138 SYNTHETIC RE-DIRECTION OF TGFβ 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 (TGFβ) 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 TGFβ exist, but the pleiotropic nature of TGFβ 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 TGFβ signaling, but also from re-direction of the signaling event toward specific cytokine pathways that activate cell function. Here we discuss novel synthetic TGFβ 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 TGFβ 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 TGFβ 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 TGFβ (rTGFβ).

Results A dose-dependent decrease in CAR-iT cell cytolytic capacity in the presence of rTGFβ was observed, with the activity of CAR-iT cells rescued in the presence of unique cytokines. We designed and tested synthetic TGFβ redirector constructs and demonstrated a rTGFβ-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 TGFβ 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 TGFβ 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 immunosuppressive signal of TGFβ 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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