



I. INTRODUCTION

A. BACKGROUND

The National Breast Cancer Coalition (NBCC) is dedicated to ending breast cancer through the power of grassroots action and advocacy. In 2010, NBCC launched **Breast Cancer Deadline 2020®** to create a paradigm shift in the world of breast cancer. The deadline refocuses resources and efforts to the areas that will lead to the knowledge needed to end breast cancer. The research component of **Breast Cancer Deadline 2020®** includes the Artemis Project®, a collaboration of various stakeholders, led by advocates, working strategically, with a mission-driven approach. The Artemis Project® focuses on two areas of research:

- 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 framework for the Artemis Project® includes catalytic workshops that address how to proceed in areas that could have a major impact toward answering these priority questions. The workshops gather academic and government scientists from multi-disciplinary fields, regulators, health care providers, researchers in biotechnology and pharmaceutical industries, and advocates. The goal is to break through the confines of the current systems that have not yet uncovered the causes of breast cancer or its spread, or have led to effective means for prevention.

The first advocate-led, collaborative effort was launched in 2011 and focuses on primary prevention. A five-year, strategic plan for the development of a preventive vaccine has been developed and is being

implemented through the Artemis Project® for a Preventive Breast Cancer Vaccine. A research plan is in place, initial seed grants have been awarded to identify vaccine targets, and regular meetings occur to assess progress and readjust plans.

This report summarizes the first meeting of the Artemis Project® on tumor dormancy, in the context of preventing metastasis.

B. MEETING PURPOSE

To catalyze efforts on the causes and prevention of metastasis, NBCC held a meeting June 10-11, 2013, in Walland, Tennessee. NBCC brought together 17 scientists and advocates to discuss launching a project around tumor dormancy as a fundamental approach for preventing metastasis. The scientists represented diverse perspectives, including physical sciences, immunology, mathematics and modeling, cancer biology, molecular biology, and biotechnology.

C. MEETING ATTENDEES

Participants

Julio Aguirre-Ghiso, PhD, Director, Head and Neck Cancer Basic Research, Director of Solid Tumor and Metastasis Research, Mount Sinai School of Medicine

Robert H. Austin, PhD, Professor of Physics, Princeton University, Principal Investigator, Princeton Physical Sciences Oncology Center

David Basanta, PhD, Assistant Member, Department of Integrated Mathematical Oncology, H. Lee Moffitt Cancer Center & Research Institute

Amy Bonoff, MBA, Advocate, NBCC

Frank Calzone, PhD, Biotechnology Consultant

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

Yibin Kang, PhD, Warner-Lambert/Parke-Davis Professor of Molecular Biology, Princeton University

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

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

Musa Mayer, MS, MFA, AdvancedBC.org

Shirley Mertz, MA, JD, President, Metastatic Breast Cancer Network

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

Josef Penninger, PhD, Senior Scientist and 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

Sridhar Ramaswamy, MD, FACP, Associate Professor of Medicine, Harvard Medical School, Tucker Gosnell Investigator, Massachusetts General Hospital Cancer Center, Associate Member, Broad Institute of Harvard & MIT, Associate Member, Harvard Stem Cell Institute

Sohail Tavazoie, MD, PhD, Senior Attending Physician, Leon Hess Assistant Professor, Elizabeth and Vincent Meyer Laboratory of Systems Cancer Biology, The Rockefeller University

Fran Visco, JD, President, NBCC

Alana Welm, PhD, Assistant Professor, Oncological Sciences, University of Utah, School of Medicine

Meeting Support

Kayla Kirsch, MS, President, Leapfrog Consulting

Marva Lewis, The Event Professionals

Shaun White, MS, MHA, Scientific Administrator, Vaccine & Gene Therapy Institute of Florida

II. MEETING AND DISCUSSION

A. DESCRIPTION

On the first day, initial presentations addressed the landscape of breast cancer and the advocate perspective on the issue of tumor dormancy—how it is defined and why it is important. This was followed by scientific presentations on the biological properties of metastatic tumor cells, and immune system regulation of tumor dormancy. This provocative discussion led to further exploration of three key areas in small group discussion: metabolism, modeling, and the immune system.

B. PRESENTATIONS

The Reality of Breast Cancer and Metastasis

Dr. Silvia Formenti

Dr. Formenti set the stage for the meeting by describing the current landscape of breast cancer for women and society. The costs are staggering, including billions of dollars for diagnosis and treatment, additional billions spent on peer-reviewed research, and the incalculable loss of economic and social productivity. The parameters for measuring progress were raised as a central issue. Dr. Formenti suggested that progress should be measured by the incidence of fatal breast cancer over time (no change since 1975), the mortality from breast cancer (modest improvement, decreased 1.9% per year over last ten years), the level of toxicity from treatments (some improvements with less invasive surgery), and cost (increasing, significant overdiagnosis).

Advocate Perspective

Laura Nikolaides

Ms. Nikolaides described how little is known about dormancy and metastasis. She defined “tumor dormancy” in clinical terms, as that period of time between diagnosis and treatment, and when the disease reappears in lethal form, a time which can span up to 25 years. Advocates want to understand how to prevent metastasis, which is what ultimately kills women. The period of dormancy may provide a window of opportunity to do so for many women and is being squandered because we don’t know what to do to prevent the metastatic process.

Laboratory tests can indicate evidence of lingering disease in women who have no evidence of metastasis, but we really don’t know what this means for metastasis or the accuracy of these tests (circulating tumor cells in blood or disseminated tumor cells in organs). What happens between diagnosis/treatment and late recurrence is a black box—there could be tumor cell dormancy, equilibrium between cell growth and death, or something else entirely. Many things could be impacting the black box, some of which are receiving research attention, but most are not. These factors may include:

- Microenvironment, pre-metastatic niche
- Physical properties of tumor cells, quantity of cells
- Immune system; immunosurveillance
- Inflammation; signals
- Evolving tumor cells
- Metastasis suppressor genes
- Epigenetics
- Blood supply, Angiogenic switch
- Metabolism, energy
- Lifestyle, diet, alcohol, exercise, stress, injury

The ultimate questions are: will women know how to prevent metastasis by 2020? Will we learn how to intervene and make a difference with current research approaches?

Could there be a need to bring together a different type of mission driven effort?

Will this happen without NBCC?

Physical Properties of Metastatic Tumor Cells

Dr. Robert Austin

Dr. Austin outlined his current hypothesis on the cause of cancer and cancer spread. He suggested that rather than the current approach of looking for genetic damage and the accumulation of mutations, the key may be to look for the accumulation of protein damage. According to Dr. Austin, one universal factor regarding metastatic cells is that they are characterized by the Warburg effect, producing energy with a high rate of glycolysis, unlike normal cells which use a low rate of glycolysis within mitochondria. He hypothesizes that the Warburg Effect is both a hallmark of cancer and metastasis, and serves as a marker, but is also a *cause* for the progression and spread of cancer. Metastatic invasion is a high risk activity for a cell and something fundamental must be driving it to do so. Dr. Austin posits there is some gradient or "order parameter" related to the Warburg effect, and the resulting protein damage, that drives the process. He suggests the pressure of accumulating protein damage and metabolites tip the balance toward tumor invasion and travel to distant sites.

The Immune System and Tumor Dormancy

Dr. Keith Knutson

Dr. Knutson proposed that the immune system, a component of the microenvironment, could have a profound influence on malignancy, both dormancy and progression. He outlined the concept of immune editing – the process that changes tumor cells as it interacts with the local environment. He described the model of tumor dormancy as immune editing that first involves elimination of tumor cells, then equilibrium of cell growth and death, followed by escape of the tumor cells. Investigators do not know if immune editing is an active or selective process, or whether there is a defect in the immune system leading to metastasis or whether it is a defect of the tumor, an accumulation of mutations that leads to a loss of antigen targets for the immune recognition. The focus is to understand what happens to tumors that evade the immune system, leading to progression and metastasis using existing knowledge of immune disorders as a reference. The discussion identified parameters to consider such as inflammation, the impact of metabolism on T-cell function, immunosuppression, etc., and demonstrated the complexity and ultimately, how little is known.

C. SMALL GROUP WORK AND OUTCOMES

Subgroups were formed to separately address three questions 1) What role might metabolism play in

tumor dormancy and the metastatic process? 2) How does our immune system affect tumor dormancy and the metastatic process? 3) How can we create models that will help us understand the characteristics of residual disease and that will inform our approach to tumor dormancy and preventing metastasis?

i. Group One: Metabolism

Robert Austin, Frank Calzone, Keith Knutson, Laura Nikolaides

Group one considered the potential role of deregulated metabolism (Warburg Effect) in maintaining tumor dormancy or driving escape. The information gaps on the metabolic status of disseminated tumor cells were acknowledged. The discussion focused on the suggestion of a connection between Warburg metabolism and the long-term accumulation of damaged proteins (carbonylation) as a potential process driving metastasis, through two possible routes. The accumulation of damaged proteins could trigger a wound healing response and the recruitment of macrophages, which then mediate growth and metastases; or the accumulation of damaged proteins that cannot be eliminated by protein turnover could eventually result in an imbalance or crisis that initiates more aggressive cell behavior; a slow process of deregulation until a threshold is reached.

This line of reasoning lends itself to several possible novel interventions: A vaccine that could target universal markers of protein damage, targeting the biochemical pathways that damage protein, agents that suppress inflammation, antioxidants, HSP90/chaperone inhibitors or safe diabetes drugs.

A fundamental knowledge gap is whether dormant cells are metabolically active and are undergoing aerobic glycolysis. Glycolysis is regulated by Akt signaling and mutations in this pathway (P13Ka, PTEN) activating glycolysis are relatively common in breast cancers. Tumors with these mutations should relapse more quickly if the damage protein hypothesis is correct. The group acknowledged that the scientific literature (in particular hallmarks of aging) probably contains much of the evidence needed to support or disprove an oxidative protein damage hypothesis of tumor dormancy.

Recommended Next Steps:

1. Explore Knowledge Gaps
 - a. Literature search on PTEN, a mutation that leads to the Warburg effect – do PTEN tumors recur more frequently?
 - b. Protein Damage + Elimination
 - c. Antioxidants
 - d. Cell Aging
 - e. Tumor Cell Kinetics
 - f. Warburg Connection
 - g. CTC/DTC Metabolic Activity
 - h. Processing and immune display of proteins
 - i. Autophagy
2. Conduct an Artemis Meeting with a Diverse Group of Experts, to Include:
 - a. A metabolic expert, “old school,” translational, perhaps diabetes area
 - b. Protein folding and turnover expert
 - c. Apoptosis expert, stress response (perhaps cell biologist who studies cell aging, neuro)
 - d. Macrophage biologist; wound healing

- e. Autophagy expert
- f. Theoretical modeler of regulation
- g. Neurodegeneration expert

ii. Group Two: Modeling

Julio Aguirre-Ghiso, Yibin Kang, Kim Lyerly, Musa Mayer, Sridhar Ramaswamy, Alana Welm

This group formed to explore the models available or needed to answer questions about dormancy and emerging recurrence of breast cancer disease. How can scientists model contributions and interactions of the host vs. the tumor cells? The group defined tumor dormancy as a period of at least five years before disease recurs. The group outlined the pathway from dormancy to emergence to metastasis and considered the vulnerable period from dormancy to emergence in terms of both intrinsic and extrinsic factors.

In considering the tumor cells during this vulnerable period, the group discussed cell cycle arrest entry; slow cycling vs. other forms of quiescence; cancer subtype differences in relation to disseminated tumor cells; location/organ specific quiescence; epigenetic changes; whether the primary tumor pre-encoded the dormancy in some way; and stress response.

Osteolytic bone markers were identified as potential biomarkers relevant to tumor dormancy (in bone) including standard serum proteins, osteoclast (OCL) mRNAs in the blood, or bone marrow. Immune system status may also predict tumor escape, markers include B/T-cells responses, such as systemic suppression or tumor-specific memory responses. Other biomarkers include tissue specific markers in the liver, lungs, etc.; factors of aging, hormones, ECM breakdown, obesity; and stress.

Interventions must prevent emergence and drive dormancy, and could be directed at intrinsic factors, possibly using repurposed treatments, or directed at extrinsic factors in the adjuvant setting, using “window of opportunity” trials.

In conclusion, the group agreed that there are not currently good models of tumor dormancy, but that a comprehensive review of clinical and model data is needed. The group volunteered to carry out this review. The key question is whether something inside or outside the cell is driving the re-emergence of the tumor, or whether it is a synergistic phenomenon.

Recommended Next Steps:

1. A comprehensive literature review of clinical and modeling data on tumor dormancy
2. A focused meeting on the clinical and molecular characteristics of residual disease
3. Create framework with resources for building knowledge about residual disease; collect cells at time of surgery and annually thereafter to create molecular taxonomy; correlate with what happens to patients

iii. Group Three: Immune System

Silvia Formenti, Josef Penninger, Sohail Tavazoie, Amy Bonoff, Shirley Mertz, David Basanta

This group focused on the immune system’s role in preventing metastasis. The group saw the goal as modulating the immune system either to maintain dormancy, or to eliminate emerging cells. However, most of the discussion time was spent on the latter, as the former seemed more difficult without having a marker for dormancy. The group did agree that a logical approach to maintaining dormancy could involve using current approaches to reduce the burden of disease as much as possible and then boosting the adaptive immune response.

In order to learn how to eliminate emerging cells, the group agreed that discovery of biomarkers to assess emergence and relapse will be critical. To eliminate the emerging cells, a logical approach would be to remove the brakes from natural killer T cells concurrently with boosting an adaptive

immune response through a vaccine or radiation. Would it be possible to develop a pill to reactivate NK cells that will find, seek out and kill cells that would become full blown metastatic cells? One setting for investigation of this approach could be women with oligometastatic disease, or disease that has spread to only one metastatic site, evaluating interventions for prevention of additional metastatic sites.

A basic understanding of the mechanisms of dormancy and emergence is needed: Is there exhaustion of immune control by the metastatic niche? Are there dormant infections? Is there a failure of the niche to suppress (Osteoclast inhibitor)? What about other niches? What are the relationships between niches? What is known about aging of the immune system and emergence of disease? Are there other appropriate models? The group thought it would be helpful to look at models of latent infections. Another suggestion was to develop a mathematical model of tumor growth to predict the best time to treat patient with immune boosting therapy.

Recommended Next Steps:

1. Understand more basic science regulating tumor dormancy
2. Literature search/investigation on aging of the immune system and emergence of disease
3. Correlate immune markers to emergence of metastatic disease/Discover biomarkers from either cell or host factors for emergence of disease
4. Literature search/investigation on the function/significance of natural killer cells, macrophages

III. ARTEMIS PROJECT®: NEXT STEPS

There was consensus among the diverse meeting participants that there is little known about breast cancer tumor dormancy, but that addressing this gap in knowledge should be a priority within the field. Participants agreed that increased knowledge could lead to an understanding of how to prevent metastasis for significant numbers of women.

NBCC is currently analyzing the meeting outcomes and recommendations in the context of current scientific literature to outline and prioritize the next steps to be taken in 2013 to further the goals of **Breast Cancer Deadline 2020®**.