245 CORD BLOOD CD34+ STEM CELLS ARE EFFICIENTLY TRANSDUCED WITH ANTI-CD19-CAR AND EXPANDED AND DIFFERENTIATED INTO VIVENK™ NATURAL KILLER CELLS WHICH DISPLAY SELECTIVE CYTOTOXICITY AGAINST B-CELL LEUKEMIA

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Background Chimeric antigen receptor (CAR)-engineered Natural Killer (NK) cells are a highly promising option for adoptive cancer immunotherapy. Glycostem Therapeutics has developed a closed, automated, and feeder-free system for ex vivo expansion and differentiation of umbilical cord blood-derived CD34+ stem cells into highly functional NK cells, currently evaluated in a Phase I/II clinical study (ClinicalTrials.gov ID: NCT04632316). The introduction of a genetic engineering step during early culture stages makes the system suitable for CAR-NK products, for the generation of billions of off-the-shelf viveNK™ cells for antigen-directed tumor targeting.

Methods To enhance the ability of NK therapies to kill resistant B-cell leukemia cells, we generated anti-CD19-CAR viveNK™ cells via lentiviral transduction with multiple second- and third-generation CARs carrying different hinge, transmembrane and intracellular domains. CAR cassettes were cloned into Glycostem's own clinically suitable lentiviral transfer plasmid; promoter analysis identified MNDU3 as the optimal transgene driver.

Results Engineered cells showed high expansion potential and fast differentiation into functional NK cells expressing specific surface markers. CAR surface expression increased up to 83% (n=8 donors) with higher Multiplicity of Infection (MOI) in a range of 1-20, but was sustained even at low (<5) MOI. CAR transgene genome integration (Vector Copy Number) and transcriptional efficiency was evaluated via quantitative Polymerase Chain Reaction (PCR). Exposure of CD19-viveNK™ cells to antigen-expressing B-cell leukemia cell lines resulted in increased degranulation and very potent antigen-specific cytotoxicity. Additionally, inherent innate NK cell phenotype and responses and the mechanism of action driving CD19-CAR viveNK™ cytotoxicity were investigated via flow cytometry-based analysis and single-cell RNA-sequencing (scRNA-Seq) of CAR-transduced vs non-transduced donors.

Conclusions Our data show how off-the-shelf, highly functional, and antigen-directed CAR-NK cells can be generated ex vivo, offering an option to target cancers which are often resistant or difficult to treat with standard immunotherapy.

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