Cytoplasmic Cyclin E is an Early Event for Progression to Invasive Breast Cancer

As depicted in this figure, we have discovered that the LMW-E isoforms are potent oncogenes that act early in the etiology of breast cancer and have developed genetically engineered mouse models that can be used to interrogate the secondary oncogenic events induced by cyclin E early on in the neoplastic process\(^1\)\(^-\)\(^5\). We have recently generated a robust inducible murine transgenic model of LMW-E mediated tumorigenesis, and have mapped some of the early events in the pre-neoplastic mammary gland that gives rise to aggressive tumors with high metastatic potential. These events include induction of DNA damage, upregulation of several genes involved in unregulated DNA replication and G2/M transition, and specific mutations in genes, such as ALK, that is readily targetable. These preliminary results have led to the following testable hypotheses and proposed aims

Hypothesis #1: Expression of LMW-E early in the pre-invasive breast cancer (i.e. ductal carcinoma in situ) results in induction of genomic alteration leading to an invasive carcinoma, to be tested by examining the role of cytoplasmic cyclin E in differentiating indolent versus high-risk ductal carcinoma in situ (DCIS).

Hypothesis #2 LMW-E in a cyclin E knockout model will result in a more aggressive phenotype than overexpression of EL, resulting in increased genomic instability, centrosome amplification and transformability in hMECs, to be tested by investigating the mechanisms of LMW-E mediated DNA damage response and centrosome amplification in the absence (via CRISPR) of endogenous cyclin E in somatic hMEC models and mouse cyclin E knockout models.

Hypothesis #3 Inhibition of ALK, a secondary oncogenic event to LMW-E induction, early in the neoplastic process can inhibit tumorigenesis and also be used as a target for the treatment of triple negative breast cancers (TNBC) expressing LMW-E, to be tested by investigating the role of ALK as a mediator of LMW-E mediated mammary tumorigenesis in the mouse LMW-E inducible transgenic model and as a therapeutic target in TNBC using patient derived xenograft models.

Translational significance: Many of the patients who succumb to breast cancer after a diagnosis of DCIS experience an invasive recurrence prior to death due to high malignant potential from the outset. Currently, there are no biomarkers that can differentiate the DCIS cases with an invasive recurrence from those which never recur. As a result, most DCIS patients undergo extensive treatments that could be avoided if a biomarker of early pre-neoplastic lesion could be used to stratify the DCIS patients at high risk of recurrence from those at low risk. The successful completion of proposed projects will delineate those early oncogenic events and provide the rationale to use LMW-E as a biomarker to identify the DCIS cases who could benefit from aggressive treatment, versus those (w/no cytoplasmic cyclin E) who can be monitored without the need for aggressive intervention. The successful completion of Aim 3 could potentially be practice-changing for the TNBC patients with tumors expressing LMW-E. We have recently reported that 70% of all TNBC patients express LMW-E. To date, there are no effective targeted therapies for LMW-E overexpressing tumors. Our studies will show, one way or the other, if ALK can be a viable target for the LMW-E overexpressing TNBC patients. Since there are already several ALK inhibitors, which have undergone Phase I-III clinical trials in malignancies other than breast, the translational of these pre-clinical studies to TNBC patients could occur readily.
Targeting Neutrophil Elastase as a Novel Therapy for Metastatic Breast Cancer.

The underlying cause of death among breast cancer patients is the metastatic spread and growth of tumors at distant organ sites. The currently available arsenal of chemotherapeutics and targeted agents is wholly insufficient to treat metastatic breast cancer. In addressing this challenge we have uncovered a targetable paracrine network that mediates breast cancer growth and metastasis in pre-clinical models, with neutrophil elastase (NE) and inflammatory immune signals at its core. The proposed project will address a major gap in knowledge centered on the mechanism(s) by which tumor-associated neutrophils promote tumor growth and metastasis, by delineating roles for NE in metastatic breast cancer. The project includes pre-clinical studies to test NE inhibitors in combination with immunotherapy for treatment of primary and metastatic breast cancer.

Tumor associated neutrophils (TANs) are associated with poor prognosis in metastatic breast cancer, yet the mechanism(s) by which TANs contribute to primary tumor progression and metastasis has been unknown. Our preliminary results indicate a direct molecular link between TANs, tumor progression and metastasis mediated by NE and, significantly, highlight a targetable pathway via NE inhibition for therapeutic intervention in metastatic breast cancer. This project will address major gaps in our understanding of how TANs enhance breast cancer growth and metastasis by testing our overall hypothesis that NE sustains breast cancer growth and metastasis through the activity of pro-tumor, immunosuppressive myeloid cells in primary tumors and lung metastatic sites (see Figure). We propose NE inhibition (e.g., via AZD9668), alone or in combination with immunotherapy, will provide a major advance in treating metastatic breast cancer. These hypotheses will be tested using orthotopic murine breast cancer models with NE-deficiency to definitively establish the role(s) for NE in primary tumor progression, metastasis and regulation of innate and adaptive immune responses. In addition, pre-clinical studies will be performed with the FDA-approved NE inhibitor AZD9668 alone or in combination with immune checkpoint blockade to evaluate therapeutic efficacy of these agents, with the long term goal of informing the design of new clinical trials for metastatic breast cancer.

**Aim 1.** Delineate the mechanism(s) by which NE regulates growth of primary PyMT tumors.

**Aim 2.** Determine the role(s) for NE in breast cancer metastasis to lung.

**Aim 3.** Evaluate the efficacy of NE inhibition alone or in combination with immune checkpoint blockade on growth and progression of metastatic breast cancer.

**Translational Significance:** The successful completion of this study will resolve fundamental mechanisms linking tumor associated neutrophils, NE and breast cancer, and delineate novel metastasis-promoting pathways in breast cancer. Moreover, the innovative therapeutic approaches using the FDA-approved NE inhibitor (AZD9668), alone or in combination with immunotherapy has the potential to be practice changing for the treatment of metastatic breast cancer, the main cause of breast cancer mortality. We propose pioneering in vivo studies to dissect the contribution of immune and tumor-associated NE in breast cancer metastasis and ways to target the NE/immune checkpoint blockade axis for its treatment.
Deregulation of the cell cycle checkpoint proteins, such as cyclin-dependent kinases CDK4 and CDK6, is a key hallmark of cancer, resulting in uncontrolled cellular proliferation and tumorigenesis. Selective CDK4/6 inhibitors, palbociclib, ribociclib, and abemaciclib, have shown promising preclinical and clinical activities in numerous solid tumors. Palbociclib (Palbo) and ribociclib (LEE011) were recently approved by the U.S. Despite these promising clinical advances with CDK4/6 inhibitors, there are three major limitations for this treatment: (i) adverse events which lead to interruption and/or discontinuation of treatment, possibly attenuating therapeutic benefit, (ii) ~16% of the ER+ cancer patients do not respond to Palbo or exhibit progression within 24 weeks, and half the patients develop clinical resistance with progression within 25 months, resulting in no overall survival benefit, and (iii) lack of reliable predictive biomarkers to identify patients with intrinsic and/or acquired resistance to Palbo.

We recently reported on the mechanism of action and pathways of resistance to Palbo. We show that at low concentrations of the drug, ER+ breast cancer cells arrest in the G1 phase of the cell cycle, but this arrest is reversible due to the activation of autophagy. However, at high concentrations of Palbo, the cells are arrested irreversibly in G1, do not undergo autophagy, and instead, undergo senescence. We also show that combining Palbo with an autophagy inhibitor (e.g., hydroxychloroquine), causes senescence at a much lower and continuous dosing of Palbo, in both in vitro and in vivo models. **We propose that Palbo activates the autophagy pathway to protect ER+ breast cancer cells from Palbo-induced senescence and inhibition of autophagy sensitizes cells to Palbo in vitro and in vivo.**

We have also generated Palbo-resistant cell lines and find that several regulators of G1 to S transition (such as low molecular weight cyclin E), EMT, DNA repair and immune response are altered in CDK4/6 resistant cells. Based on these results, we propose novel combination treatment strategies targeting these deregulated pathways and identify predictive biomarkers of response in the resistant cells that can eventually be used clinically to identify and treat patients more likely to exhibit Palbo resistance (intrinsic and/or acquired). These hypotheses will be tested through the following specific aims:

**Aim 1:** Examine the mechanisms of resistance to palbociclib and identify treatment strategies to circumvent such resistance.

**Aim 2:** Classify clinically applicable biomarkers of response to autophagy and identify patients with intrinsic or acquired resistance to palbociclib.

**Translational Significance:** There are currently no biomarkers to identify patients with intrinsic and/or acquired resistance to Palbo-based therapy. We will address these challenges by determining if autophagy is a feature of Palbo therapy, and identify clinically useful biomarkers of Palbo activity by examining key regulators of autophagy such as Beclin-1 and LC3. We will also examine if the DNA repair and CSC/EMT pathways can help identify those patients who are likely to become resistant (intrinsic or acquired) to Palbo treatment. The successful completion of these studies will provide rationale for future clinical translation to definitively test our novel combination therapy (low dose Palbo + letrozole + HCQ) that can overcome Palbo discontinuation, and positively impact survival for ER+ MBC, the subtype of cancer responsible for the most deaths—meeting a clear unmet need.

**Clinical trial undergoing IRB review currently**
Publications from the Keyomarsi lab pertaining to each of these projects


