EVALUATION AND DEVELOPMENT OF DUAL AND TRIPLE ANTIGEN TARGETING CAR-T ENGAGER PROTEINS FOR HER2-POSITIVE CNS METASTASES AND SOLID TUMORS

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Background Solid tumors display pronounced antigen heterogeneity and clinical studies have shown that antigen escape from therapy occurs rapidly, limiting the persistence and efficacy of CAR T cells. Here we present dual and triple-antigen binding proteins that bridge CAR T cells to multiple antigens, allowing a simultaneous attack on tumor antigens by a single CAR T antibody domain. We call these CAR T Engager proteins. CAR T Engager proteins can be encoded into lentiviral vectors for secretion from CAR T cells, can be encoded into oncolytic viral vectors for secretion from transduced tumor cells, or can be engineered as biologics for injection.

Methods CAR T Engagers contain a protein target for a CAR T cell, eg. an anti-CD19 CAR T cell. We have previously presented a Her2-binding CAR T Engager protein with potent in vivo activity against solid tumors. We used this CAR T Engager as the basis for building dual and triple antigen binding proteins. Specifically, we mapped antigen expression for Her2-positive solid tumors, Her2-positive metastases, and primary CNS tumors. Our analysis identified expression patterns of two and three antigens that would essentially saturate the cellular composition of specific solid tumors, greatly reducing the chance of antigen escape from therapy. We created the corresponding CAR T Engagers and have developed single, dual and triple antigen expression cells lines to model the activity and potency of these novel proteins, administered alongside CAR T cells.

Results CAR T cells plus dual antigen CAR T Engagers that recognize and target Her2 and B7H3 demonstrate potent cytotoxicity against either antigen alone, and synergistic potency (2 pM) if both antigens are expressed. Similarly, triple antigen CAR T Engagers show single antigen binding and potent cytotoxicity which is enhanced when multiple antigens are expressed on a target cell. All of the cytotoxicity is mediated through one CAR domain expressed on the primary T cells. T cells can be pre-loaded with multi-antigen CAR T Engagers and retain cytotoxic activity. Because the underlying CAR is an anti-CD19 CAR, cell persistence and fitness is further enhanced in the presence of normal B cells.

Conclusions The CAR T Engager platform is a robust and modular solution for the multi-antigen targeting of solid tumors. Diverse antigens can be readily targeted for diverse indications. Examples of other functional modalities that can be added will be presented.

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