334 CAR T ENGAGERS ENCODING IMMUNE CHECKPOINT INHIBITORS AND IMMUNE-RELEVANT CYTOKINES STIMULATE CAR T CELL ACTIVITY AGAINST HEMATOLOGIC AND SOLID TUMORS

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Background Cell therapeutics are limited by critical issues including antigen escape, antigen heterogeneity, tumor and tumor microenvironment mediated immune suppression and suboptimal T cell expansion, fitness and persistence. We originally created CAR T Engagers (CTEs) to address the issues of antigen escape and heterogeneity. Here we present novel domains added to CTEs to overcome immune suppression and enhance CAR T cell function.

Methods Our first generation of functionally enhanced CTEs are built on multi-antigen targeting modules. ALETA-001 is a biologic CTE that binds to CD20 and displays the CD19 extracellular domain (ECD). ALETA-002 is a lentiviral construct that expresses an anti-CD19 CAR domain and a CTE that binds both Her2 and B7-H3. ALETA-001 and -002 CTE protein sequences were further modified to contain additional functional domains including an anti-PD-L1 VHH, a monomeric TGFbetaR2 TRAP, the CD2-binding domain of LFA3, a T cell stimulating cytokine, and an immune system activating cytokine. CTEs that were functionally enhanced (FE) were evaluated for their ability to promote anti-CD19 CAR T-mediated cytotoxicity, to overcome immune suppression and immune escape, and to productively stimulate CAR T cells and engage with endogenous immune cells.

Results Functionally enhanced CTE's directed anti-CD19 CAR T cells to attack and kill cancer cells including B cell lymphomas (ALETA-001-FE) and solid tumors (ALETA-002-FE). Further, ALETA-001-FE-1 countered loss of CD19 antigen and provided additional costimulation via LFA3/CD2 engagement. ALETA-001-FE-2 stimulated CAR T expansion via enhanced engagement of common-gamma chain signaling. ALETA-002-FE-1 and ALETA-002-FE-2 mediated potent killing of Her+, B7H3+ and dual+ solid tumor cells. ALETA-002-FE-1 also blocked TGFb signaling with a sub-nM IC50. ALETA-002-FE-2 blocked PD-L1 activity with similar potency.

Conclusions Anti-CD19 CAR T cells demonstrate best-in-class expansion, fitness and persistence due to their ability to interact productively with normal CD19+ B cells in circulation and within secondary lymphoid organs that provide T cell nurturing signals. Anti-CD19 CAR T cell use for B cell lymphoma therapy was enhanced by limiting antigen escape and increasing costimulation via LFA3/CD2 binding Anti-CD19 CAR T cells can be redirected to solid tumor antigens by the use of CAR T-secreted CTEs. Here we used multi-antigen targeting, ie. by linking the CD19 ECD to anti-Her2 and anti-B7H3 domains, by providing productive cytokine signaling and by countering immune suppression. Notably, all these functions are incorporated into the CTE, leaving CAR T engineering as separate tool. CTEs carrying multiple domains designed to simultaneously enhance functionality are being designed and evaluated and will be presented.

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