Enabling treatment options against colorectal cancer by enhancing functionality of stem cell derived NK cells

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Key findings

- ✓ Efficient cytotoxicity in 2D and 3D CRC models for GTA002 (1-3)
- ✓ CEACAM5 is an interesting tumor antigen in various indications and is not expressed at our platform (4)
- ✓ High expression of CEACAM5 was found for LS174T cell line and intermediate expression for LOVO, HT-29 and SW48 (4)
- ✓ A high affinity scFv directed against CEACAM5 was developed showing good detection by soluble chimeric CEACAM5-Fc proteins and high avidity for CEACAM5 expressing cells (5)
- ✓ CAR specific functionality was measured in NK cells using the novel developed lentiviral transfer vector backbone from Glycostem (5)
- ✓ Improvements in CAR cassette increases CAR stability in viveNK cells (6)

Background

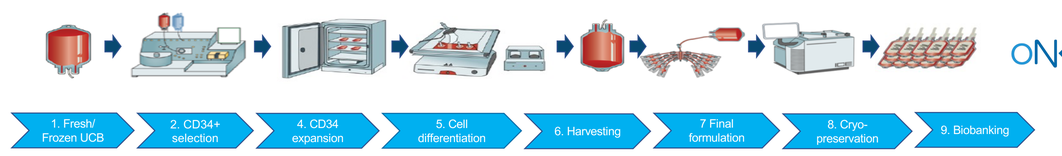
In Glycostem's manufacturing process CD34+ cells are isolated from umbilical cord blood stem cells using the CliniMacs Prodigy automated device and expanded and differentiated in bioreactors to functional NK cells. The NK cells are washed and concentrated in cryopreservation medium to generate multiple batches and preserved in liquid nitrogen for long term storage.

In this study we investigated the potency of umbilical cord blood CD34+ derived unmodified NK cell product (GTA002) against various characterized colorectal cancer cell lines (CRCs). Tumor antigen positive CRC cell lines were subsequently targeted by CEACAM5 targeting CAR NK cells.

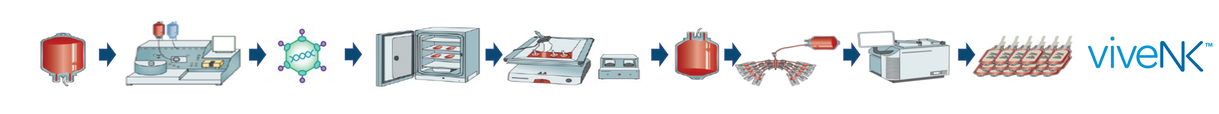
Natural cytotoxicity or antigen induced cytotoxicity of CRC cells was tested and compared either in 2D or 3D spheroid CRC cytotoxicity assays using an impendence (2D) or image-based (2D and 3D) analysis. Targeting of antigen-positive CRCs by CAR NK demonstrated significant enhanced cytotoxicity.

Results

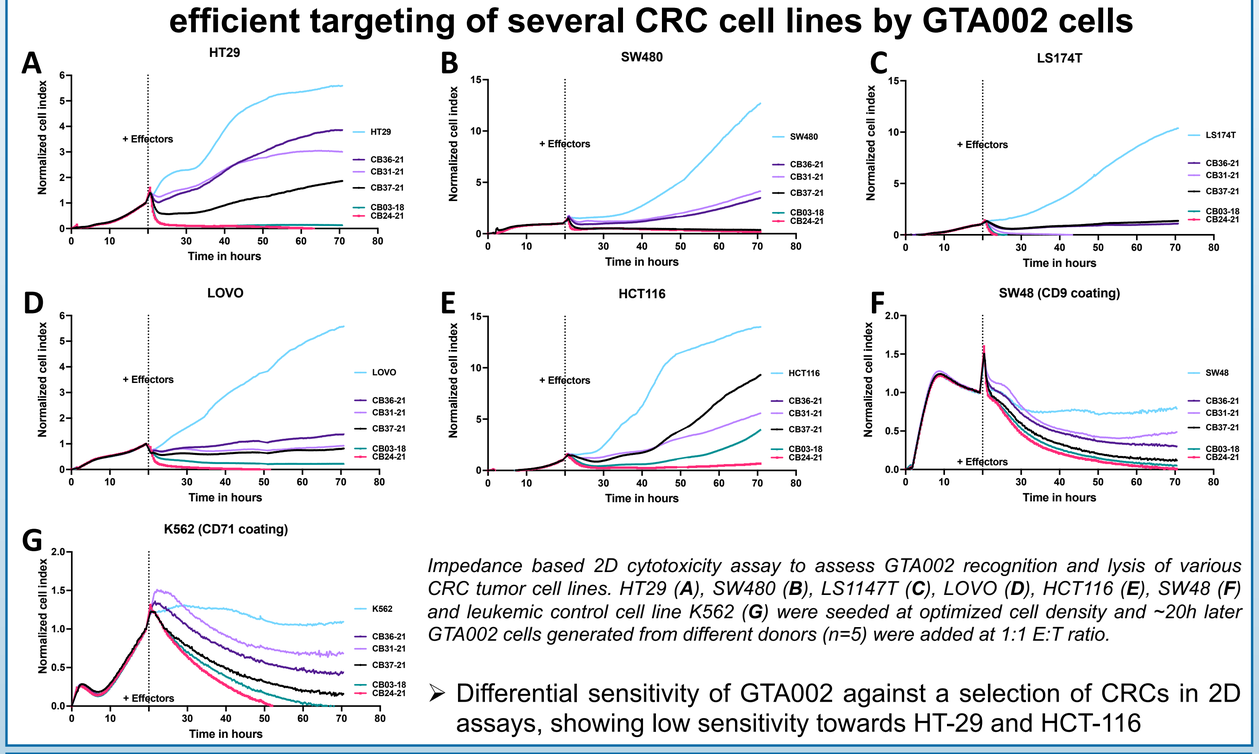
Glycostem Therapeutics' NK cell manufacturing platform uNK™



Genetically modified NK cell manufacturing platform



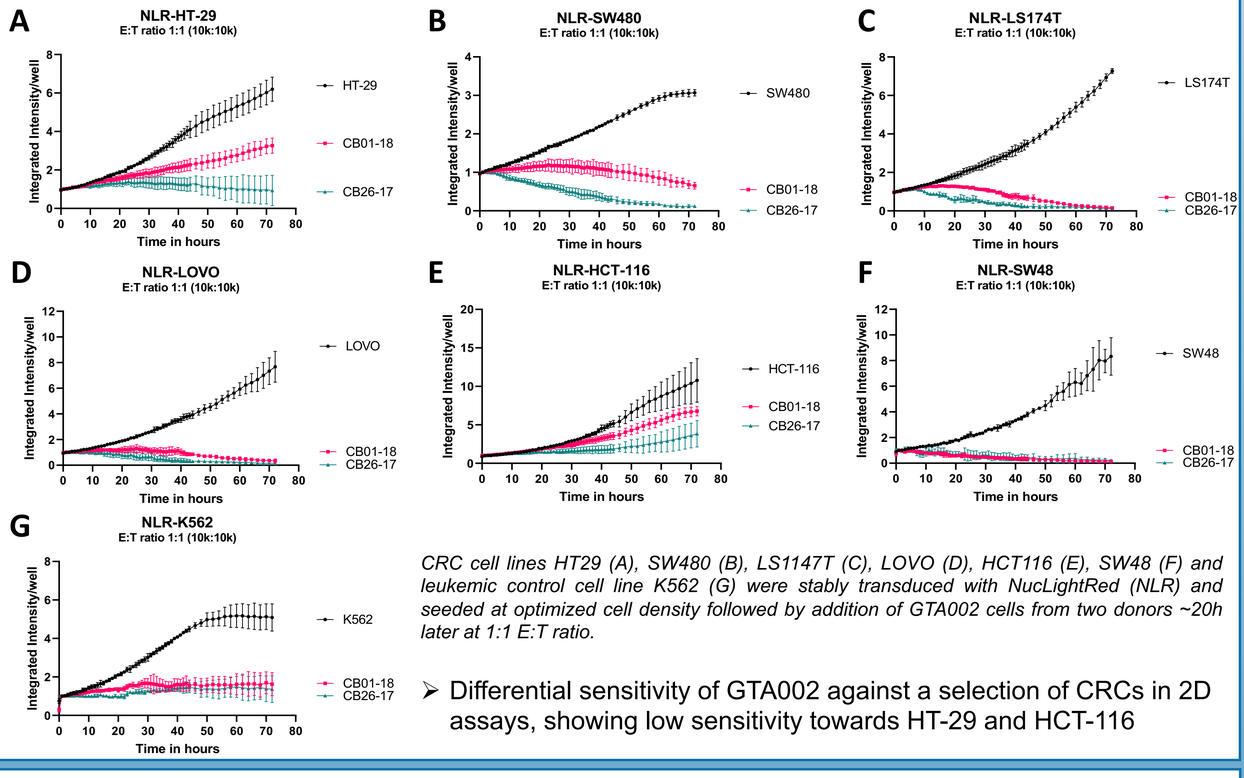
1. Cellular impedance-based (2D) assessment of cytotoxicity demonstrated efficient targeting of several CRC cell lines by GTA002 cells



*Impedance based 2D cytotoxicity assay to assess GTA002 recognition and lysis of various CRC tumor cell lines. HT29 (**A**), SW480 (**B**), LS1147T (**C**), LOVO (**D**), HCT116 (**E**), SW48 (**F**) and leukemic control cell line K562 (**G**) were seeded at optimized cell density and ~20h later GTA002 cells generated from different donors (n=5) were added at 1:1 E:T ratio.*

➢ Differential sensitivity of GTA002 against a selection of CRCs in 2D assays, showing low sensitivity towards HT-29 and HCT-116

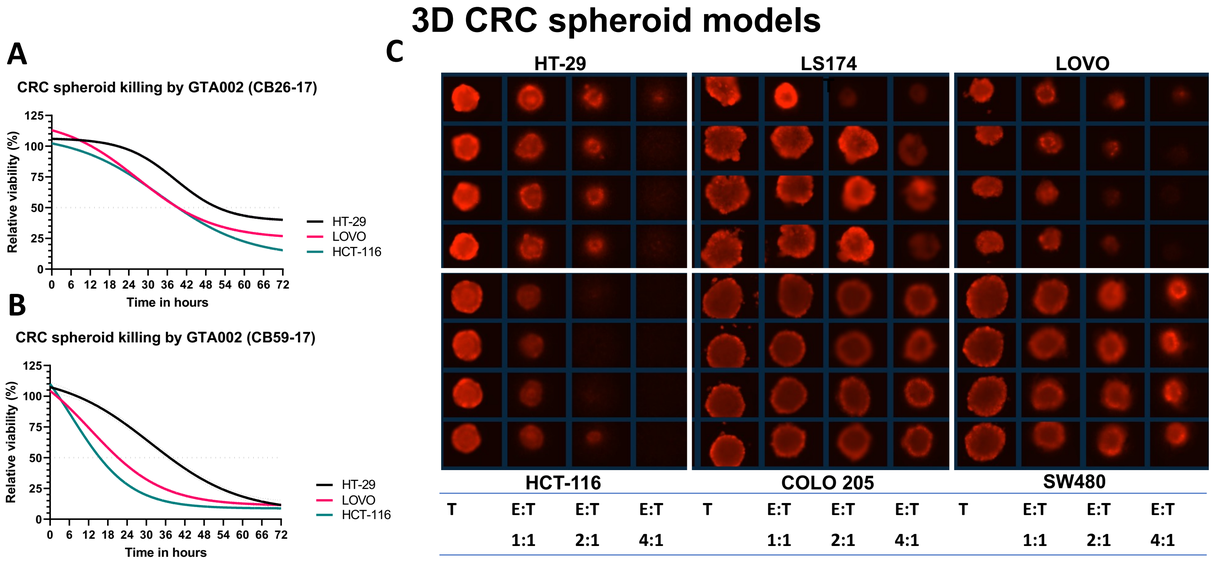
2. Imaging-based analysis of GTA002 potency against various CRC cell lines shows efficient tumor growth control and lysis



CRC cell lines HT29 (A), SW480 (B), LS1147T (C), LOVO (D), HCT116 (E), SW48 (F) and leukemic control cell line K562 (G) were stably transduced with NucLightRed (NLR) and seeded at optimized cell density followed by addition of GTA002 cells from two donors ~20h later at 1:1 E:T ratio.

➢ Differential sensitivity of GTA002 against a selection of CRCs in 2D assays, showing low sensitivity towards HT-29 and HCT-116

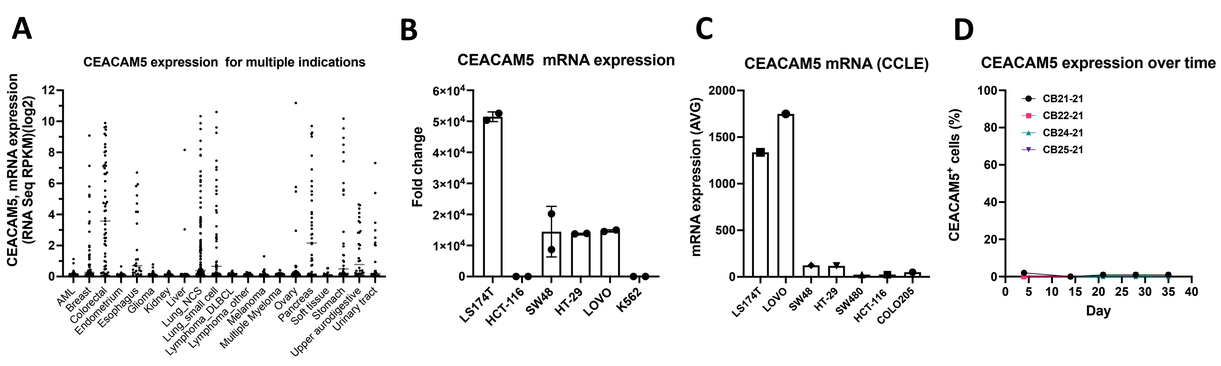
3. Efficient cytotoxicity against several CRC tumor cell lines by GTA002 in 3D CRC spheroid models



*Live cell imaging spheroid cultures (3D): NLR cell lines are seeded in ULA U-bottom plates at 10k/well (quadruplicates) and ~3 days later GTA-002 donors CB26-17 (**A**); CB59-17 (**B** and **C**) were added. Live cell imaging spheroid cultures (3D): Image taken ~48h after GTA002 addition for selection of CRCs, showing from left to right in quadruplicates: Target (T) only; T+Effectors (E) ~1:1; T+E ~2:1 and T+E ~4:1.*

- ➢ GTA002 mediated cytotoxicity exhibits differential kinetics in 3D.
- ➢ HT-29, LOVO and HCT-116 form tight spheroids and proliferate over time; LS174T, COLO 205 and SW480 spheroids do not proliferate and undergo apoptosis over time (data not shown).

4. CEACAM5 as promising multiple tumor target, and specifically verified for colorectal cancer cell lines

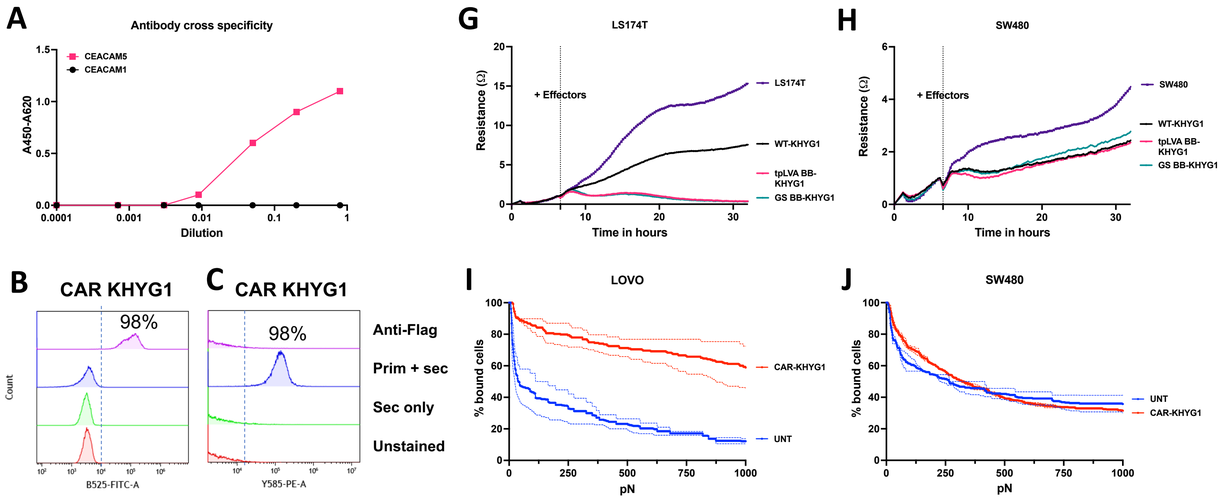


*CEACAM5 (carcinoembryonic antigen-related cell adhesion molecule 5) is a cell surface glycoprotein involved in cell adhesion, intracellular signaling and tumor progression and overexpressed in colorectal, stomach, lung, pancreatic, breast, urothelial and other cancers **(A)** CRC cell lines identified with high CEACAM5 mRNA expression (LS174T), intermediate (LOVO, HT-29 and SW48) and low/absent HCT116 **(B)**. qPCR data verifying CEACAM5 mRNA expression for a selection of CRCs **(C)**. CEACAM5 expression is absent in GTA002 (n=4) during the culture process excluding the occurrence of fratricide in CAR NK cultures **(D)**.*

5. CEACAM5 CAR NK cells efficiently target and lyse antigen positive cells

Table 1: scFv-Fc specificity for CEACAM5

Origin	Antibody clone	Format	Kd in nM	Ka in Ms-1	Kd in s-1	chi2
Library	SUW59-A8	scFv-Fc	20	4.97x 10^4	1.01x 10^-3	0.67
Yulight shuffling	Jok454-F7	scFv-Fc	3.1	6.29x 10^4	1.93x 10^-4	0.39

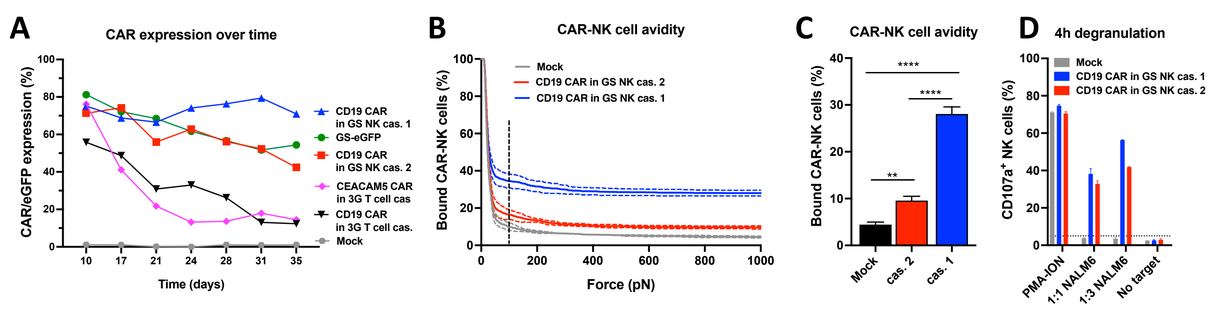


- ➢ scFv developed with 2 different affinities 20nM (low) and 3.1nM (high); presented data: Jok454-F7 (**table 1**)
- ➢ No cross specificity for scFv-Fc against CEACAM1 antigen (**A**)
- ➢ scFv binding to CEACAM5 protein (Fc chimera) (**C**) detected and comparable to mAb detection directed against tag (anti-FLAG) (**B**)

*CAR KHYG1 cells (90% vs 54% CAR and wildtype) in a 4h flow cytometry-based cytotoxicity assay against CEACAM5+ (Ag+) **(D)** and CEACAM5- (Ag-) **(E)** cell lines and K562 **(F)** at different E:T ratio's. Impedance-based cytotoxicity assay with CAR KHYG1 cells transduced with Glycostem backbone (23% CAR) vs tpLVA backbone (20% CAR) and wildtype KHYG1 against LS174T (Ag+) **(G)** and SW480 (Ag-) **(H)** CRCs at 1:1 E:T ratio. For avidity assay, CAR KHYG1 cells were added to a monolayer of LOVO (Ag+) or SW480 (Ag-) attached to the chip. Avidity curve shows the % bound cells upon application of an acoustic force ramp (z-Movi, Lumicks).*

- ➢ NK cells expressing CEACAM5 CAR show antigen specific binding in avidity assays combined with strong cytotoxicity to CEACAM5 expressing tumor cells .

6. CECAM5 CAR in 3G T cell cassette is expressed at early stages of expansion, optimized cassettes supports CAR expression and function



Different CAR cassette designs with CD19 and CEACAM5 scFvs have been tested to improve stable CAR expression over a 5-week expansion and differentiation culture, Glycostem's viveNK platform (**A**). Avidity assays with z-Movi (Lumicks) resulted in stronger binding of CD19 CAR-NK using cassette design 1 (**B** and **C**). 4h degranulation assay assessed CD19 induced degranulation of NK cells (**D**) proving that functionality is linked to antigen binding and NK cell activation.

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References