Developing a First-In-Class Universal Allogeneic SNAP-CAR NK Cell Therapy

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Abstract

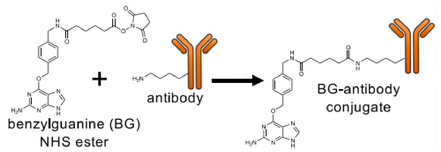
Introduction: Natural killer (NK) cells can kill tumor cells without prior activation through their complement of activating and inhibitory surface molecules. Chimeric antigen receptor (CAR) expression by engineered NK cells can improve their innate anti-tumor functions by specifically activating NK cells in the presence of tumor antigen. Compared to autologous CAR therapies, allogeneic CAR NK cells can be generated and cryopreserved for future use, do not require HLA matching and do not cause graft versus host disease or cytokine release syndrome, offering the potential for a safer, more clinically accessible and cost-effective cell therapy. Based on demonstrated successful use of a novel SNAP-CAR technology in T cells, we are developing a universal allogeneic SNAP-CAR NK cell. This first-in-class product replaces the extracellular antigen binding domain of a CAR with a SNAPtag enzyme that carries out a self-labeling reaction to covalently attach any antibody conjugated to a benzylguanine (BG) tag to create a functional antigen-specific CAR.

Methods & Results: SNAP-CAR NK cells were generated from viral vector-transduced cord blood-derived CD34+ cells that were expanded and differentiated to NK cells using a proprietary ex vivo cell culture process that utilizes cell activation by a Notch ligand. SNAP-CAR NK cells were assessed for SNAP-CAR expression, NK cell phenotype, and in vitro anti-tumor cytotoxicity using BG-conjugated antibody. The SNAP-CAR NK cells displayed viability and phenotyping comparable to the untransduced control NK cells generated in parallel. Anti-tumor cytotoxicity against a CD20+ acute lymphoblastic leukemia (ALL) cell line was enhanced when SNAP-CAR NK cells were co-cultured with a BG-conjugated anti-CD20 antibody in an in vitro assay.

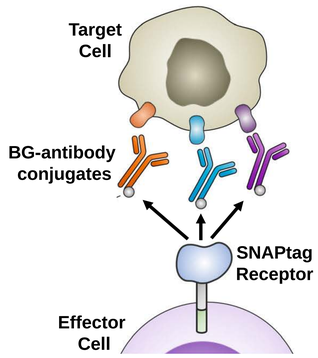
Conclusions: Our off-the-shelf allogeneic NK cell generation platform pairs exquisitely with the highly versatile nature of the novel universal SNAP-CAR platform. Tumor targeting of the SNAP-CAR NK cell therapy can be modulated by the specificity and concentration of the BG-conjugated antibodies used, allowing for one bank of SNAP-CAR NK products to be used across multiple patients and multiple tumor types. Furthermore, for a single patient, multiparametric targeting of cancer can be achieved by infusing SNAP-CAR NK cells functionalized with multiple different antibodies, used concurrently or sequentially to target the tumor microenvironment and different antigens on the same tumor, to impede tumor escape resulting from variation or loss of tumor antigens (e.g., patients who relapse after CD19 CAR T cell treatment due to tumor loss of CD19 antigen could receive follow-up treatment with CD20 and/or CD22 SNAP-CAR NK cells without additional risk of GvHD or CRS).

SNAP-CAR Technology Enables Targeting of Multiple Tumor Antigens Using a Universal SNAPtag Receptor and BG-conjugated Antibodies

A benzylguanine (BG) motif is chemically conjugated to an antibody.

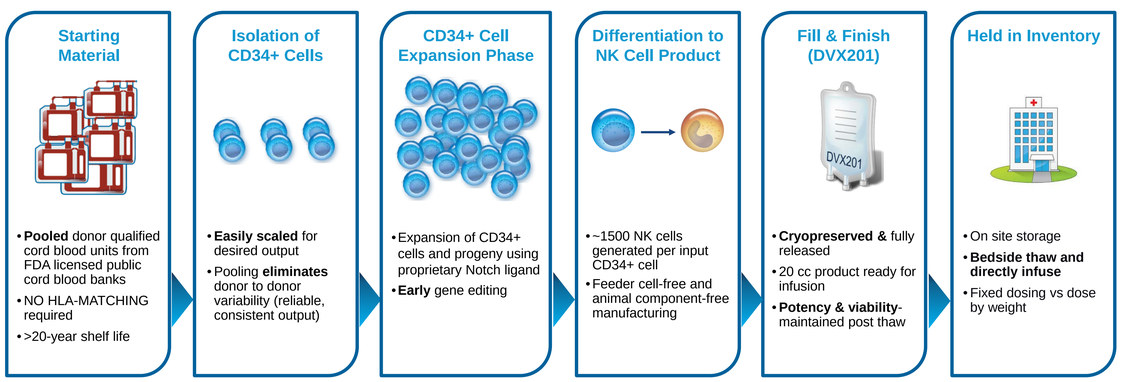


The BG-antibody conjugate covalently binds to the extracellular SNAPtag enzyme through a self-labeling reaction, forming the binding domain of the SNAP-CAR.

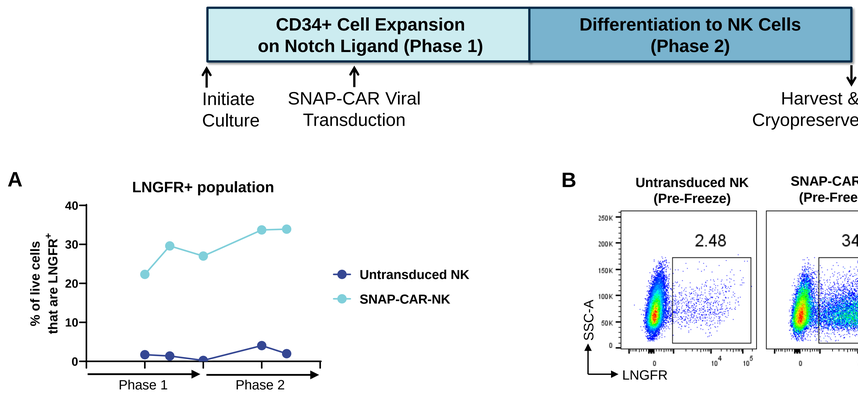


The current γ-retroviral vector contains the SNAP-CAR gene co-expressed with an LNGFR tag to identify SNAP-CAR+ cells.

Off-the-shelf Allogeneic NK Cell Product is Generated from Pooled Cord Blood-Derived CD34+ Cells Through a 2-Phase Culture Process Using a Proprietary Notch Ligand

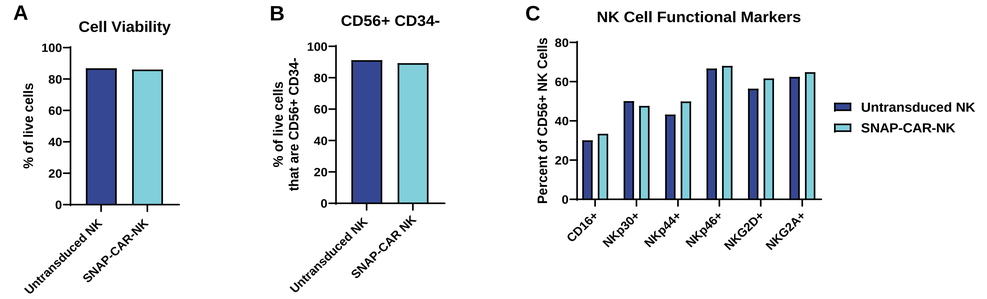


SNAP-CAR Expression is Maintained Throughout the NK Cell Generation and Cryopreservation Processes



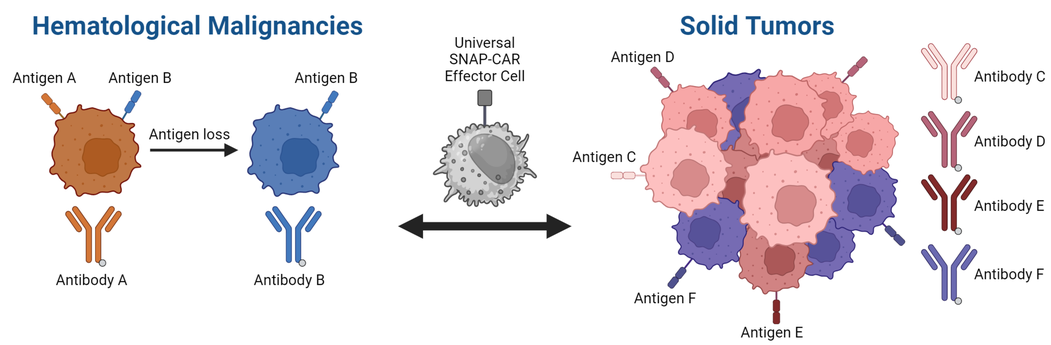
(A) Percentage of LNGFR+ SNAP-CAR NK cells throughout the expansion and differentiation phases of the *ex vivo* NK cell generation process.
(B) Representative flow cytometry scatterplots showing the percentage of LNGFR+ SNAP-CAR NK cells analyzed at the end of the culture process (Pre-Freeze) or after thawing of cryopreserved cells (Post-Freeze).

SNAP-CAR NK Cell Viability and Phenotyping Is Comparable to Unmodified NK Cells



SNAP-CAR NK cells and untransduced NK cells were analyzed by flow cytometry at the end of the culture process, prior to cryopreservation, for **(A)** cell viability, **(B)** the percentage of CD56+ NK cells, and **(C)** expression of classical markers associated with NK cell function.

Ability to Target Both Heme Malignancies and Solid Tumors with a Single Bank of SNAP-CAR Effector Cells



- We are continuing to optimize SNAP-CAR expression in our *ex vivo* generated NK cells, and to assess the cytotoxic capacity of SNAP-CAR NK cells in combination with various BG-conjugated therapeutic antibodies against the same target tumor cell type.
- We thank Dr. Jason Lohmueller and Dr. Alexander Dieters from the University of Pittsburgh for valuable scientific input and feedback.
- Figures adapted from Ruffo E et al. *Nat Commun.* (2023) 14:2463, or created with BioRender.com.