**RESULTS**

**INTRODUCTION**

We previously developed a feeder cell system (FC21) for ex vivo expansion of natural killer (NK) cells based on the K62 cell line genetically modified to express 4-1BBL and membrane-bound IL21 (mIL21). This system enabled us to robustly generate large numbers of highly active NK cells (FC21-NK) from various sources. We applied this approach in clinical trials assessing autologous and allogeneic FC21-NK products delivered with or without chemotherapy or allogeneic stem cell transplant. To date, over 300 doses of FC21-NK cells up to 10^9/kg/ dose have been delivered to over 50 patients, without any major toxicities (see abstract S284 and EPS58).

The cell line was re-engineered to enable broader academic access and the expansion process was refined through the development of a non-cellular membrane-particle preparation (PM21) to enhance safety, efficiency, scale and product uniformity for commercialization, while providing the same storm for expansion as FC21. On a preclinical scale, these results resulted in a highly similar final product, but deep characterization of the impact of these process variations in representative material intended clinical use had not yet been done.

**OBJECTIVE**

To perform deep characterization of ex vivo expanded, cryopreserved FC21-NK cells and PM21-NK cells, in order to assess uniformity of product identity and potency across production sites (GMP or large-scale process development) and platforms (FC21 (Clone9), FC21 (CSTX), and PM21) intended for use in clinical trials (PM21-NK, e.g. NCT04395092) for AML.

**METHOD(S)**

**NK cell expansion:** Up to 10 samples of each NK cell type were obtained for comparison: (1) freshly isolated NK cells from peripheral blood, (2) cryopreserved FC21-NK cells expanded with the original Clone9/mLb21L1, (3) cryopreserved FC21-NK cells expanded with the newly-developed CSTX002, and (4) cryopreserved PM21-NK cells expanded with PM21 particles from CSTX002.

All cells were expanded at large scale in the process-development laboratory or in manufacturing facilities under good manufacturing practice (GMP) guidelines and are representative for clinical materials. Cells were cryopreserved in media containing DMSO and FBI or human serum albumin. Samples were thawed and rested in media containing 5% FCS, media survival rate was 95% (II/LL2) for 48h prior to testing. Recovery was determined by total viable cells by trypan-blue staining.

**Cytotoxicity assay:** NK cells were co-cultured for 4-hours with calcein-labeled K62 at 5:1 E/T ratio. Calcein release was determined and mean percent lysis calculated (1).

**Mass cytometry (FCYT):** NK cells were fixed in Prot-1 buffer (Smart tube inc) for 10 mins at room temperature. Cells were labeled with a panel of 32 metal-conjugated antibodies against common NK cell surface markers, followed by Iridium for doublet exclusion, and acquired on a third-generation Helios mass cytometer instrument. The data was analyzed using clustering algorithms viSNE and SPADS (2,3).

**Cytokine assay:** NK cells were stimulated with PHA. After 4-hours the levels of cytokines were measured in the supernatants using 37-plex bead-based array (MACS Plex cytokine kit - Milteny).

**REFERENCES**


**ACKNOWLEDGMENTS**

We would like to thank the Behnke lab for their help with CYTOF.

**KEY**

FC21-NK (Clone9): NK cells expanded with feeder cells Clone9/mLb21L1

FC21-NK (CSTX): NK cells expanded with feeder cell CSTX

PM21-NK: NK cells expanded with PM21 particles derived from CSTX002

**NOTE:** Additional comparisons are ongoing, but could not be completed for this poster as planned due to COVID-19.

**CONTACT INFORMATION:** Prashant Trika@NationwideChildrens.org, DenaLee@NationwideChildrens.org