Background: Breast Cancer (BC) is not one disease but divided into subtypes based on pathology and molecular definitions that may allow for specific therapies. For example, targeted therapy against hormone receptors have led to long-term survival for most BC patients. In contrast, inflammatory breast cancer (IBC), the most lethal BC subtype, is still poorly defined and lacks specific therapies. At the time of diagnosis, IBC has often already spread (as metastatic disease), and women with IBC have significantly worse survival outcomes compared with those with non-IBC breast cancer subtypes. In most IBC cases, tumor cells spread through the lymphatic system in the skin as cell aggregates (clusters) that have adapted to low oxygen (hypoxia) conditions and express high levels of inflammatory mediators. Hypoxia and inflammatory signaling is well known to enhance the malignancy of many tumor types. Some progress has been made in the identification of molecular pathways that are activated or inhibited in IBC, but have not yet led to specific therapies. The current standard of care for IBC patients includes radiation therapy and chemotherapy. The rationale of these treatments is to inflict DNA damage assuming that will eventually cause cell death preferentially in tumor cells. However, the most malignant cells within a tumor are often resistant to these treatments. A new class of drugs called "histone deacetylase inhibitors" (HDACi) are directed against enzymes that remove specific protein modifications (acetylation) and lead to significant changes in gene expression patterns. Although their mechanism of action is still poorly understood, HDACi have shown promise in preclinical trials for different cancers including breast cancer.

The **Objectives** of this project are (a) to assess the role of the CCAAT/enhancer binding protein delta (CEBPD) and F-box and WD repeat domain containing protein 7 (FBXW7) in the highly malignant nature of IBC, and (b) to determine whether HDACi can be used to target the CEBPD-FBXW7 signaling pathway in IBC cell lines, which would provide a mechanistic rationale for the potential benefit of combination treatments.

Hypothesis and Rationale: We hypothesize that the CEBPD protein is a driver of the highly malignant features of IBC and that HDACi will inhibit CEBPD expression, which will therefore sensitize cells to radiation or chemotherapy. It has been reported that one type of HDACi can inhibit CEBPD expression in macrophage cells of the immune system. Our previous work showed that CEBPD promotes malignant features of cultured breast tumor cells when exposed to hypoxia, augments cell survival after DNA-damage, and supports tumor metastasis in a mouse model of breast cancer. In addition, CEBPD is well characterized as an amplifier of inflammatory pathways. CEBPD exerts these functions at least in part by inhibiting the FBXW7 protein, which is a well-known tumor suppressor for several types of cancers. Given that CEBPD can promote cell survival in response to DNA-damaging agents and promotes radiation resistance, we rationalize that inhibition of CEBPD expression may sensitize cells to chemotherapeutics and/or radiation.

Aims: The proposed research will determine if CEBPD levels are elevated in an extended panel of IBC tissues and cell lines and correlated expression with pathology or outcome. IBC cell lines will be used to determine the role of CEBPD and its target gene FBXW7 in cell proliferation, survival, growth as aggregates, invasive properties, and activation of inflammatory pathways in cell culture assays as well as mouse models. The regulation of CEBPD and FBXW7 expression in response to HDACi will be assessed.

Impact: This is a basic sciences project that will investigate the molecular mechanisms leading to the survival of metastatic BC cells, provide proof-of-principle and a potential mechanistic rationale for using HDACi to target metastatic BC cells. Most of the proposed work can be completed in one year and the results from this project will lay the ground-work for future studies to determine if down-regulation of CEBPD and/or HDACi will sensitize tumor cells to radiation and/or chemotherapy, and

therefore significantly improve the efficacy of treatment for IBC patients. Several HDACi are already in clinical studies, in combination with radiation or chemotherapy treatment, for a number of other cancers. Therefore, it is important to evaluate their potential for IBC and promising results could be readily adapted for IBC patients in the clinic. In addition, if CEBPD and/or FBXW7 signaling will be confirmed as relevant for malignant features of IBC, this pathway can be further explored to identify novel targets or strategies for therapeutic intervention. Lastly, the results of this study may also apply to other subtypes of advanced breast cancer.