

2015

2014

2013

2012

2011

National Breast Cancer Coalition

The
Breast
Cancer
Deadline

2020

Artemis Project® Second Annual Meeting March 3-5, 2012

I. ARTEMIS PROJECT®: BACKGROUND

The National Breast Cancer Coalition's (NBCC) Artemis Project® for a preventive vaccine brings together a collaborative group of advocates and scientists to take a strategic, systematic yet broad approach to the development of a breast cancer preventive vaccine within five years. With input from Project participants and others, through annual meetings and independent analyses, NBCC has identified four focus areas for the plan, identified teams of scientists and advocates for each and developed an infrastructure within which all participants interact.

The [report](#) from the first annual meeting provides an overview of the project and a description of the focus areas. After the first annual meeting in March, 2011, NBCC contracted with Science Application International Corporation (SAIC) to help prepare a detailed strategic plan for the Artemis Project®, based on the outcomes of the annual meeting and follow-up interviews with attendees. This [Project Plan](#) was completed in December, 2011. Subsequently, NBCC issued a [Call for Proposals](#) to address initial steps in antigen identification.

II. 2012 ANNUAL MEETING

The second annual Artemis Project® meeting was held March 3-5, 2012, in Calistoga, California. The purpose of this meeting was to assess progress in the field and within the Artemis Project® Plan, and to adjust teams, projects, and focus as necessary. This meeting focused on taking a comprehensive look at the first stages

of the vaccine development program outlined in the Plan, antigen identification and evaluation, and on developing strategies and models for determining what the vaccine needs to accomplish.

A. 2012 ANNUAL MEETING ATTENDEES

2012 Annual Meeting Participants

Vladimir Belyi, PhD, Institute for Advanced Study

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

Amy Bonoff, Advocate

Andy Burnett, MBA, President, KnowInnovation

Frank Calzone, PhD, Biotechnology Consultant

Brian Czerniecki, MD, PhD, Surgical Director of the Immunotherapy Program, Abramson Cancer Center, University of Pennsylvania

Yaniv Erlich, PhD, Fellow, Whitehead Institute

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

Silvia C. Formenti, MD, Professor of Medicine, Chair, Department of Radiation Oncology, New York University Medical Center

Ted Goldstein, PhD Candidate, Haussler Lab, Center for Biomolecular Science and Engineering, University of California Santa Cruz

Gabriel M. Gutierrez, PhD, Project Manager, Science Application International Corporation

Gregory J. Hannon, PhD, Professor, Investigator, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory

Pat Haugen, Advocate

Stephen Johnston, PhD, Director, Center for Innovations in Medicine, Biodesign Institute, Arizona State University

Keith Knutson, PhD, Associate Professor, Department of Immunology, College of Medicine, Mayo Clinic

Debbie Laxague, Advocate

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

Ke Liu, MD, PhD, Medical Team Leader, FDA/CBER/Office of Cellular, Tissue, and Gene Therapies

Susan Love, MD, MBA, President, Dr. Susan Love Research Foundation

H. Kim Lyerly, MD, George Barth Gellar Professor of Cancer Research, Duke University School of Medicine

Laura Nikolaidis, MS, Director of Research & Quality Care Programs, NBCC

Michele Rakoff, Advocate

Patricia Renzulli, Manager, Breast Cancer Projects, National Philanthropic Trust

Fran Visco, JD, President, NBCC

Facilitation

Kayla Kirsch, MS, President, Leapfrog Consulting

NBCC Support Staff

Aimee Near, MPH, Science Analyst

Logistics

Marva Lewis, The Event Professionals

B. 2012 ANNUAL MEETING OUTCOMES

i. Task Areas for Antigen Identification

Participants at the meeting developed plans around antigen identification in the areas of: Pathogens; Self-Encoded, Neo-Antigens; Native Self-Antigens; and Immune Profiling.

a. Pathogens (Viral and Microbial Agents)

Group Members: Frank Calzone, Vladimir Belyi, Yaniv Erlich, Pat Haugen, Susan Love, Paul Ewald

Purpose: This team will develop a strategy to systematically search for a virus or pathogen target or targets that will be safe, effective, and provide broad coverage for a diverse population of women.

In terms of known pathogens, three stand out as strong candidates with an extensive amount of background information: HPV, MMTV, and EBV. HPV already has a vaccine which makes it an attractive candidate for following-up on for breast cancer.

The development path will be clear if a pathogen is identified as a target for the vaccine. This pathogen's prevalence must be significantly greater in breast cancer than in the relevant normal breast cell-type. Such a viral candidate must be present in >10% of all breast cancers and must have subtype specificity. The development path is detailed below:

Step 1: The first step of the approach will be computational and will not require any new data. This involves validating viral vaccine candidates through screening. The source of cancer genomic data will be the Cancer Genome Atlas (TCGA).

Task	Responsible Party	Delivery date	Cost
Assemble and curate viral reference sequence set .	Virologist Genomics expert	1	
Access, assemble curate TCGA and other RNA, genomic sequences.	Genomics expert	1-6	
Query the scientific literature	Virologist Cancer Epidemiologist	1	
Assemble viral microRNA set	microRNA expert	1	
Access human cancer and normal microRNA sequences	microRNA expert	1-3	
First Pass viral genomes analysis candidate identification in breast cancer and normal if available	Genomics expert	3-9	
MicroRNA analysis	microRNA expert	3-4	
Associate Viral Candidates with breast cancer subtypes	Breast CancerExpert Genomics Expert	9-10	
Estimate Coverage	Computational Biologist	11	
Decide to pursue category 4	Team	12	
Pursue Category 4	Genomics Expert	12-24	

Step 2: The second step will be much more expensive and requires new efforts to validate viral candidates. Sequence data could be a resource for unknown viruses, but this would be very time-consuming, requiring three to four years due to necessary validation steps. This step would require nucleic acid and serology from the same individuals and could potentially add about \$0.5-1 million in costs.

Task	Responsible Party	Delivery date	Cost
Assemble Tissue Sample Cohort 100 cancers per subtype	Cohort Owner/Bank Breast Cancer Surgeons	1-6	
Viral micro array based detection assays and validation of hits	Chip Tech.	3-9	
Directed Viral Q-PCR appropriate candidates (<100) against tumor cohort.	HTP PCR Specialist Virologist	3-9	
New Unselected RNA Sequence Analysis on the cohort (See Comp Approach for tasks)	DNA Sequencing Capacity Genomics Expert	6-18	

Any candidate that is identified needs to be tested against an independent cohort or the same cohort from other labs by Q-PCR. Next, the candidates' viral expression would need to be validated by IHC or an equivalent against 100 cancers per subtype. These steps would take about nine months combined and would require a Q-OCR breast cancer screening lab and pathology lab.

b. Self-encoded, Neo-Antigens Group

Group Members: *Greg Hannon, Stephen Johnston, Ke Liu, Amy Bonoff, Ted Goldstein, Kim Lyerly*

Purpose: This team will develop a strategy for a systematic search for host-derived non-native peptides, or neo-antigens as candidate vaccine targets.

A preliminary report of candidate neo-antigens should be feasible in 6 months. The full database will be available in one year and results will be presented at the 2013 Artemis Project® meeting. When candidates have been identified, the next step will be to narrow down the list to about a dozen through validation of the targets.

Task	Responsible Party	Delivery date	Cost
Preliminary report of candidate neo-antigens	Group(s) selected by NBCC in call for proposals	6 months	
Full database of candidate neo-antigens – includes description of datasets and availability, validation of all algorithms, and a “sanity check” of 20-50 datasets annotated for the first four criteria	Group(s) selected by NBCC in call for proposals	1 year	
Launch call for proposals for DCIS and early lesions	NBCC		
Develop DCIS or early lesion dataset	Chosen group(s) and Kim Lyerly	1 year	
Launch another call for proposals for antigen validation	NBCC	Launch 6 months after first RFA is awarded	
Narrow down candidate list to about a dozen through validation of targets		1 year	

Outcomes expected for antigen identification include:

- Nature of event leading to candidate expression (e.g. RNA/DNA event)
- Sequence of candidate antigen/length of candidate neo-epitope
- Frequency (fraction of tumors by molecular subtype)
- Expression level in tumors
- HLA type of for each candidate
- Analysis of co-occurrence of events
- Frequency/expression in normal cell/tissue type
- Likelihood of cross-reaction with host
- Possibility of presentation on cell surface (e.g. by fusion to cell surface protein/signal sequence)
- Analysis of predicted MHC binding pattern
- Prediction of driver versus passenger event (e.g. pathway models)

It was suggested that another call for proposals for antigen validation be issued after the first grant is awarded. These proposals would seek to provide the following:

- Obtain data for peptide/MHC binding (e.g., recombinant or bona fide in vivo)
- Obtain evidence of functional immune recognition
 - T-cell recognition in vitro (various measures)
 - Animal models if available
 - Human data (e.g. evidence of immune responses in humans, correlation to clinical data, etc.)
- Integrate all data to design candidate vaccines (e.g. genomic, immune, frequency, HLA type etc.)

c. Self-Antigens

Group Members: *Keith Knutson, Deb Laxague, Brian Czerniecki, Gabriel Gutierrez*

Purpose: The purpose of this team is to identify known and unknown self-antigens that may be targets for a breast cancer vaccine.

The first step is to begin exploring the existing list of identified antigens and to prioritize about eight antigens that span extracellular, cell surface, and intracellular spaces. If this approach does not result in enough candidates to pool in a breast cancer vaccine, the next step would be to search for new/unknown antigens.

Once a list of candidate self-antigens has been established, the next step is to run genomics of DCIS and invasive samples to see if the selected antigens are expressed in both, and verify with IHC. A statistical plan needs to be developed, to figure out exactly how many samples are needed for IHC and genomics analysis and characterization to cover all subtypes. Then, months 6-18 will involve identifying T cell and B cell epitopes on target antigens. Once epitopes have been identified, the next step (months 18-24) is an ex vivo analysis to screen patients to see if they have T cell recall and to screen for serum antibodies. These techniques can be done in patients with breast cancer and compared to healthy controls. After 2 years, the group expects to have about 4-5 candidate antigens that activate both T and B cells, with a minimum of 2 antigens per breast cancer subtype. The next step will be testing the antigen mixture in mice to see if antigen loss variants are generated.

Task	Responsible Party	Delivery date	Cost
Examine Mac Cheever's prioritized list and choose eight candidate antigens to pursue		Month 1	
Develop statistical plan to figure out exactly how many samples are needed for IHC and genomics analysis and characterization to cover all subtypes.		Months 1-12	
Tissue analysis – acquisition of samples (identify repository, establish MTAs, ship samples for IHC and genomics analysis, pathology verification)	Kim Lyerly, new broker?	Months 1-12	
Genomics analysis	Mayo genotyping labs, Laser capture micro dissection, Biostatistician	Months 1-12	125K for genomics and IHC analysis
IHC analysis	Pathologist, staining laboratory, Biostatistician	Months 1-12	
Identify T cell and B cell epitopes on target antigens	T-cell: Keith Knutson, Nora Disis B-cell: Gary Koski, others; Complex carbohydrate group at UGA	Months 6-18	
Ex vivo analysis to screen patients to see if they have T cell recall and to screen for serum antibodies	Biostatistician B cell ELISA: Gary Koski T cell ELISPOTs: Keith Knutson, Brian Czerniecki	Months 18-24	
Test antigen mixture (4-5) in mice		Months 24+	

d. Immune Profiling to Identify Antigens

Group Members: *Leslie Bernstein, Silvia Formenti, Peter Lee, Laura Nikolaidis, Michelle Rakoff*

Purpose: This team was tasked with developing a strategy for using the immune system to identify antigens that are potentially relevant for a preventive breast cancer vaccine.

This group explored how to identify antigens that are potentially relevant for a preventive breast cancer vaccine using the immune system, natural immune surveillance, and immune dysregulation in breast cancer. This requires an understanding of the natural immune surveillance in breast cancer which does not currently exist. What are the natural target antigens, why does the immune surveillance fail in some women and work in others?

Assuming the necessary samples have been located or created, the working group decided upon a two-pronged approach to identify antigens using the immune response.

Path A: The key objective of this first path is to look at the antibody response to find potential targets. Blood and tissue samples will be run on two kinds of arrays, random peptides, and antigen candidate arrays. This approach will require about two hundred samples, pre and post breast cancer. Potential sources for these samples are:

1. Teacher's studies—How many women have blood samples before and after cancer?
2. Women's Health Initiative (WHI)—has samples, but how to access?
3. Nurse's Health Study
4. Peter Lee's samples
5. NYU—Silvia Formenti

Path B: Researchers will look at the T cell response by isolating T cells from tumor tissue or lymph nodes. First this would be done non-specifically, and then the T cells would be exposed to a set of defined antigens, including luminal and basal cells. From there, it is possible to identify T cells that are tumor reactive, and reverse engineer to determine what those T cells are seeing to get T cell targets from these patients. What are T cells targeting in different settings?

This approach is much more labor intensive than path A because it requires starting with live T cells from breast tissue and blood (fresh or viably frozen samples – which are rare). Ideally, samples would be cross-sectional from healthy, recently diagnosed patients, then at 2-3 years after diagnosis, and finally at 5-10 years after diagnosis. Serum samples from these patients will also be necessary to correlate T cell targets with the antibody response.

Task	Responsible Party	Delivery date	Cost
Obtain blood and tissue samples and run on two kinds of arrays, random peptides, and antigen candidate arrays to find candidate targets.		Months 1-12	\$100,000
Pre-clinical work		Months 12-24	
Phase I trial		Months 24-36	
T-Cell Approach		Months 1-24	\$1 million

ii. Immune Evaluation of Identified Antigens

After candidate antigens have been identified, quantitative considerations of the vaccine would be considered. The presence of an immune response to the vaccine is not enough. Certain thresholds of response must be met, and the cancer properties that drive immune thresholds, such as doubling time, diversification rate (leading to escape mutants), acquisition of immune evasion/suppression mechanisms, and the rate of metastasis, must be considered. The tumor cell population needs to be eliminated before the doubling time, before the acquisition of immune evasion/suppression mechanisms and before the rate of metastasis.

The magnitude and quality of immune cells are key determinants of protection. This is what is needed in terms of quantitative considerations:

- Minimum threshold for protection: 1-2% per antigen
- High avidity
- Location: within breast tissue (Tem) and draining lymph nodes (Tcm)
- Antibodies: minimum titer needed
- Other cells types: dendritic cells, macrophages, natural kill cells, [Tregs]
- Decay rates of memory cells -> boosts needed

After identification of antigens, candidates are advanced through required product development steps. The vaccine will be composed of high affinity DNA, and an adjuvant (GM-CSF, CPG, etc.). Strategies to elicit high

avidity cells need to be tested in parallel to antigen discovery. Many adjuvants have already been used in cancer immunotherapeutic vaccines. However, the potential adjuvants need to be evaluated side-by-side with the vaccine design. Ideally, the end result will work for all HLA-types. Considering immunodominance and how many antigens can effectively be combined in a final product will also be important.

III. ARTEMIS PROJECT[®]: NEXT STEPS

During 2012, NBCC will continue to create an infrastructure for the project, encompassing means for communication and data sharing. Researchers will begin the first steps within each task area to identify antigen targets, and advocates will compile a list of prioritized breast cancer self-antigens. In parallel to antigen discovery, team members will begin thinking about animal models, necessary immunological responses, and assays to evaluate the developing list of antigen candidates.

The third annual Artemis Project[®] meeting will be held in Calistoga, California, March 8-11, 2013.