NATIONAL BREAST CANCER COALITION

ARTEMIS PROJECT

ANNUAL MEETINGS
MARCH 3-6, 2022

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 2022 annual Artemis meeting, which had 19 participants, including advocates and scientific expertise ranging from immunology, biophysics, biomedical engineering and genetics to molecular biology, radiation oncology, and clinical oncology.

2022 ANNUAL MEETING PARTICIPANTS

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

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

Jayanta Debnath, M.D. Distinguished Professor and Chair, Department of Pathology, University of California San Francisco; Member, Helen Diller Family Comprehensive Cancer Center

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

Andrew Ewald, Ph.D. Professor and Director, Department of Cell Biology, Johns Hopkins University School of Medicine

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

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

Andrei Goga, M.D., Ph.D. Professor, Department of Cell and Tissue Biology, University of California, San Francisco; Co-Leader, Breast Oncology Program, UCSF Helen Diller Family Comprehensive Cancer Center

Pat Haugen, B.A. Advocate, Minnesota Breast Cancer Coalition

Judi Hirshfield-Bartek, R.N., M.S., O.C.N. Advocate, Dr. Susan Love Foundation for Breast Cancer Research

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

Christopher Li, M.D., Ph.D. Research Professor, Epidemiology Fred Hutchinson Cancer Research Center

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

Michele Rakoff Advocate, Breast Cancer Care and Research Fund

Wade Shen, M.S. Chief Program Officer, Actuate

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

Xiang (Shawn) Zhang, Ph.D. William T. Butler, M.D., Endowed Chair for Distinguished Faculty and Professor of Molecular and Cellular Biology, Baylor College of Medicine

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 Thursday evening, March 3, which was set aside for introductions, an NBCC update, Artemis' background and general scientific discussion. As is tradition, participants then began by identifying and discussing the burning questions they believe are key in breast cancer at this time.

The meeting then moved to the session on "Primary Prevention, Preventive Vaccine," which was held from Friday, March 4, 2022, to noon on Saturday, March 5, followed by the session on "Prevention of Metastasis," which concluded after lunch on Sunday, March 6.

III. ARTEMIS PROJECT ON PRIMARY PREVENTION

March 4-5, 2022

BACKGROUND PRESENTATIONS

Review of the Vaccine Landscape

Michelle Tregear

In the early years of Artemis, the vaccine trials were mostly therapeutic. This year, there are two prevention vaccine trials registered on www.ClinicalTrials.gov. One is a preventive vaccine out of the University of Pennsylvania for individuals with BRCA mutations, and the other is an alpha-lactalbumin vaccine out of the Cleveland Clinic for women with triple-negative breast cancer. There are new therapeutic vaccine trials, one of which is headed into Phase 3. In addition, there are three breast cancer vaccine trials sponsored by the National Cancer Institute's (NCI's) PREVENT program. Posters of the vaccine landscape from over the years were presented.

Mapping the Current Territory

Participants began to map the current territory in breast cancer and identified and discussed trends, drivers and innovations in the landscape that could change how we approach primary prevention and the eradication of breast cancer.

Vaccinating in the Face of Antigenic Variation

Danny Douek

Douek presented results from a study looking at whether boosting with Omicron-matched mRNA increases immunity and protection against Omicron challenge in nonhuman primates compared to using the currently approved Moderna vaccine. He found that a boost from the Omicron-specific vaccine and the original Moderna vaccine equivalently increased neutralizing antibodies against Omicron and all variants of concern as well as equally mediated lower airway protection against Omicron challenge.

He then presented five concepts to help select future vaccines in the face of antigenic variation. Instead of using phylogenetics, in which the genetic distance between variants is mapped to reveal virologic relationships, the selection of vaccines could use antigenic cartography to map the immunologic distance between the variants. This immunologic map can then be envisioned to reside within an imaginary virologic space, within which the SARS-CoV-2 spike can only mutate so far while still retaining ACE2 binding and function. The vaccine is concerned only with virus variants within this virologic space and exploits the concept of original antigenic sin to recall prior immune memory.

Antigenic cartography can inform the "direction" for vaccine design. It is a proactive approach to predicting the future by measuring the present.

The concepts could be applied to breast cancer vaccine design. Antigenic cartography could be used to map tumor antigens within the mutational space; self-antigens would be the analogous original antigenic sin; therapeutic vaccines are how we provide protection to people who already have tumor antigens (breast cancer); and prophylactic breast cancer vaccines are how we would prime and boost immunologically naïve people.

The discussion then involved issues of viral evolution, application to prevention in breast cancer, surveillance and engineering an artificial immune system.

Revisiting Seed and Soil: What Is the Role of Nontransformed Cells in Tumor Initiation and Discrimination?

H. Kim Lyerly

The traditional theory of cancer progression is that mutations accumulate until a threshold event. However, even in the earliest stages of cancer, there is heterogeneity. Lyerly suggested that we revisit the idea of a single-driver mutation in early cancer progression and rethink the role of cell-intrinsic heterogeneity. Disseminated tumor cells (DTCs) have been shown to be the basis of metastases; however, the mere presence of DTCs is not sufficient for disease progression and lethality. A niche is also needed for metastasis development, indicating that cancer progression is not completely cell intrinsic.

Lyerly presented data from a study[1] looking at ductal carcinoma in situ (DCIS) progression to invasive breast cancer. Using high-throughput gene expression analysis with pathologic evaluation, DCIS samples with and without subsequent invasive breast cancer were compared to create a pseudo-timeline of DCIS progression. The predominant hallmark signatures that varied along the timeline of DCIS progression were proliferation and epithelial to mesenchymal transition (EMT).

Lyerly was able to de-convolute the heterogeneity in the HER2 CRAINBOW mouse model, developed with Josh Snyder at Duke, in which tumors form from one of three human oncogenes, specifically human HER2. The mouse model allows epithelial cells to express one of the three transgenes (either wild-type HER2, the HER2 isoform d16 or the C-terminus HER2 p95). As previously reported, the human wild-type HER2 rarely forms tumors, but the two oncogenic HER2 isoforms not only develop tumors as expected, but unexpectedly develop unique epithelial cell malignant trajectories, with the p95 expressing cells being more invasive with early dissemination and the d16 expressing cells being more proliferative, invading and metastasizing much later. Using tissue clarification and whole-gland 3D imaging, Lyerly traced the heterogeneity of both transformed and nontransformed epithelial cells from development through tumorigenesis. Using a newly developed technique called Mouse Paint, he was able to map tissue heterogeneity visualizing the epithelial cells to demonstrate the dynamics of early change and retractable cell populations.

Discussion among participants involved issues of whether there is immunity in the breast, the level of antigen presentation, the timeline of breast development and limitations of animal models.

Artemis Seed Grant: Prevention Vaccine Project Update

Keith L. Knutson

Knutson presented the history of the Artemis preventive vaccine, which is described in detail in prior Artemis annual reports. He described the population for the Phase I trial as patients with any ER/PR/HER2 breast cancer treated solely with endocrine therapy. There will be two dose levels and three cohorts. The first safety lead-in cohort will be comprised of six Stage IV breast cancer patients. The second safety lead-in cohort also will be comprised of six Stage IV breast cancer patients. And the expanded cohort will be comprised of 30 Stage III breast cancer patients. The primary outcomes are safety and tolerability, along with immunity. The secondary outcomes are the ability to traffic to breast mucosa and persistence.

Artemis participants then discussed safety and endpoints, mRNA vaccines in this context, and the need to have Phase 2 endpoints mirror Phase3

Knutson then presented work on identifying neoantigens in breast cancer and presented a trial design for neoantigen vaccination in combination with anti-PD1. This study was approved in January 2022 by the U.S. Food and Drug Administration (FDA) and is open to all cancers.

Participant discussion included the issues of computational predictions, validation and computer predictions, and the possibility of an overlap with Douek's immunological mapping.

[1]PREPRINT: Creating a 'Timeline' of ductal carcinoma in situ to identify processes and biomarkers for progression towards invasive ductal carcinoma. Clare A. Rebbeck, Jian Xian, Susanne Bornelöv, Joseph Geradts, Amy Hobeika, Heather Geiger, Jose Franco Alvarez, Elena Rozhkova, Ashley Nicholls, Nicolas Robine, Herbert K. Lyerly, Gregory J. Hannon. bioRxiv 2022.03.01.482529; doi: https://doi.org/10.1101/2022.03.01.482529.

WORKING GROUP TOPICS, DISCUSSIONS AND ACTION PLANS FOR THE NEXT 12-18 MONTHS

After discussion and debate, participants agreed on four primary prevention topics for working group activity. The groups were chosen and convened to discuss each topic, followed by large-group discussion and subsequent small-group discussion. Working groups then outlined 12- to 18-month action plans for each topic area.

Breast Cancer Shield: Rational Regional Therapies for Risk Reduction

Andrew Ewald, Andrei Goga, Simon Knott, H. Kim Lyerly, Michele Rakoff

Goal: To make the female breast impervious to the development of breast cancer without mastectomy.

Preliminary observations:

- There is an overall silencing of transcriptional and translational activity in the breast following systemic testosterone in the premenopausal breast, along with a molecular shift consistent with male versus female breast tissue.
- Only cells with two DNA damage "hits" (i.e., BRCA carriers and PALB) are sensitive to PARP inhibitors.
- Myoepithelial cells are a barrier to invasion.

Approach: Identify safe and tolerable locally deliverable agents to make the breast impervious to cancer; this could be synergistic with vaccination and occur one step earlier.

What could be achieved in the next 12-18 months?

- Generate evidence for reprogramming the breast luminal epithelium with locally delivered therapies:
  - Anti-hormone (SERM/SERD/testosterone)
  - Synthetic lethality (PARP inhibitors)
  - Altered development (myoepithelial shield)
- How will we do it?
  - Perform reduction mammoplasty, treat for 24-72 hours, and perform FACS-based analysis and single-cell RNA sequencing to show cell-intrinsic effects of the interventions. Use breast organoids as an alternative.
  - Use wild-type mouse models to look for changes in development and undesirable off-target/systemic effects (e.g., uterine horn regression indicating hormonal changes). Possible cancer models include PyMT (luminal), MTB-TOM (triple-negative) and HER2.

During large-group discussion, it was explained that the vision was to do a short-term liquid culture with quick analysis. A suggestion to consider the population of women with ER+ breast cancer, many of whom will have bilateral mastectomy and are already receiving systemic hormonal treatment. We should be able to get approval for local intraductal endocrine therapy administration, enabling a look at the immediate effects on the breast in women and comparison with the healthy breast. The group agreed that this would be a good idea once the initial demonstration was done in explants.

Artemis Atlas: Improving Risk Prediction

Silvia C. Formenti, Judi Hirshfield-Bartek, Keith L. Knutson, Christopher Li, Wade Shen

The group discussed how to identify modifiable determinants of the risk of lethal breast cancer, in part to better select a population for a preventive vaccine.

Background: There are large databases of longitudinal screening images and tissue biopsies (benign and malignant). How could these existing data sources be used to address breast cancer primary prevention, and is there new technology that could yield a discovery of risk?

Goals/outcomes:

- Demonstrate the ability to improve risk prediction with untested approaches.
- Can we learn from exceptional survivors?
- Can we learn from the patients with a good prognosis who die?

Key tasks/milestones:

- Select 50 DCIS or benign breast disease (BBD) patients who remained disease-free for more than 15 years:
  - Perform deep interrogation of DCIS or BBD samples and compare to adjacent normal tissue.

- Select 50 DCIS or BBD patients who were then subsequently diagnosed with invasive breast cancer and died within three years (lethal invasive):
  - Perform deep interrogation of invasive cancer samples and compare to adjacent normal tissue.
- Use sequencing and artificial intelligence (AI) to compare the imaging data from these different groups and partner with different bioinformatic and AI experts to identify patients with DCIS who have a high risk of developing invasive lethal breast cancer versus those who have a very low risk of their DCIS progressing.
- Identify components of the microbiome that could be associated with risk and potential targets for intervention.
- Apply modern technologies such as COSMX NanoString, radiomics (mammogram, MRI), 16S (microbiome), RNA-Seq, metabolomics and epigenomics.

In response to a question about the benefit of studying extremes, Li responded that by using extremes, we would be able to see if the technology could even identify differences. This proof of concept is needed to be able to see if it would work for the middle group—to see whether new technology can provide new answers. Discussion also centered around how this approach would differ from existing studies on DCIS.

Sin Nombre: Engineering an Anti-Breast Cancer Immune System

Frank Calzone, Danny Douek, Pat Haugen, Alana Welm, Shawn Zhang

Building on the antigenic cartography presentation earlier, this group discussed vaccinating against all driver mutations and developing cartography based on epitope screening. The goal would be to test for binding and activation of T cells. Large-group discussion focused on whether there would be negative selection for cross-reactivity in every patient and when this could work in people.

The working group defined a goal to develop a proactive strategy to eliminate breast cancer before it develops by using a comprehensive primary prevention vaccine specific to oncogenic driver mutations.

Rationale: If a vaccine is developed against driver mutations, the immune system will eliminate the transformed cells as they appear and cancer will not develop.

Action items/timeline:

- List hot-spot mutations and driver genes relevant to breast cancer (one month).
- Consult with Steve Elledge/T-Scan on a polycistronic mRNA design (one month).
- Develop assays and perform T-cell receptor (TCR) cartography/screens: mutated versus wild type.
- Develop a vaccine: mRNAs encoding validated epitopes as a cocktail (as many as possible).
- Test the vaccine: initial testing among Stage IV breast cancer patients for safety and feasibility and to see if it elicits an immune response.
- Develop for the future: a prevention trial.

During large-group discussion, participants discussed a predetermined library with proof-of-concept testing among Stage IV breast cancer patients. The group discussed that the vaccination would be against multiple anticipated future mutations, not necessarily what is already present.

Stand Your Ground

Michele Atlan, Jay Debnath, Cyrus Ghajar

The group started the discussion about the fact that one of the earliest signs of emerging cancer is the loss of tissue architecture and the breakdown of the basement membrane and myoepithelium. Is there some way of intercepting initial breaches of the basement membrane and loss of myoepithelium to stop the process that leads to invasive cancer?

Discussion also involved the results of a small study that showed distinct differences between the basement membrane and myoepithelium in patients with DCIS who progress to invasive breast cancer versus those who do not. Building on the Operation Prairie Justice approach described in previous Artemis meetings, the group discussed expanding the approach to ducts.

The goal would be to develop an intraductal agent to patrol for the first sign of cancer, utilizing a switch based on metabolite presence in the duct to turn the system on. Normal apical proteins would keep the second gene turned off, but when they disappear, the cell would turn on and kill the first signs of atypia. A third gate would be when a cell escapes from the luminal epithelium and the stroma becomes activated.

Goal: To engineer an intraductal, logic-gated trafficking agent that can eliminate the first atypical cells. The group identified the challenges in this approach and agreed to the following aims:

- **Aim 1:** Development of a luminal chimeric antigen receptor (CAR)
- **Aim 2:** Development of a basal CAR

The group identified the appropriate model and endpoints for Aims 1 and 2.

- **Aim 3:** Reverse engineering of wound healing cessation: to engineer "stromal reversal" into a breast cancer surveillance mechanism in mouse and human models in parallel

The group identified a timeline, milestones and needed resources.

During large-group discussion, participants asked about model selection and intraductal injection, distinguishing between early cancer signals and the normal estrus cycle, and the residual CAR effect.

IV. ARTEMIS PROJECT ON PREVENTION OF METASTASIS

March 5-6, 2022

BACKGROUND PRESENTATIONS

Defining DTCs and Their Vulnerabilities

Alana Welm

Welm discussed the challenge to better understand dormancy so that either dormant cells can be killed or cells can remain dormant and to identify risk factors or markers of cancer recurrence. She described the Grand Challenge proposal developed by Artemis participants, including the planned approach to exploit four hallmarks of DTCs: 1) regulation by the vascular niche, 2) chemoresistance, 3) immune evasion and 4) oxidative stress. Core resources include human specimens, led by Alana Welm and Christopher Li, along with an advocate panel to determine how biospecimens could be used; integration of high-dimensional data, led by Simon Knott; and immune-competent models of dormancy and metastasis, led by Alana Welm and Cyrus Ghajar.

The D-4-DTC collaboration is comprised of the following five work packages:

1. Immune mechanisms and DTC targeting
2. Targeting the DTC microenvironment
3. Identifying DTC metabolic vulnerabilities
4. Defining and leveraging the tumor microbiome
5. Addressing DTCs with nanosystems

Welm gave an update on the rapid autopsy program previously discussed at Artemis meetings. The purpose of the program is to determine if DTCs can be detected in healthy tissue regions on rapid autopsy, characterize DTCs and their microenvironment and determine how they are different than active tumors, and identify targets on DTCs that could be used to eliminate them during adjuvant therapy to prevent recurrence.

Welm then presented updates on a few scientific follow-up questions, including whether bacteria are present in and around dormant DTCs and/or micrometastases. In large-group discussion, participants raised questions about whether the cells hanging around for a long time are relevant to dormancy, whether the Ki67 presence in primary tumors is significantly correlated with risk of metastasis and if that might differ across breast cancer subtypes, and the bacteria found in primary tumors.

Metabolic Requirements for Tissue Colonization

Cyrus Ghajar

A rapid autopsy study from 2008 showed that out of 432 breast cancer patients, none of them were found to have metastases in the skeletal muscle. Working with the BROCADE rapid autopsy program, co-led by Alex Swarbrick, Ghajar was able to confirm that in healthy people, there are no ER/PR receptors in skeletal muscle; however, among patients with ER/PR+ breast cancer, several single tumor cells were identified in skeletal muscle.

Using mammary fat pad injections to test whether DTCs traffic to muscle spontaneously, Ghajar found that DTCs frequently reside within skeletal tissues, indicating that they disseminate to muscles spontaneously. Subsequent tissue-clearing experiments suggest that dissemination to muscle is not a rare event but that colonization is.

Ghajar then discussed possible metabolic adaptations required for colonization of skeletal muscle. In a reactive oxygen species (ROS)-centric view, tissue-specific means of countering oxidative stress must be employed for metastases to successfully emerge. Alleviating oxidative stress enabled tumor colonization of skeletal muscle. Sustaining this redox imbalance (oxidative stress) may be a novel way to keep DTCs dormant. But furthering this imbalance may push DTCs over the edge and kill them altogether.

Ghajar identified some key questions around ROS and metastasis. Participants discussed tissue-specific scenarios and the role of myoglobin.

T-Cell Targets in PD-1/PDL-1 Inhibitor Responsive Breast Cancer Patients

Simon Knott

Knott presented results from a study that performed single-cell resolution profiling of triple-negative breast cancer patients throughout their immunotherapy response.

Three patient categories emerged from the spatial profiling. One group was heavily enriched in patients who did not have a pathological complete response and were found to have very few immune cells (nonresponders). Among the 70 percent of patients who did have a pathological complete response, one group had a high level of B cells and separation of the immune cells from the cancer-dominant epithelial cell community (R1), while the second group of patients did not have B cells and had less separation of the immune cells from the epithelial cell community (R2).

These patient immune categories were found to predict tumor cell elimination rates. By the time of the third biopsy, the R1 group had no tumor cells, while the R2 group still had residual tumor cells. In addition, metastases have started to occur during follow-up: Two have occurred among patients in the nonresponder group and two among patients in the R2 group.

Knott looked at TCRs in the tumor and using the GLIPH software to cluster TCRs between patients to find common targets, he revealed that TCR targets are not well shared between patients. Screening of T cells based on "NeoTCR" signatures from T cells previously shown to target neoantigens also predicted that the T cells targeting tumor antigens in this study were follicular helper and effector T cells in R1 and R2 patients and regulatory T cells in nonresponders.

The next steps include refining the list of TCRs based on neoTCR predictions for subsequent T-scan screening; continuing to screen self-antigens in additional patients; screening Elledge's neoantigen library and integrating patient DNA sequencing data; incorporating CD45- single-cell multiome data, CosMX, single-cell peripheral blood mononuclear cell (PBMC), and metabolomics to understand the drivers of patient subtypes; and continuing the analysis of the ER+ cohort where response is only 35 percent.

Participants discussed the effect of the presence of B cells in the R1 responder group, the "immunological factories" that appear to be tertiary lymphoid structures (TLS), and the timing of the tumor/TLS formation and evolution.

WORKING GROUP TOPICS, DISCUSSIONS AND ACTION PLANS FOR THE NEXT 12-18 MONTHS

Three topics for preventing metastasis were identified, some of which came out of the background presentations and discussion that followed. Working groups convened to discuss each topic, followed by large-group discussion and subsequent small-group discussion. Working groups then outlined 12- to 18-month action plans for each topic.

TLS: Tertiary Lymphoid Structures

Danny Douek, Andrew Ewald, Silvia C. Formenti, Pat Haugen, Keith L. Knutson, Wade Shen, Alana Welm

The group coalesced around Knott's presentation and how to create the characteristics of the R1 group such as activating B cells, enhancing B-cell/T-cell crosstalk and inducing TLS. The group developed a plan to address whether it is possible to induce better immunity to the primary tumor, in order to prevent metastasis, as follows:

- Summarize the literature to determine the frequency of TLS in primary breast tumors and any correlation with survival and tumor-infiltrating lymphocytes(TIL) after standard chemotherapy and immunotherapy.
- Use preclinical models to examine whether we can induce TLS in mice and whether having TLS functionally protects from metastasis:
  - Within a year, different labs could be testing the induction of TLS in different models
  - CXCL13, adjuvant TLR agonist and others?
- Identify the general state of the immune system during the window of therapy before surgery and perform a systemic cytokine profile of breast cancer and the transcriptome of PBMCs.

During large-group discussion, participants raised the issue of immune phenotype and creating a score of immune fitness that is predictive. Discussion included how to identify the appropriate samples and models to address the question.

Filtering Cancer Cells/Mobilizing DTCs – MASS EVICTION

Cyrus Ghajar, H. Kim Lyerly, Shawn Zhang, Michele Atlan, Judi Hirshfield-Bartek, Fran Visco

The group explored the idea of mobilizing DTCs from various metastatic sites, which can be done in bone using granulocyte colony-stimulating factor (G-CSF) and inhibitors of the chemokine receptor CXCR4. Is it possible to mobilize DTCs from other metastatic sites (e.g., lung, liver, brain)? And if it is, once mobilized and in the blood, might they die or be targeted for killing (by a prooxidant)? Concern was also raised about whether we want to mobilize DTCs. It might be more dangerous than keeping them asleep in their metastatic niches. Another question that was raised included whether mobilized DTCs are different from those that are left behind. This might be addressed by profiling DTCs.

The group also discussed the fact that available clinical data likely exist to answer this question. For example, many patients have been treated with G-CSF. Patients who are treated with dose-dense chemotherapy are generally given G-CSF prophylactically to prevent neutropenia. And there are likely early clinical trial (safety and efficacy) data that might be evaluated to discern whether there is a difference in the clinical outcomes for patients (either good or bad) from the use of G-CSF.

Another concept discussed was the stickiness of DTCs—that is, perhaps single DTCs alone aren't the problem. But when they begin to form small clusters and stick together, they become more dangerous and have more potential to migrate and further metastasize.

The group ultimately came up with four key questions to address in parallel:

1. G-CSF can mobilize hematopoietic stem cells in bone marrow. Can it also be used to mobilize tumor cells from other sites such as liver, lung and brain? And if so, how many cells are released into the blood, and what is the timeline for peaking?
2. If this process is then repeated a number of times, would this increasingly deplete cells from the tissues?
3. Based on tissue-specific, metastasis-free survival, is there a therapeutic benefit to mobilizing tumor cells to the blood and then treating with prooxidants (or antioxidants) to deplete cells from the blood?
4. Looking retrospectively at chemotherapy-only trials and G-CSF additions, what is the effect of G-CSF on metastasis-free survival and other health-related side effects?

Neighborhood Watch: Metastatic Site First Responders

Frank Calzone, Jay Debnath, Andrei Goga, Simon Knott, Christopher Li, Michele Rakoff

The group began by discussing points of DTC metabolic vulnerabilities, tissue reaction to a DTC and how to make DTCs more immunogenic. The three key questions they were trying to address were:

1. How does the epithelium in one tissue respond to an epithelial cell arriving from another tissue?
2. Are there resident intraepithelial immune populations responsible for tissue repair that could be co-opted to eliminate DTCs?
3. What are the different metabolic stressors that tumor cells experience?

The group focused on changes that occur in the host tissue when it interacts with DTCs or cells from another organ as well as whether vulnerabilities exist within the tissue that could then be exploited to change the DTC phenotype and prevent overt metastasis from developing. The group identified both physiological and pathological situations where cells are in the wrong place.

Proposed experiment:

Interloper (donor cells)	Neighborhood (host)
Normal cell (breast)	Normal host
Fetal cell	Tumor-bearing host (pre-metastatic niche)
DTC (from tumor model)	(2!)
Clusters of tumor cells	(4!)

24 combinations x different organs

Sequential combinations for follow-up studies to see if one cell type primes the microenvironment for a second cell type:

Normal→DTC

Fetal→DTC

DTC→Cluster or activated "DTC"

The primary endpoint would be persistence and/or outgrowth of the secondary DTC introduced into the host. Depending on the amount of DTC persistence in each condition, one could conduct immune profiling (spatial profiling) of the host microenvironment in which the DTCs reside.

Why is this important?

- What is required in the microenvironment to allow the DTCs to survive?
- Is the fetal cell tolerance and survival program used for a DTC to survive in a foreign tissue?
- Can we short-circuit these programs to change the microenvironment in a way that no longer allows DTCs to survive in the host?

What will we achieve?

- Generate foundational knowledge of a program that promotes DTC persistence.
- Determine whether such program can be reset. Are there human correlational studies?

How will we do it?

- Search literature to learn more about fetal tolerance and persistence of cells beyond delivery of the fetus and talk to experts on fetal cell persistence in the mother (three to six months).
- Inject cells (normal, fetal, DTCs) into normal and tumor-bearing hosts (host = mouse).

During large-group discussion, participants suggested experts who could be involved and appropriate models.

V. CONCLUSION

More than a decade into its existence, the Artemis Project has fostered the establishment of numerous fruitful and long-lasting collaborations among diverse researchers and advocates who would have likely otherwise never crossed paths. The annual meetings continue to generate bold ideas and work plans on novel approaches for preventing breast cancer and preventing metastasis, many of which are brought to life through collaborative research efforts throughout the year.

Artemis continues to make progress on a preventive vaccine for breast cancer. In 2020, NBCC's proposal was accepted by the NCI's PREVENT program to advance the vaccine to a Phase 1 clinical trial with manufacturing support. And though progress has been slowed because of the COVID-19 pandemic, a clear path has been outlined for production for the Phase I safety trial in late 2022 to early 2023.

This year, in primary prevention, distinct topics explored included risk prediction and reduction strategies as well as immune system enhancement mechanisms such as a vaccine against neoantigens, harnessing the knowledge gained from the development of mRNA COVID vaccines. During the Artemis Meeting for the Prevention of Metastasis, modulation of the human immune system continued as a key theme as well as ways to mobilize and destroy latent DTCs. The group also spent time discussing the state of the sciences and exploring how emerging technologies might be leveraged to prevent breast cancer and end deaths, and be incorporated into the goals of the Artemis Project.

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