PUBLIC ABSTRACT

Although approaches for detecting and treating localized breast cancers have dramatically improved overall patient survival, 5-year survival rates for patients diagnosed with metastatic breast cancer remain low at 24%. All patients are at risk for developing metastatic breast cancer. Because metastasis remains the leading cause for morbidity and mortality of breast cancer patients, there is a critical need for developing novel approaches that reduce established lesions and inhibit the formation of future metastatic lesions. Significant efforts are underway to develop targeted therapies that inhibit metastatic lesions. While some agents have demonstrated promising pre-clinical results, few have led to positive results in the clinic. Patients with estrogen receptor-positive (ER+) cancers often develop resistance and suffer late recurrences, up to 5-20 years after initial therapy for the primary tumor. One potential mechanism of therapeutic resistance in ER+ patients is the presence of slow growing well-differentiated cancer cells which eventually result in clinically detectable metastases after a prolonged latency; clinical trial data show that neither chemotherapy nor hormonal therapy eliminates them. Thus, there is an unmet need to develop therapeutic strategies to target and eliminate established metastases and such micrometastatic, slow growing cells. Immunotherapy is one such approach.

Recently, immunotherapy which leverages cells of the body's immune system to kill cancer cells has been highly successful in some metastatic solid cancers such as melanoma, lung, and renal cancers. Most of the current immunotherapy drugs serve to activate a type of white blood cells (immune cells) called T cells to kill tumors. A class of these T cell directed therapies are called immune checkpoint inhibitors that block the activity of proteins that function normally to downregulate immune responses. Development of such drugs resulting in increased activation of the immune system, has led to new immunotherapies for melanoma, non–small cell lung, and renal cancers. A second type of T-cell drugs is the chimeric antigen receptor (CAR) T-cells but these have to be engineered for each patient. To respond to T-cell based immunotherapy, cancers must have high mutational activity. Unfortunately, breast cancers in particular ER+ ones, have not responded to these T-cell based immunotherapy drugs as they are not generally known to display high mutational rates. Our objective is to increase the response rate of established metastases and slow growing well-differentiated micrometastatic cancer cells to immunotherapy using another type of cells of our immune system called **natural killer** (NK) cells. NK cells are a type of immune cells in our body that normally function to fight infected cells and cause cell killing. We will train the NK cells to attack and kill the metastatic breast cancer cells.

The **objective** of this proposal is to develop an off-the shelf immunotherapeutic drug that will eliminate metastatic breast cancers and make them responsive to immunotherapy. We have shown a key role for a growth factor called IGF receptor (IGF1R) in breast cancer metastasis. The expression of IGF1R is highly correlated to ER function in ER+ breast cancer. IGF1R is a cell surface marker on slow growing micrometastatic ER+ breast cancer cells and triple negative (TN) breast cancer cells and we will use this marker to train NK cells to kill the breast cancer cells**.** We have also previously studied numerous antibodies against IGF1R and demonstrated that an IGF1R single chain antibody (IGF1R scFv-Fc) binds to IGF1R on breast cancer cells. We will use a platform that directs NK mediated cancer cell killing through trispecific killer engagers (TriKEs). In this proposal**,** we seek to develop TriKEs that contains small portions of two well-characterized antibodies in the same drug, one of which will bind to NK cells to activate them and the second will stick to the surface marker (IGF1R) on breast cancer cells. The drug will also include a small factor called interleukin-15 which will cause the NK cells to expand at the site of established metastases and eradicate them and the slower growing micrormetastatic cells to inhibit late recurrences. Thus, in aim 1, we will develop a natural killer cell based immunotherapeutic drug that will train NK cells to kill and eliminate breast cancer cells. The effect of this drug on function of NK cells from blood of healthy volunteers and breast cancer patients, and on breast cancer cell killing will be tested. In Aim 2, the efficacy of IGF1R TriKE in treating established bone and lung metastases in mouse models will be tested. Further, in aim 2 we will also utilize our novel MRI method of sweep imaging with Fourier transformation (SWIFT) to monitor inhibition of metastasis by the TriKEs. We recently, were the first in the world to demonstrate that SWIFT is more sensitive in detecting metastases of breast cancer.

This proposal will develop a novel drug that will train NK cells to eliminate established metastases and micrometastatic cells. This work will help patients with stage IV ER+ as well as triple negative breast cancer. This work is directly applicable to patient care and also uses a novel MR imaging to monitor inhibition of metastasis. As patients with metastases already undergo imaging we can translate the novel drug and the MR technology to the clinic in 2 to 2.5 years. The success of this study will have an impact on taking the initial step towards ending mortality from breast cancer and directly impact patients with stage IV breast cancer.