NATIONAL BREAST CANCER COALITION

ARTEMIS PROJECT

National Breast Cancer Coalition
The Breast Cancer Deadline 2020

MARCH 11-14, 2016

I. INTRODUCTION

A. BACKGROUND

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 supports its mission. In 2010, NBCC launched the **Breast Cancer Deadline 2020®** campaign, a strategic plan of action that is designed to identify by 2020, the knowledge, approaches and tools, needed to end breast cancer. This unprecedented campaign includes a research component, known as the **Artemis Project®**, a collaboration that involves researchers, advocates, and other key stakeholders who set priorities and design and implement research plans that focus on two areas:

- **Primary Prevention:** How do we stop people from getting breast cancer?
- **Prevention of Metastasis:** How do we stop people from dying of breast cancer?

The history of the Artemis Project is laid out in the various reports from previous annual meetings, found at (http://www.breastcancerdeadline2020.org/about-the-deadline/artemis-project.html). This report is a summary of discussions and recommendations made at the 2016 annual Artemis meeting. This meeting included more than 40 participants including advocates and scientific expertise ranging from immunology, biophysics, and genetics, to molecular biology, and clinical oncology.

2016 ANNUAL MEETING PARTICIPANTS

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

Frank Calzone, PhD, Biotechnology Consultant

Michael Clarke, MD, Professor, Stanford University School of Medicine, Member, Bio-X

Brian Czerniecki, MD, PhD, Rhodes-Harrington Professor in Surgical Oncology, University of Pennsylvania, Co-Director, Rena Rowan Breast Center

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

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

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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

Michael Goldberg, PhD, Assistant Professor, Cancer Immunology and Virology, Dana-Farber Cancer Institute

Jennie Grimes, Advocate, Metup

Pat Haugen, BA, Advocate, Inflammatory Breast Cancer Research

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

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

Alexander (Sasha) Kamb, PhD, Senior Vice President, Research, Amgen

Helen Kim, PhD, Director of Technology, Amgen

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

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

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Debbie Laxague, RN, Advocate, BCSSC

Mark Lee, MD, PhD, GRAIL

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Vivian Lee, Advocate, Breast Cancer Connections

Ke Liu, MD, PhD, Chief, Oncology Branch, Division of Clinical Evaluation, Pharmacology and Toxicology, Office of Cellular, Tissue and Gene Therapies, Center for Biologics Evaluation and Research (CBER), FDA

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

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

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

Rick Michaelson, MD, Chief Medical Officer for Oncology, St. Barnabas Medical Center

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

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

Michele Rakoff, Advocate, CABCO

Jill Slansky, PhD, Professor of Immunology and Microbiology, University of Colorado Anschutz Medical Campus

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

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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, Assistant Professor, Lester and Sue Smith Breast Center, Baylor College of Medicine

MEETING SUPPORT

NOTE TAKERS:

Najme Faham, PhD, Postdoctoral Researcher, Huntsman Cancer Institute, University of Utah

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

Tim Marsh, BA, PhD Candidate, University of California, San Francisco

Linda Horton, Senior Research Specialist, Department of Cancer Biology, Vanderbilt University Medical Center (Ret.)

FACILITATOR:

Kayla Kirsch, MS, President, Leapfrog Consulting

LOGISTICS:

Marva Lewis, The Event Professionals

ARTEMIS PROJECT ANNUAL MEETING

MEETING DISCUSSION

The session on Prevention of Metastasis began Saturday, March 12 to Sunday, March 13, followed by the session on Primary Prevention, Preventive Vaccine. Friday evening, March 10, was set aside for introductions, background and general scientific presentations.

A. BACKGROUND PRESENTATIONS

Review of Vaccine Landscape

Debbie Laxague, RN, Advocate, BCSSC

A review of the vaccine trials landscape over the past twelve months revealed that there are a few new vaccines being tested in the post-neoadjuvant setting, mostly in triple negative breast cancer. There are several new trials enrolling, mostly in the metastatic setting, combining vaccines and immunomodulation. In the past few years, there has been a small shift toward more therapeutic trials in the adjuvant and post-neoadjuvant setting, and a wider variety of targets in these trials (not just limited to HER+ women). There is still no information on the proposed preventive trial for healthy women that was reported to be upcoming (in 2014). There remains a problem with lack of timely reporting and publication that continues to waste resources and to compromise evidence-based decision making.

Potential of Neo-Antigens for Immune Response

Alexander (Sasha) Kamb, Amgen Research

Tumors are distinguished from normal tissue by the frequency of somatic mutations. A number of publications suggest that total mutation load predicts (frequency/MB) the efficacy of immune checkpoint inhibitors such as CTLA4, PD1 and PDL1 antibodies. Other factors such as PDL1 expression and immune cell infiltration also appear important for antitumor efficacy. New immune therapies are being developed against a wide range of new targets. The drug modalities include mono- and bispecific antibodies, small molecules, oncolytic viruses, and engineered T-cells and vaccines; most of which will be combined with existing checkpoint inhibitors and tested in the context of progressive disease. The overall number of mutations in breast cancers is low relative to cancers that are more likely to respond to checkpoint inhibitors (melanoma, bladder, lung). New approaches are needed to promote the ability of the immune system to recognize and reject breast cancer. These include combination of checkpoint inhibitors with other types of antitumor agents (cytotoxics, kinase inhibitors) and vaccines.

B. RESEARCH UPDATES

ARTEMIS SEED GRANTS

DNA.Land

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

DNA.Land has received a seed grant from the Artemis Project to develop a large scale resource for studying breast cancer genomics.

The increased availability of personal genome analysis has opened the door to understand the genetics of disease. In breast cancer studies, several attempts to identify genetic factors that contribute to breast cancer have been made, however these studies have largely not been successful in reliably identifying genes that are involved in breast cancer development, recurrence, or progression.

This project involves the development of a database of at least 25,000 individuals by crowd sourcing the collection of genotype/phenotype data by leveraging DNA information from direct to consumer (DTC) genomics companies such as 23andMe, AncestryDNA, or FamilyTreeDNA. Participants will upload their genomic information to the database and answer a series of clinical questions. The participants will also be able to upload their family tree and share information about breast cancer of other family members. The clinical questionnaire will link a thorough set of clinical information with genomic data. This will allow researchers to ask questions about breast cancer recurrence and progression in new ways. The clinical questionnaire is being developed by NBCC advocates, Daniel Speyer, New York Genome Center and Peter Fasching, MD, Associate Professor of Gynecology and Obstetrics, Department 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, CA.

A cohort of tens of thousands, may not include many actual patients with metastatic breast cancer; however, many will be relatives of people with metastatic breast cancer, thus allowing genome-wide association studies by proxy.

Investigating Immune Recognition of Quiescent Disseminated Tumor Cells

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

The idea for this project, funded with an Artemis seed grant, arose out of the 2015 Artemis meeting. Among the possible strategies for metastasis prevention, two are to maintain dormant cells in a dormant state or to target them for elimination, possibly by immunologic mechanisms. Not much is currently known about how the immune system interacts with dormant tumor cells. Questions presented included: do T cells traffic near dormant disseminated tumor cells (DTCs) in the bone marrow where they are thought to be dormant in niches; can antigen specific T cells, which would be capable of eliminating DTCs, recognize antigen presented by the DTCs; for example, the antigens may be present, but their presentation as peptides within the MHC complex

on the cell surface can be altered. One example could be that the MHC complexes themselves are generally altered in quiescent cells. Another factor that could prevent immune recognition and elimination could be the local microenvironment, which could have profound impact on the function of T cells. DTCs may be found in a perivascular niche which provides signals to the tumor cells. It is not known if these same signals have a direct effect on the T cells. Therefore, we need to know the role of cyotokines and chemokines found at high concentrations in the perivascular niche in immune recognition of disseminated tumor cells. Alternatively, do these signals on DTCs cause them to secrete their own immune-suppressive factors? This question can be addressed by asking if quiescent cells secrete immune-suppressive factors.

This work will include a focus based on the knowledge that most disseminated tumor cells reside along the vascular and/or lymphatic endothelium.

C. GENERAL RESEARCH PRESENTATIONS

NEW APPROACHES

Tools for Exploring the History of Autoimmunity and Viral Infections in Human Populations

Steve Elledge, PhD, Gregor Mendel Professor of Genetics and Medicine, Harvard Medical School

The Elledge Lab has developed two technologies to profile antigens and epitopes recognized by the complex antibodies mixtures that exist in human circulation. The first method combines T7 phage display with high-through-put DNA sequencing (Phage IP Sequencing – PhiP-Seq). Protein sequences are encoded as a series of overlapping or tiled DNA created by highly parallel DNA synthesis. The DNA are inserted into the T7 10b gene for display on the phage capsid. The specific peptides captured by antibody immunoprecipitation are identified by HiSEQ 200 DNA sequencing. The VirScan (T7-Vir-Pep) method has 56 amino acid fragments tiled every 28 amino acids and has been used to profile viral antibodies in ~700 human serum samples using a little as 1 ul for immune capture. The evidence validates T7-Vir-Pep as a method to identify viral exposure or specific immune responses associated with disease. A T7 Phage library representing 23,959 human open reading frames (ORFs) as 413,611 x 36 amino acid peptides tiles has been synthesized to explore the human proteome reactivity in the antibody repertoire of human populations. The second antibody profiling method is called PLATO or Parallel Analysis of Translated ORFs. ORFs are expressed by in vitro transcription and translation. Specific ORF are captured by immune precipitation of ribosome/mRNA complexes via nascent proteins and identified by DNA sequencing or qRT-PCR. PLATO may be more sensitive to conformational epitopes by virtue of encoding longer ORF. Aneuploidy predicts two hallmarks of cancer: cell proliferation and immune infiltration. The immune signature is significantly reduced in high aneuploidy tumors and it appears to be associated with lower survival. Application of Phip-Seq and PLATO may help define the interaction of the immune system and human breast cancers and lead to more effective immunotherapy. Whether or not antibody profiling can identify women at risk for breast cancer and identify antigens for a prevention vaccine is a subject for further investigation.

Breast Cancer Pathogenesis and Specifi Changes in Protein Glycosylation

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

Protein glycosylation refers to an extremely diverse group covalently linked protein-sugar structures including single mono-saccharides, small 0-linked glycans, extended and highly complex N-linked glycans, and long proteoglycan chains. Essentially all secreted proteins, integral and peripheral membrane proteins, and ECM proteins are glycosylated. The predictive value of changes in protein glycosylation for breast cancer recurrence and survival, detected by plant and animal lectins which bind specific sugars or sugar structures was first described many years ago. The secreted and transmembrane mucins are a family of high molecular weight, heavily glycosylated proteins that constitute the mucous barrier protecting epithelial cells bacterial and viral infection. MUC1 is a common breast cancer vaccine antigen and tumor-specific alterations of MUC1 glycosylation have been the target of a number of antibody therapeutics. Plasmodium falciparum infected erythrocytes present the malarial protein, VAR2CSA, which binds a distinct type chondroitin sulfate (CS) normally exclusively placental. This CS modification is present on a high proportion of malignant cells including breast cancer and it can be specifically targeted by

recombinant VAR2CSA (rVAR2) in various antitumor configurations. New technologies for molecularly characterizing glycosylation (glycomics) are raising the profile of this post-translation modification for cancer therapeutics and vaccines. This approach has been used to define a novel sugar code for ricin, one of the most potent of all known toxins, and also to describe a glycoproteomic profile of the highly metastatic mouse breast cancer 4T1 cell line. Glycomics combined genomics and proteomics applied to tumor stem cells, dormant cells, immune infiltration and other samples may lead to more selective vaccine target and new therapeutics.

The presentation generated a lengthy discussion about aneuploidy and neoantigens and whether the amount and types of viruses affect breast cancer progression and development.

SMALL GROUP SESSIONS

Artemis participants identified and discussed various topics to be addressed in order to prevent metastasis. The group agreed to break into smaller groups to discuss the following topics and present recommendations.

1. CLINICAL TRIALS

This group decided to view metastatic breast cancer as a distinct disease from primary breast cancer, as an "orphan disease," in order to get attention from Pharma. Their goals were to set criteria to identify the best agents that prevent metastatic relapse/expansion, and design a Phase II Trial targeting Stage 4 patients with resectable oligometastatic disease. Multiple agents would be tested simultaneously/sequentially and compared to the control arm. This patient population is at very high risk for developing new metastases so there is a window in which agents to prevent development of metastasis could be tested.

Agents would be found (criteria to be set) by repurposing drugs or vaccines that were considered to have "failed" in metastatic breast cancer and other diseases, because they were ineffective at shrinking established metastases. These agents could possibly prevent initial establishment of metastasis.

Criteria Could Include:

i. Requirement for potential agents would include: basic dose limiting toxicity/safety already determined in a previous trial (can be anything, vaccine, drug, immune regulator)

ii. Need strong preclinical rationale/data for promoting dormancy or preventing outgrowth without affecting dormancy

iii. Develop a patient accrual strategy and evaluate commercial barriers, e.g. IP and cost

iv. Trial has pretreatment and post treatment tumor/bm/blood profiling; recurrent tumor profiling

This is considered different from most approaches because this approach does not assess primary tumor or shrinking of existing metastases as an outcome; rather it looks at preventing new metastasis.

Six month plan:

- Identify agents fitting criteria
- Determine feasibility of trial design/develop pipeline
- Define criteria for success to move into prevention trial

2. ISOLATE AND CHARACTERIZE DTC'S

Some breast cancer patients (especially ER+) experience metastatic relapse at some point during the course of their life after completion of primary treatment. It is believed that disseminated tumor cells (DTCs) are responsible for dormant stage of metastasis progression, wherein cancer cells stay quiescent in distant niches. Given the rare population and difficulties in detecting disseminated tumor cells, knowledge about tumor dormancy is extremely limited. Characterizing these cells at the genomic and transcriptomic level should give interesting insight for identification of new mediators that can be targeted in DTCs to prevent them from transitioning to macrometastatic stage.

There were several questions raised by the group:

1. Do DTCs have preference to go to a specific niche (like bone marrow vs. liver or lung)?

2. What are the features/molecular profiles of the DTCs that grow and develop macrometastatic lesions vs the ones that do not grow?

3. Do DTCs first go to bone marrow and then to other niches? Do the DTCs in bone marrow have the same features as the ones in other niches like liver or lung? Can DTCs in bone marrow be considered as representative of DTCs residing in other organs?

Recommended Next Steps

Molecular profiling of dormant DTCs including genomic and gene expression signature.

The group suggested two cohorts initially to define dormant vs. active metastatic cells using next generation sequencing:

1. Rapid autopsy from the breast cancer (BC) patients that have died of breast cancer and compare it to the autopsy from BC patients that haven't died of breast cancer. (Days after death work fine for getting cells and DNA, but for RNA analysis sampling needs to be rapid.)

2. Do prospective study and get samples from patients over the course of their disease during or after completion of treatment. Blood and bone marrow biopsies will be done on metastatic and non-metastatic patients. CTCs will be analyzed from blood and DTCs will be analyzed from bone marrow. **(Assumption: DTCs from bone marrow are representative of DTCs residing in other organs).

The group presented the DISS-MET study: To define the molecular determinants within DISSeminated tumor cells that drive dormancy versus METastatic recurrence in women after adjuvant hormone- and chemo-therapy.

Impact

- Identify therapeutic targets in DTCs (drugs or vaccines) to prevent emergence of metastasis
- Improve our ability to stratify patients' risk of recurrence to reduce overtreatment of breast cancer
- Prevent death from metastatic breast cancer
- Understand the biology of late metastatic recurrences, in the most common type of breast cancer (ER+)

Description:

- Post-menopausal ER+/PR+ patients with a high risk of recurrence (high grade, node involvement, etc...)
- Can be enrolled anytime during or after their treatment (chemo/hormonal therapy)
- Two arms: compare disseminated tumor cells (DTCs) and circulating tumor cells (CTCs) in patients who develop metastasis vs patients that have been well up to 10 years or more (94% of cancer survivors with no evidence of disease have detectable DTCs upon autopsy)
- Collect CTCs (blood), DTCs (BM core), and metastatic biopsies if applicable (any site)
- Ideally, primary tumor will be archived and available for research
- Sequence DNA and RNA; measure cytokines in blood

3. CIRCULATING TUMOR DNA (CTDNA)

The group questioned whether ctDNA is a sensitive surrogate for early evaluation of clinical efficacy. The group began to design a prospective, observational study to develop a ctDNA test for monitoring progression to clinical metastasis; unique to each patient. This work is preliminary to designing an intervention trial to prevent clinical presentation of metastasis, in those patients where a rise in ctDNA is noted.

i. Population will be TNBC that did not have a pathological complete response. 3 groups: no ctDNA after treatment (no recurrence), ctDNA no change after treatment (fast recurrence), and ctDNA that decreases after treatment, but doesn't completely disappear (delayed occurrence of metastasis). One suggestion was for a pilot project to prove the sensitivity is there to detect these ctDNA.

ii. Procedure: biopsy at surgery; exome sequence; identify mutation profile (90 to 100 mutations)

iii. Continuing monitoring plasma DNA collection; mutation sequencing for specific patient; longitudinal measurements (once per month) to monitor ctDNA increases

iv. Residual signal at end associated with early recurrence; no residual signal associated with delayed recurrence

4. LYMPH NODE NICHE

An Artemis participant posited that lymph node metastases (LNM) is biologically different than organ metastases. A subgroup discussed this issue, identifying a hypothesis that LNM do not go on to form organ mets but function in the lymph node to promote immune suppression and allow other metastatic cells to colonize and outgrow to distant organs. This raised the following questions:

i. Do DTC's receive instruction in lymph node niche that controls dormancy?

ii. Do DTC's instruct the immune system in the lymph node to influence dormancy? Cause reduction in anti-tumor immunity (immune supression)?

iii. Are peripheral T&B cells relevant to immune recognition "locally" in tumors?

Recommendations:

To test hypotheses: Analyze tumor draining lymph nodes from pts who relapsed within 5 years compare to matched pts not relapse more than 10 years.

Metastasis Triggers

A few groups discussed identifying and understanding various triggers for metastasis. They were identified as:

1. Stress
   a. Cell
   b. Systemic (nervous system) (psychoimmunity)
2. Wounding
   a. Chronic
   b. Acute
3. Anesthesia
4. Operative and post operative inflammation

5. CMV VIRUS - DORMANCY

One group questioned if there is a viral etiology of metastatic disease. It was suggested that CMV drives metastasis or changes the tumor microenvironment in such a way that tumors can progress. (CMV is apparently present in many metastatic tumor samples) Is it possible to do a study of viral presence in metastatic lesions?

Recommendations:

1. Can now study women treated with gangcyclovir; Varicella Zoster vaccine
2. Neoantigens role in dormancy

ARTEMIS PROJECT FOR A PREVENTIVE BREAST CANCER VACCINE

A. RESEARCH UPDATES

ARTEMIS SEED GRANTS

DCIS Project: A Genomic Approach to Identify Antigens

Simon Knott, PhD, Postdoctoral Fellow, Cold Spring Harbor Laboratory, Kim Lyerly, MD, FACS, George Barth Geller Professor for Research in Cancer and Professor of Surgery, Duke University Medical Center

An update on ongoing work funded through an Artemis seed grant and work funded through a DOD grant, looking at a Molecular Framework of Early Breast Cancer was provided. The Duke group had collected DCIS biopsies through all stages. 165 had DCIS in the core, including patients with higher stages of disease. The TCR repertoire in invasive breast tumors and DCIS was done comparing PFtissue and fresh core biopsies.

The basic question to be resolved is whether DCIS is the precursor lesion of cancer and can be a vaccine target. DCIS is categorized on the basis of pathological appearance. Gene Expression Analysis by Microarray showed that DCIS is nearly identical to invasive BC. A library of drug-linker-contrast agent was developed (chemical probe). A bit of autofluorescence was noted, but tumors still detectable. HS-131 was used for "proof of principle". Increasing wavelength leads deeper penetration so a variety of these chemical probes was developed.

Key questions:

1. What is the biology of the early disease?
2. Which DCIS lesions are a danger?
3. How is DCIS linked to known breast cancer?

Analysis of other tissue types would include normal stroma, invasive ductal carcinoma, solid DCIS, papillary DCIS, stroma adjacent to tumor or DCIS, etc. RNAseq analysis show samples tend to cluster well by tissue type. An initial analysis shows a good correlation between replicates. Genomic information from core biopsies that have been stored for over 10 years was obtained. This is a "real time" presentation and a lot of data is "in process".

Prevention Vaccine Project: Proposal Update

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

An overall report of the background and path to a preventive vaccine in breast cancer was presented, as was the criteria to be applied to choose targets

and the basis for those choices to date in the Artemis project. The explanation for the rationale of vaccination was presented, as was a description of various mechanisms of tolerance. Issues raised included whether the mucosal immune system should be looked at, as most responses occur in mucosal tissue. Staining for CD45, CD8, and CD11c are all evident in mucosal tissue. In addition, the usefulness of a mouse model and the need to look at off target effects were discussed. Questions were also raised as to why GM-CSF is currently used in many vaccine preparations on the basis that if you immunize with CD4 peptides but want to activate CD8 cells, you would want to create a vaccine that performs both functions. A viral vector vaccine that would serve this purpose was suggested.

An outline of the Path to a Preventative Vaccine was Laid Out Listing Possible Topics for Small Group Discussion:

- FDA Interactions
- Safety/toxicity (murine modeling)
- Pre-IND meeting request
- What would the first clinical trial look like?
- Antigen Design
- Removal of tolerance inducing epitopes
- Vector choice
- Immunization Strategies
- Intraglandular
- Viral prime/Vector boost
- Differentiation/Adjuvant
- Multiple INDs
- Comparing two (or more) different strategies
- Preclinical safety and immunologic efficacy
- Antitumor data required?
- Phase I trial design
- Primary safety testing
- Vaccine production
- Composition
- Cell bank
- Lot release
- Potency assays
- Stability assays

SMALL GROUP DISCUSSIONS

1. CLINICAL TRIAL DESIGN

In order to determine the efficacy of a preventative vaccine for breast cancer, this group discussed the design of a clinical trial. They had some reservations about the design, as the vaccine itself has not yet been made and preclinical safety data unavailable. There was avid discussion about the safety risks of targeting self-antigens potentially leading to autoimmune side-effects. There are current trials investigating self-targeted cancer vaccines and the group agreed that the trials might be useful in determining the overall safety of this approach.

Questions for this group are what safety data or information can be provided that would make it reasonable to start with a healthy population and does this information already exists in the literature.

As for the initial trial, one possibility discussed was an early phase (i.e. phase I) study with the primary aim of dose/safety and secondary aims of efficacy or immunogenicity, in a population of women with no invasive breast cancer but at high risk (e.g., DCIS) for developing breast cancer in the future. This would be followed by a confirmatory phase II study with the primary aim of reducing BC incidence and a secondary aim of safety.

Another possibility the group discussed is whether to test preliminarily in a population of Stage IV patients to determine dose/safety/efficacy, acknowledging that the patients may be immune compromised and would be a barrier to knowledge. In regards to immune compromise the possibility of targeting the metastatic BC population in PD1 trials was considered, since there is a PD1 vaccine boosting effect at the time of vaccine priming.

After continued discussion, the group ultimately determined that a series of clinical trials should be done, however the group was split on the initial cohort of patients. On one hand, administering the vaccine to subjects with ductal carcinoma in situ (DCIS) destined for prophylactic mastectomy would be advantageous because of the increased chance of therapeutic benefit in this setting. On the other hand, the majority of patients with DCIS will not experience fatal breast cancer and they run the risk of autoimmunity because the vaccine is targeting self-antigens, in which case the initial trial should be in high-risk stage IV patients.

There was also a long discussion with the representative from the Food and Drug Administration's Center for Biologics Evaluation and Research about safety data needed to initiate a pre-IND package and initial cohort selection for early phase trials.

Recommended Next Steps Discussed Included:

1. Decide on a cohort for the early phase trials
2. Determine measures needed to measure immunological endpoint, safety and efficacy for early phase trials

2. PRE-IND PACKAGE

The consensus from the group was that a dialogue with the FDA be the first step to advise the Artemis Project what would be needed.

The Artemis Project had previously selected the initial antigens to be tested. One basis for the choice of antigens was that the individual target components have been tested in humans, which yielded safety information. However, it was noted that the combination has not been tested which could be the basis for safety problems. A fundamental question raised is how to get the toxicity data necessary for a pre-IND package? There was much discussion about the use of transgenic mouse models (with high risk for developing breast cancer) and whether it would be a viable option. Antigen homology of murine vs. human would be a consideration. It could constitute the safety feature before "first-in-humans" vaccine trial. It was decided to look in the literature to determine how these autoantigens have already been used in other cancers or diseases, what safety information was available and what is the length of time before immunity developed?

It was concluded that a Pre-IND Information Package should be developed early on with the assumptions that individual constructs are made for each antigen; each will be full length; some will be secreted and some intracellularly expressed.

The Pre-IND Package Would Include the Following Elements:

1. A safety, pharmacology, and toxicology section
2. A proposed safety trial section
3. A chemistry manufacturing and composition section

Background Assumptions for the Pre-IND package Are:

1. Preventative vaccine that protects against breast cancer for all women.
2. The intent is to vaccinate women who have not been diagnosed with breast cancer.
3. The target antigens will be the well characterized and tested list (already used in humans): HER2/neu; Mage 3; Muc1; Survivin; Mammoglobin A; and hTERT (in stem cells).
4. Prevalence must be 100% in primary tumor.
5. That mutations cannot be targeted because there are insufficient numbers of recurrent mutations for a preventative vaccine. Along these lines, the group discussed other possible targets such as PIC3CA which has 3 unique amino acids and is found in 35% of primary breast cancers.

It was also concluded that the Artemis Project continue making a list of antigens highly expressed in breast cancer (very low in normal) and request Paul Spellman to compare with known proteomics and new programs, need further understanding of the biology of antigens, compare normal breast to all other normal tissues. It was also noted that while an interest in tumor associated carbohydrate antigens was expressed, it was acknowledged that less is known about these targets, for example, are the sugars expressed in early tumors? It was recommended to review the literature to determine the present state of knowledge in this area.

OTHER TOPICS

Anti Rank Ligand (Clinical Trial)

Due to the biological relevance of RANK ligand (RANKL), specifically the absent development of breast tissue in RANKL knockout mice, the concept of targeting RANKL as a breast cancer prevention strategy has been considered. There is considerable evidence that anti-RANKL antibodies currently used clincally in preventing osteoporosis have an anti-cancer benefit. In addition, it is possible that an anti-RANKL antibody, provided at the appropriate time, would reduce the development of breast cancer, perhaps by limiting the development of ongoing breast cancer precursors.

The potential long-term cancer prevention benefit from a defined course of anti-RANKL antibody therapy may be observed in patients receiving anti-RANKL therapy for osteoporosis. This is an attractive option, as the long term toxicity from anti-RANKL therapy is known. One limitation is that chronic therapy with anti-RANKL antibodies is needed, which may be impractical. One alternative could be a vaccine approach, in which polyclonal anti-RANKL antibodies were generated by a vaccine. Overall, these anti-RANKL antibodies may lead to a reduction in breast cancer.

The idea of an anti-RANKL antibody generating vaccine could be tested in an experimental animal model. Anti-RANKL vaccine constructs will be generated, and immunogenicity in appropriate animal models will be tested. The ability to prevent breast cancer development can then be tested and compared in appropriate mouse models.