

**Background.** Deaths from breast cancer are not caused by the primary tumors because they are surgically removed in the majority of patients. Breast cancer kills patients by generating secondary tumors in vital organs such as the lung, bones, brain, or liver. These secondary tumors are called “metastases” and represent Stage IV cancer. *How do these secondary tumors form?* Some tumor cells acquire special characteristics that allow them to escape from the primary breast tumor, enter a blood vessel (a process called “*intravasation*”), travel through the bloodstream (“*dissemination*”), and then exit the blood vessels in another organ (“*extravasation*”) where the tumor cells grow to form secondary tumors.

**Clinical problem and critical barrier to progress in the field of metastasis.** 90% of cancer deaths are due to metastasis. Despite the progress that has been made in cancer treatments, we are still unable to effectively block metastasis. *Why is this so?* Because until now current therapies have been designed to target only the growth of primary and/or secondary tumors. While these therapies can slow, or even reverse tumor growth, tumor cell dissemination cannot be treated with current therapies, and this leads to a continual spread of tumor cells throughout the body and hastens the death of Stage IV metastatic patients. This gap in our anti-cancer arsenal is due to a lack of understanding of dissemination, which is technically difficult to study. Therefore, *new technologies are needed to study the mechanisms underlying tumor cell dissemination so that new therapies can be developed to target this important step and address this clinical problem.*

**Rationale for the proposed project.** Our laboratory has been successful in studying tumor cell dissemination from primary breast tumors. We have been pioneers in a cutting-edge technology called intravital microscopy which allows us to observe the behavior of tumor cells in real time, in living animals. Using this technology, we discovered that tumor cell intravasation occurs through “doorways” in the tumor blood vessels that we have called **P-TMEM (Primary-Tumor MicroEnvironment of Metastasis)**. These structures are formed when three particular cell types touch each other: a tumor cell that over-express a protein called Mena, a macrophage (a type of immune cell), and an endothelial cell (a type of cell that forms blood vessels). These doorways cause an opening into the blood vessel through which tumor cells are able to enter the circulatory system, travel throughout the body, and exit in a second organ such as the lung. Rebastinib, a specific inhibitor of P-TMEM doorways, is currently in phase Ib clinical trial in metastatic breast cancer patients (NCT02824575) and drastically reduces circulating tumor cells in patients. Recently, we have also found the presence of TMEM doorways at secondary sites, in human lung metastases of breast cancer patients. We have named these **S-TMEM (Secondary-Tumor MicroEnvironment of Metastasis)** doorways. Therefore, based on these preliminary data, ***we hypothesize that, in patients with stage IV disease, metastatic dissemination occurs, even after the resection of the primary tumor, because S-TMEM doorways (in the lung) allow tumor cells to re-intravasate and re-disseminate to tertiary sites (e.g. bone, brain, liver). We further hypothesize that P-TMEM and S-TMEM doorways utilize the same molecular mechanisms. Consequently, by systemically blocking TMEM doorways with Rebastinib, we will be able to stop/prevent metastatic spread in Stage IV breast cancer patients.***

**Goal of the proposed project.** The goal of this proposal is to understand the mechanism of tumor cell re-dissemination from secondary sites in order to stop/prevent further metastatic spread in Stage IV breast cancer patients. The long-term goal is to eliminate mortality associated with metastatic breast cancer.

**Scientific approach.** To test our hypotheses, we will conduct experiments to answer the following questions: **(i)** Are S-TMEM doorways sites of tumor cell re-intravasation and re-dissemination to tertiary sites? **(ii)** Does Rebastinib block tumor cell re-intravasation and re-dissemination to tertiary sites? **(iii)** Do S-TMEM doorways use the same molecular mechanisms as previously identified in P-TMEM doorways? We will test our hypothesis in several breast metastatic cell lines and animal models using a pharmacological approach to deplete macrophages (immune cells) that assemble TMEM sites, along with our cutting-edge technologies.

**Clinical benefits for Stage IV metastatic breast cancer patients.** This project has a high clinical impact for Stage IV metastatic breast cancer patients because it will: **1)** Show that blocking S-TMEM doorways is an effective strategy for preventing further spread in Stage IV metastatic breast cancer patients; **2)** Give us confidence that the current clinical trial will effectively work in Stage IV metastatic breast patients with resected primary tumor; and **3)** Generate new knowledge of the mechanism of tumor cell re-dissemination that may lead to the development of novel therapeutic strategies for those patients who do not respond to current treatment. Therefore, this project will demonstrate that it is ***never too late to treat Stage IV metastatic breast cancer patients*** and, in line with METAvivor mission, will giving hope that metastatic cancer may be “*transformed from a terminal diagnosis to a chronic and manageable disease*”.