NATIONAL BREAST CANCER COALITION

ARTEMIS PROJECT

National Breast Cancer Coalition
The Breast Cancer Deadline 2020

ANNUAL MEETINGS
MARCH 9-12, 2018

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 including 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 of 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 2018 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.

2018 ANNUAL MEETING PARTICIPANTS

Alex Aravanis, MD, PhD, Scientific Co-founder and Head of Research and Development, GRAIL

Frank Calzone, PhD, Vice President, Research, REMD Biotherapeutics and Biotechnology Consultant

Joe Camardo, MD, PhD, 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, PhD, 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, Chief Science Officer, Myheritage.com, Assistant Professor of Computer Science and Computational Biology, Columbia University, Core Member, New York Genome Center

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

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

Suzanne Fuqua, PhD, Professor, Medicine and Molecular and Cellular Biology, Baylor College of Medicine

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, Minnesota Breast Cancer Coalition

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

Stephen A. Johnston, PhD, Director, Center for Innovations in Medicine (CIM), 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, Biomedical Sciences and 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, Director, Mayo Clinic Florida Cancer Research Program

Debbie Laxague, RN, Advocate, BCSSC

Tracy Leduc, JD, Advocate, NBCC

Mark Lee, MD, PhD, Global Head for Personalized Healthcare, Product Development, Genentech/Roche

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

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 of Cancer Research, Professor of Surgery, Associate Professor of Pathology and Assistant Professor of Immunology, Duke University Medical Center

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

Donald McDonnell, PhD, Glaxo-Wellcome Professor of Molecular Biology and Chairman, Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Co-Director, Women's Cancer Program, Duke Cancer Institute

Elizabeth Ann Mittendorf, MD, PhD, Robert and Karen Hale Distinguished Chair in Surgical Oncology and Director of the Breast Immuno-Oncology Program, Brigham and Women's Hospital, Dana-Farber Cancer Institute

Josef Penninger, PhD, Scientific Director, Institute of Molecular Biotechnology of the Austrian Academy of Sciences

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

Xiang (Shawn) Zhang, PhD, Associate Professor, Lester and Sue Smith Breast Cancer, Baylor College of Medicine

MEETING SUPPORT

NOTE TAKERS:

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

Giselle Hicks, MPH, Advocate, NBCC

Kamila N. Naxerova, PhD, Postdoctoral Researcher, Harvard Medical School

Medha Sutliff, MS, Director, Scientific Affairs, NBCC

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 10 to Sunday, March 11, noon, 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

Debbie reported there were fewer vaccine and more checkpoint inhibitor trials this year. Of the 13 new vaccine trials, 6 were in combination with a checkpoint inhibitor. Most were small Phase I trials with dose-escalation and safety with some immune read-out. None were in the prevention setting.

An alpha-lactalbumin vaccine received DoD funding for a Phase Ia and Ib trial. The Phase Ia trial is targeted for triple-negative breast cancer patients recovered from the current standard of care therapy, and looks at safety and dosage. The Phase Ib trial looks at safety among high-risk women who have had prophylactic mastectomy.

Eight vaccine trials were conducted in the metastatic setting with five of those not breast cancer-specific, but rather target-specific. Two trials were randomized: a dendritic cell vaccine versus WOK-VAC in the post-neoadjuvant setting for HER2+ patients, and an antigen DNA vaccine with and without durvalumab. There were several non-randomized Phase II trials.

There were 35 new immunotherapy trials including breast cancer. Many included other solid tumors, and the settings ranged from pre-neoadjuvant to late-stage refractory metastatic breast cancer. Some were monotherapy, and many were in combination with checkpoint inhibitors. There were 14 CAR-T trials that included breast cancer, although only three were breast cancer-specific. Each targeted expression of different candidates.

Converting Breast Cancer into an In Situ Vaccine

Silvia C. Formenti, MD

Silvia presented on a study she is conducting combining fractionated stereotactic radiosurgery with pembrolizumab, a checkpoint inhibitor, to treat brain metastasis among women with any subtype of breast cancer. Most breast cancer tumors are "cold," with few tumor-infiltrating lymphocytes (TILs). Such tumors can be stimulated, perhaps with DNA damaging agents, to jump start the process and become more pro-inflammatory. By treating only 1-2 metastatic lesions in the brain, these lesions can then be used as an "in situ vaccine" to develop a sustained immune response against even the untreated lesions. This study indicates that radiotherapy with immune checkpoint blockade can trigger a systemic immune response.

Local immunization has become the popular approach to avoid systemic side effects while breaking tolerance in a cost-effective way. If the patient can build an immune response herself, the response could be for life. The key will be finding the correct dosage and timing that stimulates an immune response without killing all the t cells.

Additional issues raised during discussion included what is needed to turn this data into a Phase II study, whether the sequence of immune therapy and primary tumor treatment is important and the relevance of the fact that the immune response changes over time.

Grand Challenge

Alana Welm, PhD

Although not an Artemis seed grant, a global project proposal on how tissue microenvironments might support or interfere with tumor dormancy was submitted to the Cancer Research UK (CRUK) Grand Challenge, based on discussions held at prior Artemis meetings. The hypothesis is that the microenvironment around disseminated tumor cells (DTCs) supports their survival and helps facilitate re-awakening upon initial release. In situ profiling is required to help understand how DTCs exist in their niche, and to potentially reveal therapeutic targets that could prevent metastasis. The proposal included a global patient advocate network to create and maintain a biobank; MERFISH and metabolic profiling; identification of DTC (neo) antigens and the development of strategies to eliminate them; and analysis of systemic factors that predict escape from dormancy and relapse.

III. ARTEMIS PROJECT ON PREVENTION OF METASTASIS

SEED GRANT UPDATES

Genetic Determinants of Metastasis: DNA.Land

Yaniv Erlich, PhD

Yaniv provided an overview on the current popularity of direct-to-consumer genetic testing with over 12 million people worldwide having taken a direct-to-consumer genetic test, thus enabling crowdsourcing of genomes. Yaniv also provided updates on the progress of gathering genetic data through DNA.Land and the related NBCC breast cancer questionnaire. DNA.Land is partially funded by a seed grant from the Artemis Project, and is a website for consumers to upload their DNA test results and enables "case-control association mapping by proxy" using family history of disease.

Currently, 90,000 people have uploaded their genomes to DNA.Land, averaging about 200 people each day. An NBCC survey was launched in 2017 asking about respondents' breast cancer history and that of their immediate family members. As of the Artemis meeting, of the 20,000 surveys started, 16,000 were completed with 2,300 people indicating breast cancer in their family. Only 400 people did not complete the consent, and almost 12% provided an email address so that researchers could make follow-up contact.

Participants discussed how the DNA.Land dataset differs from the other large consortium datasets with both survival data and signatures associated with survival. It was suggested that NBCC cohort data could become a part of the Breast Cancer Association Consortium (BCAC), with the advantage of being able to ask questions electronically and follow-up quickly.

Lastly, the group raised the question on how many genomes are needed to be able to answer questions about the genetic contribution to dormancy. Yaniv estimated that 10,000 people with a family history of breast cancer would be needed to create an informative Manhattan plot. To address potential shifts in survival over time among the different disease subtypes, one could look at ten-year increments of breast cancer deaths to see the composition of histology changes by decade.

Investigating Adaptive Immune Recognition of Dormant Disseminated Tumor Cells

Cyrus Ghajar, PhD

Cyrus presented an update to the project designed to understand tumor dormancy and the immune response to disseminated tumor cells (DTCs). Unchanged from last year, there is still only one relevant paper on quiescent cells. To test the underlying assumption that t-cells are required to maintain dormancy, primary tumor cell growth was compared in two different mouse models. Metastasis-free survival was identical in the two models, and although t cells did not seem to directly regulate dormancy they can still be leveraged to help. Mammary tumor DTCs do not grow out in immune suppressed mice.

A variety of models were discussed, but immune-competent mouse models are needed to research the specific aims identified last year. After establishing that quiescent DTCs isolated from bone marrow are capable of growing, it was suggested that after identification of antigen specific t-cells, CAR-T cells could be used to assess whether self-activated t-cells are able to eliminate tumor cells.

PREVENTING METASTASIS WORKING GROUPS

After discussion, participants identified four topics for further focus and broke out into small working groups to discuss an action plan on what teams, targets/key milestones, 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.

1. DNA.LAND: DATA COMMONS

Judi Hirshfield-Bartek, Frank Calzone, Yaniv Erlich, Kim Lyerly, Josef Penninger, Paul Spellman

The group discussed marketing strategy and a variety of ways to potentially increase outreach to include electronic outreach to NBCC advocates to help spread awareness of the project and generate interest among people who are not yet tested. The challenge is overcoming people's wariness of sharing personal information through either their interest in genealogy or providing incentives such as identifying lifestyle modification factors that could reduce their risk of breast cancer.

Action Steps and Budget NEXT 12-18 MONTHS

Marketing and promotion to increase uploaded data

- NBCC to continue promotion to coalition via social media, email and webinars
- Outreach to survey participants who have not yet uploaded genetic data
- Strategize ways to encourage people to get a DTC DNA test
- Cost estimate: $300-500k for another 10,000 participants

The group discussed two other strategies to increase survey numbers and awareness of DNA.Land:

1) Advertising at high-risk clinics to enrich the data for information needed

2) Providing incentives for individuals to update their data if they were diagnosed after testing

Several ideas for utilization of the DNA.Land platform were introduced:

- To facilitate "pushing" the data out: Dream Challenge
- To increase interest or "pull" of the data:
  - "Resilience" Project: Why do certain women with BRCA not develop disease? Use historic records to identify families that despite mutation, don't develop disease, or if they do develop disease it doesn't develop metastasis
  - Follow the women with breast cancer for relapse and see if they have genetic markers that predict variants
- Utilization of international projects beyond US industry (e.g., Canadian Genome Quebec)

2. DESIGNING AN ANTI-METASTASIS CLINICAL TRIAL

Alex Aravanis, Joe Camardo, Suzanne Fuqua, Stephen Johnston, Tracy LeDuc, Peter Lee, Stuart Martin, Asad Umar, Kim Lyerly

The group discussed the current treatment process in some breast cancer patients with no evidence of metastasis who are initially treated with surgery and adjuvant chemotherapy followed by repeat chemotherapy if metastasis develops. They asked whether it would be possible to find the existence of earlier indicators of recurrence to allow metastasis treatment prior to detection by imaging. One suggestion was to reclassify overt metastasis to circulating tumor DNA (ctDNA) in order to initiate earlier treatment. It was noted that the lead time between radiographic detection of a lesion and ctDNA is 9-12 months.

Several concerns were raised:

- As half of the patients who recur were ctDNA negative after initial treatment, ctDNA in breast cancer is "suggestive" but not highly predictive
- The vast majority of breast cancers do not product much ctDNA
- Adjuvant therapy, such as chemotherapy, generally reduces the ctDNA yield

A possibility discussed by participants was the development of a ctDNA-based vaccine that could be combined with an immune activator such as a checkpoint blocker. This could be offered as a treatment option and a peptide vaccine against, perhaps, PI3kmut/ESR1mut in estrogen receptor positive (ER+) patients.

The group also discussed the frustration over mouse models often focusing on primary tumor growth and that "anti-metastasis" clinical trials in humans should evaluate metastasis formation and not primary tumor response. Rather than having the "ideal" clinical trial, the group agreed that there is a need for a development plan.

Study Design: Early Vaccination Against PI3K/ESR1 Public Mutations with ctDNA Monitoring

The objectives of this project include:

- Phase II Study to extinguish smoldering metastasis
- Use ctDNA levels as a "quick" clinical endpoint to shorten trial time (proof of concept)
- Patients could switch therapies before metastasis becomes a "raging fire"

Action Steps and Budget NEXT 12-18 MONTHS

- Pharmacology/toxicology study ($1m)
- Develop trial design white paper on how to use vaccines to prevent metastasis laying out all the evidence for such an approach
- File IND application ($1m)
- Safety trial in metastatic patients ($2m)
- Start the Phase 2 trial with 50 patients with Stage III ER+ breast cancer (these patients are high-risk, and 40% will recur within 6 years) ($5m/3 yrs)
- Early peptide vaccine with PI3Kmut/ESR1 given to all patients after surgery (while undergoing radiotherapy, but before AI therapy since this is when mutations arise)

The group outlined several identifiable success factors in the study:

- Low toxicity, high immunogenicity, safe
- Recruit ER+ Stage III patients
- Same trial could be performed in 100 ER+ dogs ($1m)
- Enables testing of different vaccine formulations to see which ones delay the onset of ctDNA re-surfacing

Several concerns were discussed among the participants:

- Most women (70%) who would receive the vaccine may never recur. Some participants felt that the toxicity profile would be favorable, and that this would still be a worthwhile strategy.

- Vaccine might not "stick" during radiotherapy due to the memory t cells and circulating lymphocytes. This may require boosts.
- The concept of "pre-emptive" vaccinating against likely mutations that will drive resistance is different and has not been done.

Additional suggestions proposed included stratifying patients by MHC/HLA to ensure the peptide is presented and using a DNA-encoded protein vaccine instead of a peptide vaccine since the marker is ctDNA.

3. GRAND CHALLENGE: DISSEMINATED TUMOR CELLS- IN SITU

Jay Debnath, Danny Douek, Cyrus Ghajar, Pat Haugen, Simon Knott, Chris Li, Michelle Rakoff, Sohail Tavazoie, Alana Welm, Donald McDonnell

The primary objectives of the group were to focus and prioritize the proposal that was submitted for the CRUK Grand Challenge on tumor dormancy and to identify action items that can be achieved in the next 1-2 years.

Key points discussed include:

- Feasibility regarding whether metabolomics can be analyzed from the samples
- Consideration for host factors that can trigger a break from dormancy and what should be included in the probe panel to address these
- Alternative approaches to MERFISH for learning about how DTCs interact with the microenvironment
- Hormone-related factors that might regulate dormancy and changes in the microenvironment surrounding dormant cells
- Logistics of developing and testing MERFISH
- How to identify DTCs within sections to determine which tissue sections should be analyzed with MERFISH
- Patient population for a prospective study designed for collection of bone marrow core biopsies (to assess DTCs and microenvironment) and blood (systemic factors)

Group agreed that ER expression and signaling in DTCs should be investigated in the proposed study. The group discussed whether or not DTCs express ERs, since it is unclear whether DTCs that have experienced endocrine therapies downregulate expression of ERs.

The highest priority work packages were the prospective bio specimen collection and MERFISH analysis. Several participants agreed that collection and analysis of multi-organ samples via rapid autopsy is a good place to start for several reasons. If resources were unlimited, the group concluded that both rapid autopsy specimens and prospective bone marrow cores could be analyzed.

Study Design: Profiling the Local and Systemic Environment of DTCs to Prevent Metastasis

Objectives: Prevent systemic metastasis by eradicating DTCs or keeping them dormant

- DTC microenvironment factors that promote survival, sustain quiescence, and promote re-emergence
- Accrual of 50 specimens for longitudinal analysis relatable to recurrence
- Systemic factors associated with dormancy and recurrence
- Pre-clinical validation of DTC targets

Action Steps and Budget NEXT 12-18 MONTHS

- Set up MERFISH microscope and protocol (develop, test, validate)
- R21-level funding to cover resources and personnel to set up MERFISH and develop the probe set
- Profile dormant DTCs versus metastases (transcriptomics)
- Retrospective analysis (chemo/cytokines, hormones, metabolites, microbiome)
- Obtain prospective bone marrow biopsies to use them to validate MERFISH
- Apply for pilot funding from cancer centers
- Obtain single cell RNAseq on rapid autopsy specimens (lung, brain, bone metastasis)
- Obtain IRB approval/surgical agreements to get core biopsies from a small prospective cohort (10-20 patients with untreated Stage II/III breast cancer)
- Cd19/20 MERFISH on human leukemia core biopsies (proof-of-concept biopsy to ensure MERFISH works well on human bone marrow core biopsy samples)
- Identify/define the 1,000 probe set using data from the RNAseq on rapid autopsy samples
- Begin testing a 10 probe set. Upon successful completion, build up to a 100 probe set.

The main challenge identified by the group was funding the project, estimated to be $4.3m for all four objectives (about $1m each)

4. VACCINE AGAINST NEO-ANTIGENS TO PREVENT METASTASIS

Frank Calzone, Stephen Elledge, Peter Fasching, Silvia Formenti, Keith Knutson, Debbie Laxague, Susan Love, Donald McDonnell, Xiang Zhang, Kim Lyerly

Initially the Artemis Project screened The Cancer Genome Atlas (TCGA) for epitopes. Studies are now using bioinformatics and HLA algorithms to identify antigenic epitopes to better understand which allele variants are immunogenic.

Various approaches and challenges were discussed including:

- Conduct a trial in both primary and metastatic breast cancer to investigate which epitopes might have t cells; blood draws for b cells and dendritic cells, followed by a biopsy, would enable ranking of neo-epitope patterns and isolation of t cell clones that could form the basis for a vaccine
- Obtain a core biopsy of the primary tumor to develop a signature of immune suppression
- In the neo-adjuvant setting, ER+ patients with no indication for chemotherapy can be given an AI for 6 months to generate an immune response. Neo-antigens can then be identified for the vaccine to be built. Multiple core biopsies to assess tumor response and to identify neo-antigens is preferable.
- Look at ER mutants to assess whether the neo-antigens in the primary tumor are related to dormant cells; determine how many types of ER mutants result in resistance and possibly use a vaccine to elicit a response from antigens that don't induce a response.
- Re-focus on immune therapy in breast cancer; learn from conventional treatment immune-mediated response and from survival on anti-hormonal therapies in the adjuvant setting. One advantage is that data already exists, it just has to be mined.

Key question: Can you generate an immune response against the primary lesion to create a durable, persistent immunity to protect against relapse?

Study Design: Prevention of Metastasis Trial

Objective: After proof of principle, conduct a large adjuvant trial for hormone receptor positive breast cancer patients to prevent metastasis

Trial design was outlined as follows:

- 2 x 2 factorial design with four arms: AI alone, AI with vaccine, AI plus pembro, AI plus pembro with vaccine
- Radiation boost for all, 8Gy x 3
- Tumor size criteria is 1 cm

The group identified needed team/resources including a clinical team to utilize existing trial networks (e.g., Neo-Orb study sites), a scientific team (Artemis scientists), D1/PDL1 through pharma for investigator-initiated trial and assistance with vaccine and manufacturing, again possibly academia or pharma.

Action Steps and Budget NEXT 12-18 MONTHS

Stage I includes protocol write-up and funding. Trial budget estimated to be $1.2m for trial and $600,000 for genotyping and bioinformatics. This does not account for drugs, vaccine manufacturing or additional funding for scientific projects. Stage II would include the IND, companies, preparing the clinical trial, CRO and academic sites. Stage III would begin with activation of study sites and beginning of patient recruitment.

IV. ARTEMIS PROJECT ON PRIMARY PREVENTION

SEED GRANT UPDATES

Prevention Vaccine Project

Keith Knutson, PhD

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. 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.

Since last year's Artemis meeting, a pre-IND package was submitted to the FDA for a multi-antigen vaccine for the prevention of breast cancer. The antigens included in the vaccine are HER-2/neu, MUC1, Mammaglobin-A, Survivin, hTERT, and MAGEA3. These six antigens are low abundance proteins in normal tissues, and have been tested individually among hundreds of patients in other vaccine trials. Keith led a teleconference in early March 2018 with the FDA to discuss the FDA's responses to several questions included in the pre-IND package.

The group discussed how to choose individuals with metastatic breast cancer for the Phase I safety trial design, and how to identify "high risk" individuals for future efficacy trials.

Participants discussed different ways to define a high risk population, (BRCA and the GAIL model may not be ideal) and identified SNPs have not been validated. Length of follow-up for safety data was also discussed. Most adverse events are seen in first month, and the included antigens have all been previously tested indicating the possibility that autoimmunity might not be a big concern.

A Molecular Framework for Understanding DCIS

Gregory J. Hannon, PhD

Greg presented an overview of data from the SPORE tissue bank. To date, 197 cases of DCIS and DCIS/IDC have been selected and annotated. 150 cases of DCIS have been annotated pathologically with 9,000 tissue samples collected by LCM and stored for analysis. 59 cases have been sequenced resulting in almost 400 libraries, and 1,300 RNAseq libraries have been sequenced. Some libraries were removed from analysis due to poor quality, but a three replicate strategy has been useful.

Looking across different tumor types for patterns of mutations, the data identified six mutational signatures. The signatures prevalent in DCIS are much less prevalent in IDC. Ongoing analysis includes subtype classification, expression differences in DCIS and IDC, and expression differences between mutational signatures. Over the next six months, 200 cases will be completed and research to address which mutations drive the transition between DCIS and IDC can begin. A group from Cambridge is working with Simon Knott to analyze the DNA.

BACKGROUND AND DISCUSSION

Early Breast Cancer Detection Test Based on Circulating Cell-Free Nucleic Acids

Alex Aravanis, MD, PhD

Alex shared the purpose of GRAIL, which is early cancer detection, using cell-free nucleic acids (cfNAs) as a direct measure of cancer. The GRAIL approach is to combine high-intensity sequencing to detect cfNAs shed by tumor cells in the blood with a large clinical trial program to generate evidence of clinical utility and data science to classify patients by presence, type, and severity of cancer from a large and complex dataset.

The STRIVE Women's Cohort is a prospective, observational cohort study collaboration with Mayo Clinic and Sutter Health. Women are enrolled at the time of mammography (age 50 years and up) and followed for five years. Using blood draws at the time of mammography, and sequencing those women who develop cancer and comparing

them to randomly selected women with no cancer events, GRAIL aims to show that you could have anticipated which women would develop breast cancer. Of 120,000 mammograms, 108,000 will be normal and 12,000 abnormal. Specificity will need to be high, but by improving the diagnostic work-up, the number of biopsies and morbidities could be reduced and it could save the system money.

Group discussed the possibility of sequencing all women in the study to show there really can be early detection and that women have better survival outcomes? Would early detection make a difference in outcomes in these patients?

Alex further shared data on a Norwegian study that suggests up to 25% of breast cancers regress naturally, and if so, this type of test could result in over-diagnosis. Mark Lee stated that over-diagnosis has been a concern from the beginning, and that by monitoring incidence in a randomized interventional study you should see a spike at the beginning that decreases in year 2. Participants discussed whether the test could detect dormant disease, or distinguish between indolent and non-indolent breast cancer.

Microbiome

Daniel Douek, MD, PhD

Danny began his presentation by discussing how the microbiome could be associated with cancer through multiple mechanisms, including the direct toxic or inflammatory action of bacteria on host cells, infection of host cells, and indirect effects on host metabolism. A Science paper in 2017 used a colon cancer mouse model to demonstrate that bacteria in the tumor mediate resistance to chemotherapy, and that antibiotics eliminate the effect. Three more recent papers in Science show that the gut microbiome influences response to checkpoint blockade drugs, and that bacterial transplant can transfer sensitivity to checkpoint inhibitors to previous non-responders. The results were conflicting, however, with different bacteria associated with the same outcome in different studies and the same bacteria associated with different outcomes in different studies. This was, in part, due to a biased sequencing approach, poor quality bioinformatics, and the use of fecal samples which may not reflect the gut microbiome.

Danny shared another example of the microbiome's role in disease. Among HIV-infected patients, markers of gut inflammation and gastro-intestinal dysfunction predict mortality independent of CD4 counts and viral load, even among patients on anti-retroviral drugs with zero viral loads. A study in Uganda analyzed the "plasma" microbiome among HIV-infected patients and found a high abundance of proteobacteria, worms, and GBVc (formerly Hepatitis G). Patients with a higher abundance of proteobacteria were found to have significantly lower abundance of worms and GBVc.. Once these patients began anti-retroviral therapy, the balance of the three organisms changed and was correlated with changes in cytokines.

One concern raised was differentiating causation and association between the microbiome and risk, as evidenced by changes in HLA regions. Danny responded that all microbiome-mediated findings would result in something that could be detected by signaling.

DCIS Study: Artemis Antigens

H. Kim Lyerly, MD, FACS

The goal is to determine if DCIS is in fact the precursor for invasive breast cancer using RNAseq.

Kim looked at the relative up-regulation of the Artemis antigens in DCIS as compared to expression in normal epithelium. Multiple lesions from each patient were analyzed, although it was noted that core biopsies prior to diagnosis differ from many samples during resection and "normal epithelium" may not necessarily be normal since it is from a core biopsy. There was variability within patients, and one patient without any expression of the six antigens. Survivin was nearly unexpressed in the normal breast. Participants suggested using a continuous heat map to visualize expression differences, instead of a 4-fold threshold which obscures 2- and 3-fold increases. The Artemis antigens were then mapped against the Cheever list of antigens and a genome-wide list of antigens, and compared DCIS up-regulated antigens to those of invasive disease. The antigens appeared stable. The changes in gene expression in DCIS seemed predictive of what is seen in invasive disease, although an alternate explanation could be that they are both derived from the same precursor. One suggestion was to randomize the pairing to see if the correlation changes. Another suggestion was to analyze stromal tissue because if DCIS and invasive tumors have similar gene expression then a change in the stroma might dictate invasiveness. Kim responded that both epithelial and stromal tissues were analyzed, and that the preliminary data indicate that the stroma may differ.

PRIMARY PREVENTION WORK GROUPS

Participants identified three topic areas for focused discussion in work groups: moving the IND forward and clinical trial design, questions to ask with Simon Knott's data (Artemis Vaccine 2.0), and the role of the microbiome in risk stratification and immune analysis. A fourth work group continued discussion from the prevention of metastasis meeting to establish action items related to the Grand Challenge and these outcomes were included in the Prevention of Metastasis meeting summary.

1. IND and Clinical Trial

Joe Camardo, Jay Debnath, Cyrus Ghajar, Pat Haugen, Stephen Johnston, Keith Knutson, Peter Lee, Kim Lyerly, Stuart Martin, Michele Rakoff, Paul Spellman, Asad Umar

Group discussion was focused on identifying what is needed for moving forward with the FDA. The following challenges were identified:

Keith discussed the flexibility of the project at this stage as the product has not yet been developed. If during the first round of testing results show two of the antigens elicit no immune response, then they could be could swapped out for others. Concerns advanced included the need to identify credentials for antigens to be swapped in? Would it be a problem if there was an antigen never previously tested? Could one of the DCIS antigens be used, or would that not work with the IND timeline?

Participants recommended dose escalation and staying with the DNA-based vaccine versus adding a peptide-based vaccine.

The group also discussed the possibility of bringing this vaccine into the NCI PREVENT Cancer Program.

Keith stated that September 4, 2018 is the proposal submission deadline. To date, Keith has contacted a company in France to do the MVA production (Modified Vaccinia Ankara vector) and companies that develop clinical grade plasmids. The NCI program has a contract with Bavarian Nordic. Lastly, Keith expressed some cost concerns. Keith's estimate for covering both products is $3m, but with an accelerated time frame the cost could increase to $5m. The goal is to vaccinate the first patient in January 2020.

In the second work group session, participants were asked to develop a specific strategic plan laying out action steps for the next 12-18 months:

Strategic Action Plan: NEXT 12-18 MONTHS

Months 0-9

- Product production
  - Cost for GMP and MVA vector estimated $5m (Bavarian Nordic/Transgene)
  - Mode of delivery (Biojet)
  - Antigen construct sequence (antigens are all validated and over-expressed in breast cancer)
  - First construct is 3 antigens (HER2, MUC1, MAGEA3)
  - Second construct adds 3 antigens to initial construct (Mammaglobin-A, hTERT, Survivin)

Months 10-15

- Pharmacology/toxicology (potentially optional)
  - Dosing animals with product to determine safety, not to obtain auto-immune safety information
  - Cost estimate is $1m
- ND application
  - After FDA approval of IND, it will take at least 6 months and $1m

Months 16-24

- Site activation and protocol approval
  - IRB approval cannot start until after FDA approval
  - Sites are City of Hope and Mayo Clinic

Months 24-36

- Phase I study – Cost estimate is $2m
  - **Accrual strategy:** 31 stage IV patients, relatively healthy with a small burden of disease and no chemotherapy, except for selective estrogen antagonists
  - Vaccinate first patient in January 2020
- Initial blood draw followed by vaccination with plasmid based vaccine.
- At 30 days, second blood draw followed by final immunization with MVA-based vaccine.
  - Follow patients for at least one month following final vaccination to gauge safety and immunogenicity
- Th1/Th2
- Avidity

- Response of 2/3 patients (antibody titer)
- IgG T-cell response
  - Dose escalation (proposed option)
- **Stage 1:** Nine patients receive 3 antigens at differing doses to identify which dose maximizes the immune response
- Low dose (3 patients), medium dose (3 patients), high dose (3 patients)
- **Stage 2:** Twenty-two patients receive 6 antigens
- Phase 2 study – TBD
- Phase 3 study – TBD

2. Artemis Vaccine 2.0

Stephen Elledge, Simon Knott, Debbie Laxague, Tracy Leduc, Mark Lee, Kim Lyerly, Josef Penninger [Alana Welm and Stephen Johnston joined for the second discussion]

The goal of this discussion was to define a "Plan B" for the second iteration of the Artemis vaccine. The purpose is not to change the current vaccine or identify a specific product, but rather to create a development framework and process for selecting new antigens for vaccination in humans. The main criteria for antigen selection were modeled on Cheever's paper The Prioritization of Cancer Antigens.

Some participants felt that somatic mutations in PIK3CA would be a good antigen to add to the 6-antigen panel since it is mutated in a large proportion of breast cancers and DCIS found in the molecular framework of DCIS project. Another recommendation was to analyze tumors from immunotherapy responders to identify antigens that drive an effective immune response that leads to tumor regression and long term survival. For example, if all long term survivors to immunotherapy have a T cell response to antigen X, then a vaccine against antigen X would be attractive.

Additional suggestions were to take into account the HLA genotype of the patient to be vaccinated since some MHCs are better at presenting certain peptides than others, to look for differential glycosylation patterns in DCIS/IDC versus normal tissues to identify new antigens, and to focus on antigens involved in tumor biology.

Gene candidates for an Artemis 2.0 vaccine would be selected according to the following rules:

1) Overexpressed/presented as peptide in MHC in a certain percentage of DCIS (and also in the corresponding IDC). Determine whether peptides are presented on MHC in tumor versus normal tissue

2) Not expressed in most normal tissues, except for ovaries, testes, and possible brain

3) "Driver" status (look at DNA sequencing data in DCIS, e.g., mutations, frameshifts, splice-site mutations)

Test whether t cells actually recognize and lyse tumor cells presenting the peptide, but not normal cells

3. Exploring Aspects of the Microbiome and Risk Stratification

Danny Douek, Peter Fasching, Silvia Formenti, Judi Hirshfield-Bartek, Peter Lee, Susan Love, Beth Mittendorf, Sohail Tavazoie, Cyrus Ghajar and Christopher Li

The discussion began on bacteria metabolites and their effect on inflammation and the immune system. The group raised the following issues:

Can the microbiome and other assays be incorporated into the preventive vaccine or be used to identify a high-risk population?

What is the role of the microbiome in metastasis progression or breast cancer incidence?

The breast microbiome does not match the stool microbiome, and it is very unlikely that the breast microbiome comes from the gut. How does stool microbiome affect the blood microbiome? And while stool is easier to collect, nipple aspirate is more direct to the tissue getting cancer. However, all samples are indicators of the systemic state.

Peter Fasching described a prevention trial identifying people at higher risk using genetic factors, mammographic density, previous biopsies, and proneurotensin (serum marker). The study could also look at factors influencing immune therapy efficacy such as the microbiome. There are serum samples in Hamburg of over 1,000 healthy women observed over ten years.

Danny also discussed a registration trial across multiple sites among women at high risk for awakening metastasis to define what is high risk for interrupting dormancy. This would involve risk stratification by inflammation and microbiome factors. Next would be to create a repository for women to donate stool and blood once a month until they recurred. At recurrence, samples could

be analyzed to see what breaks dormancy. Triple-negative, node-positive breast cancer patients with no pathologic response would be the subset of breast cancer patients waiting for recurrence.

In the second work group session, participants were asked to develop a specific strategic plan laying out action steps around the hypothesis that the patient's inflammatory state is related to their response to immune therapies. The goal being to generate a biobank of samples that can be used for good analysis downstream

Participants acknowledged leveraging four existing clinical trials to collect samples to enable microbiome analysis. **ACTION REQUIRED:** each researcher to provide samples from 50 patients.

1) Peter Fasching

   a. Anti-PD-1 therapy in the neoadjuvant setting

   b. **Endpoint:** Primary tumor complete response and tumor-infiltrating lymphocytes

2) Silvia Formenti

   a. Anti-PD-1 therapy plus radiation therapy in the metastatic setting

   b. **Endpoint:** clinical response

3) Beth Mittendorf

   a. HER2-targeting vaccine among DCIS patients (HER2 expression not required), randomized 2:1 vaccine: GM-CSF alone

   b. **Endpoint:** Peripheral immune response as determined by E75 reactive t cells, tumor-infiltrating lymphocytes, DCIS by pathology

4) Susan Love

   a. Nipple aspirates from patients with DCIS of both ducts with DCIS and ducts without DCIS

IMMEDIATE ACTION: Danny shared the following information to the four researchers:

- Paragraph describing what tests should be done and why (blood cytokine profile, inflammatory markers, stool analysis), and their cost
- Information on the stool collection kit

In addition, Danny will investigate whether the NIH crowdsourced microbiome project is collecting breast cancer data. Silvia can also to reach out to colleagues about the possibility of collaboration and sharing of existing samples.

Action Steps and Budget: NEXT 12-18 MONTHS

- Purchase reagents, secure funding, amend protocols, train personnel for collection
- Execute trials and perform interim analysis at Month 12
- Correlate with immune or tumor, survival, diagnosis, response with inflammatory treatments
- Validate specific findings with a prospective study (future trial for primary or secondary prevention)

The cost per patient, per time point and per sample would be about $1,000. This would include plasma microbiome analysis with the inflammatory MDS assay, RNAseq sorting, and metabolites profiling. The total estimated cost for kits and analysis is $1.5 million.

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. 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. A five-year, strategic plan for the development of a preventive vaccine was launched in 2011 and is being implemented through the Artemis Project® for a Preventive Breast Cancer Vaccine. In 2018, the vaccine development plan was presented to the FDA and received positive feedback. The project now moves forward to the next critical step: a Phase I safety trial. 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.

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.


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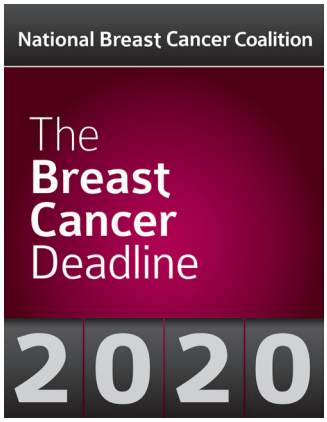

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