Estrogen Receptor (ER-positive) breast cancer is the most common form of breast cancer. A new class of drugs called CDK4/6 inhibitors have recently been introduced for the treatment patients with ER-positive, metastatic breast cancer. These drugs, which are often used in combination with hormone therapies, have become the first line treatment in such patients. However, nearly all patients eventually become unresponsive to these drugs resulting in progression of their cancer. Treatment options outside of chemotherapy are limited in such patients. Therefore, there is an urgent need to study why metastatic breast cancer patients become unresponsive to CDK4/6 inhibitors and develop new and effective drugs for treating such patients.

In this proposal, we develop model systems for studying CDK4/6 inhibitor resistance (unresponsiveness). Our first model is based on the observation that some of the metastatic breast cancer patients who develop resistance to CDK4/6 inhibitors acquire a mutation in a tumor suppressor gene called retinoblastoma (*RB1*). We used gene editing technologies to delete *RB1* from a panel of three ER-positive breast cancer cell lines. We have demonstrated that these cells, which were originally responsive to CDK4/6 inhibitors such as palbociclib and ribociclib, become completely resistant to these drugs after deletion of *RB1*. As a second model, we used ER-positive breast cancer cells that have been made unresponsive to ribociclib by prolonged exposure to increasing concentrations of the drug. By screening these resistant cell lines against a panel of drugs, we have identified that OTX015, a new drug under development which blocks the BET (bromodomain and extraterminal domain) family of proteins, is highly effective in stopping the growth of these cells. We have also demonstrated that a number of alterations in the resistant cells that may contribute to their unresponsiveness to CDK4/6 inhibitors (such as increase in protein levels of c-Myc and CDK6) are blocked by OTX015. In this study, we will carry out detailed investigations to characterize the alterations in breast cancer cells that make them unresponsive to CDK4/6 inhibitors and study how OTX015 blocks such alterations. We will also test the activity OTX015 in mice that have been injected with ER-positive breast cancer cells that have become unresponsive to CDK4/6 inhibitors or implanted with tumors derived from patients who have become unresponsive to the drug. We will also inject these unresponsive cancer cells into the blood circulation of mice to establish a widely metastatic breast cancer model and will evaluate the efficacy of the drug in controlling the disseminated cancer. The findings of this study could potentially establish a new approach for treatment of patients with highly aggressive metastatic breast cancer who have become unresponsive to standard agents used in clinic.

It is estimated that there are over 150,000 women living with metastatic breast cancer in the United States. This number is expected to grow by 30% by 2020. A vast majority of these women harbor ER-positive breast cancer and will be treated with CDK4/6 inhibitors, often in combination with hormone therapies. Nearly all of these patients will eventually become unresponsive to both these agents. In this study we propose a novel agent, OTX015, for treatment of patients with these highly aggressive cancers. OTX015 has already completed a toxicity evaluation in humans and has been shown to have a favorable safety profile. Therefore, successful completion of goals outlined in this proposal could quickly introduce a new drug for the treatment of many metastatic ER-positive breast cancer patients that have become unresponsive to CDK4/6 inhibitors. Thus, our study has high potential to substantially improve the care of metastatic, ER positive breast cancer patients and increase their survival.