413 GENETIC DELETION OF TIGIT ENHANCES CAR-NK CELL FUNCTION IN THE SOLID TUMOR MICROENVIRONMENT

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Background Natural killer cells (NKs) expressing chimeric antigen receptors (CAR-NKs) were successful in hematological malignancies.[1] However, solid tumors resist CAR-NKs via a tumor microenvironment (TME) that includes myeloid derived suppressor cells (MDSCs) and M2 macrophages (M2s).[2] We demonstrated that ex vivo manufacture of therapeutic CAR-NKs significantly upregulated **T** cell immunoreceptor with **Ig** and **ITIM** domains (TIGIT), an inhibitory NK receptor. Analysis of pediatric neuroblastoma and sarcoma patient tumors confirmed high expression of TIGIT ligands on tumor cells and intra-tumoral MDSCs and M2s. ***Our main objective*** was to determine influence of TIGIT on CAR-NK function in the TME. Current TIGIT-targeting approaches using antibodies are handicapped by poor bioavailability and transient binding in the TME. ***We hypothesized*** that genetic deletion of TIGIT on CAR-NKs will lead to a more profound and durable anti-tumor response within the TME.[3]

Methods TIGIT knockout (KO) CAR-NKs expressing a GD2.4-1BB.zeta CAR were generated by concurrent CRISPR/cas9 and retroviral transduction of expanded primary human NK cells. Degranulation (CD107a) and IFN-γ by $TIGIT^{KO}$. GD2.CAR-NK were assessed in short-term TME co-cultures containing LAN-1 neuroblastoma and human MDSCs. A novel long-term TME co-culture wherein human monocytes and CHLA255 neuroblastoma pre-established a suppressive TME for 72 hours prior to addition of $TIGIT^{KO}$ GD2.CAR-NK was used to assess tumor growth and CAR-NK proliferation over 96 hours using Incucyte. We assessed phosphorylated mTOR (pMTOR) in CAR-NKs by intracellular flow cytometry.

Results CRISPR/Cas9 and retroviral transduction generated stable $TIGIT^{KO}$.CAR-NKs (>90% TIGIT deletion; 60% GD2.CAR expression). $TIGIT^{KO}$ enhanced GD2.CAR-NK cytokine secretion but not degranulation in short-term TME co-cultures. In long-term tumor co-cultures, $TIGIT^{KO}$.GD2.CAR-NKs eliminated more tumor vs control NKs (<10% viable tumor vs 35% viable tumor, $p<0.01$; n=4 donors). In TME co-cultures, $TIGIT^{KO}$.GD2.CAR-NK rapidly proliferated and controlled tumor compared to $TIGIT^{wt}$.CAR-NKs (37% viable tumor vs 57% viable tumor, $p<0.05$; n=4 donors). $TIGIT^{KO}$ did not increase CAR-NK degranulation or Fas ligand expression, nor did it alter CAR-NK surface expression of DNAM-1, NKG2D, NKG2A, TIM-3, PD-1 or LAG-3. While $TIGIT^{wt}$.CAR-NKs massively downregulated pMTOR within the TME, $TIGIT^{KO}$.CAR-NKs maintained pMTOR expression. Ongoing studies using a novel in vivo TME xenograft model of neuroblastoma will determine benefit of $TIGIT^{KO}$ vs. TIGIT antibody therapy on CAR-NK activity.

Conclusions We defined a role for TIGIT in inhibition of CAR-NK function and suggest TIGIT deletion as a novel NK therapeutic platform to evade immune suppression in the TME. This highlights the potential of gene-edited CAR-NKs to improve clinical outcomes in patients with solid tumors.

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