DEVELOPMENT OF WU-NK-101, A FEEDER CELL-FREE EXPANDED ALLOGENEIC MEMORY NK CELL PRODUCT WITH POTENT ANTI-TUMOR ACTIVITY

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Background Allogeneic Natural Killer (NK) cells are emerging as a safe and effective modality for the treatment of cancer, overcoming several limitations associated with adoptive T cell therapies. Cytokine induced memory-NK cells offer several advantages over conventional NK cells, including enhanced functional persistence, efficacy, and metabolic fitness. Additionally, unlike iPSC and cord blood derived NK cells, they do not require engineering to enable functionality. Here we describe the use of WU-PRIME, a GMP-grade fusion protein complex to generate memory NK cells, and WU-EXPAND, a feeder cell free expansion system to expand memory-NK cells and create WU-NK-101. Further cryopreservation enables the large-scale, off-the-shelf manufacture of memory NK for cancer immunotherapy, with high anti-tumor activity.

Methods NK cells derived from healthy donor leukopheresate were either activated with WU-PRIME and then expanded with WU-EXPAND to form WU-NK-101 or immediately expanded with WU-EXPAND as controls and then cryopreserved. We compared NK cell expansion as well as post-thaw NK cell functionality as assessed by cytokine secretion and short-term and long-term anti-tumor functionality, long-term persistence in NSG mice, as well as anti-tumor activity in vivo.

60). Notably, this functionality is maintained long-term upon repeated challenge. In vivo, WU-NK-101 cells, compared to expanded NK cells have improved in vivo persistence (figure 1; 50,290 v. 9,623, $p < 0.0001$). In vivo anti-tumor activity was also assessed in leukemia models, where Memory NK cells demonstrate superior anti-tumor activity compared to expanded NK cells.

Conclusions The data demonstrate that WU-NK-101 generated using a feeder cell-free expansion system has a memory phenotype and improved in vitro and in vivo anti-tumor activity compared to conventional NK cells. This activation and expansion platform will enable the development and clinical translation of multiple allogeneic NK cell therapies.

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Results NK cells activated with WU-PRIME followed by WU-EXPAND (WU-NK-101), expand robustly in large-scale reactions, over 250-fold in 14 days. The cells maintain durable expression of CD25 after expansion, as well as several other hallmarks of the memory-NK phenotype as assessed by mass cytometry. As compared to cells expanded with WU-EXPAND only, WU-NK-101 cells have improved in vitro activity against K562 cells, as well as AML cell lines (TF-1, THP-1, and HL-