

## Public Abstract

Brain metastasis develops when cancer cells spread from a primary tumor to the central nervous system. It is a common complication in patients with advanced cancer. Of the 300,000 patients newly diagnosed with breast cancer in the United States annually, up to 20% will develop symptomatic brain metastases. In fact, the incidence of breast cancer brain metastasis is rising for several reasons. First, brain imaging technology has made it possible to detect brain metastases at an earlier stage. Second, successful new treatments for breast cancer, such as targeted therapy and immunotherapy, have prolonged patient survival, which gives more time to establish brain metastasis. Third, many drugs that effectively treat primary breast tumors cannot cross the blood-brain barrier and therefore cannot eradicate small, asymptomatic metastases in the brain. Unfortunately, brain-metastatic breast cancers do not respond well to current treatments, and fewer than 20% of patients with brain metastases survive for longer than 1 year. However, in the new era of successful targeted therapies and immunotherapy, which control the systemic disease well, more attention is turning to brain metastasis. Patients with a limited number of brain metastases stand to benefit from effective combination treatment of their brain lesions, with both improved quality of life and longer survival. Consequently, there is an urgent need to identify therapeutically targetable factors that promote breast cancer brain metastasis. Identifying these factors will guide discovery of more effective treatments for brain metastasis.

Tumors transform the tissues around them, creating their own tumor microenvironment (TME), which they use to establish a supply of nutrients and evade the body's immune system. In the brain, the TME supports the seeding, colonization, and outgrowth of metastatic cancer cells. Therefore, treatments that can modulate the TME to inhibit metastasis growth are effective for brain metastasis. However, just how cells in the brain TME, especially immune cells, contribute to metastasis has not been well studied. We have found that when metastatic breast cancer cells colonize the brain, they increase the secretion of the signaling molecule CCL2. This attracts immune cells called tumor-associated CCR2+ myeloid cells into the brain. Mice with breast tumors in which CCL2 secretion was genetically suppressed lived longer and had fewer CCR2+ myeloid cells in the brain than did mice with high CCL2 because tumor cells in the brain metastatic lesions with CCL2-knockdown grew more slowly and even died. These data indicate that myeloid cells are essential for the growth of brain-metastatic breast tumor cells in mice and that suppressing CCL2 or CCR2 may help to slow or stop the growth of metastases.

Importantly, CCL2/CCR2 can be suppressed not only with genetic modification, but also with drugs that can be used in humans. Therefore, we **hypothesize** that CCL2/CCR2 promotes breast cancer brain metastasis and that drugs that inhibit CCL2/CCR2 may be effective new treatments for breast cancer brain metastasis. We propose 2 Specific Aims to investigate whether targeting CCL2/CCR2 will effectively treat breast cancer brain metastasis. In Aim 1, we will use multiple breast cancer brain metastasis mouse models to further confirm that CCL2 promotes brain metastasis and to better understand the biological mechanisms by which it does so. In Aim 2, we will conduct preclinical trials in mice to test the efficacy of targeting CCR2 with a clinically in-development drug named PF-04136309 for the treatment of breast cancer brain metastasis. Because CCR2+ myeloid cells make brain metastases less sensitive to the current immunotherapy approach, e.g. immune checkpoint blockade (ICB), we will also test whether PF-04136309, by blocking tumor-associated CCR2+ myeloid cells from infiltrating the brain, may make brain metastases more sensitive to ICB. Specifically, we will test whether combining PF-04136309 with ICB can inhibit the growth of brain metastases and prolong mice survival. If these studies are successful, our findings could be translated to clinical trials in the near future.

This research will produce novel and deeper insights into how tumors metastasize by altering the brain's immune system and may bring about a novel and effective immune-modulating therapy for breast cancer brain metastasis. Ultimately, we expect that it will lead to new and better treatments for breast cancer brain metastasis, which will prolong patients' survival and improve their quality of life.