

Targeting Creatine Kinases to Inhibit Metastatic Breast Cancer (MBC)

Tiffany N. Seagroves, Ph.D.
Associate Professor of Pathology
Center for Cancer Research, University of Tennessee Health Science Center

Laboratory Objective: Breast cancer deaths are due to spread, then growth, of tumor cells that have left the breast and grow as lethal lesions in other organs (“metastasis”). Our laboratory’s long-term goal is to find safe, effective and specific “targeted” drug therapies to block pathways that drive metastasis. We are interested in drugs that block metastatic potential, even if primary breast tumor growth is unchanged. We strive to use pre-clinical mouse models that are the most representative of human metastatic breast cancer (MBC), i.e., those that consistently and reproducibly metastasize from the breast throughout the body.

Project Background and Rationale: In response to very low oxygen (“hypoxia”), cells try to restore balance through regulation of cellular energy sources, formation of new blood vessels and executing decisions between survival or cell death. Solid tumors frequently contain oxygen-starved regions that have bypassed the normal checks and balances regulating these processes, leading to emergence of adapted, more aggressive tumor cells. The Hypoxia-Inducible Factors (HIFs) are key proteins that control how cells react to low oxygen by turning on or off hundreds of genes in a network known as the “hypoxic response”. Hypoxia is clinically relevant since hypoxic regions of tumors are enriched with cells resistant to radiation and chemotherapy. In addition, over-expression of the HIF proteins directly correlates with poor prognosis and decreased survival. Yet, there are several roadblocks to developing HIF inhibitors for clinical use. First, most “HIF” inhibitors are not specific to the HIF proteins and have “off-target” effects. Second, loss of HIF function is lethal in mice, therefore, side effects of HIF inhibition are to be expected. To address these challenges, we employed a transgenic mouse model of highly metastatic breast cancer (MBC) known as MMTV-PyMT to screen for genes that are increased in a HIF-dependent manner and for which chemical inhibitors were available. One gene identified was creatine kinase, brain isoform (CKB). CKB is over-expressed in several types of cancers relative to normal cells. There are well-characterized chemical inhibitors that block creatine kinase activity, including cyclocreatine (cCr), and a next-generation cCr derivative known as LUM-001 (Lumos Pharma, Austin, TX). This class of drugs is well-tolerated in animals, and can cross the blood-brain barrier, which may be useful for inhibiting brain metastases. cCr was also recently shown to block liver metastasis in mice with colon cancer. Our preliminary data show that CKB directly mediates ATP production, cell invasion and lung metastasis, and that either deletion of the *Ckb* gene or cCr drug therapy blocks metastasis of breast cancer cells to the lungs. We also show that cCr therapy represses the growth of small metastases into large, lethal metastatic lesions; therefore, cCr therapy may benefit patients who have already developed metastatic disease.

Aims/Goals: The goals of this proposal are to test if cCr therapy is effective as a single agent or is synergistic with conventional chemotherapies and to test the *in vivo* anti-metastasis efficacy of cCr/LUM-001 using multiple models of metastatic breast cancer (MBC), including state-of-the-art patient-derived xenograft (PDX) models that express CKB protein and that *efficiently* metastasize to distant vital organs from the breast.

Aim 1: To demonstrate that CKB or CK activity (CK^{act}) is required downstream of HIF to mediate formation of invadopodia (local invasion) and anoikis resistance (survival in suspension) to enhance metastatic potential. The contribution of CK^{act} to invadopodia formation will be investigated using PyMT CKB loss- or gain-of-function approaches, or with cCr therapy. In addition, whether CK activity or CKB expression is required for survival in suspension conditions will be tested.

Aim 2: To test the anti-metastasis efficacy of cCr/LUM-001 in xenograft models of MBC. ER+ models will be used to compare cCr therapy to standard of care regimens and to evaluate in pilot studies whether blocking CK activity ameliorates osteolytic bone metastases.

Expected Outcomes: Completion of this work is expected to demonstrate that cCr is an effective anti-metastatic compound when used alone or in combination with conventional therapies, and to reveal molecular mechanisms of how CKB promotes metastatic potential in breast cancer.

Clinical Benefits and Impact: CKB is expressed in all subtypes of breast cancer; therefore, it has potential for broad use in MBC. cCr and its derivatives may be less toxic and more durable treatment options for patients with stage IV disease who relapse from acquired resistance to either general cell killing (cytotoxic) or receptor-targeted (estrogen receptor, ER, or HER2) therapies. Overall, if successful, our studies would provide strong rationale for testing cCr/LUM-001 in new clinical trials for breast cancer patients within the next 5 years (by 2020).