## Lay Abstract

Breast cancer remains the second leading cause of cancer death among women. With primary breast cancer largely manageable by surgical resection, chemotherapy, and radiotherapy, metastatic breast cancer (MBC) remains a major threat and is resistant to most of conventional therapies. To date, there is still no cure for MBC, motivating the development of new therapies for treating MBC. Immunotherapies have shifted the paradigm for cancer treatment in the past decade, especially with the success of checkpoint blockades and chimeric antigen receptor (CAR) T cell therapy. However, the limited patient response rate to checkpoint blockades, poor efficacy against solid tumors for CAR T therapy, and severe side effects in both have limited their wide applications. Therapeutic cancer vaccine is one of the most promising modalities for treating MBC, by inducing the presentation of MBC antigens on antigen presenting cells (e.g., dendritic cells (DCs)) in the body to generate persistent MBC-specific cytotoxic T lymphocyte (CTL) response. However, the therapeutic benefit of existing cancer vaccines is still limited in general. Hurdles for developing potent cancer vaccines include the lack of available tumor antigens and sub-optimal modulation of DCs. Tumor-secreted nano-sized extracellular vesicles (EVs) are a good source of tumor antigens and have been widely explored as cancer vaccines in preclinical studies and clinical trials. However, the antitumor efficacy is far from satisfactory, likely as a result of poor CTL response. **The primary goal of this project is to develop a strategy that can well integrate tumor EVs and adjuvants for the development of potent MBC EV vaccines**. To achieve this, we utilize a metabolic glycan labeling approach to label MBC cells with chemical tags (e.g., azido groups), and hypothesize that EVs secreted by these labeled MBC cells will carry chemical tags. The chemically tagged MBC EVs can covalently conjugate adjuvants and other immunomodulators via efficient click chemistry, for improved activation and antigen presentation of DCs and enhanced CTL response. This project will be organized around three specific aims. In *Aim 1*, metabolic labeling of mouse and human breast cancer cells, including surgically resected or biopsied human MBC, with azido groups and subsequent generation of azido-labeled EVs will be studied and optimized. The conjugation efficiency of adjuvants to breast cancer EVs will also be studied. In *Aim 2*, adjuvant-conjugated breast cancer EVs will be evaluated for DC activation, CTL response, and antitumor efficacy in murine MBC models. In *Aim 3*, the CTL response and antitumor efficacy of EV vaccines derived from surgically resected or biopsied human MBC will be evaluated in $CD34^+$ humanized mice. Successful completion of this project will yield potent and safe EV vaccines for treating MBC. Our EV tagging technology can be fabricated from biopsied human MBC within 3-7 days. The proposed TLR agonists and EVs have been extensively tested in clinical trials with favorable safety profiles. These confer high translation potential for our platform.