Public Abstract

Breast cancer metastasis is the spreading of cancer cells from the breast to other tissues in the body. Metastasis is the main cause of death for breast cancer patients, and once breast cancer has metastasized, it is considered incurable. Therefore, new treatments for metastatic breast cancer are needed. This proposal evaluates inhibiting the RON tyrosine kinase signaling pathway as a new strategy to treat established metastases in metastatic breast cancer patients.

RON tyrosine kinase is a receptor expressed on multiple different cell types including tumor cells, bone-resorbing osteoclasts, and immune cells such as macrophages. Macrophage-stimulating protein (MSP) is the ligand that activates RON, which leads to downstream signaling within the cells. Increased levels of the RON/MSP signaling pathway are seen in 40% in breast cancers. Importantly, high levels of the RON pathway are associated with increased metastasis and decreased survival in breast cancer patients. In mice, RON promotes metastasis in a number of ways. High levels of RON and/or MSP in cancer cells cause cancer cells to metastasize or spread from the mammary gland to clinically-relevant sites, such as bone, lung, and lymph node. Additionally, a role for host RON in promoting bone metastasis has been identified, and RON function in the immune system allows tumor cells to escape the immune defenses that normally eliminate them. We have tested RON inhibitors in each of these contexts and found that inhibiting RON could effectively treat metastasis. Importantly, RON inhibitor treatment worked when given either before or after metastasis developed, indicating RON inhibitors may be effective at treating patients with established metastatic disease. **Based on these data, we hypothesize that RON inhibitors can effectively be developed as a new treatment for metastatic breast cancer patients.** In support of this hypothesis, data from early clinical testing of the RON inhibitor BMS-777607/ASLAN002 supported continued investigation of RON inhibitors for use in metastatic breast cancer patients. Unfortunately, the RON inhibitor used for this study is no longer available for us to pursue for these purposes, though these data do provide proof-of-concept for use of RON inhibitors in patients. Therefore, there is a need to identify new RON inhibitors for use in metastatic breast cancer.

We have identified two novel compounds (TGN-HCI-1003 and TGN-HCI-1005), which inhibit RON in biochemical and cell-based testing. **The purpose of this proposal is to test effectiveness and safety of TGN-HCI-1003 and TGN-HCI-1005 in *in vivo* preclinical models of established breast cancer metastasis to prepare for early clinical testing in metastatic breast cancer patients.**

In Aim 1, these inhibitors will be tested in immunocompetent mouse models of metastatic breast cancer, which have previously been show to recapitulate many aspects of the human disease. Two approaches will be taken. In Aim 1a, mouse mammary tumor cells with the ability to metastasize spontaneously will be injected into the mammary gland, and mice will receive RON inhibitor treatment once metastases are established and detectable. In Aim 1b, tumor cells will be introduced directly into the metastatic sites of the bone or lung, and RON inhibitor treatment will begin once bone or lung tumors are detected. In Aim 2, RON inhibitors will be tested in human models of metastatic breast cancer, using a spontaneously metastasizing human breast cancer cell line and spontaneously metastasizing patient-derived xenograft models in immunodeficient mice. Aims 1 and 2 are complementary to one another, as Aim 1 will allow us to evaluate the inhibitors in the context of a fully functioning immune system, and Aim 2 will utilize human breast cancer lines. In both aims, metastatic burden, anti-tumor immunity (Aim 1 only), bone turnover, and survival will be evaluated to assess effectiveness. Safety will be evaluated by monitoring mice for changes in weight, appearance, behavior, and histology of kidney, liver, and RON-expressing tissues.

The goal of this proposal is to translate our extensive pre-clinical findings to clinical application as quickly as possible. Our pre-clinical and early clinical data already show that RON inhibitors can help the immune system eliminate metastatic breast cancer cells, interrupt a pro-metastatic cycle in the bone, and target the metastatic cells themselves. Data from this proposal are anticipated to provide support for the key next step: transitioning novel RON inhibitor compounds into early phase clinical trials. It is anticipated that patients with high RON signaling in their tumors may benefit from RON inhibitor treatment, as well as patients without high RON signaling in their tumors, due to effects of RON inhibitors on the host microenvironment.