NATIONAL BREAST CANCER COALITION

ARTEMIS PROJECT

ANNUAL MEETINGS
MARCH 6-9, 2020

NATIONAL BREAST CANCER COALITION • ARTEMIS REPORT

I. INTRODUCTION

The National Breast Cancer Coalition (NBCC) was formed in 1991 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. Launched in 2010 to support the NBCC's mission-oriented research goals, the Artemis Project®, under NBCC leadership, brings together leading researchers and trained advocates 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?

Artemis Project reports from previous annual meetings, found at www.stopbreastcancer.org/what-we-do/research/artemis-project/, lay out the history of the Artemis Project. This report provides a summary of discussions and recommendations made at the 2020 annual Artemis meeting. While the coronavirus pandemic was just beginning to emerge in the United States in early March, the 2020 Artemis meeting went on as planned. Although not all invited participants were able to attend as a result of travel restrictions, this meeting had 22 participants, including advocates and scientific expertise ranging from immunology, biophysics, biomedical engineering, and genetics to molecular biology, radiation oncology and clinical oncology.

2020 ANNUAL MEETING PARTICIPANTS

Michele Atlan, Vice President, Breast Cancer Care & Research Fund

John C. Bischof, Ph.D. Director, Institute for Engineering in Medicine, University of Minnesota

Frank Calzone, Ph.D. Vice President, Research, REMD Biotherapeutics and Biotechnology Consultant

Joe Camardo, M.D., F.C.P.P. Head of Medical Affairs, ADC Therapeutics

Tara Deans, Ph.D. Assistant Professor in Biomedical Engineering, University of Utah

Daniel Douek, M.D., Ph.D. Chief, Human Immunology Section, Vaccine Research Center, NIAID, NIH, DHHS

Stephen J. Elledge, Ph.D. Gregor Mendel Professor of Genetics and Medicine, Harvard Medical School

Peter Fasching, M.D. 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

Cyrus Ghajar, Ph.D. Director, Laboratory for the Study of Metastatic Microenvironments, Fred Hutchinson Cancer Research Center

Simon Knott, Ph.D. Assistant Professor, Biomedical Sciences and Associate Director, Center of Bioinformatics and Functional Genomics, Cedars-Sinai Medical Institute

Keith L. Knutson, Ph.D. Professor, Department of Immunology, College of Medicine, Mayo Clinic, Director, Mayo Clinic Cancer Center's Cancer Immunology and Immunotherapy Program

Debbie Laxague, R.N. Advocate, NBCC

Tracy Leduc, J.D. Advocate, Alamo Breast Cancer Foundation

Susan Love, M.D., M.B.A. Chief Visionary Officer, Dr. Susan Love Research Foundation

H. Kim Lyerly, M.D., F.A.C.S. George Barth Geller Professor of Cancer Research, Professor of Surgery, Associate Professor of Pathology and Assistant Professor of Immunology, Duke University Medical Center

Garry Nolan, Ph.D. Rachford and Carlota A. Harris Professor, Department of Microbiology and Immunology, Stanford University School of Medicine

Ben Ho Park, M.D., Ph.D. The Donna S. Hall Professor of Medicine, Vanderbilt University Medical Center, Co-Leader Breast Cancer Research Program, Associate Director for Translational Research, Director of Precision Oncology, Vanderbilt-Ingram Cancer Center

Daniel Sarewitz, Ph.D. Co-Director, Consortium for Science, Policy & Outcomes, Professor of Science and Society, School for the Future of Innovation in Society

Stephen Shiao, M.D., Ph.D. Basic Science Director, Radiation Oncology, Cedars-Sinai Medical Center

Fran Visco, J.D. President, NBCC

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

Min Yu, M.D., Ph.D. Assistant Professor of Stem Cell Biology and Regenerative Medicine, Keck School of Medicine, USC

SPONSOR REPRESENTATIVE

Douglas Wall, Volcano Capital and Vance Wall Foundation

MEETING SUPPORT

Jaime Fornetti, Ph.D. Postdoctoral Researcher, Huntsman Cancer Institute, University of Utah

Giselle Hicks, M.P.H. Advocate, NBCC

Kayla Kirsch, M.S. President, Leapfrog Consulting

Marva Lewis McKnight, The Event Professionals

Michelle Tregear, Ph.D. Chief Programs Officer, NBCC

II. BACKGROUND

The Artemis meeting began Friday evening, March 6, which was set aside for introductions, background, and general scientific discussion and presentations.

The session on Prevention of Metastasis was held Saturday, March 7, to noon on Sunday, March 8, followed by the session on Primary Prevention, Preventive Vaccine.

III. ARTEMIS PROJECT ON PREVENTION OF METASTASIS

March 7-8, 2020

BACKGROUND PRESENTATIONS

Review of the Vaccine Landscape

Debbie Laxague

Laxague presented an overview of the vaccine landscape covering trials posted since the 2019 Artemis meeting (trials opened or registered since 03/19). No new breast cancer prevention vaccine trials have been listed in ClinicalTrials.gov over the past year. It was noted, however, that some early phase 1 breast cancer vaccine trials may ultimately be intended for primary prevention, although they are currently being tested in the metastatic setting. Most of the vaccines in current testing continue to be add-ons to current breast cancer therapy.

Patient populations continue to include all stages of breast cancer, with most trials primarily in the neoadjuvant or post-neoadjuvant setting and in HER2+ or triple-negative breast cancer (TNBC) subtypes. Two landscape posters were presented that provide more detail: one for trials with vaccines (plus standard therapy) and one for vaccines and/or checkpoint inhibitors or other immune modulators (plus standard therapy).

Participants also discussed whether there had been any vaccine successes in other organ cancer types that could be drawn from, such as the cervical cancer vaccine and PROVENGE. Participants also discussed outside viral antigen vaccines versus personal antigen vaccines and how they are performing.

An Update on Metastasis-Associated Genomic Alterations

Simon Knott

Knott shared results from a 2019 *Nature* paper that provides an analysis of whole-genome sequencing data for 2,520 pairs of tumor/normal tissue for 20 different metastatic cancers, surveying more than 70 million somatic variants. Across cancer types, the most highly amplified regions of the metastatic cohort contain established oncogenes such as the epidermal growth factor receptor (EGFR). For tumor suppressor genes, these results strongly support the Knudson two-hit hypothesis (i.e., that most tumor suppressor genes require both alleles to be inactivated, either through mutations or through epigenetic silencing, to cause a phenotypic change), with 80% of all tumor suppressor gene drivers found to have biallelic inactivation by genetic alterations. Participants noted that one major weakness of this study was that there was no comparison of the primary with the metastases, and there was no mention of immune-related genes.

Knott also shared data from a preprint of an article by Curtis, which presents whole exome sequencing data for 457 paired primary and metastatic tumor samples from breast, colorectal, and lung patients with both treated and untreated metastases (now published: www.nature.com/articles/s41588-020-0628-z).

Participants discussed Peter Lee's work on lymph node immunology and Amanda Lund's work on cross-presentation of lymphatic epithelial cells as a way to get broader immune suppression. Cancer treatments may be creating clonal bottlenecks,

but by potentially rotating through different treatments (even if they are working), we might be able to avoid the constant selective pressure and prolong progression-free survival (PFS). Advocates made the point that PFS is not a clinical benefit. Participants discussed the need for a systematic approach to these analyses where a cohort of patients with primary tumor samples and matched metastases are analyzed by treatment type.

Challenges identified were the need to plan genomic studies years in advance, new technology development that requires changes to sample storage techniques over time, and the lack of studies designed to look at overall survival.

Project Update: Enhancing Immune Recognition of Dormant Disseminated Tumor Cells

Cyrus Ghajar

Late recurrences account for a considerable proportion of metastatic breast cancers, with more than half of ER+ breast cancer recurrence occurring after five years—indicating that endocrine therapies are just delaying metastasis. This suggests that disseminated tumor cells (DTCs) are surviving over time, evading the immune system and eventually causing metastases. Single-cell sequencing has shown that dormant DTCs globally down-regulate human leukocyte antigen (HLA) molecules (cellular components of the immune system associated with tumor recognition) and that breast tumor cells exhibit reduced HLA-I expression upon dormancy induction. Chimeric antigen receptor (CAR) T cells function in a major histocompatibility complex (MHC)-independent manner and, thus, have the potential to eliminate DTCs in vivo regardless of their proliferation state.

In previous presentations, Ghajar illustrated that CAR T cells were able to target proliferative and dormant cells presenting a model antigen with equal efficacy. This year, he presented data demonstrating that this effect carried over in vivo, in a small cohort of mice that will be expanded drastically. Namely, CAR T cells eliminated metastases and single cells in bone/bone marrow of mice harboring metastases prior to CAR T infusion. Next, they will expand these studies and also contrast them with T cell receptor-mediated approaches using model neoantigens. Participant discussions focused on various models that could be utilized.

Rapid Autopsy Program

Alana Welm

Welm presented her plan for rapid autopsy studies to identify DTCs in their natural environment and to compare DTCs or dormant micrometastasis to active metastatic tumors to better understand drivers of tumor dormancy. Due to the generosity of a metastatic patient and her family, Welm was able to conduct one such study and used imaging data from before death to direct tissue collection. Three hundred samples from the brain, lungs, bone, bone marrow, and skin were obtained, including involved (metastasis) and uninvolved (distant to metastasis) tissue from each site. Welm described the analyses she performed. Some DTC samples were also sent to Simon Knott to test with multiplexed error-robust fluorescence in situ hybridization (MERFISH) techniques and Cyrus Ghajar for additional analysis. Welm was able to successfully identify DTCs in all tissues examined and is currently pursuing molecular analysis.

Artemis participants then discussed the various aspects of rapid autopsy studies, including the microenvironment, inflammation, and methods of sample preservation and storage.

Purifying DTCs From Breast Cancer Patient Bone Marrow

Cyrus Ghajar

Ghajar described the preliminary results of an Artemis pilot study examining strategies to find targets on DTCs. This pilot was a collaboration with several other Artemis participants (Danny Douek, Alana Welm, Simon Knott, Pat Haugen, Fran Visco and Chris Li). Ghajar reviewed current methods for isolating DTCs from patient bone marrow. Based on an in silico analysis of preexisting data conducted by Knott, they developed a scheme involving extensive "dump" gating to remove unwanted cells and incorporation of additional markers to preserve DTCs, and they tested it on commercially purchased bone marrow spiked with breast cancer cells. They also tested the gating scheme on a sample from uninvolved bone received in a rapid autopsy sample from Welm's lab and found that in the presence of bone metastasis, many more cells make it through the gates. Ultimately, once these cells are isolated, they can be sequenced to identify cancer driver mutations in the transcriptome to confirm their identity, and ultimately, potential DTC neoantigens and antigens. During this pilot study, Ghajar identified and addressed several technical issues.

Participants discussed whether this approach to isolating DTCs would work in TNBC based on an epithelial cell adhesion molecule (EpCAM) and whether it would be more inclusive with a known marker for bone marrow cells added. Ghajar responded that they are trying to broaden the negative selection and that such an analysis was done in the process of identifying the three gates. Participants also discussed examining intracellular markers. Ghajar noted that he is obtaining and analyzing bone marrow aspirates and bone marrow cores in parallel. A focus on patients at surgery with stages 2-3 breast cancer would increase the likelihood of DTCs, facilitating answers sooner.

Microbiome Signatures in Cancer Biopsies

Danny Douek

Douek reviewed the literature on how the gut microbiome affects the response in humans to immune checkpoint blockade (ICB), how the immune system and the microbiome are intimately related, and evidence that bacteria in tumors are not contamination. He also showed that almost all human tumors sampled to date contain bacteria.

Douek has developed a pipeline to look at the effect of the tumor microbiome on vaccine efficacy. Tumor RNA sequencing was performed on two different mouse models: one that models human stage 4 highly metastatic breast cancer and another that models a less metastatic medullary breast adenocarcinoma. Prioritized neoantigen discovery was done by comparing the tumor sequencing data with whole genome sequencing data on normal healthy tissue. Using MHC Class I binding scores and transcript abundance, candidate neoepitopes were selected. Various strategies were used to examine the effect on tumor growth in animal models. The next step will be microbial manipulation of the tumor environment. Participants discussed how tumor cells collect bacteria, especially since it is a common strategy of cancer cells to manipulate the environment, and the potential symbiotic relationship that might exist between cancer cells and bacteria. Participants suggested that since different colonies of mice may have different microbiota, future experiments should be replicated in multiple colonies.

WORKING GROUP DISCUSSIONS

Four topics were identified by participants for the first breakout discussions. Participants were asked to think about what we should be doing and what needs to be done to stop breast cancer in the context of preventing metastasis—but without other constraints.

TECHNOLOGY PROJECT

Tara Deans, Steve Elledge, Keith Knutson, Kim Lyerly, Tracy LeDuc, Sue Love and Garry Nolan

The group discussed quantum entanglement and bacteria as possible avenues to visualize metastasis in real time, longitudinally.

Quantum entanglement works using paired photons: an idler and a detector. The idler stays put while the detector is sent out, and when the detector sign changes, so does the idler sign. This technology could possibly be used to provide a deeper, noninvasive imaging technique for visualizing metastasis and dormant tumor cells in biological tissues. Without needing to know the exact location of each DTC, quantum entanglement could be used to define cell surface markers, a specific phenotype surface of tumor cells or even an entire protein complex. We could correlate the quantity of DTCs with metastasis. This approach would depend on identifying and selecting unique markers; however, it wouldn't be able to distinguish lethal tumor cells. Caution about early detection and its unintended consequences was also strongly noted.

Another idea was to alter the microenvironment when metastasis starts to grow. Bacteria could be used as a "fantastic voyager." Concerns were raised about triggering chronic inflammation unless inert, impotent, synthetic bacteria were used. One way to alter the microenvironment could be to modify the tissue to become resistant to metastasis development. We could look at the features of organs that never develop metastasis like the spleen, which has a great blood supply yet never develops breast cancer metastasis. In addition, different breast cancer subtypes tend to metastasize to different organs. We could evolve bacteria to live in a particular tumor environment. By hooking them up to T cells, which can go everywhere, we could build a combinatorial logic circuit for the bacteria to only replicate under certain circumstances. The bacteria could act as a

minion, changing the microenvironment, laying extracellular matrix, turning certain genes on, injecting proteins into cells, creating a biosensor to set up an alarm or activating cell destruction.

In order for the quantum entangled photons to react with DTCs or metastatic cells, we will need a unique marker to distinguish them from the surrounding tissues. Bacteria could be engineered with logic circuits as sentinels to detect these cells and then used in imaging, to be induced to deliver molecules, to secrete something to engage the immune system or to act as bombs to destroy the cells. Participants discussed incorporating tissue engineering technology that creates new tissue when tissue is damaged.

The group suggested the following areas of focus over the next 12-18 months:

Test quantum entanglement and its ability to detect DTCs:

- Reach out to a quantum entanglement expert.
- Test the detection of individual DTCs in a mouse model using quantum entanglement:
  - Set up a cell surface marker or internal detectable marker akin to a green fluorescent protein (GFP) for positive control that should be detectable beyond current limits of detection with an isotope, nIR or MRI scans.
  - Conjugate an antibody with material for use in the detection of cell surface markers.

Develop a phenotype DTC cell surface by using databases:

- Identify cell surface markers for dormant tumor cells from Artemis data.
- Use nanobody or antibody libraries to bind to the surface and identify anything unique relative to controls; unbiased library detection would also detect unique combinations of markers that wouldn't be identified using mass spectrometry or transcriptome analysis.

Engineer bacteria to act as sentinels for DTCs:

- Combinatorial logic gates could be created with bacteria or nanoparticles.
- Bacteria could be engineered with material for use in detection, like magnetosomes or iron, and programmed to only survive in specific locations
- Bacteria could be filled with magnetic material and then used in imaging. They could be injected into breast ducts to induce an immune system reaction that could eliminate DTCs elsewhere.
- Engineered bacteria, if properly localized, could be programmed to respond to radiation such as microwave to activate killing mechanisms or to heat themselves to destroy nearby cells.

EPIGENETICS, AND VACCINE AND CHECKPOINT BLOCKADE FOR METASTASIS PREVENTION

John Bischof, Joe Camardo, Simon Knott, Debbie Laxague, Ben Ho Park, Stephen Shiao and Min Yu, with Keith Knutson and Kim Lyerly joining during the second half of the discussion

The group began discussing how to leverage epigenetic modification effects on cell behavior to prevent metastasis and to change a metastatic cell into a nonmetastatic cell. There are some drugs that could be delivered chronically; however, we do not yet know what specific genetic modifications could accomplish this. The focus of this group then turned toward personalized vaccines with an immune checkpoint blockade that could identify cells with metastatic markers and focus the immune system to eliminate those specific cells.

Artemis participants discussed what new technology would be needed to develop the diagnostics to identify patients highly likely to have metastases present at the time the local tumor is observed. Cell-free DNA could be one such marker. Combining a PD-1 inhibitor with the administration of a vaccine specific for a "metastatic antigen" would stimulate the immune system to destroy cells with metastatic potential. Adjuvant chemotherapy is delivered as a way to eliminate any possible remaining tumor cells. This is not a specific or directed approach, it is not always effective, and it has serious side effects.

Goal: Create an adaptive trial framework, not a specific trial, to encompass multiple scenarios with standard metrics to compare which vaccine approaches work best for metastasis prevention.

Elements:

- Test a variety of vaccine models, including targeted antigens, Tvax and personalized vaccines.
- Make the backbone of the trials a checkpoint blockade combined with vaccine.

- Identify high-risk patients—those likely to develop metastasis (not those with metastasis already).
- Identify surrogate endpoints relevant to metastasis, such as pathologic complete response and cell-free DNA, and standardize across all trials.
- Note that all trials under the Artemis aegis would be comparable, with Artemis providing scientific oversight on the types of vaccine approaches and surrogate endpoints.

One-trial schema (window of opportunity):

- Diagnosis (biopsy)
- Surgery upfront (one to three months) and cell-free DNA measurement
- Vaccination and treatment with PD-1
- Standard of care (hormone therapy, chemotherapy, targeted therapy)
- Booster vaccine administration

Next steps:

- Conduct a feasibility assessment over the next year, including the mechanics of infrastructure, institutional review board and correlatives.
- Identify the teams needed: vaccine experts and manufacturing; clinical trial development; and a literature review for trial population, monitoring metrics and a vaccine platform.

INSTITUTIONALIZING ARTEMIS

Frank Calzone, Dan Sarewitz and Fran Visco, with Kim Lyerly and Peter Fasching for part of the discussion

This group discussed how to maintain the Artemis Project and expand its capacity to support collaborative research—not just as a think tank, but to also implement ideas and strengthen the research network.

Participants looked at examples such as the Defense Advanced Research Projects Agency (DARPA) and the Howard Hughes Medical Institute (HHMI) as well as the Stowers Institute in Kansas City. It was agreed that this could not be a bricks-and-mortar institute but some type of virtual model that is a hybrid of DARPA, HHMI, etc. This approach will require good technology to enable collaborative work across institutions.

The existing annual meeting would still be the core of the Artemis Institute and where Artemis participants would set the agenda. From a researcher's point of view, part of the prestige is driving the project and not just being told what to do. Flexibility and academic freedom are essential, along with a self-sustaining ecosystem of a shared mission, a focus on values and continued active involvement with advocates. The Artemis Institute would have a focused mission with shared values and will be integrated with what we do at the annual Artemis meeting.

To build and maintain the prestige and credibility of an Artemis Institute, several well-known and highly respected investigators and leaders in the business arena would need to be involved. Their role would be to help draw funding and people as well as to serve as oversight and guidance.

Various possibilities were discussed, including an Artemis academy constituted by senior leaders that would "tithe" a proportion of their funding to the academy, with some funding going to researchers and some toward full-time Artemis staff. The academy infrastructure would be coordinated by NBCC with dedicated funding and full-time staff for Artemis. It could be a consortium model with universities contributing. And although funding was not explicitly discussed, beyond this suggestion, it was in the background as an issue to be addressed.

Researchers would need to apply to become involved. Currently, Artemis participants are invited by NBCC through recommendations of the Artemis Executive Committee and others involved in the Artemis Project.

The Artemis Institute will need dedicated full-time staff at NBCC to help keep the collaborations across institutions active, follow up on the annual meeting next steps and help coordinate research advocate involvement. Periodic meetings throughout the year, perhaps quarterly, would be needed.

The group agreed that the goals would be:

- End breast cancer deaths through primary prevention and prevention of metastasis.
- Keep the Artemis Project alive—not just as a think tank, but also to implement ideas and strengthen research network.

Elements:

- It would be led by NBCC with advocate involvement infused at every level.
- The core would be the annual meeting with sub-meetings throughout the year.
- There would not be a bricks-and-mortar location; rather, collaboration would be virtual.
- It would be modeled on HHMI, DARPA and the Gates Foundation.
- An oversight board would be created and include a few prestigious scientists, a business person and a majority of advocates.
- Three NBCC staff would be dedicated to the Artemis Institute to engage with researchers on technical issues.
- Researchers could be under contract to do a percentage of their work for Artemis.
- The contract could be between a researcher's institution, NPT and NBCC.

Next steps:

- Create a case statement about why this needs to happen and what makes this effort unique.
- Interview Artemis participants on why they participate and keep coming back.
- Create a marketing plan to establish an endowment.
- Identify oversight board members.
- Note that Peter has offered to help set up a virtual connection among participants.
- Identify relevant policy issues, such as intellectual property, and plan to address.

DTCs

Michele Atlan, Danny Douek, Cyrus Ghajar and Alana Welm, with Peter Fasching joining later

The discussion was primarily on identifying important questions about DTCs and methods to address those questions. The group focused on using single cells from the tissue obtained from Alana's rapid autopsy study, testing candidates experimentally in Cyrus' immune competent models of dormancy (4T1 Balb/c mouse model and E0771 C57Bl/6 mouse model), utilizing Danny's pipeline to identify neoantigens and capitalizing on Peter's clinical trial—all with the overall goal of understanding the biology and markers of DTCs.

Questions to be addressed ranged from whether we know for certain that dormant DTCs are actually the cells that form metastasis to whether there are differences between the single cells that seed the tissues early in the course of the disease versus the ones that seed the tissue later and whether and how the microenvironment influences DTCs. Participants also discussed the need to investigate intracellular bacteria that might influence DTC behavior.

The group reviewed various current techniques that could be used to profile the DTCs and the microenvironment in the rapid autopsy samples from fresh, fresh-frozen or fixed tissue.

- Analyze tissue sections by:
  - Single-cell laser capture microdissection (LCM) to determine the human and bacterial transcriptome
  - ATACseq to look at the chromatin structure and methylation status
  - Whole genome sequencing (WGS) to compare the tissue samples to primary tumor samples, if available
  - CODEX to identify tumor cells and their microenvironment, including endothelium, fibroblasts, immune cells, extracellular matrix (ECM), bacteria, p16/senescence and tissue-specific cells that may play an important role in that specific microenvironment
- Analyze cells by:
  - Sorting out the tumor cells for HER2+ disease
  - 10x genomics sequencing, which allows these different technologies to be used concurrently
  - Transcriptomics, ATACseq, and WGS, as listed above in the tissue analysis

This information can then be used in the immune competent mouse models to answer specific questions. It would be important to correlate observations in women with DTCs and those without. Large, pooled databases can be used to compile information on drugs received, physiology and time-dependent events near metastasis, in addition to comparing DTCs and active metastases within the same individual.

The final discussion centered around what samples could be collected from Peter Fasching's SURVIVE study, being conducted in Germany, that might benefit the question of dormant tumor cells and what additional samples we would want from that study.

The final proposal design presented was:

- A total of 3,500 breast cancer patients who have received primary treatment accrued over the next 18 months (SURVIVE study)
- Patients stratified into high surveillance (imaging) or no surveillance groups based on circulating tumor cells, cancer antigen 15-3, and possibly DTCs in the bone marrow
- A longitudinal follow-up to determine whether increased surveillance affects outcomes
- A sample collection for insights into DTCs, which includes:
  - Primary tumor preserved in formalin-fixed paraffin-embedded (FFPE) tissue (already included in the study design with 90 percent of specimens preserved):
    - Whole genome sequencing
  - Bone marrow aspirates:
    - Sorted for DTCs and analyzed by SMARTseq for whole transcriptomes
    - 10x genomics for the full transcriptomes of bone marrow cells (T cells, myeloid cells, etc.)
    - Microbiome and plasma exosome multiomic analysis
  - Liver biopsy (voluntary option with consent):
    - Fixed for histology and then used for laser capture microdissection

IV. ARTEMIS PROJECT ON PRIMARY PREVENTION

March 8-9, 2020

BACKGROUND PRESENTATIONS

Review of 2019 Artemis Primary Prevention Meeting

Alana Welm

Welm reviewed the main ideas that came out of the 2019 meeting. These included ways to keep anti-estrogen therapy local to the breast and minimize systemic side effects, differentiation therapy to block cell proliferation in the breast and engineering an estrogen "sink" using bacteria that naturally live in the breast. Another idea was to leverage the depolarization of the epithelium and subsequent exposure of apical proteins in the tissue as a way for T cells to recognize and eliminate breast cancer cells in "Operation Prairie Justice."

The group discussed the natural breast microbiome and the limitations of most microbiome studies, which are only looking at chunks of breast tissue and not taking into account breast anatomy. Sue Love reported she has looked at the microbiome in nipple breast fluid with Delphine Lee and found that people with cancer had a different microbiome than those without cancer.

Artemis Project for a Preventive Breast Cancer Vaccine: Update

Keith Knutson

The Artemis preventive vaccine for breast cancer is ready for the phase 1 safety trial in patients with low-volume, stable metastatic breast cancer. Knutson continues to develop immune monitoring strategies and noted that the 25-patient sample size is powered sufficiently to see elevated immunity pre- and post-vaccination. The primary outcomes of the phase 1 trial will be the safety and immunogenicity of the vaccine and not the direct cancer response to the vaccine since, ultimately, the vaccine is intended for prevention.

The following milestones were presented but may be affected by the current emergency response and restrictions surrounding the COVID-19 pandemic:

- **June/July 2020** – Plasmid manufacturing to begin
- **August 2020** – Final approval of the investigational new drug (IND) by the U.S. Food and Drug Administration (FDA)
- **November 2020** – Vaccine to be administered to the first patient in the phase 1 safety trial
- **January 2021** – Data assessment of the first three trial participants completed

- **May 2021** – Last trial participant to receive the last vaccine dose
- **September 2021** – Clinical study report completed

WORKING GROUP DISCUSSIONS

There were two primary prevention topics identified by all participants for the breakout discussions. There were time constraints on the discussion, and as such, only two breakout sessions occurred, including the reports on action plans.

ENGINEERING BACTERIA/ OPERATION PRAIRIE JUSTICE

First Session: Frank Calzone, Tara Deans, Danny Douek, Steve Elledge, Cyrus Ghajar, Tracy LeDuc, Sue Love, Stephen Shiao and Alana Welm; Second Session: Cyrus Ghajar, Tracy LeDuc and Stephen Shiao

Participants began discussing how to use the loss of polarity of the epithelial cells to detect and eliminate breast cancer cells early in the cancer process when they begin to lose their architecture.

The initial discussion centered around CAR T cells versus engineered bacteria as a "patroller" within the breast ducts that would ignore tight junctions. A number of concerns were identified and debated, including toxicity. The feasibility of engineering bacteria vs. CAR T cells was discussed, along with ideas of how to program bacteria to die if they leave the ducts.

Another idea was to "tune" the breast microbiome (e.g., targeting with immunoglobulin A [IgA]) to make it more sponge-like for estrogen. Participants suggested characterizing the ductal fluid to see if there is a relationship between antibody composition and bacteria. Participants raised concerns about the assumption that tumors are estrogen dependent prior to clinical presentation and engineered bacteria's ability to bind estrogen. One approach could be to replace a microbiome that is not good at binding estrogen with one that is through a microbiome "transplant."

Ideal properties identified for the bacteria include a patrolling function along the ductal tree, quorum sensing to maintain a stable population, a kill switch, the ability to sense or bind and the ability to kill or deliver a payload. Participants discussed whether to focus on a killing function versus a repair function, payload options and whether the payload options would require intracellular or extracellular bacteria. Various options were debated. The pros and cons of synthetic viruses were discussed as well as an inactivated virus that could be injected into the breast ducts after childbearing and engineered to kill the entire mammary epithelia.

During a large-group discussion, it was reiterated that we need to characterize the microbiome in the breast among healthy women, women with DCIS and those with breast cancer. The target population for this prevention strategy is women at high risk (e.g., genetically at risk).

During the second session, two smaller subgroups formed to continue the discussion.

SUBGROUP 1: OPERATION PRAIRIE JUSTICE

Cyrus Ghajar, Tracy LeDuc and Stephen Shiao

One group explored the possibility of generating a basement membrane that is unbreakable and insensitive to the proteases that are secreted by epithelial cells so that tumor cells could never escape or get out of the duct. They designed a project to address this possibility.

Uncleavable basement membrane project:

1. Identify matrix metalloproteinase (MMP) cleavage sites in the two major components of the basement membrane in mammary epithelium: collagen IV and laminin 111 (we know they can be cleaved by MMP-9).
2. Perform a site-directed mutagenesis with CRISPR. Introduce the necessary mutations to make the cleavage sites uncleavable, although still able to secrete things and be properly organized.
3. Test the function. Make a K14-driven basement membrane mutant and use a mouse model with an uncleavable basement membrane.
4. Cross the K14-driven basement membrane mutants with MMTV-PyMT and MMTV-HER2 mice.
5. In parallel, create mosaics of intrauterine transduction with basement membrane mutants to develop mammary glands with a proportion of an uncleavable basement membrane.
6. Determine how to limit basement membrane mutation—intraductal injection of a viral particle that would target the ductal epithelium to change the basement membrane locally.

During a large-group discussion, participants commented that it may be sufficient to reconstitute part, rather than all, of the ductal network. Moreover, we would need to test and demonstrate

experimentally if immune cells use the same proteases to cross the basement membrane and how the immune system would operate in this setting. Various existing approaches to achieve these goals were discussed.

SUBGROUP 2: DELIVERY/DUCT TAPE

Frank Calzone, Tara Deans, Danny Douek, Kim Lyerly and Alana Welm

This was a breakout group from the first session, Operation Prairie Justice. The focus was on how to capitalize on the transcytosis mechanism to deliver bacteria or nanoparticles that degrade estradiol or change the breast microbiome locally to effect primary prevention.

The first approach discussed was removing estrogen locally. One idea was to inject nanoparticles conjugated to an estrogen-degrading enzyme to locally deplete estrogen in the breast. This could be tested among women waiting to have a mastectomy, although some concerns about this approach were discussed.

Another idea for removing estrogen locally was to use bacteria to change the breast microbiome into one that better binds estrogen. The bacteria would need to be engineered to enter cells and then lyse to release a protein that would bind endogenous estrogen or degrade the estrogen receptors using an engineered PROTAC. One suggestion was to load up a micro-vesicle inside the bacteria and piggyback on the ubiquitin pathway. However, additional concerns remained about the bacteria getting into the bloodstream and estrogen degradation, possibly being genotoxic.

The second approach discussed was killing transformed cells in the duct and prompting the duct to repair itself, like "duct" tape. The group identified four categories of bacterial triggers from putative transformed cells as a next step for investigating the approaches discussed.

PROTECTIVE EFFECTS OF PREGNANCY

Michele Atlan, Joe Camardo, Peter Fasching, Simon Knott, Keith Knutson, Debbie Laxague and Min Yu, with Kim Lyerly and Dan Sarewitz for the first session only

The main goal of the group was to explore ways to learn about the mechanisms behind the protective effect of pregnancy on breast cancer. The group ended up focusing on designing two large-scale epidemiology studies to:

1. Address changes in the breast before and after pregnancy.
2. Compare breasts of women who have and have not been pregnant.

The group roughly outlined two studies: one that would be a case control in which the breast tissue from women with a previous pregnancy would be compared to age-matched breast tissue from women without a previous pregnancy and then molecularly profiled. The other would be a longitudinal study for which recruitment would occur before pregnancy. Biomaterials would be collected pre-pregnancy, post-pregnancy, and at the time of weaning.

Based on the feedback after the first breakout session and discussion at dinner, group members questioned the utility and amount of information that could be gained from the previous day's proposed trials. Also they extended their thinking to consider many factors associated with breast cancer risk, not just pregnancy.

It was proposed that their group should focus on developing a wide-scale program to characterize the normal breast and determine how each of these factors is related to breast cancer risk. The discussion centered around how existing tissue banks could be used to determine how breast cancer risk factors associate with molecular changes in the breasts of women who don't have cancer, and that may be associated with future cancer development.

Action items:

1. Review literature to determine what questions have been asked already and what that data show.
2. Compare molecular changes in the breast tissue of women with the maximum number of risk factors to breast tissue from women with the minimum number of risk factors to determine if there is anything molecularly associated with breast cancer risk.

The group proposed the following study: Examine the molecular differences in healthy breast tissue according to extreme risk factor patterns.

Examples of extreme risk factors:

- Number of pregnancies >4 versus no pregnancy
- First full-term pregnancy <25 years of age versus >35 years of age
- Body mass index (BMI) >35 versus <25
- Mammographic density >50 percent versus <10 percent

All comparisons would be made in premenopausal and postmenopausal women.

It was estimated that there would need to be 160-320 samples available for this study.

The samples would be analyzed via single-cell sequencing, digital spatial profiling and microbiome analysis.

During a large-group discussion, participants asked about the power of the study and whether there was consensus on how big an effect each of the factors have on breast cancer risk. Another suggestion was to compare the stroma of women with BRCA mutations who developed cancer to those who did not.

Participants also discussed the applicability of pregnancy-induced breast cancer in animal models and whether we could generate hypotheses from the data that already exists. Suggestions included characterizing organoids for nulliparous and multiparous women. There might also be existing mammographic data from radiology departments available to address this type of question.

V. CONCLUSION

For the past ten years, the Artemis Project has continued to bring a focus to two overarching questions that are not receiving sufficient attention: primary prevention (How do we stop women and men from getting breast cancer?) and preventing metastasis (How do we stop them from dying of breast cancer?). In 2020, NBCC once again brought together a diverse collection of researchers and advocates, many of whom would not have otherwise met, to discuss progress, brainstorm and plan new strategies, and form new and ongoing collaborations that persist after the meeting in pursuit of answering these questions.

Important progress continues to be 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 strategic plan for the development of a preventive vaccine, launched in 2011, resulted in the funding of the Artemis Project for a Preventive Breast Cancer Vaccine. This project is now months away from a phase 1 clinical trial in collaboration with Dr. Keith Knutson and Dr. Sara Chumsri of the Mayo Clinic Cancer Center and the National Cancer Institute's PREVENT program.

Additional avenues for primary prevention continued to be explored in 2020, focusing on better understanding mechanisms behind the protective effect of pregnancy on breast cancer as well as engineering strategies locally within the breast to detect and eliminate breast cancer cells early in the cancer process.

For the prevention of metastases, immune-based approaches continue to be a focus area of Artemis participant research, as well as characterizing DTC and identifying new neoantigens for a vaccine 2.0. Further, new technology-based strategies leveraging physical properties such as quantum entanglement are also being discussed.

Lastly, there was a special focus this year among participants on the necessary steps for institutionalizing Artemis. After ten years, NBCC has shown that the Artemis Project works as demonstrated in part by the many collaborations coming out of the project and the forthcoming phase 1 vaccine trial. Artemis works because of the advocate leadership, the dedication of visionary scientists, and the strategic, creative approach to achieving its goals. While Artemis is not and never will be a bricks-and-mortar structure, all of the components of an institution that is innovative, value driven, and mission oriented exist in it today. It is time to transform the Artemis Project into a sustainable, virtual collaboration institute to bring it to the next level and fulfill the extraordinary possibilities it presents.

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