**Background**

The efficacy of CAR-NK cellular therapies in solid tumors is limited by insufficient survival and expansion within the suppressive tumor microenvironment (TME). To augment CAR-NK survival and anti-tumor function, CAR-NK cells are typically administered with exogenous cytokines or other signal modifiers that converge on signal transducer and activator of transcription-5 (STAT5) activation, an essential signaling node for NK survival and function. However, systemic administration of these cytokines or signal modifiers is associated with toxicity, unintended bolstering of TME components, and CAR-NK rejection. Thus, novel strategies to enhance CAR-NK function within the TME are needed. To promote CAR-NK survival and function in the TME while bypassing the need for exogenous signal dependency, we genetically modified GD2-specific CAR-NK cells to express a constitutively active IL-7 receptor complex, termed C7R (C7R.GD2-NK), that intrinsically confers persistent STAT5 activity within NK cells.

**Methods**

To mimic challenges of the TME, we created an *in vitro* TME model by preconditioning CD14+ monocytes with CHLA255 neuroblastoma cells for 72 hours, converting them to an inhibitory M2-TAM phenotype expressing suppressive cytokines and ligands. 4 days after the addition of the C7R.GD2-NK cells to this immunosuppressive milieu, we assessed NK survival, expansion, cytokine secretion, and anti-tumor function.

**Results**

Resting C7R.GD2-NK cells exhibited 3.4-fold (± 0.7; n=4 donors, p<0.005) higher phosphorylated STAT5 (pSTAT5) compared to resting GD2-NK cells. C7R.GD2-NK cells alone in long-term culture expanded for 14 days without exogenous cytokine support but not indefinitely. In short-term co-cultures with GD2+ CHLA255 cells, C7R.GD2-NK demonstrated similar IFNγ levels as GD2-NK stimulated with exogenous IL-2 or IL-15 (mean% GD2-NK IFNy+ of 26.1, 21.2, and 22.4, respectively). In the *in vitro* TME model, C7R.GD2-NK cells expanded less than IL-15- or IL-2-stimulated GD2-NK cells (mean fold expansions of 2.8-, 4.5-, and 4.3-fold, respectively) but demonstrated similar anti-tumor activity. Furthermore, cell culture supernatants from C7R.GD2-NK treated conditions exhibited similar levels of NK effector cytokines IFNγ, TNF-α, and GM-CSF to exogenous cytokine-stimulated GD2-NK cells. Ongoing experiments in an *in vivo* TME xenograft model will assess the benefit of C7R in CAR-NK activity.

**Conclusions**

C7R drives STAT5 activity in GD2 CAR-NK cells within TME conditions without the need for exogenous cytokines. The NK-intrinsic STAT5 signal is sufficient to promote NK survival, expansion, and function within the TME, potentially without the side effects of exogenous cytokines or cytokine modulators. Thus, C7R may be a viable alternative therapeutic strategy to augment CAR-NK efficacy in solid tumors.

**REFERENCES**
