

The National Breast Cancer Coalition's (NBCC) Artemis Project® 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. The NBCC has created an innovative, mission driven model which ensures appropriate focus on the end result. The Artemis Project is not simply facilitating work in progress, but actually creating the infrastructure for collaboration around development of the vaccine. Advocates are the conveners and leaders of this project, bringing together regulators, providers, scientists and others to develop and implement the strategic plan.

The Artemis Project<sup>®</sup> has developed a strategic plan through a series of meeting, the most recent of which was held in March 2011. Through these collaborative meetings, four themes have been developed:

- **1.** Identification of Targets of Prevention through a Genomic Approach to Prioritizing Preventive Vaccine Candidates
- 2. Immune System—Variations in Breast Cancer
- 3. Development Plan for Efficacy
- 4. Development Plan for Safety

NBCC contracted with Science Application International Corporation (SAIC) to provide assistance in preparing a strategic plan for the development of a preventive breast cancer vaccine for executing the

first two themes of the Artemis Project<sup>®</sup>: (1) identification of targets of prevention and (2) immune system variations in breast cancer. The project plan presented in this report was developed using the following resources:

- The draft report from the March 2011 NBCC Artemis Project<sup>®</sup> meeting in Calistoga, California. This meeting brought together 17 participants who reflected a broad range of expertise, including breast cancer advocacy, epidemiology, immunology, clinical cancer care, biotechnology product development, and the federal regulatory drug approval process.
- Per NBCC direction, interviews were held with the following expert consultants:

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

Frank Calzone, PhD, Scientific Executive Director, Hematology and Oncology Research, Amgen, Inc.

Nora Disis, MD, Professor, University of Washington School of Medicine

Peter Fasching, MD, PhD, Department of Medicine, Division of Hematology and Oncology, University of California at Los Angeles

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

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

Stephen Johnston, PhD, Center for Innovations in Medicine, Biodesign Institute, Director, Biological Design Graduate Program, Arizona State University

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

Peter P. Lee, MD, Associate Professor of Medicine and Hematology, Stanford University School of Medicine

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

H. Kim Lyerly, MD, Duke Comprehensive Cancer Center

- SAIC's previous experience in developing project plans for vaccines and managing similar research and development efforts.
- Review of resources recommended in interviews and published literature.

# I. INTERVIEW PROCESS AND SUMMARY RECOMMENDATIONS

SAIC developed an outline for a project plan using the draft report from the March 2011 Artemis Project<sup>®</sup> meeting; the outline and resulting plan only reflect the first two Artemis Project<sup>®</sup> themes that address target identification and immune system variations. The outline served a basis for the Project Plan described in this report; it was annotated with questions and distributed to interviewees to focus interviews on the work and resources required to develop a breast cancer preventive vaccine in five years. During interviews, SAIC guided discussions so that they focused on those elements of a project plan required to meet the Artemis Project<sup>®</sup>'s primary goal of developing a breast cancer preventive vaccine in five years.

In the initial outline, two areas of the plan were not well developed and required particular attention during interviews. First, the report included several concepts and proposed areas of study important in understanding immune system variations in breast cancer. However, it was unclear which of these recommendations from the March meeting addressed the primary path of identifying a breast cancer prevention vaccine in five

years and which recommended studies were more basic science in nature. Interviewees confirmed Dr. Peter Lee was the primary source for many of these ideas; thus in the interview with Dr. Lee he provided clarifications on the focus of these recommendations. Second, there was minimal detail in the report about steps required between identifying target candidates and animal testing, i.e., methods and the decision process for confirming candidates. These details were added to the plan and included an emphasis on the immune system assays.

Several interviewees made global recommendations that impacted multiple components or critical components in the project plan:

- Opinions varied substantially on the role of an infectious agent<sup>1</sup> in breast cancer. One interviewee thought the likelihood of an infectious agent being causative was virtually non-existent while another felt strongly that an infectious agent would be identified as the cause. Regardless of their opinions on the likelihood of an infectious agent being responsible, all agreed that a systematic reevaluation was warranted to either identify an infectious agent or eliminate infectious agents as a potential cause.
- One interviewee did not feel there was consensus on focusing on using a genomic approach to identify a candidate. Rather, she felt that more data needed to be generated on immune system variations. As she was the only one to voice this concern, these recommendations are noted in this report as potential options to pursue as alternate means to feed a pipeline of vaccine candidates.
- Antigen genomic mining campaigns should be initiated with pilot projects. This would serve the purpose of confirming feasibility of an approach and commitment of laboratory.
- Two interviewees expressed concern about duplicating current neo-antigen and self-antigen genomic mining studies. Thus, it was recommended that a meeting be held prior to initiating laboratory efforts to determine gaps in current approaches.
- Sufficient financial support should be invested in genomics screening to ensure success; a "shoestring" budget will not get the attention of necessary experts.
- While the plan in this report focuses on two Artemis Project<sup>®</sup> themes, target identification and immune system variation, a few interviewees cautioned that planning efficacy trials should be considered as early as possible. Specially, determining appropriate surrogate markers for clinical trials should be identified well in advance.
- In order to facilitate access to tissue and serum from women with breast cancer, should a vaccine to prevent progression be pursued concurrently?
- Concerns were noted about focusing efforts on a prevention vaccine for young women, e.g., do we sufficiently understand breast development to ensure there will not be an unintended side effect?

The majority of expert consultant interviewees agreed with the general outline for the project plan and provided input on how to meet each component. One interviewee voiced concerns over the focus of the effort, she felt there was not consensus on a genomics screen and that additional work on understanding dormancy and innate immune responses was warranted prior to initiating this campaign. A second interviewee was concerned about the potential duplication of efforts in the pursuit of a neo-antigen or self-antigen target; she was however supportive of pursuing an infectious agent. Specific recommendations for each component in the project are included later in this report.

Prior to going into the full plan, additional background information will be provided on infectious agents as potential breast cancer carcinogens.

<sup>1 &</sup>quot;Infectious agent" includes viruses, virus-like agents, mobile genetic units, bacteria, or parasites. Interviewees often had strong opinions on the likelihood of an infectious agent being identified and those opinions typically correlated with the type of infectious agent that might be identified. For simplicity, "infectious agent" is used throughout this report. However, the type of genomic data screened will vary depending upon the agent sought, e.g., virus versus bacteria.

# II. INFECTIOUS AGENTS: A POTENTIAL BREAST CANCER CARCINOGEN

Infectious agents as a potential cause, trigger, and/or contributing agent in breast cancer have been an often queried but never proven, nor disproven factor(s), in breast cancer. Given this lack of a definitive answer and recognizing that an infectious agent would be an ideal candidate for a preventive vaccine, the NBCC is particularly interested in investigating the role of infectious agents in breast cancer. Prior to addressing the best approach to investigate the role of infectious agents in breast cancer, the role of infectious agents in cancer will be briefly summarized.

The current understanding that infectious agents are capable of causing cancer dates back to 1911, when Peyton Rouss discovered that a virus was responsible for sarcomas in chickens (1). It is now generally accepted that nearly 20% of all cancers<sup>2</sup> worldwide are caused by an infectious agent with the majority of these cases in developing countries; in the United States only one out of 12 cancers are attributed to a virus, bacteria, or parasite (2). To date several infectious agents have been identified as carcinogens/probable carcinogens (Table 1). In addition, the human immunodeficiency virus 1 (HIV 1) has been shown to be an indirect risk factor associated with increased cancer rates primarily because the weakened immune system can no longer fight infection from some of these agents or help clear out any abnormal cell growth from the body (3, 4).

The transformation of a normal body tissue into a cancerous tumor is a progressive, complex process of successive genetic changes (5). There are two characterized mechanisms by which an infectious agent can promote and maintain tumor formation: chronic inflammation and viral-induced transformation (2, 4, 6). In chronic inflammation, genetic changes do not usually result from immediate mutagenesis but come as a result of tissue damage as a result of an immune response to a persistent infection. This is the case with the liver flukes, Schistosoma, and helicobacter infections, as well as viral infection with HBV and HCV (1, 6). Viral-induced transformation requires the insertion of a viral genome in a cell. Tumorigenic viruses are classified into two groups depending on the mechanism underlying the disease. Acute transforming viruses rapidly induce transformation by potent expression of viral oncogenes which in turn up-regulate host cell protooncogenes by gene amplification, chromosomal translocation, or mutagenesis, leading to uncontrolled cell proliferation. In contrast, slow transforming viruses do not carry oncogenes. Tumorigenesis occurs from viral DNA insertion into the host genome leading to recruitment of promoter/enhancer sequences to activate host proto-oncogenes or results in genomic mutations which affect the transcription of neighboring genes (4).

Given the broad diversity in infectious mechanisms proving causality of an infectious agent can thus be challenging. If the goal was simply to identify an infectious agent as a direct cause in a disease than Koch's postulate might be applied, i.e., the infectious agent is found in diseased individuals, but not healthy individuals; the isolated agent causes disease when introduced into a healthy subject; and the agent can be reisolated from the inoculated, diseased experimental subject. However, this has not been possible in cancer as most infectious agents do not induce cancer in a different host species, i.e., a human cancer virus does not usually cause cancer in monkeys. Thus, in general, identification of infectious agents as the primary causes of their respective cancers have come as a result of exhaustive epidemiological studies combined with molecular approaches to reveal the nature of the infectious agent. The approach used for HPV (cervical cancer) and HBV (liver cancer) and are briefly described below.

In the early 20th century, epidemiologists observed that cervical cancer behaved like a sexually transmitted disease. Although HPV was not characterized until the 1940s, cervical cancer was generally thought to be

<sup>2</sup> Note, percentages are for total cancer incidence not types of cancer, with the majority linked to EBV, HBV, HCV, and HPV.

Infectious Agent		Associated Cancers
	Epstein-Barr virus/human herpes virus 4 (EBV/HHV 4)	<ul> <li>Burkitt lymphoma</li> <li>Hodgkin lymphoma</li> <li>Non-Hodgkin lymphoma</li> <li>Nasopharyngeal carcinoma</li> <li>NK/T-cell lymphoma</li> </ul>
	Hepatitis B virus (HBV)	<ul> <li>Hepatocellular carcinoma (HCC, a type of liver cancer)</li> </ul>
	Hepatitis C virus (HCV)	<ul><li>Hepatocellular carcinoma</li><li>Non-Hodgkin lymphoma</li></ul>
Viruses	Human papilloma virus types 16, 18, and others (HPV)	<ul> <li>Anal cancer</li> <li>Cervical cancer</li> <li>Oral cancer</li> <li>Oropharyngeal cancer (cancer of the base of the tongue, tonsils, or upper throat)</li> <li>Penile cancer</li> <li>Vaginal cancer</li> <li>Vulvar cancer</li> </ul>
	Human T-cell lymphotropic virus 1 (HTLV 1)	<ul> <li>Adult T-cell leukemia/lymphoma</li> </ul>
	Kaposi sarcoma herpes virus/human herpes virus 8 (KSHV/HHV 8)	<ul><li>Kaposi sarcoma</li><li>Primary effusion lymphoma</li></ul>
	Merkel cell polyoma virus (MCPV)	<ul> <li>Merkel cell carcinoma (skin cancer)</li> </ul>
Bacterium	Helicobacter pylori	Gastric cancer
Parasites	Liver flukes	<ul> <li>Cholangiocarcinoma (a type of liver cancer)</li> </ul>
	Schistosoma haematobium	<ul> <li>Bladder cancer</li> </ul>

# Table 1: International Agency for Research on Cancer's List of Carcinogenic or Probably Carcinogenic Infectious Agents and Cancer (Modified from reference 3)

caused by some form of herpes virus until the 1980s when DNA from a biopsy sample of cervical cancer was shown to contain sequences loosely matching those of various HPV types. Subsequently, the newly identified HPV type 16 was shown to be present in over 60% of cervical cancers (7, 8).

Hepatocellular carcinoma ranks in prevalence and mortality as one of the top 10 cancers worldwide. Originally it was believed that damage to the liver (e.g., alcoholic cirrhosis, exposure to environmental toxins such as aflatoxin) was responsible for the majority of cases. However, after the HBV was identified in the 1960s, a prospective general population study of 22,707 Chinese men demonstrated that HBV was the primary cause of hepatocellular carcinoma (9, 10). Of note prospective studies to demonstrate this link took almost 20 years.

Modern genomic and sequencing techniques have greatly facilitated the identification of foreign DNA samples contained within a human tissue. The most promising approach to detect the presence of a microbial genome in a human tissue sample is subtractive representational difference analysis (RDA). This technique, which was used to detect KSHV in Kaposi's sarcoma in 1994, is based on the removal of all known human sequences from a tissue sample leaving behind any presumed foreign DNA (1). Variations of this technique have been subsequently developed and applied. Digital transcript subtraction (DTS) involves high-throughput sequencing to identify the presence of RNA and DNA from viruses and was successfully used to identify the Merkel cell polyoma virus (12, 13). In 2009, Digital Karyotyping Microbe Identification (DK-MICROBE) was used to identify human herpes virus 6 (HHV 6) in various human cancer samples (14). More recently, biome representational in silico karyotyping (BRISK) was shown to identify over 41 microbial species from the oral mucosa of two human subjects, including EBV from a nasopharyngeal carcinoma sample; of these 41, over 60% were previously unannotated meaning that their identity is still to be determined (15).

In general, once an agent is identified as being associated with a cancer it can be considered a potential carcinogen. Large epidemiological studies may be required to demonstrate a potential carcinogen is a carcinogen, as was the case for HBV. The need for an epidemiological study will depend on the data generated in the identification process. If an infectious agent has a clear pattern in breast cancer vs. non-breast cancer a limited epidemiological study or survey may be necessary. Further, if sufficient and appropriate tissue samples are available, a retrospective analysis of tissue specimens may be sufficient to demonstrate a link.

Unlike all the previously mentioned infectious agents and their associated forms of cancer, the information supporting an infectious agent as a potential cause for breast cancer is at best controversial. Since mouse mammary tumor virus (MMTV) is largely responsible for breast cancer in mice, similar viruses have long been sought, without a definitive answer, as potential causes for human breast cancer. Epidemiological studies have demonstrated that women residing in certain countries have a six-fold increase in breast cancer incidence compared to the general population. Given this difference disappears within one generation after women migrate from a "high incidence" to "low incidence" areas, the implication is that an environmental factor<sup>3</sup> contributes to the higher risk (16, 17, 18). Some viruses, such as HPV, HBV/HCV, and EBV have been identified in human breast cancer samples, but their low incidence does not support a causal role. Recently, bovine leukemia virus (BLV), a cancer causing virus of cattle which can be transmitted via milk, has also been implicated. A recent study found that 75% of humans tested positive for antibodies against BLV. Further in a study of 211 women, significantly more breast cancer tissue from women with breast cancer tested positive for BLV DNA (62%) than women with no history of breast cancer (23%). However, none of these demonstrate a causative effect, but merely establishes a correlation for future studies (19).

# III. OVERALL PROJECT PLAN

The Artemis Project's<sup>®</sup> goal is to develop a breast cancer prevention vaccine in five years. To meet this goal, SAIC outlined a vaccine development plan that evolved during the course of the interviews (**Figure 1**). The plan emphasizes the primary goal, steps required to develop a breast cancer prevention vaccine for use in a Phase 1 clinical trial. Overlaid on the project plan are the four themes for the Artemis Project<sup>®</sup>. As illustrated in Figure 1, the four themes are integrated through many steps in the overall project plan and, in general, apply to multiple steps in the project plan. Since the project plan is a step wise process for candidates to progress through in a vaccine development campaign, this report is organized in accordance with those steps.

To facilitate aligning this report with the previous March 2011 recommendations, a brief summary of where the Artemis Project<sup>®</sup> Themes intersect with the Vaccine Development Project Plan is summarized in this section.

<sup>3</sup> Environmental agents can be an infectious agent but also could be diet, local pollutants, or a combination of agents.

### A. IDENTIFICATION OF TARGETS OF PREVENTION THROUGH A GENOMIC APPROACH TO PRIORITIZING PREVENTIVE VACCINE CANDIDATES

Target identification, also known as antigen discovery, spans work from identifying potential candidates (e.g., genomic screens) through testing candidates in appropriate assays to confirm their suitability for incorporation in a vaccine. The Artemis Project® target identification working group focused their discussions/recommendations on the types of antigens that should be identified, i.e., infectious agents, self-antigens, and neo-antigens. Regardless of the target antigen, parallel efforts using similar genomic approaches can be applied. However, not all candidates identified in genomic screens will be effective in preventive vaccines because not all candidates will induce an appropriate immune response (e.g., anti-tumor, not anti-self). Thus, an assay funnel has been detailed in the plan to refine the selection of targets. Through the appropriate molecular and immunological screening assays, the potential thousands of candidates identified with genomic screens can be refined to confirm expression in breast cancer tumors and ability to induce effective immune responses.



**Figure 1:** Overview of a Project Development Plan for a Breast Cancer Prevention Vaccine— Target Identification through Phase 1 Clinical Trial In addition to the steps required to bring a candidate to clinical trial, the four Artemis themes and two types of targets are overlaid on the primary pathway. In the March report, the target identification working group also suggested that serological screens might be used as a potential alternate screen. This approach was not recommended as a screening method during any of the interviews for several reasons, including the lack of serum banks and lack of an existing high-throughput assay. However, use of serological screens, for medium/low-through-put assays is feasible so this approach has been incorporated into down-selection assays.

### **B. IMMUNE SYSTEM—VARIATIONS IN BREAST CANCER**

In the Artemis Project<sup>®</sup> Immune System working group covered many areas critical in vaccine development. These included, but were not limited to studies on: tumor-associated T cells, T-Cell CD8+ profiles, immune surveillance mechanisms (in particular those enhanced by mucosal vaccines), immune dysregulation in breast cancer, immune response to breast tumors and breast tumor subtypes, reasons for dormancy, regenerative cross-talk with immune system. While all of these studies will provide extremely valuable insights on breast cancer, they may not lead to a breast cancer vaccine in five years. However, the aforementioned studies could be pursued as alternate lines for candidate identification and/or provide surrogate markers of protection in a clinical trial. One significant advantage of the plan summarized in Figure 1 is it's adaptability; potential breast cancer prevention vaccine candidates identified by any approach can be run through the testing funnel and the product development plan. Further, establishment and qualification of this testing funnel could become a valuable asset to the community at large as a standard for in vitro efficacy testing and identification of a correlates of immunity.

Immune system assays play a critical and central role in vaccine development campaigns. Assays are initially used in screens to either identify and/or down-select candidates. Many of these assays are further developed and qualified for use in downstream product development efforts (e.g., efficacy models, during process development, stability testing). Subsequently, many of the assays used for product selection will be adapted, qualified, and/or validated for use in development and stability studies. The Artemis Project<sup>®</sup> can save significant resources (time and money) if the assays used for screening/down-selection address immunological readouts are suitable for an investigation new drug (IND) application. Further, it should be noted that the funnel described herein should not be considered static. Testing funnels should be carefully monitored and adjusted as new assays become available and adapted for specific targets/candidates.

### C. PLAN FOR EFFICACY

A Efficacy Plan is not part of the current report. However, efficacy should be considered during the development of immunological assays. Whenever possible, assays for screening should also be considered as potential means to test efficacy. Interviewees also emphasized their concern that plans for testing efficacy and the identification of potential surrogate markers should be a high priority.

### **D. PLAN FOR SAFETY**

Similar to the Plan for Efficacy, a Safety Plan is not part of the current report. However, concerns for safety of a prevention vaccine were raised by interviewees. These are noted for use in future planning as well as consideration in development of appropriate assays. Interviewees noted the following concerns and considerations during discussions:

- Vaccines with self-antigens or mutated self antigens could induce and/or self-tolerance. These
  possibilities can be examined in appropriate assays early in the project plan; data can subsequently
  be used to guide the safety plan.
- Concerns about administering the vaccine to young women were voiced by several interviewees. In these instances, concerns were almost always followed up with a recommendation to perform clinical trials to prevent recurrence.

# **IV. TARGET IDENTIFICATION**

Figure 2 illustrates the first step in the Project Plan, identification of candidates.During the interview process, two screening strategies for identifying targets were developed. The first, a genomics approach, was outlined in the meeting report. The second, a microarray screen for an infectious agent, was touched on the report. This second approach can be performed in parallel to the genomics mining.



**Figure 2:** Target Identification Infectious agents (Path A) can be identified using genomics mining and screening breast microbiome. Neo and self-antigens can be identified via genomics mining.

### A. GENOMICS MINING

One mechanism to investigate breast cancer heterogeneity and subsequently identify antigens is to utilize genome-based approaches. These approaches allow for the identification and assessment of the mechanistic role of gene mutation in the initiation and progression of cancer. Given recent advances (e.g., sample processing techniques, protein biomarker discovery, and antibody-based profiling arrays) proteomics may offer a viable alternative to the potential limitations of genomic approaches. There are a variety of genome-based techniques available which allow for the characterization of genes associated with the development of various breast cancer-related phenotypes. The two most utilized techniques are RNA interference (RNAi) and cDNA and miRNA expression libraries.

#### RNA interference (RNAi):

In mammalian cells, small double stranded RNAs such as short interfering RNAs (siRNAs) regulate gene expression and can be utilized to target individual or large numbers of genes (20). Utilizing techniques such as gene transfer via lipid-based transfection, electroporation or viral vector-based methodology, siRNAs can induce gene suppression to identify genes relevant to specific cancer-related phenotypes. The use of viral vector-based methods is advantageous in that it allows for a wide range of cell types to be studied including primary cells, non-dividing cells, and cells within an organism (20). In general, a disadvantage of using RNAi to screen for genes/antigens associated with breast cancer is that it is not yet possible to perform saturating genetic screens in mammalian models (used to uncover every gene that is involved in a particular phenotype in a given species). Further, primary screens require the use of multiple controls to validate datasets therefore resulting in increased cost and labor expenditures.

#### cDNA and miRNA expression libraries:

Overexpression systems derived from cDNA libraries (i.e., DNA generated from messenger RNA (mRNA)) of specific cells and tissues have been utilized to identify oncogenes (21). However, the utility of libraries pooled from reverse transcription of mRNA is limited due to the unequal representation of genes resulting from differential expression in donor cells/tissues following reverse transcription (20). Similar to siRNAs, are also short pieces of RNA that regulate gene expression. To date, expression libraries of miRNAs have also been developed and are currently being used to investigate cancer-related phenotypes.

These approaches could be used to identify breast cancer antigens, such as infectious agents or neoantigens. A multi-component vaccine may also incorporate new antigens as well as previously identified neoantigens **(Table 2)**.

Antigen	Model	References
P-selectin	In vivo: murine, rat	22-25
E-selectin	In vivo: murine	26
ICAM-1	In vitro	27-29
Fas	In vitro	30
GRP78	In vivo: murine	31

#### Table 2: Breast Cancer Neoantigens

#### Recommendations during interviews to execute genomics screen included:

- Concurrently pursue both the Path A, identifying infectious agents candidates, and Path B, identifying neo and self antigens concurrently.
- Use concurrent seed/pilot projects at multiple laboratories to demonstrate feasibility and computational analyses. Pilot projects would offer an opportunity to set up a group, perform modeling, and test assumptions posited by the Artemis Project members.
- Thousands of potential candidates may be identified via mining campaigns. Algorithms to identify candidates will be critical.
- A sufficient number of candidate/targets should be identified such that ultimately a mix of approximately 100 immunogens can be included in a multi-antigen vaccine that would prevent breast cancer in a diverse population. (Note: One immunologist questioned this approach. Alternatively a more selective screening approach could be employed that results in smaller pool of self- and neo-antigens that would drive an antigen spreading response.)
- Strong statisticians in the computational groups will be critical.
- Several laboratories were identified as potential performers (Table 3)
- No interviewee was aware of genomics mining being performed to identify an infectious agent. However, concern was voiced over potential duplication of effort if genomics mining for neo and self-antigens. Thus, a meeting to convene currently funded investigators was recommended

prior to initiating this mining campaign. Investigators currently performing these studies are listed in **Table 3**.

Laboratory	Status	Considerations
Alan Ashworth Institute of Cancer Research (ICR) http://www.icr.ac.uk/research/ team_leaders/Ashworth_Alan/ index.shtml	<ul> <li>Could provide genome wide RNAi screens</li> </ul>	<ul> <li>Provides complementary approaches to others on list</li> </ul>
William Gillanders & Mark Ellis Washington University in St. Louis http://blogs.riverfronttimes. com/ dailyrft/2011/04/ susan_g_ komen_ breast_cancer_cure_ gillanders.php	<ul> <li>Currently working on a breast cancer recurrence vaccine with Susan G. Komen funding</li> </ul>	<ul> <li>Large team effort with experience sequencing and and identifying candidates.</li> <li>Focus appears to be on vaccines for recurrence not prevention</li> </ul>
<b>Greg Hannon</b> Cold Spring Harbor Laboratory http://hannonlab.cshl.edu/	<ul> <li>Has considered this work.</li> <li>During interview he gave no indication he has started a systematic re-evaluation for targets</li> </ul>	<ul> <li>Has strong mathematics and statistics team</li> </ul>
Stephen Johnston Arizona State University http://www.biodesign.asu.edu/ people/stephen-johnston	<ul> <li>Has already identified ~100 potential neo-antigen candidates</li> <li>Limited screening of early stage or DCIS genomes</li> </ul>	<ul> <li>Data is unpublished and to date, it appears data have not yet been peer reviewed</li> <li>At this time, identified neo- antigens do not appear to be intellectual property (IP) burdened</li> </ul>
Daniel Mirl Broad Institute, Boston http://www.broadinstitute.org/ scientific-community/science/ platforms/platforms	<ul> <li>Biostatistics group</li> </ul>	<ul> <li>Broad Institute has numerous genomic platforms that have been applied to other diseases and can be applied to breast cancer.</li> </ul>
Michael Stratton Wellcome Trust Sanger Institute's Cancer Genome Project http://www.sanger.ac.uk/ research/faculty/mstratton/	<ul> <li>Conducts high throughput, systematic genome wide searches for somatic mutations in human cancer</li> </ul>	<ul> <li>Maintains a database of cancer somatic mutations, COSMIC (Catalog of Somatic Mutations in Cancer)</li> <li>May have access to additional gene banks</li> </ul>

Table 3: Computational Bioinformatics Groups Identified as Potential Performers for Conducting Genomics Screens

#### Potential technical limitations that could impact this genomics mining effort include:

- Different expertise are required for self-antigens, neo-antigens, and infectious agents.
- No source of DCIS genomic data was identified.
- Previously identified target antigens could carry intellectual property (IP) that may impede efficient incorporation in a vaccine
- One source of cancer genomic data was repetitively cited by interviewees, The Cancer Genome Atlas (TCGA).

The TCGA is the most commonly accessed source of breast cancer genomic data. It has been previous screened for potential targets and will continue to be screened. Strengths and potential limitations of this data set are summarized in **Table 4**. Of note, while published data refers to other sources of breast cancer data, much of this appears to be held at individual laboratories.

Stengths	Potential Limitations
<ul> <li>In September 2011, data from 827 total breast samples was available. Of these 537 were invasive breast carcinoma tumor samples</li> <li>Contains two genomes: breast lobular and ductal carcinomas</li> <li>Genomic and RNA data is available, inclusive of case-matched controls</li> <li>Cancers are well documented</li> </ul>	<ul> <li>Does not contain DCIS data</li> <li>Most US institutions tend to heavily rely on this set of data for genomics mining. Thus any limitations in the data will be reflected in all analyses performed with this data.</li> <li>A meeting with TCGA should be held to fully appreciate limitations. For example, one interviewee was aware of a suspicion that ovarian data was not aligned.</li> <li>Significant delays in accessing data should be considered; in one case it took 8 weeks to receive a response to a request for data from TCGA</li> </ul>

**Table 4:** The Cancer Genome Atlas (TCGA) – A Source for Breast Cancer Genomics Data (http://tcga-data.nci.nih.gov/tcga/)

In addition, there are several databases available that provide useful resources for the study of immune responses against tumors, examples are provided in **Table 5**.

Database	Description	Reference
CT Database	A repository of cancer-testis antigen data	http://www.cta.lncc.br/
CAPD	An analysis system for cancer related data www.bioinf.uni-sb.de/CAP	
Cancer-immunome Database	A repertoire of antigens eliciting antibody responses in cancer patients	http://ludwig-sun5.unil.ch/ CancerlmmunomeDB/
Cancer immunity Peptide DatabaseFour data tables containing 129 tumor antigens with defined T-cell epitopeshttp		http://www.cancerimmunity.org/ peptidedatabase/Tcellepitopes.htm
TANTIGEN	A human tumor T-cell antigen database	http://cvc.dfci.harvard.edu/tadb/

#### **Table 5:** Tumor Antigen Databases

## **B. MICROARRAY SCREEN OF BREAST MICROBIOME FOR AN INFECTIOUS AGENT**

During the interview process several discussions addressed the importance of identifying an infectious agent that could be a cause or trigger for breast cancer. It was noted in several discussions that there is still a lack of a basic understanding of the normal flora in the breast. During the later interviews, a plan was proposed and subsequently developed that bore many similarities to the sero-epidemilogical approach described in the March 2011 meeting. An outline of the basic plan is illustrated in **Figure 2** and summarized in **Table 6**. This approach involves the collection of normal breast tissue and/or fluid followed by screening for infectious agents. This screening campaign was not vetted with all interviewees and requires further development. It has been incorporated in the plan as a potential parallel path for identification of infectious agents.

Step	Description	Considerations
1A	Collect breast fluid via lavage	<ul> <li>Likelihood of getting cells is low, thus intercellular agents would not be detected.</li> <li>Processes need to be carefully worked out to ensure breast fluid and not agents on the skin are collected</li> <li>Storage conditions need to be addressed.</li> <li>Dr. Susan Love is initiating work using this type of approach.</li> </ul>
1B	Collect biopsy and/or breast reduction samples (Alternate or additional source to breast fluid)	<ul> <li>Women undergoing biopsies are often amenable to a providing a second adjacent sample from non-affected tissue.</li> <li>Setting up a protocol to bank material at two to four regionally diverse clinical sites was recommended.</li> </ul>
2	Screen for agents via microarrays	<ul> <li>Lawrence Livermore Laboratories has developed a microarray chip/probe to screen for bioterrorism agents (https://ipo.llnl. gov/?q=technologies-microbial_detection_array). The newest version has the capability to screen for ~6,000 viruses, ~15,000 bacteria as well as fungi and protozoa organisms.</li> <li>Other microarray chips might also be used for this screening (http://www.abbott.com/news-media/press-releases/2011-sept29.htm or http://us.plex-id.com/index.html).</li> </ul>

Table 6: Screen of Breast Microbiome for an Infectious Agent

# V. CANDIDATE DOWN-SELECTION: ASSAY FUNNEL

A key aspect of product development strategies is the process of screening /down-selecting candidates that are identified during the target identification/discovery phase. Candidates identified via screening efforts can be confirmed and/or down-selected via a assay testing funnel. SAIC noted that while assays were listed in the Artemis Project<sup>®</sup> report, they were not organized in formalized process for use in confirming, optimizing, down-selecting, and characterizing potential candidates. SAIC adapted a general model that is routinely used its other vaccine/drug discovery efforts and received comments on the approach. This testing funnel strategy was presented to the majority of interviewees; two early interviewees did not see this scheme in a formalized document, the majority of interviews were supportive or very supportive of the concept and provided specific recommendations, and one interviewee was

neutral to non-supportive. It was unclear if the lack of support was due to concerns with pursuing a 100-antigen vaccine candidate versus development vaccine that induces antigen spreading or specific concerns with some assays.

Importantly, the funnel described herein can be utilized to test many breast cancer prevention target candidates, regardless of the source. Candidates identified via genomic screen, a microarray screen of the duct tissue/fluid, or other non- Artemis Project<sup>®</sup> supported efforts could all be tested in the same funnel. Of note, as the Artemis Project<sup>®</sup> evolves and other lines of investigation are pursued, e.g., further studies on the immune system variations in breast cancer, new candidates may be identified that can be tested using the same general scheme in **Figure 3** and detailed in **Table 7**. Further as science evolves, assays can be updated or replaced and as necessary.



In refining and finalizing assays in a testing funnel, several issues should be taken into consideration:

- Assays should be specific, robust, and precise.
- Cost and throughput should be considered. Assays later/lower in the funnel can cost more and take more time; assays high in the funnel may have a higher false positive rate, but they should have a relatively high throughput.
- Prior to running potential candidates through each assay, a threshold acceptance criteria to progress to the next level should be pre-defined.
- Intellectual property (IP) should be considered. Many assay owners or inventors request reachthrough royalty payments if their assay was used to identify or characterize a product is ultimately sold.
- Assays should be selected for which the necessary reagents/resources are available.

Screen	Assay	Descriptions/Considerations
Primary Screens	Molecular Assay	<ul> <li>A medium to high throughput assay to screen the anticipated 500-1,000 potential candidates identified via genomic mining or microarray screening.</li> <li>Ideally this should be performed on freshly isolated specimens as opposed paraffin embedded tissues.</li> <li>Obtaining fresh tissue may be a challenge, thus alternatives may need to be considered and developed.</li> <li>Depending upon the source of tissue, these assays can be performed at contract research organizations (CROs) or by a breast cancer research group.</li> <li>A threshold acceptance criteria for a candidate to progress to the secondary screen needs to be established prior to initiation of screening. For example, a candidate may need to be advanced to secondary screen.</li> </ul>
condary Screens: HLA Binding	HLA Prediction Algorithm HLA Binding Assay	<ul> <li>To induce immune responses, HLA binding is critical.</li> <li>Prediction algorithms will be used initially to identify high avidity and candidates that bind multiple HLA types.</li> <li>Prediction algorithms will also guide the choice of specific amino acids to be used, i.e., adding or subtracting a few amino acids to the N or C terminus can significantly increase/decrease binding.</li> <li>At least one peptide sequence for each candidate will also be tested in a binding assay.</li> <li>Peptides will be generated and tested in a binding assay</li> <li>Numerous CROs offer these services.</li> <li>A threshold for binding affinity should be established prior running this assay.</li> </ul>
Tertiary assays are prused to identify cance		<ul> <li>Note, antigens that have a high frequency of expression in primary assay may require multiple runs with varying peptides through this assay to identify optimal sequences.</li> <li>obably the most critical assay in the funnel. A positive response in either assay will be lidates for full characterization and incorporation in a vaccine candidate. In addition,</li> </ul>
assays measure humoral and cellular responses will be critical late stage product de assessments of drug product used in clinical trials. These assays may even serve as s immune protections, e.g., a long-term sustained T cell response in a clinical trials.		oral and cellular responses will be critical late stage product development and stability product used in clinical trials. These assays may even serve as surrogate markers of e.g., a long-term sustained T cell response in a clinical trials.
Tertiary Screens	Serological Screen	<ul> <li>Given the limited availability of well-characterized serum sources, this assay will likely be conducted concurrently on multiple candidates. For example, custom chips with target peptides may need to generated to test screen multiple candidates against a single serum sample.</li> <li>A potential limitation of this approach is that an effective immune response may be generated in individuals who do not have breast cancer and using serum from breast cancer patients may not be selective for protective candidates.</li> </ul>
	In Vitro T-cell Assay	<ul> <li>The choice of assay may be adjusted based upon the antigen. For example, a cytotoxic T cell assay may be selected for an intracellular infectious agent while a T helper assay may be more appropriate for neo- and self-antigens.</li> <li>Several assays are available or can be adapted for this effort.</li> </ul>

### Table 7: Vaccine Candidate Down-Selection Testing Funnel

Screen	Assay	Descriptions/Considerations	
ys	Characterization assays do not generally result in Go/No Go decisions for progression of a candidate into a vaccine. They are however essential to understanding the mechanism of action, stability, the nature of the active pharmaceutical ingredient (API), all elements critical to the FDA in an IND. A sample list of probable assay is included in this section.		
Assa	Avidity	<ul> <li>Avidity may be used to tweak/refine a candidate in order to improve</li> </ul>	
Antigen Characterization Node T Ce Response Mouse Challe Model	Draining Lymph Node T Cell Responses	<ul> <li>An in vivo correlate in humans that demonstrates clinical relevance of a candidate will likely be requested by the FDA. Thus, planning for an assay such as draining lymph node T cell responses early in the product development plan would be prudent.</li> </ul>	
	Mouse Challenge Model	<ul> <li>A mouse challenge model will be critical in demonstrating potential efficacy. All candidates will be screened in models prior to full product development.</li> </ul>	
		<ul> <li>A mouse model will also be used later in development to test final combinatorial vaccine candidates.</li> </ul>	
		<ul> <li>An interviewee noted that several mouse models are available and are suitable for these studies. A list of models is pending.</li> </ul>	

 Table 7: Vaccine Candidate Down-Selection Testing Funnel (continued)

### A. RESOURCES REQUIRED FOR ASSAYS

To execute the assays outline in **Table 4**, specific sequences (RNA, DNA, or peptide) will be used as probes or serve as targets. Utilizing either a genomics mining or microarray screens, it is anticipated that most candidates will be 15 to 25 amino acids in length regardless of type, i.e., neo-antigens, self-antigens, or infectious agents. As the anticipated candidate size is relatively small, RNA, DNA, and peptides can be efficiently synthesized by CROs.

Unlike the target candidates, breast cancer specific material for use in confirming the presence of target antigens in breast cancer may pose greater challenges. Most assays will require specific breast cancer source material, serum or tissue: potential sources are listed in **Table 8**.

When selecting sources, some general recommendations were made:

- Given the diversity in anticipated responses, consider using serum from ethnic groups with the highest incidence instead of all ethnic groups
- Most banks lack DCIS samples.
- Need to focus on early cancers in screening for infectious agents, but cancer stage may not matter as mush in neo- or self-antigen assays.

Resource	Notes
Breast Cancer Biomarker Discovery Project http://www.womenscancerresearchfund.org/bios/ http://www.fhcrc.org/about/ne/news/2005/10/04/ biomarker.html	<ul> <li>This Entertainment Industry Foundation funded effort identify biomarkers that would reveal the presence of breast cancer in the blood to provide early detection, predict the potential for metastasis and guide therapeutic response</li> <li>Potential source of serum and tissue</li> </ul>
Patient Tumor Bank of Hope	<ul> <li>Contains 4,000 breast cancer samples</li> </ul>

#### Table 8: Assay Reagent Resources

Resource	Notes	
Laura Esserman/Athena Project	<ul> <li>Collection of tissues collected through University of California system</li> </ul>	
University of Indiana	<ul> <li>Contains well documented normal and cancer tissue.</li> </ul>	
Mayo Clinic	<ul> <li>Contains well documented normal and cancer tissue.</li> </ul>	
MD Anderson	<ul> <li>Contains well documented normal and cancer tissue.</li> </ul>	
Michael Press	<ul> <li>Contains well documented normal and cancer tissue.</li> </ul>	
SEER Tissue Repositories at University of Iowa, University of Hawaii, and University of Southern California	<ul> <li>Well-annotated biorepository of paraffin tissue.</li> <li>NCI funding was discontinued, resource may not be available for much longer.</li> </ul>	
Nurse's Cohort Study and Teachers Cohort Study	<ul> <li>Two cohort studies with well-documented serum.</li> <li>Reagents are limited and "guarded." They might be available for screening in a qualified confirmatory assay.</li> </ul>	
Army of Women	<ul> <li>Dr. Susan Love Research Foundation has already lined up 370 women who may be a source for serum.</li> </ul>	

Table 8: Assay Reagent Resources (continued)

# VI. DOWN-STREAM PRE-CLINICAL DEVELOPMENT

After identification of antigens, candidates are advanced through required product development steps. These are illustrated in Figure 1. As these steps are largely involve Efficacy and Safety planning, they are not included in this report. However, a few details should be considered when planning and executing target identification and selection as they impact later development.

### A. VACCINE DESIGN AND CONSTRUCTION

In Figure 1, vaccine design and construction is illustrated as a nanoparticle/virus like particle or DNA. These illustrate two likely platforms for use in delivering a100-antigen vaccine. Both DNA vaccines and nanoparticles have been approved by the FDA for other vaccines and both have been shown to induce T cell responses. Thus the would provide an inexpensive platform with limited regulatory hurdles for delivery.

### **B. IN VIVO CHALLENGE MODEL**

An in vivo challenge model will be a critical element for testing vaccine efficacy prior to clinical trial. In the current figure it is shown after vaccine design. But is also likely to be used after adjuvant selection/ formulation.

### C. ADJUVANT FORMULATION

In the last several years, numerous new adjuvants have been developed. Many have already been used in cancer immunotherapeutic vaccines. Further, novel adjuvants often turn a mediocre antigen into a strong immune-stimulating vaccines. Prior to vaccine formulation, potential novel adjuvants should be considered and if possible evaluated side-by-side with vaccine design.

### D. PRODUCT MANUFACTURE THROUGH CLINICAL TRIAL

Product manufacture through clinical trial should be carefully planned as early as possible by the efficacy and safety groups. While many of these steps are strictly regulated by the FDA, pre-emptive meetings and discussions with the FDA can save time by ensuring all studies are performed as early as possible. This planning should be conducted in the context of knowledge and lessons learned from ongoing clinical trials. To date, there are a number of clinical trials ongoing that are aimed at assessing the efficacy of breast cancer vaccines. These vaccines have been characterized as dendritic cell (DC), peptide, whole tumor cell, or viral in nature.

#### **DC Vaccines:**

As their name imparts, DC vaccines utilize DCs that when activated express high levels of MHC class I and class II molecules in addition to co-stimulatory molecules and cytokines required for T-cell activation (33). DCs can be obtained from peripheral blood and once obtained can be subsequently loaded with antigens in different forms (peptide, proteins, whole-tumor cells or tumor cell lysates) (34). One caveat to the utility of DCs however is that the frequencies of these cells in peripheral blood are often low, with peripheral DCs often being functionally defective in breast cancer patients (35,36). Despite this caveat there are currently two ongoing Phase II clinical trials investigating HER2 and p53 based DC vac¬cines.

#### **Peptide Vaccines:**

Peptide vaccines utilize antigenic peptides derived from tumor-associated antigens (TAAs) to induce peptide-specific immune regulators that rec¬ognize and lyse tumor cells expressing the immunogenic peptide on their surface as well as CD4+ T cells that stimulate anti-TAA immune responses (34). Both adjuvant and human leukocyte antigen (HLA) restrictions need to be carefully planned for peptide vaccines. To date, there are two Phase II peptide vaccine trails ongoing for patients with breast, both of which target HER2.

#### Whole Tumor Cell Vaccines:

Whole tumor cell vaccines can be composed of allogeneic (isolated from multiple established tumor cells) or autologous tumor cells (isolated tumor cells from an individual patient). Allogeneic tumor cell vaccines are advantageous in that they can be used as an antigen source for a broadly applicable vaccine as tumors from multiple cell lines have variable overlapping expression profiles (32, 35, 37). In addition, autologous tumor cell vaccines are beneficial in that they allow for the delivery of antigens that may be specific for an individual's tumor as they are produced from an individual patient's isolated tumor cells (35). A caveat to the utility of whole tumor cell vaccines is that the amount of immunogenic antigen delivered is attenuated as a result of tumor cells being composed of normal antigens. Thus far, clinical trials are being conducted investigating granulocyte-macrophage colony-stimulating factor (GM-CSF) allogeneic vaccines as well as a large multivalent immunogen (LMI) vaccine constructed from SKBr3 breast cancer cells.

#### Viral Vector Vaccines:

Viral vector vaccines exploit the natural ability of virus' to generate an immune response and the efficiency of viral mediated gene transduction. Previous investigations incorporating the carcinoembryonic antigen (CEA) and mucin-1 (MUC1) TAAs in poxyviral vaccines have demonstrated the utility of these

vaccines for initial inoculation via stimulation of neutralizing host antibodies (38, 39). Alternatively, fowl pox vectors were identified as being useful for subsequent vaccinations to allow for a progressive immune response to encoded TAAs (38,39). Currently, viral vector based vaccines expressing CEA and a triad of T-cell costimulatory molecules to include B7.1, ICAM-1 and lymphocyte function-associated antigen 3 (TRICOM) are underway.

# VII. A PROJECT DEVELOPMENT PLAN AND SCHEDULE FOR IDENTIFYING AND SELECTING A CANDIDATE

The required screening and characterization of target antigens has been incorporated into a MS Project Schedule (**Figure 4**). This high level schedule illustrates estimated time required to complete each step, which steps need to proceed others, and which activities can be performed in parallel.

# VIII. SUMMARY

Eleven breast cancer researchers and clinicians who attended the March 2011 Artemis Meeting were interviewed to provide additional details for a Project Plan to develop a Breast Cancer Prevention Vaccine in five years. Interviewees were in 100% on the importance of this effort. Even though some interviewees felt the likelihood of finding an infectious agent was "extremely" low, all interviewees were supportive of systematically investigating the possibility of an infectious agent being causative. In general, at least one interviewee disagreed with each item (e.g., choice of an assays) in the plan outlined in this report. However, the development plan presented herein represents the majority opinion (usually 9-10 of the interviewees). Since interviews were done one-on-one no single opinion or voice dominates this report and likely reflects a general consensus.



Figure 4: MS Project Schedule

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# **National Breast Cancer Coalition**

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