Targeting Activated Progesterone Receptors in Metastatic Breast Cancer
Lay Abstract (one-page limit)

Breast cancer is the most frequently diagnosed cancer in women. Despite advances in both early detection and therapeutics, metastatic and/or recurrent breast cancer, often manifesting many years after the conclusion of initial therapy, remains a major threat to breast cancer survivors as the second leading cause of cancer-related deaths in women. Approximately 75% of breast cancers (luminal subtype) have proteins that interact with specific sex hormones, called steroid hormone receptors (SR) such as the estrogen receptor (ER) and/or progesterone receptors (PRs). These luminal (ER+) breast cancers depend on SRs and their ovarian sex hormone signals (estrogen and progesterone) to promote tumor growth. Hormone exposure is an important breast cancer risk factor. Women who are taking hormone replacement therapy that contains estrogen(s) and progestin(s) (i.e. synthetic progesterone analogues or drugs that interact with PRs) at the time of breast cancer diagnosis typically have larger and more aggressive (luminal B type) tumors and experience worse outcomes.

ER and PR proteins are abundant in breast and reproductive tissues and are required for normal health and development. Following hormone stimulation of the cell, these receptors enter and are retained in the nucleus where they interact with DNA to "turn-on" (express) or "turn-off" (repress) genes that promote normal cell processes. ER and PRs often work together as balanced "yin and yang" collaborators or co-dependent partners that orchestrate normal reproductive cycles and breast health. These receptors also work outside the nucleus in the cell cytoplasm to activate another class of proteins called kinases. Protein kinases are enzymes that chemically modify other proteins including SRs, and play a key role in normal cell "communication" pathways that precisely instruct cells when to "STOP" or "GO" (cell signaling). Protein kinases are also frequently increased in breast cancer, where they provide constant GO signals. Abnormal signaling in the cytoplasm of the cell can disrupt normal SR-driven events and cause uncontrolled hormone action in the nucleus, the site of gene regulation in our DNA. We know this chain of events ultimately affects SR activity at SR-regulated genes to support uncontrolled breast cancer cell growth. Thus, traditional therapeutics for ER+ breast cancer patients include anti-estrogens such as tamoxifen (a drug that inhibits ER, sending a STOP signal) or a class of drugs that block ER action by inhibiting estrogen hormone production in the body (such as Letrozole/Femara). Similarly, SRs collaborate with cell signaling pathways to support advanced metastatic breast cancer and can evade these ER-blocking therapies in up to 41% of lymph node-positive patients. However, this process as a whole is not well understood, especially in the context of therapy-adaptive cancer cell *ESR1* (ER) gene mutations that cause ER to turn-on or express genes even without estrogen; *ESR1* mutations primarily arise during endocrine therapy and reside in recurrent breast cancer metastases. The gene known as *PGR*, which encodes two related types of PR proteins, is highly expressed in breast cancers harboring an active mutant ER.

We recently discovered that when PRs are chemically modified by protein kinases (i.e. phosphorylated) in breast cancer cells, they interact with ER to promote specific gene programs associated with breast cancer cell survival, dissemination, and metastasis. In particular, we showed that phosphorylated PRs drive breast cancer stem cell self-renewal or "stemness" phenotypes, a process believed to be required for the formation and maintenance of new metastatic tumors. Relative to hormone-responsive (luminal A type) primary breast tumors, we find that the controlled yin and yang relationship between ER and PRs is also disrupted in metastatic breast cancer. Instead, these receptors adapt a competitive relationship and acquire new independent roles, in which phosphorylated PRs co-opt the GO signal, especially when ER is therapeutically blocked. Metastatic *ESR1*-mutant breast cancers express high levels of PR that is always phosphorylated. We **hypothesize** that phosphorylated PRs "feed" metastatic ER+ breast cancers, allowing them to renew themselves and maintain a therapy resistant state. The **objective** of this proposal is to uncover how PRs, when phosphorylated, can support, maintain, and renew the metastatic cascade in ER+ breast cancer. A complete understanding of the significance of SR cooperation between activated ER and PRs, as well as their interactions with abnormal cell signaling pathways is urgently needed if we are to improve upon existing treatment strategies for metastatic breast cancer.

Impact on women with metastatic breast cancer: The studies in this proposal will yield a clear understanding of PR actions and the associated abnormal signaling events that can be collectively targeted in the clinic as part of new modernized endocrine therapies that account for both ER and PR actions. New biomarkers will be discovered to identify women whose late-stage metastatic breast cancers are PR-driven. New antiprogestins that block phosphorylated PRs are now in clinical trials. The information gained from these preclinical studies of human mER+ tumors grown in mouse models can also be used to design clinical trials of new drug combinations that block uncontrolled and abnormal signaling events that fuel phosphorylated PRs in ER+ metastatic breast cancer. We anticipate that the routine addition of PR-blocking strategies to existing standard-of-care therapies will dramatically improve health and extend lifespan for women suffering from metastatic ER+ breast cancer.