar to the immunotherapies group idea. The difference is that this design is not pulling antigens out, but rather assuming that patients already have the correct antigens and immune cells to fight the tumor. The goal is to prevent metastasis by keeping the dormant cells and niches in check with minimal toxicity by boosting the patient's intrinsic anti-tumor immune response.

IV. ARTEMIS PROJECT ON PRIMARY PREVENTION

SEED GRANT UPDATES

Prevention Vaccine Project

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

The goals of the Artemis Project for a preventive breast cancer vaccine are to develop a safe and cost-effective vaccine that targets all three major subsets of breast cancer, reduces the incidence of breast cancer, and prevents death from breast cancer. Although there is the possibility of identifying the role of CMV, or Epstein-Barr virus (EBV) or a variety of neo-antigens, the vaccine will be based on non-mutated self-antigens (also known as subdominant neo-antigens). The discussions and steps from previous Artemis Project meetings are laid out in prior annual reports.

The vaccine will include the following six antigens: HER2/neu, MAGE3, MUC1, survivin, mammaglobin A, and hTERT. A number of therapeutic studies have shown the importance in function (biology) and frequency (abundant in breast cancer while not in most differentiated cells) of these antigens.

The vaccine will be DNA based using a prime boost strategy. The initial prime will be with plasmid DNA and electroporation followed by virus-encoded antigen. A phase I safety trial may include 25 patients previously treated for DCIS, and assess both safety and immunogenicity. The participants discussed a concern that combining all six products together in a single vaccine could result in competition and antigen exclusion. Delivery

may have to be through injection at separate sites instead of a single injection.

In December 2016, NBCC and the Mayo Clinic signed a master service agreement plan to keep the intellectual property rights with NBCC. The goal is to prepare a pre-IND package for submission to FDA in 2017 with a rationale for why pre-clinical studies are not needed. A teleconference meeting with FDA will be held to discuss clinical trial transitioning from "disease state" to normal healthy individuals, the choice of vectors, and manufacturing.

Genomic Characterization of DCIS

Gregory J. Hannon, PhD, Professor, Investigator, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory and H. Kim Lyerly, MD, FACS, George Barth Geller Professor for Research in Cancer and Professor of Surgery, Duke University Medical Center

The Hannon lab received 150-200 samples of ductal carcinoma in situ (DCIS) with and without invasive ductal carcinoma (IDC) from Kim Lyerly at Duke University. Samples from 62 patients have been pathologically annotated and dissected resulting in 973 libraries sequenced by RNA-Seq and 215 libraries sequenced by whole genome DNA. They have deeply characterized and sequenced 18 patients with a near complete set of DCIS and IDC lesions and two "good" replicates that have passed quality control and expect to do 50 within the month. For maximal use of the samples, both the DCIS regions and the adjacent stroma were included. Similarity analysis showed that DCIS and IDC are very similar and overlap with normal epithelia, while stromal cells are very different.

They will next look more broadly with more patients and construct an evolutionary tree of samples to see how similar DCIS is to current invasive disease, whether it is a precursor, and whether they can trace invasive disease to particular DCIS lesions.

During discussion, participants addressed the possible differences among stromal cells surrounding DCIS and those around invasive tumor cells. Greg mentioned the difficulty with creating a high quality library from stromal cells due to sample non-uniformity, and requested any thoughts on how the stroma might be changing to help direct what his team is looking for (markers, antibody sets, panels of mRNA). Another participant asked about the feasibility of using MERFISH with these DCIS samples. To discuss further how best to interact and collaborate, a face-to-face meeting in New York City with Greg was planned.

VACCINE WORKING GROUPS

Participants identified three topics for focused discussion: vector systems and a vision for the FDA, a design for the next clinical trial and follow up to DCIS data.

1. Vector Systems

Jayamta Debnath, Daniel Douek, Paul Ewald, Stephen Johnston, Debbie Laxague, Kim Lyerly, Keith Knutson, Stuart Martin, Sohail Tavazoie

The efficacy trial will include three DNA primes 2-4 weeks apart followed by a viral boost 4-6 months later. Immunity will be measured one week after each dose, and again three and six months after the boost, and a full blood work-up will be done annually. Administration could be intramuscular, although a skin patch was also mentioned. Concern with the skin patch was a lack of immunity.

Participants discussed commercialization. NBCC has been in talks with non-profit vaccine firms and industry representatives from global medicine divisions and has received some pro-bono assistance from BCG. Participants recommended using an infectious disease vaccine model, and noted that there is different licensing for the U.S. compared to other markets.

There was a suggestion that the vaccine could be tested therapeutically in dogs. Efficacy data could be obtained over a few months if tested in Europe, and it would not be expensive because it would be owner-enrolled. FDA would look favorably on a positive effect, such as tumor shrinkage after diagnosis, in dogs.

Vaccine cost will involve both research, to establish different configurations and minimize the number of constructs, and production work. Testing the different constructs to ensure good expression of all the genes would cost an estimated $300,000-500,000. A minimal construct would take $100,000 and 4-5 months. Synthesizing the plasmids and testing them in mice would take about $25,000 and one month.

The participants discussed a concern that the FDA may want pre-clinical studies to show that the vaccine generates immunity and is not immune-toxic. The data show that each individual component is safe, but the combination could result in an autoimmune response. One approach is to do a similar construct with the mouse counterpart, then to do a tissue analysis.

The FDA may also want a demonstration to show that the vaccine will work therapeutically in breast cancer and that the participants discussed responses to that issue.

Another approach is to start testing the vaccine in a specific age group, such as 65 years and older, to create a safety profile in a large population. However, larger Phase IV clinical trials will still be required – to perform long-term surveillance for safety issues that are uncommon, but perhaps of sufficient magnitude to preclude widespread use.

Action Steps and Budget NEXT 12-18 MONTHS

A CRO can do the initial testing and vaccine synthesis.

- Creating/testing vaccine construct configurations and expression levels: $300,000-500,000
- Synthesize plasmids (not GMP): 1 month, $25,000
- Test plasmid configuration and confirm viral expression: 4-5 months, $100,000
- GLP-like grade product for 25 patients and stability testing: 1 year, $400,000

A remaining concern is that FDA will want more mouse modeling to determine if and when immunity is generated. Keith Knutson offered to share the pre-IND package with any interested participants.

2. Clinical Trials

Leslie Bernstein, Joe Camardo, Silvia Formenti, Judi Hirshfield-Bartek, Mark Lee, Joseph Pickrell, Michele Rakoff, Paul Spellman, Asad Umar

The group agreed that the study population should have a higher incidence of breast cancer than the general population, and discussed whether the study population should be women with DCIS or BRCA carriers. Concerns with identifying BRCA carriers include the cost and the prevalence of prophylactic surgery. Among patients with DCIS treated with surgery, the risk of recurrent DCIS or invasive breast cancer developing is 1-2% per year. Conducting an initial therapeutic trial among 25 women may help get to a preventive trial. The vaccine may also prevent other cancers in these women in the therapeutic trial. Any reduction in cancer occurrence, breast or other would take years to see.

A "window of opportunity" study was suggested with the vaccine administered prior to surgery, and then when surgery is performed, examination of DCIS to use as evidence that the vaccine is having an effect. This trial may not generate sufficient evidence to document patient benefit, or to serve as the data to receive FDA marketing approval. However, there is precedent with a trial of colorectal cancer vaccines in which vaccine administration after adenoma removal showed prevention. The trial only enrolled 120 patients with a recurrence rate of about 1% per year.

Study design: Prevention of Invasive Breast Cancer

- Powered at 30% decrease in risk for vaccinated group
- Patients with DCIS treated with surgery alone, and then randomized to vaccine (could include BRCA carriers in Europe; DNA.Land to identify high risk people in US)
- 2,500 participants followed for 3 years would cost $15m ($2,000/person/year) and excludes the cost of making the vaccine
- **Endpoint:** recurrence of DCIS or invasive breast cancer in either breast
- If vaccination prior to surgery, could also look at biological activity of the vaccine

Action Steps: NEXT 12-18 MONTHS

Since Artemis is not a company, the following are challenges for building the infrastructure for a Phase II clinical trial:

- Manufacturing the vaccine
- What is the regulatory path? Internationally? Regulatory representative?
- Site management and operations (feasibility of getting patients, finding sites, establishing contracts)
- Data operations and monitoring
- Oversight and project management team
- Legal and business development, contacts

Building the infrastructure could take up to 18 months, and a three-year study could cost upwards of $20m.

Assuming the challenges are overcome, then NBCC should establish an advisory board to discuss strategies to de-risk the Phase II/III study, to address safety, and to determine what to measure for interim markers

- Interim analysis strategy: immunogenicity analog? Go/No-Go?
- Surrogate markers of activity and toxicity
- Integrate "window of opportunity" into Phase I trial
- Intact DCIS -> vaccine -> surgery

3. Follow Up To DCIS Data

Frank Calzone, Cyrus Ghajar, Pat Haugen, Peter Lee, Christopher Li, Fran Visco, Alana Welm

The group focused on what to do with the data that is being generated from the Artemis seed grant to Greg and Kim, which includes epithelial, stromal, DCIS and invasive breast cancer tissue. Laser capture techniques enable analysis of neighboring regions of epithelial tissue from the biopsy samples. The data sequencing and variant calling will be performed at Cambridge, while Simon Knott is participating in the computational analysis of Greg's data for biological questions, such as whether RNA-Seq data are identical in DCIS and invasive breast cancer and how much variation would be considered "normal" or within experimental error. Hannon's group is currently focused on the 3D cell model.

Clinical outcome data, including ten years of follow-up, are in a research database at Cedars Sinai with Kaplan-Meier curves, and are available for the 50 deeply characterized patients. It was recommended that a third party adjudicate the protocol and a scientific advisory committee devise the research questions. Formalin-fixed paraffin-embedded (FFPE) samples could serve as confirmatory data, along with a second cohort such as Chris Li's cohort of DCIS RNAseq progression data.

The group re-focused the discussion on the Artemis Project priority of identifying antigens in DCIS, and asking whether DCIS is the obligate precursor to cancer, like in cervical cancer. The data may also help distinguish which DCIS is indolent versus that which will grow. The dormant tumor cell project is a second separate project (Simon Knott, Cyrus Ghajar, Alana Welm).

Chris Li offered to take the lead on the indolent versus bad DCIS question, and also use his second dataset as a validation set for factors identified in the Hannon data to prioritize the six vaccine targets in the 50 patient samples analyzed so far. The DCIS data may be useful for primary prevention and should be included in the pre-IND package.

Participants then discussed the metastasis prevention project. Specifically, the use of in situ MERFISH of DTCs in bone to investigate DTCs that are dormant versus those that are not and understanding active metastasis versus inactive DTCs in the same patient. Alana offered to take the lead on communication for this project.

4. Other Topics

Discussion Of New Prevention Concepts

Participants were asked to think about a way to end breast cancer without drugs and primary prevention without the Artemis prophylactic vaccine.

- Breast cancer prevention as a public health policy, with the example of heart disease having no single solution
- Shift focus to preventing breast cancer that kills, 70% of women diagnosed with breast cancer will not die of breast cancer
- Need to understand the mechanisms to produce better interventions
- Microbiome activity and interactions
- Stratify population to identify individuals at high risk of developing cancer within 3-5 years; higher event rate would progress prevention trials faster
- Use autopsy data to identify incidence of indolent cancer and risk factors of breast cancer developing between cases and controls

V. CONCLUSION

The Artemis Project has produced a number of effective collaborations among diverse researchers and advocates. The Project participants continue to focus on primary prevention and the prevention of metastasis. As can be seen by this and prior reports, much of the discussion and a number of the plans bring immunology and genomics to the fore. While the two broad areas of focus are distinct, there is overlap across approaches. As this became clear, all participants were invited to attend the meetings of both projects, further developing the multidisciplinary and innovative thinking that has been a hallmark of the Artemis Project.

The Artemis Project continues to rely on an innovative model of cooperation, in which individuals aligned toward a common goal can interact and engage with others with complementary talents, skills, and expertise. In contrast to more conventional strategies, the Artemis Project enables advocates, physicians, scientists and other stakeholders to interact and develop activities that collectively contribute to a highly complex strategic plan that would ordinarily be supported by significant financial resources, or a large corporate organization. Using this approach, important progress has been made in the critical activities needed to develop and test a preventive vaccine for breast cancer and to understand the process of metastasis and how to stop it.

Overall, the Artemis Project continues to advance the concept that a breast cancer prevention vaccine is feasible, and its development continues to be pioneered by Artemis. During the past five years, remarkable progress has been made in the general field of cancer immunotherapy. New immune-based treatments have been developed and approved for some cancers. There is evidence that these forms of immune therapy have promise in breast cancer, but it is currently not clear. The general and broad advances in the immune therapy field have lent credibility to the vision setting goals of the Artemis Project, which focused on a cancer vaccine based on the available science in years predating these current successes. These advances provide additional justification and credibility to pursuing the scientific goal of the Artemis Project in developing and testing a breast cancer prevention vaccine.

Immune approaches are also discussed in the prevention of metastasis Artemis project. Participants continue to focus on tumor dormancy, with an emphasis on determining which disseminated tumor cells result in lethal metastasis and the strategy to pursue once they are identified. In 2017, approaches to analyzing existing immune based therapies in a breast cancer population were also discussed and prioritized. In addition, the group discussed how data would be best used to identify targets for preventing lethal disease and risk reduction.

In addition to these directed activities, participants in the Artemis Project are continuously reevaluating the state of the sciences to ensure that alternatives, or additional opportunities to prevent breast cancer and end deaths are being considered, and appropriately incorporated into the goals of the Artemis Project.