SYNTHETIC RE-DIRECTION OF TGF β RECEPTORS AS A NOVEL STRATEGY TO ENHANCE THE ANTI-TUMOR ACTIVITY OF CAR-T CELLS IN SOLID TUMORS

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Background Transforming growth factor beta (TGF β) is an immuno-suppressive cytokine present in the tumor microenvironment (TME) that creates considerable challenges for the treatment of solid tumors. Small molecule inhibitors targeting TGF β exist, but the pleiotropic nature of TGF β signaling suggests that a more targeted approach is preferential, especially in the context of cellular therapy. We hypothesized that primary T cells and iPSC-derived chimeric antigen receptor-T cells (CAR-iT cells) would benefit not only from blockade of TGF β signaling, but also from re-direction of the signaling event toward specific cytokine pathways that activate cell function. Here we discuss novel synthetic TGF β redirector constructs that overcome TME limitations and enhance CAR-iT cell function for improved efficacy in treating solid tumors.

Methods To identify activation pathways for redirection of TGF β signaling, we screened a panel of cytokines for their effect on the anti-tumor activity of CAR-iT cells. We then developed synthetic redirector receptors where a TGFBR2 ectodomain was fused to the top selected cytokine receptor endodomains. Redirection of TGF β signaling was confirmed by phospho-flow of key signaling proteins. Anti-tumor activity of CAR-iT cells expressing these synthetic redirector constructs was tested in serial restimulation assays in the absence of cytokine support and in the presence of recombinant TGF β (rTGF β).

Results A dose-dependent decrease in CAR-iT cell cytolytic capacity in the presence of rTGF β was observed, with the activity of CAR-iT cells rescued in the presence of unique cytokines. We designed and tested synthetic TGF β redirector constructs and demonstrated a rTGF β -dependent increase in pSTAT5 positive cells (2.8-fold over control). The serial stimulation assay was then used to test CAR-iT cells engineered with synthetic TGF β redirector receptors. After three rounds of restimulation, an increase in tumor cell numbers for non-engineered and dominant negative TGFBR2 CAR-iT cell controls was observed (41-fold and 32-fold increase over base input, respectively). In contrast, the synthetic TGF β redirector receptor improved the ability of CAR-iT cells to control tumor cell growth with remarkable efficiency, limiting tumor growth to only 1.5-fold over three rounds of restimulation.

Conclusions These studies demonstrate that a novel synthetic construct comprised of fusion of cytokine endodomains to a TGFBR2 ectodomain can be deployed to hijack the immuno-suppressive signal of TGF β often found in the TME and activate CAR-iT cells for enhanced anti-tumor activity in solid tumors. Additional studies are underway to assess the temporal expression and activity of these synthetic redirector receptors in various preclinical models which will be further discussed.

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