Background T cells that are genetically modified to express chimeric antigen receptors (CARs) show promising results for treating hematological tumors, however CAR-T cell therapy have thus far demonstrated limited anti-tumor activity in solid tumors.\(^1\) The immunosuppressive tumor microenvironment (TME)\(^2\) and T cell dysfunction, driven by chronic antigen exposure in solid tumor, likely contribute to the CAR-T resistance. In order to advance the CAR-T therapy into patients with solid tumors, we need models which accurately represent the TME to evaluate CAR-T efficacy at the discovery, preclinical and translational stages of R&D.

Methods Using a proprietary 3D hydrogel patterning technology,\(^3\) a 3D in vitro tumor model was generated in 96-well plates utilizing breast cancer cells and human dermal fibroblasts to reflect the tumor and stromal compartments, respectively, of the tumor microenvironment. Specifically, the commercially available HER2-positive breast cancer cell line, JIMT-1, and the triple-negative patient-derived xenograft (PDX) cell line MAXFTN 401, were utilized in these 3D TME models and subsequently interrogated with HER2-specific CAR-T cells as well as untransduced T cells. 5,000, 10,000, or 25,000 T cells were added to each well of the 3D in vitro models and apoptosis via Caspase 3/7 staining was analyzed at day 4 endpoint using high content imaging.

Results T cell-mediated killing in the respective models was highly dependent on their HER2 status – HER2-positive JIMT-1 demonstrated a dose dependent effect in apoptosis (Caspase 3/7 marker) and up to 42% of JIMT-1 cells underwent apoptosis in response to HER2-specific CAR-T cells, while less than 5% of HER2-negative MAXFTN 401 showed a response to any therapeutic dose of the CAR-T cells. Moreover, inclusion of fibroblasts in the 3D TME model enhanced the CAR-T mediated tumor killing in JIMT-1 model. Finally, the untransduced T cells demonstrated negligible effects (<2% in the JIMT-1 model), highlighting the specificity of the HER2-targeting CAR-T cells.

Conclusions A novel 3D in vitro tumor model platform has been described for assaying CAR-T efficacy. In this case, the platform demonstrated the highly specific nature of the CAR-T cells in targeting HER2-positive tumors cells in a translationally relevant 3D in vitro TME model.

REFERENCES