Engineered NK Cells for Optimal Anti-tumor Immunotherapy

Challice Bonifant
Johns Hopkins University School of Medicine, USA

Background: For most indications, engineered cell therapies have yet to become standard of care. Because of manufacturing complexity, specialized toxicity management, and highly regulated use, even early-stage clinical trials testing these agents are not available to all patients. To allow for increased access and product uniformity, allogeneic therapies are desirable. Natural Killer (NK) cells have powerful anti-tumor effects, can safely be collected from healthy donors, manipulated *ex vivo*, and infused to patients.

Results: To identify characteristics that define an optimal NK cell product, we investigated the surface receptor, transcriptional, and methylation phenotype of NK cells activated *ex vivo* using feeder cells expressing IL15 or IL21 and cultured with IL2 or IL2+TGFβ. We distinguished NK cell subsets by multiparameter flow and found that expanded NK cells had higher expression of activation markers (NKG2D, DNAM, NKp30, CD69), TIGIT, and CD96 that correlated with transcriptional expression. Whole genome bisulfite sequencing revealed genome-wide hypomethylation in expanded NK cells with single cell RNAseq illuminating characteristic compositional NK cell subsets per activation condition. We engineered *ex vivo* expanded NK cells to express chimeric receptors with 2B4 and CD3ζ intracellular signaling domains targeting the cancer-associated antigens CD123 (IL3Rα) and CD276 (B7-H3). CD123- and CD276-CAR NK cells have specific anti-tumor effect evident in *in vitro* and *in vivo* models. While anti-tumor activity in AML and brain tumor xenografts is associated with improved survival, a lack of CAR-NK cell persistence prevents cure. To increase persistence, we designed chimeric cytokine receptors (CCRs) based on the heterodimeric IL15 receptor using sequences from the native IL15Rβ and the common gamma chain and anti-CD123 or anti-CD276 binding moieties. Cytotoxicity of CCR-NK cells was tested in co-culture assays and *in vivo*. NK cells expressing CCR chains targeting CD123 or CD276 have powerful cytotoxicity against cancer cell lines that is dependent on intact CCR signaling. Anti-CD123 CCR-NK cells have increased long-term survival and continue to kill target through multiple rechallenges, with sustained survival and lower toxicity when compared to anti-CD123 CAR-NK cells and NK cells secreting IL15.

Conclusion: In summary, we have evaluated the determinant programing of *ex vivo* activation methods and tested NK cells expressing chimeric cytotoxicity and cytokine receptors in models of poor prognosis cancers. CD123-targeted CCR-NK cells have powerful and sustained anti-tumor activity and are being further evaluated for clinical translation as cancer immunotherapy.