Universal prospects of cryopreserved “off-the-shelf” umbilical cord blood CD34⁺ progenitor cell-derived NK cell therapeutics: Clinical and preclinical evaluation of GTA002 and genetically modified candidates

Anna-Maria Georgoudaki[1]*, Adil D. Duru[1]*, Nina Lamers-Kok[1], Didem Özkazanc[1], Amanda van Vliet[1], Ella Peters[1], Kasia Nowek[1], Greis Sahini[1], Denise Vodegel[1], Danielle Steenmans[1], Denise Panella[1], Arianna Micciche[1], Dirk Geerts[1], Virginie Bonnamain[1], Monica Raimo[1], Lucia Kucerova[1], Kai Pinkernell[1], Jan Spanholtz[1].

Affiliations: [1]Glycostem Therapeutics, Oss, The Netherlands.

Key findings

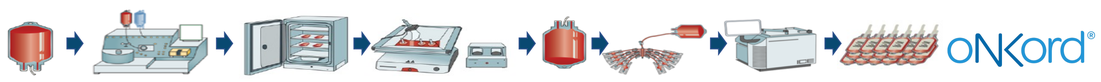
- GTA002 NK cells target hematological and solid tumors like AML and MM as well as GBM[1], melanoma; (**Poster #29** by Amanda van Vliet**)** and colorectal cancer (CRC); (**Poster #100** by Nina Lamers-Kok)
- WiNK trial (NCT04632316) initial results show a good initial safety profile of GTA002. MRD and chimerism analysis reconfirm the excitement to further investigate this treatment strategy for disease control in AML in the upcoming cohorts and with repeat infusions.
- Lentiviral-based chimeric antigen receptor (CAR) transduction is implemented to a closed system for stable expression of functional CAR and investigated in multiple models (CD19- and Her2-specific).
- Preclinical and clinical performance of cryopreserved “off-the-shelf” GTA002 cells as well as CAR-NK cells demonstrates great potential for multimodal targeting of various cancers.

Background

- Glycostem Therapeutics developed a platform to expand and differentiate CD34⁺ hematopoietic stem cells (HSCs) from umbilical cord blood (CB) into highly active NK cells, GTA002[2].
- Safety and tolerability of a predecessor non-cryopreserved product was already demonstrated in an earlier Phase I trial in elderly AML patients[3].
- A fully closed, semi-automated manufacturing platform was developed by Glycostem, to generate GTA002, an “off-the-shelf” (allogeneic), cryopreserved NK cell preparation, currently tested in AML patients, in the WiNK trial (NCT04632316).
- Cancer specific products are engineered with lentiviral vectors, implemented to the closed system, using a novel design of a clinically compatible LV backbone with optimized promoter and CAR cassette.

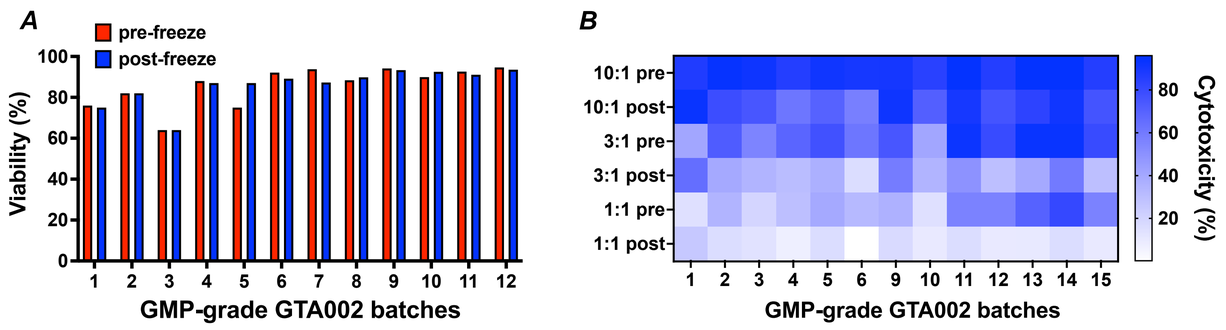
Results

Glycostem Therapeutics’ NK cell manufacturing platform uNK™



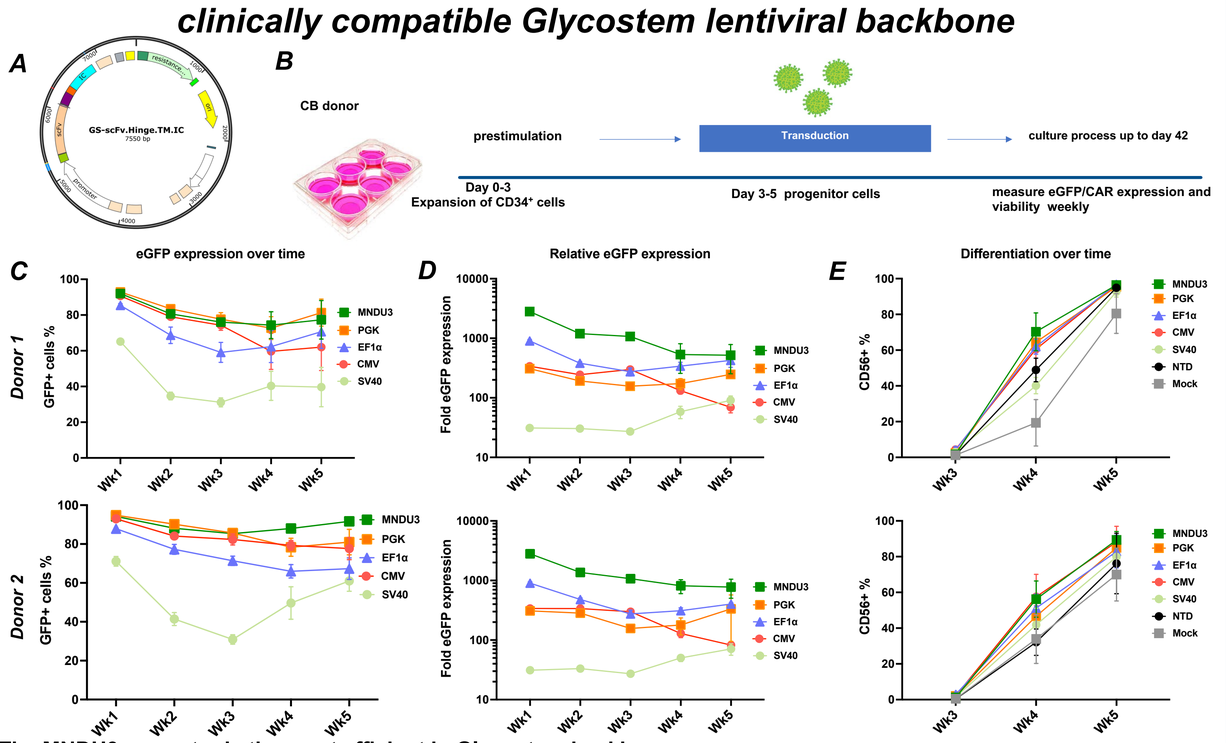
Genetically modified NK cell manufacturing platform

Cryopreserved GTA002 retain high viability and potent anti-tumour activity



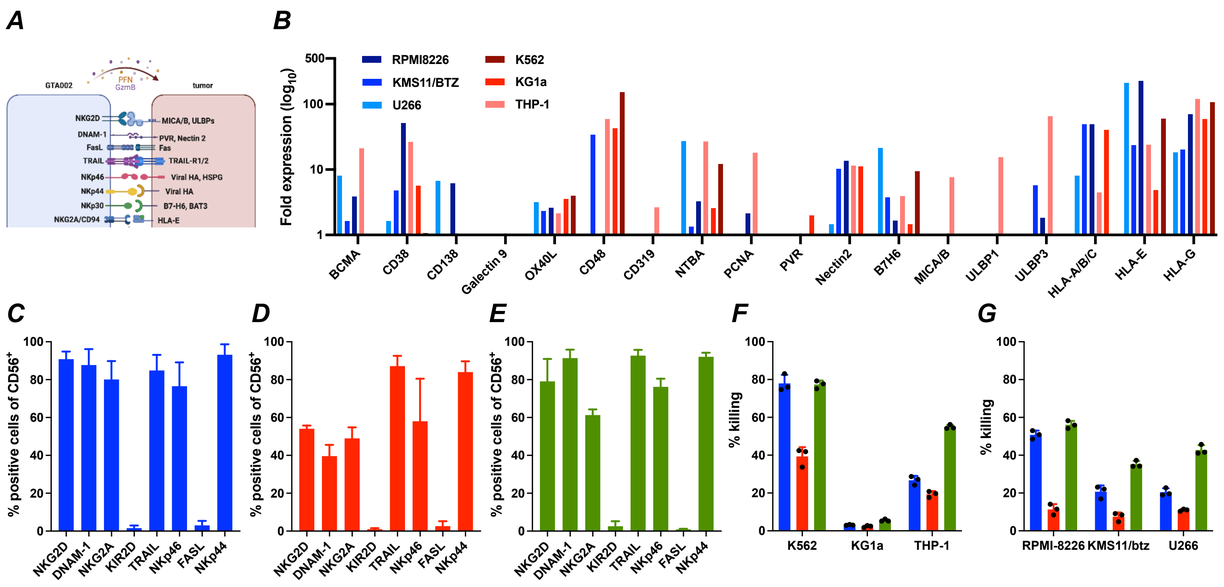
Cryopreserving GTA002 cells does not affect their viability (**A**) and GTA002 cells retain their capacity to lyse K562 cells after cryopreservation (**B**). NK progenitor cells are expanded from UCB-derived CD34⁺ cells within 2 weeks of static culture. Progenitor cells are then transferred to a rocking bioreactor to differentiate into NK cells. Finally, oNKord® infusion doses are cryopreserved in bags using a controlled rate freezer and then banked until use.

MNDU3 promoter induces optimal and stable gene expression in the clinically compatible Glycostem lentiviral backbone



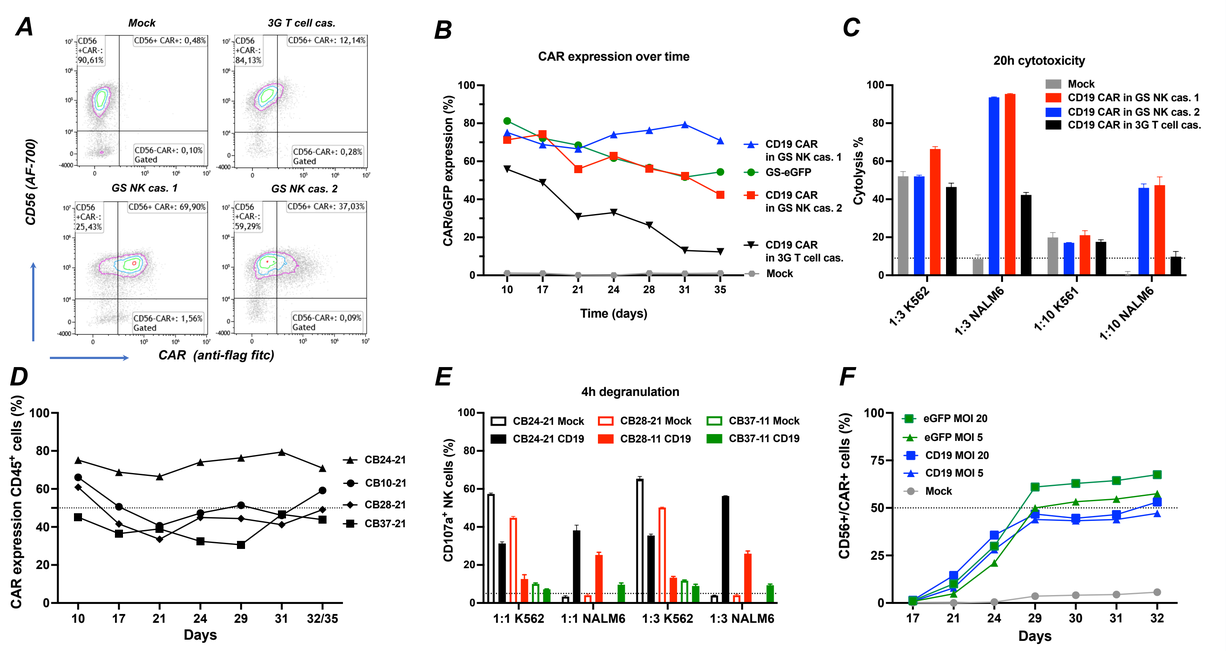
The MNDU3 promoter is the most efficient in Glycostem backbone.
Layout of Glycostem LV backbone (**A**) and LV transduction process (**B**). eGFP expression analysis throughout the expansion and differentiation process: GFP positive cells in percentage (**C**); relative fold expression of eGFP (**D**); NK cell differentiation (**E**).

GTA002 exert potent antitumor responses against AML and MM cell lines



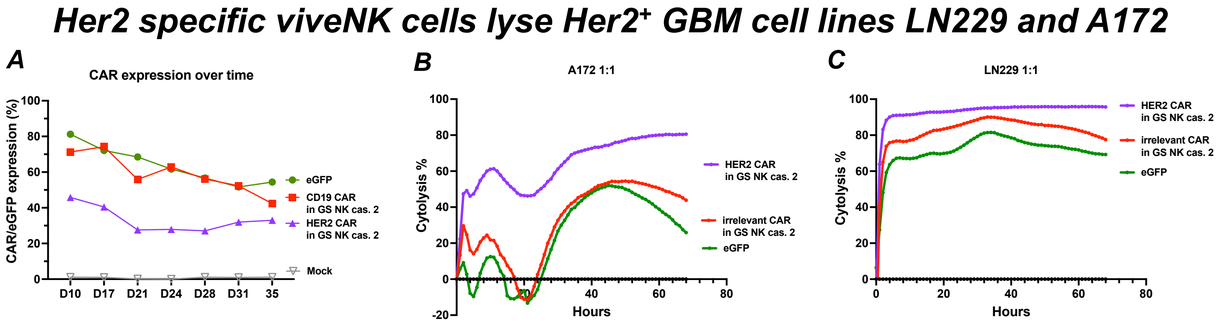
NK cell cytotoxicity is regulated by inhibitory and activating receptor ligand interactions (**A**). Various AML and MM cell lines express multiple NK cell ligands and GTA002 cells are equipped with diverse NK cell receptors targeting these ligands, such as NKG2D, DNAM-1 NKp44, NCRs or death receptor/ligands (**B**), assessed by flow cytometry and shown as relative expression compared to unstained control. Receptor profiling of representative GTA002 NK cell products (n=3) (**C,D,E**) by flow cytometry for confirmation of identity at day 35 of expansion/differentiation, assessed in experimental triplicates, data shown as mean and SD. Most interestingly, GTA002 cells efficiently lyse the majority of these cell lines at low E:T ratios (1:1), except for KG1a (**F** and **G**). GTA002 cytotoxicity against Leukemic **(F)** and MM **(G)** cell lines was assessed at E:T 1:1 after 20 hours in experimental triplicates, data shown as mean and SD.

viveNK cells express stable and functional CD19 CAR



Optimized CAR cassette embedded in GS backbone facilitates efficient and stable CAR expression even at low MOIs. CD19 (FMC63) viveNK cells efficiently target and lyse antigen positive cells. Superior CAR expression (GS NK cas.1) on NK cells at d35 transduced with different CAR cassettes (MOI20) (**A**). CAR expression is assessed by anti-FLAG antibody overtime (**B**). CD19 viveNK responses against CD19-negative K562 cells and CD19-positive NALM6 cells assessed by flow cytometry-based 20-hour cytotoxicity assays at different effector:target ratios (**C**). GS NK cassette 1 based CD19 CAR expression overtime is followed on 4 different donors (MOI20) (**D**). CD19 specific degranulation of GS NK cassette 1 based CD19 viveNK cells assessed using CD19-negative K562 cells and CD19-positive NALM6 cells (**E**). CAR expression on viveNK cells transduced with GS NK cassette 1 at different MOIs (5 and 20) during expansion and differentiation process (**F**).

Her2 specific viveNK cells lyse Her2⁺ GBM cell lines LN229 and A172



Her2 (FRP5) CAR transduced cells (MOI 20) show stable CAR-expression overtime, as assessed by anti-FLAG antibody (**A**). Most importantly Her2 viveNK cells efficiently target and lyse Her2+ cell lines A172 (Her2 medium) (**B**) and LN229 (Her2 high) (**C**) as assessed by Axion’s Maestro Z impedance platform, demonstrating the potential of viveNK platform for both haematological and solid cancers.

Clinical trial (NCT04632316): GTA002 for the Treatment of Acute Myeloid Leukemia Patients With Measurable Residual Disease is *ongoing & safe*

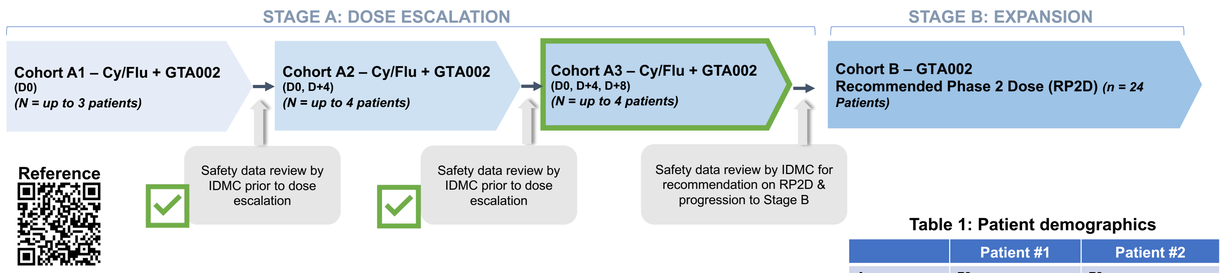
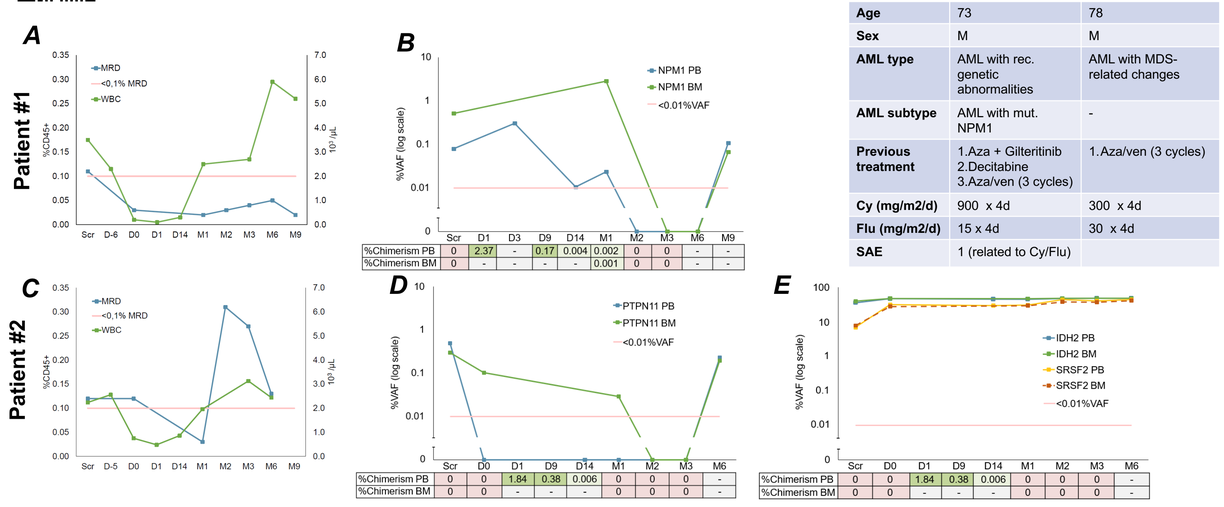


Table 1: Patient demographics

	Patient #1	Patient #2
Age	73	78
Sex	M	M
AML type	AML with rec. genetic abnormalities	AML with MDS-related changes
AML subtype	AML with mut. NPM1	-
Previous treatment	1.Aza + Gilteritinib 2.Decitabine 3.Aza/ven (3 cycles)	1.Aza/ven (3 cycles)
Cy (mg/m2/d)	900 x 4d	300 x 4d
Flu (mg/m2/d)	15 x 4d	30 x 4d
SAE	1 (related to Cy/Flu)	



GTA002 show a good initial safety profile (Heuser et al, 2021, **table 1**), with only 1 SAE reported. MRD assessments show clearance by MFC (multiparametric flowcytometry and WBC count) at 9 (**A**) and by NGS (Thol et al. 2018) at 6 months (**B**) after a single dose infusion with GTA002, and clearance of a PTPN11 mutated clone (6 months in PB (**C**), at 4 months in the BM (**D**)). The MRD results coincide with the detectable presence of GTA002 in PB by chimerism analysis for 9 days. Results raise excitement to further investigate this treatment for disease control in AML at the upcoming higher dose levels and with repeat infusions.

Anna-Maria Georgoudaki, PhD
Expert in Cancer immunobiology and Tumor microenvironment modulation with 14 years of experience in development of antibody-based and cellular cancer immunotherapies, working with human primary tumors and animal models of human cancers. Experience in several academic and industry-sponsored projects at various institutions including Karolinska Institutet, the Rockefeller University and Nova Southeastern University. Since 2020, focusing on mechanistic functional aspects of stem-derived NK products & combination therapies as Research manager at Glycostem Therapeutics.

anna@glycostem.com

Glycostem Therapeutics BV.

References