Applicant: Andrew J. Ewald, Ph.D.
Title: Targeting the survival pathways required by established breast cancer metastases

Significance of the Proposed Research: Early stage breast cancer can generally be successfully treated. Most mortality from breast cancer instead arises when cancer cells escape the primary tumor, enter blood vessels, and grow into large new tumors in distant vital organs, a process that scientists refer to as metastasis. METAvivor is a national leader in funding research on metastatic breast cancer. This emphasis is important as metastasis is incompletely understood, relatively little research is conducted at metastatic stages, few drugs even attempt to target metastasis, and few treatments work well enough for patients with established metastases. I have organized my laboratory and launched a major new program in the Sidney Kimmel Comprehensive Cancer Center to overcome each of these challenges and identify novel targets for anti-metastatic therapy.

Background and Preliminary Data: Our recent research reveals that metastatic breast cancer cells express a common "basal" molecular "toolkit" as they escape the tumor and travel through the body (Cell 2013), that they rely upon the tumor microenvironment to invade (PNAS 2012, Biomaterials 2013), that groups of epithelial cells are better at sensing spatial information than single cells (PNAS 2016a), that normal myoepithelial cells actively resist breast cancer invasion (JCB 2018), and that breast cancer cells travel in multicellular clusters to found multiclonal metastases (PNAS 2016b, Science 2016). Taken together our studies support a new conceptual framework for metastasis, in which groups of cancer cells invade and disseminate collectively, while remaining stuck to each other through specialized adhesive molecular connections. We next demonstrated that cancer cells rely upon these adhesive connections, mediated through the protein E-cadherin, to survive in the circulation and as they seed distant organs (Nature 2019). Inspired by the focus of the Metavivor Foundation and by our interactions with metastatic patients, we next disrupted these adhesive connections in established metastases and found that the metastases shrunk (unpublished). Our discoveries reveal that metastasis is a highly inefficient and molecularly stressful process for breast cancer cells. Put simply, our specific aims seek to determine which subtypes of human breast cancer most rely upon these adhesive survival mechanisms, to use genetic techniques in mouse models to identify precisely when we can intervene to disrupt existing metastases, and to utilize our 3D culture models of metastatic growth to identify pharmacologic strategies to control or eliminate metastatic tumors.

Approach: My laboratory has developed new 3D culture and animal models of the late stages of metastasis, after the cancer cells have arrived and started to grow in distant organs. Our new methods enable us to culture, genetically manipulate, image, and molecularly analyze "organoids" isolated from mouse or human tumors, from either the primary or metastatic site. As described above, we recently used these methods to identify how invasive ductal breast cancers use E-cadherin-dependent molecular signals to survive the circulation and grow into metastatic tumors. If we disrupt these survival signals, the cancer cells die, even if they are already part of a growing metastasis. I seek the Founder's Award from the Metavivor Foundation to exploit these assays and develop practical ways to disrupt survival signals in metastatic cancer cells in patients. We have already identified multiple strategies to disrupt these survival signals and propose to:

Aim 1: *Utilize patient derived xenograft (PDX) models of breast cancer metastasis to identify which subtypes of breast cancer are most dependent on E-cadherin mediated survival signals.* We will analyze the dependence on E-cadherin mediated signals in patient tumor tissue from diverse metastatic PDX models. All reagents and mice are available.

Aim 2: *Test the hypothesis that disruption of E-cadherin mediated adhesive signals will cause regression of large, established metastases.* We will use an inducible genetic switch to delete E-cadherin in progressively later stages of metastasis to identify the time window for targeting this cancer cell survival pathway. All reagents and mice are available.

Aim 3: *Screen an existing library of FDA approved drugs for agents that can eliminate growing metastases.* Our novel 3D culture assay is highly scalable (2.5 million clusters per 2 cm tumor) and we have begun adapting it for drug screening. We will begin by screening the FDA approved drug library to test if existing agents could help current metastatic patients and to lay the foundations for screens of larger and more diverse libraries, here at Johns Hopkins.

Impact on Patients: Most research in metastasis has focused on the molecular tools used by tumors to invade into the surrounding breast tissue. However, we know that patient outcomes are instead determined by whether cancer cells can survive in the circulation and grow in the distant organs. Our proposal focuses on these later stages and will generate deeper insights into the molecular mechanisms by which breast cancer cells survive in distant organs, such as the lungs and liver. We will then leverage those insights to develop novel therapies.