PUBLIC ABSTRACT.

Rationale. In 2019, the FDA approved the first immunotherapy for breast cancer. The clinical trials that led to this approval showed, for the first time, that the body's immune response can be directed to kill breast cancer and delay disease progression. These immunotherapies are also known as immune checkpoint inhibitors. They work by stimulating T cells, an immune cell type that loses the ability to come into contact with and kill tumor cells as the tumor grows. Even though there was a lot of excitement surrounding the use of immune checkpoint inhibitors in metastatic breast cancer, immunotherapies are currently only used in some people with metastatic triple-negative breast cancer.

More than half of breast cancers spread to the liver, a metastatic site that often has the poorest response to immune checkpoint inhibitors. Liver metastases may be unresponsive to immunotherapy because they are 'immune cold,' meaning they have fewer T cells than other breast tumors. However, it is unclear why liver metastases have few T cells. *To overcome this knowledge gap*, we looked at immune cells in human breast cancer liver metastases and found that tumors could be divided into two different groups. One group we called immune hot (or immune infiltrated) because the tumors had a lot of immune cells. The other group had very few immune cells, which we called immune cold (or immune ignored) tumors. Comparison of immune hot and immune cold liver metastases showed that several factors in the tumor were different. One different factor was anterior gradient-2 (AGR2) that was higher in immune cold compared to immune hot tumors. AGR2 is a factor that can function inside tumors cells. AGR2 can also function outside of tumor cells after it is released or secreted by the tumor. Preliminary studies from other groups showed that secreted AGR2 decreased the number of immune cells in tumors. We showed that AGR2 is high in about half of liver metastases from all breast cancer subtypes. We also showed that AGR2 treatment increased the distance between T cells and tumor cells in cell culture model systems.

Objective. The overall goal of this proposal is to identify factors that are made by breast cancer liver metastases so they can block T cells from getting into the tumor, such as AGR2. Addressing this goal will include using established animal models of breast cancer liver metastasis and developing novel patient-derived models. In future work, we hope to develop antibodies to target these identified factors, starting with AGR2. Our long term goal is to develop these antibodies for clinical use and test them in clinical trials as new immunotherapies in metastatic breast cancer.

Approach and expected outcomes. In this application, we propose to test if targeting AGR2 increases the number of T cells in breast cancer liver metastases. The first scientific goal is to determine if tumor production and secretion of AGR2 prevents T cells from entering the tumor. We expect that blocking AGR2 will decrease this effect and increase the number of T cells in mouse models of breast cancer liver metastasis.

Our second scientific goal will be to create better cancer models for liver metastasis that more closely represent real tumors. This will be completed using the exceptional tissue collection resources at the University of Pittsburgh and UPMC Hillman Cancer Center. By partnering with the 'Hope for OTHERS' tissue donation program, we plan to develop additional patient-derived models of breast cancer liver metastasis. Development and characterization of these models will allow our group and others to identify more therapeutic targets for breast cancer liver metastasis.

Clinical impact on stage IV breast cancer. We anticipate that these studies will provide the data required to further develop AGR2 antibodies as a new therapy for metastatic breast cancer. These AGR2 antibodies would be a similar treatment approach to Herceptin or Keytruda. However, unlike Herceptin or Keytruda, we predict that AGR2 antibodies will be able to target metastases from all breast cancer subtypes. Additionally, we will make the patient-derived models we develop and characterize available to the public upon request. We expect that this will lead to significant advancements in the field of breast cancer liver metastasis research. Together, the proposed aims will have a profound impact on the development of new treatment approaches for breast cancer liver metastasis.