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

Ishwar Navin*, Matthew Dysthe, Corrine Baumgartner, Robin Parihar. Baylor College of Medicine, Houston, TX, United States

Background: Natural killer cells (NKs) expressing chimeric antigen receptors (CAR-NKs) were successful in hematological malignancies. However, solid tumors resist CAR-NKs via a tumor microenvironment (TME) that includes myeloid-derived suppressor cells (MDSCs) and M2 macrophages (M2s). 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.

Methods: TIGIT knockout (KO) CAR-NKs expressing a GD2.4-1BB.ζ CAR were generated by concurrent CRISPR/Cas9 and retroviral transduction of expanded primary human NK cells. Degranulation (CD107a) and IFN-γ by TIGITKO. 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 where human monocytes and CHLA255 neuroblastoma pre-established a suppressive TME for 72 hours prior to addition of TIGITKO.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 TIGITKO.CAR-NKs (>90% TIGIT deletion; 60% GD2. CAR expression). TIGITKO enhanced GD2.CAR-NK cytokine secretion but not degranulation in short-term TME co-cultures. In long-term tumor co-cultures, TIGITKO.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, TIGITKO.GD2.CAR-NK rapidly proliferated and controlled tumor compared to TIGITwt.CAR-NKs (37% viable tumor vs 57% viable tumor, p<0.05; n=4 donors). TIGITKO 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 TIGITwt. CAR-NKs massively downregulated pMTOR within the TME, TIGITKO.CAR-NKs maintained pMTOR expression. Ongoing studies using a novel in vivo TME xenograft model of neuroblastoma will determine benefit of TIGITKO 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.

REFERENCES