3D *in vitro* Tumor microenvironment models for screening CAR-T cell therapy efficacy.

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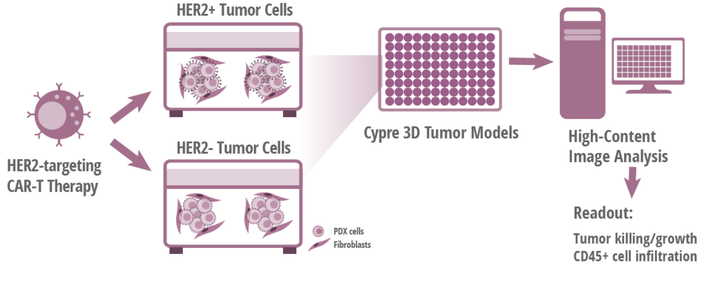
1 INTRODUCTION

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 (Rodriguez-Garcia et al., 2020). The immunosuppressive tumor microenvironment (TME) (Pitt et al., 2016) 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.

2 METHOD



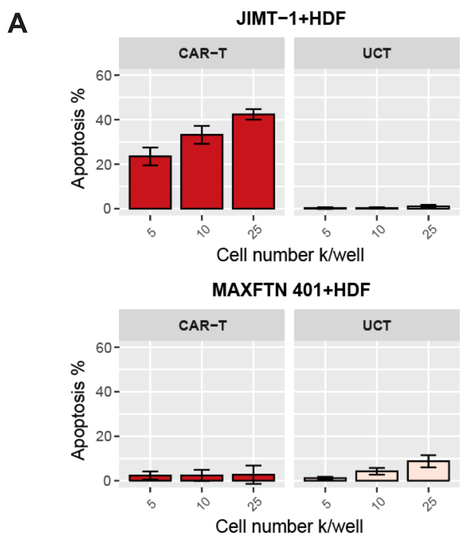
Setup of the Cypre 3D tumor model for assaying CAR-T cell therapy. HER2-targeting CAR-T cells were added into 3D *in vitro* tumor models comprising breast tumorspheres co-cultured with human dermal fibroblasts in a 3D hydrogel matrix. HER2-positive breast cancer cell line, JIMT-1, and the triple-negative patient derived xenograft (PDX) cell line, MAXFTX401, were utilized in this particular study. Responses were analyzed on day 4 endpoint using high-content imaging for tumor apoptosis (Caspase 3/7 substrate staining) and immunofluorescent CD45+ cell infiltration.

4 CONCLUSIONS

- **Cypre 3D hydrogel *in vitro* tumor models rapidly screen CAR-T cell therapies for critical endpoints such as tumor killing and immune cell infiltration with high content image analysis *in situ*.**
- **HER2-targeting CAR-T showed selective killing of HER2-positive tumor model, compared to untransduced T cells and the HER2-negative control.**
- **Rapid and scalable 3D *in vitro* models for screening cell therapy may be leveraged in early discovery, pharmacology and translational research.**

3 RESULTS

Fig 1. Tumor killing analysis of HER2-targeting CAR-T therapy in Cypre 3D tumor models using Apoptosis.



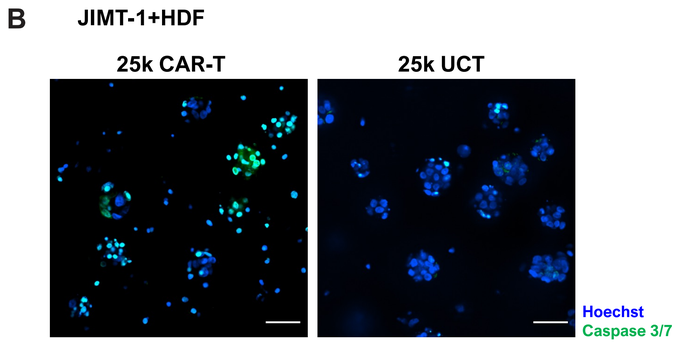
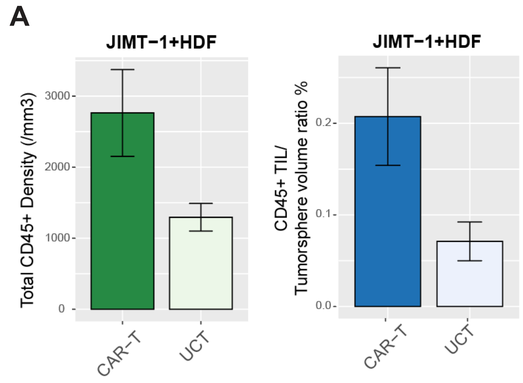


Fig 1. 3D *in vitro* hydrogel 3D CAR-T assay showing the on-target killing of HER2-positive tumor cell line, JIMT-1 by the HER2-targeting CAR-T therapy. 5k, 10k and 25k of HER2-targeting CAR-T cells and untransduced T cells (UCTs) were added to the 3D-culture. Only HER2-positive JIMT-1 cells demonstrated a dose-dependent response in apoptosis to HER-2 targeting CAR-T. A) Quantitation of the percentage of apoptotic tumor cells (Caspase 3/7 substrate staining). B) Representative images of JIMT-1 treated with 25k CAR-Ts or UCTs. Scale bar = 100 µm.

Fig 2. Immune infiltration of CAR-T cells in Cypre 3D tumor models using CD45+ immunofluorescence.



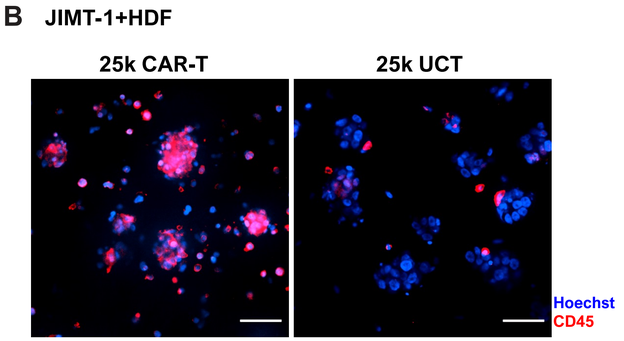


Fig 2. 3D immune infiltration of HER2-targting CAR-T cells in the HER2+ Cypre 3D model for JIMT-1.
A) Quantitation of total CD45+ immune cells infiltrated into hydrogel, left, or CD45+ immune cells infiltrated into tumorspheres, right. B) Representative images of JIMT-1+HDF co-culture treated with 25k CAR-T or UCT cells labeled with anti-CD45 by immunofluorescence staining, showing CAR-T infiltrate into the hydrogel and tumorspheres. Scale bar = 100 µm.

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