GENERATION OF IMMUNE-EVASIVE ALLOGENEIC CAR T CELLS BY INACTIVATION OF THE HLA TRANSCRIPTIONAL REGULATOR RFX5 AND DISRUPTION OF THE IMMUNE SYNPSE

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Background Autologous CAR T cell therapies have revolutionized the treatment of hematologic malignancies but have inherent disadvantages that hinder widespread access, including complex logistics and manufacturing limitations. These challenges may be overcome with off-the-shelf allogeneic CAR T cells derived from healthy donor T cells. Although allogeneic CAR T cells provide immediate availability to patients and the convenience of scalable manufacturing, they may be susceptible to immune rejection and may therefore have reduced persistence, limiting clinical responses. Immune evasion strategies centered on deletion of β2-microglobulin may avoid rejection by CD8 T cells but may elicit strong NK cell reactivity. Moreover, induction of HLA class II expression upon CAR T cell activation may increase the risk of rejection by CD4 T cells. We previously showed that inactivation of RFX5, a transcriptional regulator of HLA class I/II genes, resulted in effective resistance to T cell rejection and induced low NK cell reactivity. Here, we describe an additional anti-rejection strategy by inactivating CD58 and ICAM-1, key components of the immune synapse required for effective recognition and lysis by alloreactive T/NK cells. Knockout of either gene in allogeneic CAR T cells reduced alloreactivity and gave greater survival benefit in combination with RFX5 KO.

Methods CRISPR/Cas9 technology was used to knock out RFX5, B2M, CD58, ICAM-1 and/or TRAC. Survival of gene-edited CAR T cells in the presence of HLA-mismatched allogeneic T cells and NK cells was assessed in mixed lymphocyte reaction (MLR) assays. CAR T cell cytotoxicity was assessed in a serial stimulation assay.

Results CAR T cells with targeted deletion of RFX5, CD58 and ICAM-1 demonstrated enhanced survival, whereas unmodified CAR T cells were quickly eliminated by HLA-mismatched T cells (p<0.0001). Combination of CD58 KO with RFX5 KO potentiated evasion in MLR assays (p<0.0001), whereas unmodified control and B2M KO cells were eliminated by allogeneic T cells and NK cells, respectively. Expression of HLA molecules was unaffected in CD58 KO and ICAM-1 KO CAR T cells and as a result, allogeneic NK cell reactivity was not elicited. Importantly, inactivation of CD58 or ICAM-1 did not affect cytotoxic activity or elicit IL-2 independent CAR T cell expansion.

Conclusions Targeted deletion of CD58 or ICAM-1 effectively reduces T cell rejection of allogeneic CAR T cells without triggering NK cell rejection or impacting effector function and works additively with RFX5 KO. Off-the-shelf immune-evasive CAR T cells have the potential to resist rejection and achieve improved therapeutic responses.

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