**METAvivor Progress Report: Lay Description and Important Outcomes**

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Breast cancer (BC) is one of the most diagnosed cancers in women. Despite successful treatment when diagnosed early, resistance to therapy is expected, constituting a significant bottleneck for improving BC patient prognosis. Therefore, developing novel therapies is required to treat BC effectively, particularly for metastatic BC (mBC). Immunotherapies using monoclonal antibodies (mAbs) targeting HER2 in HER2-positive BC or immune checkpoint inhibitors in triple-negative BC can improve patient prognosis; however, the heterogeneity of mBC subtypes, grades, and stages prevents using one treatment that fits all patients, emphasizing the need for personalized medicine, particularly mAbs, to treat mBC effectively.

Since the discovery of mAbs, their use has revolutionized modern medicine. Today, the discovery and screening of mAbs is enabled by microscale technologies and their high-throughput (HT) capabilities. However, these technologies are exclusively protein-based and not cell-based, thus preventing the screening and discovery of mAbs specific for antigens that are either novel, rare, highly expressed, or expressed as a conformational isoform on the cell surface. Therefore, we developed a novel cell-based micro-ELISA platform for the HT screening of mAbs against unknown antigens.

This novel cell-based micro-ELISA platform consists of a microwell array containing antibody-secreting hybridoma cells sandwiched with a glass slide covered with a monolayer of target cells. The microwell array, containing 143,360 microwells, was fabricated with standard soft lithography. We achieved a single-cell seeding efficiency of 40%, effectively screening 57,344 single hybridoma cells per assay. We also rapidly generated uniform monolayers of cells expressing high levels of target antigens (96 ± 4%) and confirmed that uniform target cell monolayers on coverslips could detect mAbs at a high resolution. Lastly, we confirmed that our cell-based sandwiched micro-ELISA platform detected mAbs secreted by single or multiple hybridoma cells. This platform demonstrated that we detected 31 ± 7% contained a single hybridoma cell, and 5 ± 1% contained more than one hybridoma cell. Empty microwells did not demonstrate false positivity, and microwells containing hybridoma cells showed very low (<5%) false negativity. The outcomes of this work resulted in a manuscript in preparation/finalization entitled "Micro cell-based ELISA platform for the high-throughput screening of monoclonal antibodies."

We presented a novel cell-based micro-ELISA platform for the high-throughput screening of monoclonal antibodies. This novel platform could offer a robust pipeline for the efficient screening and discovery of mAbs specific for novel, rare, and conformational antigens expressed on the cell surface. This technology platform could be applied to rapidly isolate mBC-specific mAbs in a personalized manner using biopsy tissues, thus accounting for the heterogeneity of mBC subtypes, grades, and stages. A shift towards personalized therapies has great promise in addressing the limitations of current immunotherapies and achieving complete remission in mBC patients. It is anticipated that mAb-based therapies tailored to individual patients should selectively target individual patients' BC cells and potentially improve the prognosis of patients with mBC.