

PUBLIC ABSTRACT

Significance: Breast cancer (BC) can be treated with systemic therapies administered intravenously or orally, but drug resistance remains a significant bottleneck for improving BC prognosis. Immunotherapies, using monoclonal antibodies (mAbs) targeting HER2 in HER2-positive BC or immune checkpoint inhibitors (ICI) in triple-negative BC, have improved prognosis, particularly in metastatic BC (mBC). The heterogeneity of mBC subtypes, grades, and stages prevents using one treatment that fits all, emphasizing the need for personalized medicine to treat mBC effectively. Efforts to develop individual patient-specific mAbs, generated from the immunization of patient-derived mBC cells, have not been successful yet, although their potential benefit for mBC patients is evident.

Impact: This proposal will demonstrate the practical application of personalized medicine for treating mBC and allow for a deeper understanding of the breadth of the immunogenicity and antigenicity of mBC cells. Successful completion of this project will hold great promise to increase the efficiency of immunotherapies by using personalized mAbs in conjunction with current ICI therapies. While dramatically reducing the time to discover new mAbs, this platform will allow to test the mAbs' efficacy *in vitro* and *in vivo*, accelerate their translation to clinical trials, and eventually deliver them to the population. Lastly, this platform can be easily applied to promote personalized therapies for BC and other malignancies.

Background/Rationale: BC is one of the most commonly diagnosed cancers in women. Invasive BC (iBC) is a heterogeneous disease with different subtypes that are successfully treated when diagnosed early. However, nearly 30% of women diagnosed with early-stage iBC will develop mBC. mBC is treated with cytotoxic, hormonal, and immunotherapeutic agents, yet resistance to therapy is expected. Therefore, the development of novel treatments is required to treat mBC effectively. Targeting mBC cell surface markers using mAbs is promising; however, the heterogeneity of BC prevents the generation of one mAb that fits all. Personalized mAbs, generated against an individual's BC tumors, can improve the prognosis of mBC; however, current manufacturing processes of mAbs remain impractical and costly for personalized medicine applications. Microengineered systems can accelerate the development of mAbs. The minimization of cell culture volumes enables the rapid concentration of mAbs in cell culture supernatants. High-throughput (HT) microscale systems increase the speed of screening (from weeks to hours), the number of single cells per screen (~10,000 fold), and the number of epitope specificities (~10 fold). These platforms offer practical solutions to develop BC personalized mAbs. Therefore, this proposal aims to develop a HT screening platform to rapidly generate BC personalized mAbs libraries and demonstrate the application of microscale systems for personalized medicine.

Approach: The combination of microwells, microarray, and multiplex technologies will allow for the effective development of a robust pipeline for the efficient generation of BC-specific personalized mAbs using a microscale platform. To accomplish this task, we propose a 2-step strategy described in **Figure 1**.

Aim 1. Develop a HT sandwich microarray platform for rapid mAbs selection. We will fabricate a sandwich microarray platform consisting of single hybridoma cell-containing microwell arrays covered by a glass slide micropatterned with target cells. We will optimize single-cell immobilization and micropatterning efficiencies, cell viability, limits-of-detection, antibody secretion rates, and single-cell recovery rates.

Aim 2. Generate BC personalized mAbs libraries using the HT platform. In a CD34+ humanized mouse, mAbs raised against different mBC cell lines will be selected against mBC cells and their healthy counterparts using the HT screening platform. BC-specific mAbs will be isolated, their target antigen identified, and their effector function characterized using a three-dimensional mBC spheroid model.

Completion of this project holds great promise to accelerate the discovery of melanoma-specific personalized mAbs with therapeutic potential. This platform can be easily applied to other types of cancer.