iPSC-derived CAR-γδT with Novel Combinatorial KO Demonstrated Extended Longevity and Profound Anti-tumor Efficacy Without Cytokine Support in Preclinical Studies

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The induced pluripotent stem cell (iPSC)-derived cell therapy bears unparallel competitiveness in the value proposition across the cellular medicine landscape. iPSC allows a given cellular therapeutic to be produced from a single cell clone that harbors all the desired genetic manipulations, thus ensures absolute homogeneity of the gene edits at the single cell level. The subsequent uniformed differentiation and expansion methodologies further safeguard the consistency of the therapeutic products and eliminate patient-to-patient or donor-to-donor manufacturing inconsistencies commonly seen in the autologous and allogeneic donor-derived cell therapies. These features will not only dramatically reduce the cost-of-goods but also enhance the affordability and accessibility, hence democratizing the application of the cell therapy globally.

The gamma delta (γδ) T cell possesses numerous unique characteristics that are advantageous as a cellular therapeutic in the clinic, such as manifesting minimal CRS, GvHD, and ICANS etc. Moreover, activated γδT cells could target a broad range of tumor cells and demonstrate the capacity for tumor reduction in murine xenotransplant tumor models. However, it is well known that the responses of the allogeneic T cell therapeutics are hampered by the lack of persistence and durable anti-tumor activity, which could dim the prospects of utilizing the γδT cell as an effective cancer therapeutic. Here, to overcome these shortcomings, through a hypothesis-driven, combinatorial KO of SOCS family genes, we successfully enhanced the durable killing capacity of iγδT by creating a “conditional” activation scenario that allows protracted and amplified signal transduction events induced by activating cytokines. We also lengthened the longevity of the iγδT by depleting pro-apoptotic genes to bolster effector’s intrinsic survival capability, while eliminating B2M/CIITA and increasing the expression of HLA-E to evade host immune surveillance. Further, a proprietary signal converter was designed to transform the soluble factors generated during tumor-effector engagement into the inducers of productive signal events that drive iγδT proliferation and activation. Altogether, these genetic engineering efforts that aim to achieve the goldilocks signaling balance could strengthen the efficacy and longevity of the iγδT, while avoid the possible dysfunction, like exhaustion, caused by introducing constitutively active cytokine receptors as many others have attempted. Building on this engineered platform, through incorporating chimeric antigen receptor (CAR) constructs against specific tumor associated antigens (TAAs), we further demonstrated that these engineered CAR-

iγδTs exhibited robust long-term durable killing (more than 2 weeks) without any supporting cytokines in the tumor rechallenging studies *in vitro*, accompanied by the significantly increased tumor lysis enzyme secretion. In addition, these CAR-iγδT cells, either single dosed or repeated, eliminated the engrafted tumor cells *in vivo* with persisted cellular kinetics and boosted expansion in the selected B cell- and myeloid-malignancy models.

Altogether, the summation of aforementioned data elevated our confidence of applying BeiGene's engineered CAR-iγδT in the clinic in the near future. Combining with the robust iPSC differentiation and expansion methodologies to derive γδTs, we believe that arriving to an inflection point of turning cell therapy into a massively produced medicine should not be far.