

## **Convergent transcriptional states in resistant ER+ metastatic breast cancer**

### **Public Abstract.**

Estrogen receptor positive (ER+) metastatic breast cancer (MBC) remains the most common cause of breast cancer death, resulting in more than 20,000 deaths in the U.S each year. Though we have made many advances in the treatment of ER+ MBC using agents that target the estrogen receptor, patients nearly always develop resistance to these therapies. Over the past few years, the strategy of combining agents that target the estrogen receptor and novel agents called CDK4/6 inhibitors have shown to have significant benefits in patients with ER+ MBC. However, although combining estrogen receptor treatments and CDK4/6 inhibitors benefits a large percentage of patients, nearly all of these tumors eventually develop resistance to the combination, resulting in continued growth of the cancer. In addition, some patients never respond to these treatments at all. The mechanisms of resistance to these treatments are not well understood. To improve survival for patients with ER+ MBC, it is critical to develop an understanding of this resistance.

Over the past several years, our lab has identified multiple ways that tumors become resistant to ER and CDK4/6 inhibitors by performing *whole exome sequencing* (a technique that characterizes the DNA of more than 20,000 genes) on metastatic tumor biopsies from patients who developed resistance to these drugs. However, although we have identified multiple ways that tumors can develop resistance, each individual mechanism of resistance is relatively rare in patients. For this reason, developing individualized approaches to target each resistance mechanism present in each patient's tumor will be challenging – since there are so many different ways that tumors can evade therapies. An alternative strategy is to develop more “universal” approaches to targeting resistance so that more patients can benefit from each therapy. We propose that this can be done by identifying key targetable commonalities (that we call “nodes”) that might exist in multiple rare resistance mechanisms.

Most studies of cancer resistance have focused on identifying genetic resistance mechanisms using DNA sequencing, leading to lists of diverse (and rare) gene alterations. Here, we propose a different approach: transcriptome sequencing, also known as RNA-seq (a technique that characterizes the RNA of more than 20,000 genes) of over 500 resistant tumors that we have collected from patients, as well as RNA-seq of resistant ER+ breast cancer cell lines, to group the rare genetic resistance mechanisms into these common “nodes”. This approach will allow us to recategorize resistant tumors into fewer categories. In addition, this approach will allow us to classify tumors in which no clear mechanism has previously been identified by DNA sequencing.

The overarching goals of this research are i) to characterize the ways in which ER+ MBC develops resistance to ER and CDK4/6 inhibition by identifying resistance “nodes” in resistant tumor biopsies, and ii) to develop specific therapeutic combination strategies to target these “nodes”. The guiding hypothesis is that the numerous rare resistance mechanisms to ER and/or CDK4/6 inhibition will converge to a relatively small number of common “nodes” or pathways, and therefore might be targeted by a few “universal” therapeutic regimens. To test our hypothesis, we will engineer ER+ breast cancer cell lines to express each of the candidate genetic resistance mechanisms identified in patients in our studies and others. We will characterize each of these cell lines and create a cell “map” corresponding to specific genetic resistance mechanisms in each cell line. In parallel, we will analyze RNA-seq data from 500 resistant ER+ metastatic tumor biopsies that we have already collected to identify “nodes” associated with clinical resistance to ER and CDK4/6 inhibition. Finally, we will develop combination therapeutic regimens to target ER, CDK4/6, and candidate resistance “nodes”, focusing on resistance mechanisms we and others have described – MAPK, AKT, CDK2, and AURKA – as well as new candidate resistance pathways.

If successful, this approach could lead to a shift in how we think about resistant tumors in ER+ MBC and other cancers, establishing relatively few nodes of resistance, enabling the classification of more resistant tumors, and setting the stage for more universal therapeutic strategies that more patients will benefit from. At the completion of the project, knowledge gained from this study should lead to improved understanding of ER/CDK4/6i resistance and will enable: i) the development of clinical biomarkers of resistance, ii) identification and validation of novel targets, and iii) the development of new treatment regimens for patients with resistant ER+ MBC. By the end of the grant term, we expect to be poised to design clinical trials of novel therapeutic approaches and rational drug combinations in patients with ER+ MBC. While these trials will initially aim to overcome resistance in patients who have already progressed on ER and CDK4/6i therapy, over time these strategies may be moved upfront to be able to prevent all potential mechanisms of resistance, ultimately leading to durable responses or even cures in patients with ER+ MBC.