SINGLE-CELL TRANSCRIPTOMICS, PROTEOMICS AND IN VITRO CYTOTOXICITY OF ALLOGENEIC CRYOPRESERVED NATURAL KILLER CELL THERAPY GTA002 IDENTIFY POTENT EFFECTOR SIGNATURES

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Background Glycostem Therapeutics has developed a closed, automated, and feeder-free system (uniK™) for ex vivo expansion and differentiation of umbilical cord blood-derived CD34+ stem cells into highly functional, cryopreserved, off-the-shelf GTA002 NK cells, currently evaluated in a Phase I/II clinical study in AML, WiNK (NCT04632316).

Methods Extensive characterization of cell therapies is pivotal to advance product understanding and relate it to treatment outcomes. The identification of clinically relevant features such as product heterogeneity, mechanism of action, its interaction with target tumor cells is challenging and cannot be fully understood with the traditional metrics used in cell therapy, namely flow cytometry. Single-cell sequencing technologies enable unprecedented high-resolution analysis of the cell therapy molecular landscape.

Results In this study, we used scRNA-Seq to investigate the transcriptome and surface proteome of GTA002 clinical batches, which identified distinct sub-populations of NK cells. Molecular marker analysis distinguished clusters of potent effectors, characterized by high expression of cytotoxic genes, and of regulatory effectors, characterized by high expression of cytokine/chemokine receptors and other trafficking and homing signals. Notably, only minimal, non-T cell impurities were observed, in line with the excellent safety profile of NK cell therapies.

Aiming to link transcriptomics data to product attributes measurable in the wet lab and applicable to quality control analysis, we performed surface and intracellular proteomics of NK cell batches. Interestingly, we identified that signature genes from RNA-seq analysis were also able to distinguish highly cytotoxic effectors at the protein level. Furthermore, effector signatures correlated with in vitro killing of AML.

Conclusions In summary, this research contributes to the advancement of NK cell therapies by generating a deep understanding of the cells’ mechanism of action, and by linking it to features that can be measured during product manufacturing and QC. Notably, such pipeline is applicable to NK cell therapies from different sources, as well to genetically engineered products.

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