

## Public/Lay Abstract

Estrogens are the primary female sex hormones. They function by diffusing into cells and binding to the estrogen receptor (ER), which subsequently binds to specific regions of the genome and leads to the regulation of a group of genes that play important roles in a wide range of cellular functions.

About 75% of breast cancers rely on estrogen to grow, so they are known as ER-positive cancers. Breast cancer treatments that suppress estrogen production or binding to ER are referred to as endocrine therapies, which are widely used and effective in many patients. However, about 30% of patients do not respond at all, and 40% of those who do initially respond develop resistance later on, mostly progress to metastatic lesions.

One major mechanism of the acquired resistance that has been recently identified is the frequent mutations in the gene encoding ER (gene name ESR1), resulting in structural changes that enable this receptor to remain active without estrogen, and rendering the cells resistance to the traditional endocrine therapies. Importantly, these mutations are almost exclusively found in metastatic lesions, pointing to the importance of this finding for metastatic diseases. Among those mutations, the most prominent one, Y537S mutation, has shown the most resistance to existing treatment. In addition, metastatic breast cancer patients with this ESR1-Y537S mutation showed shorted progression free survival in several clinical trials. Therefore, effective treatment targeting the most dominant and resistant ESR1-Y537S mutation for metastatic breast cancer is still lacking.

However, developing novel therapies for these mutated metastatic lesions are limited by technical hurdle of difficulty in sampling tumor cells from these tissues. Intriguingly, tumors also shed so-called circulating tumor cells (CTCs) into the blood stream. Although in low quantity, these CTCs can be shed from actively growing metastatic lesions and isolated from peripheral blood of patients. We have previously pioneered a method to isolate live CTCs from metastatic breast cancer patients and expanded them in the laboratory. We identified a CTC line that carries the most common ESR1-Y537S mutation, showed that it forms a high prevalence of bone metastases in metastatic assay in mice, recapitulating the bone metastatic status of the corresponding patient. These results now provided us with a great model to study bone metastasis and developing novel targeted therapies for patients with mutant ER driven metastatic disease.

To find the mechanism underlying this mutant ER driven bone metastasis, we analyzed where does the mutant ER bind in the genome and compared to the normal ER conditions. We identified a unique genomic region that specifically bound by the mutant ER which activates a gene that has been previously shown to play a role in breast cancer progression and bone metastasis. Based on our exciting preliminary results, we will test our hypothesis that targeting this mutant ER induced downstream factor could be a novel targeted therapy to treat bone metastasis. We also aim to further elucidate the molecular mechanism that enables the interaction of this factor with mutant ER to contribute to therapy resistance and progression in bone metastasis.

Bone metastasis is the most prominent, and often the first site of, metastasis in breast cancer patients. Patients with bone metastasis suffer from high morbidity and mortality. However, there are not enough effective bone metastasis specific targeted therapies, besides the bone symptom relieving drug, bisphosphonates. The proposed study will significantly advance the development of effective means to target mutant ER induced bone metastasis, and will lead to improved treatment strategies to benefit metastatic breast cancer patients with these mutations in general.

Furthermore, despite increasing amount of evidence on the critical role of organ-specific tumor microenvironment, most of the current cancer therapies are not really targeting organ-specific metastasis, which is an area with significant unmet need. Completion of this proposal will provide important data and conceptual proof of the importance of considering both tumor cell properties as well as the organ site in designing organ-specific metastatic treatment, which could contribute to paradigm shift in future drug development for metastasis.