A clinical-grade manufacturing process using CliniMACS Prodigy® for CAR-NK cell-based immunotherapy

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Introduction

In recent years, great progress has been made in the use of natural killer (NK) cells for cancer immunotherapy. Their biological and safety properties increase the interest in expanding the therapeutic potential of NK cells by chimeric antigen receptors (CAR). Here we present a straightforward manufacturing process to facilitate the generation of CAR-NK cells for clinical applications. This fully automated process was developed to generate CAR-NK cells under good manufacturing practice (GMP)-compliant conditions in a closed system with the CliniMACS Prodigy platform. Starting with highly efficient NK cell purification, the process enables activation, transduction, and expansion of NK cells. We further developed a standardized in-process control / quality control (IPC/QC) analysis including flow cytometry panels and automated gating strategies for harmonized CAR-NK cell analysis using Express Modes.

Methods

1 Experimental setup and workflow

The CliniMACS Prodigy LP-3-56 System enables a fully automated depletion of CD3$^+$ cells with or without subsequent enrichment of CD56$^+$ cells by using the CliniMACS Prodigy TS 320. Additionally, depletion of CD3$^+$ cells can also be performed with process buffer, simplifying downstream applications (fig. 1A).
After depletion of CD3$^+$ cells, the CD56$^+$ NK cells are enriched using a CliniMACS Prodigy TS 520 in combination with CliniMACS Prodigy PD-56 Engineering (fig. 1B).
The CliniMACS Prodigy NK Cell Transduction enables the automated generation of engineered NK cells by combining the CliniMACS Prodigy LP-3-56 Separation and PD-56 Engineering processes. NK cells are separated, transduced, expanded and the final CAR-NK cell product is reconstituted in CliniMACS® Formulation Solution (fig. 2).

Figure 1

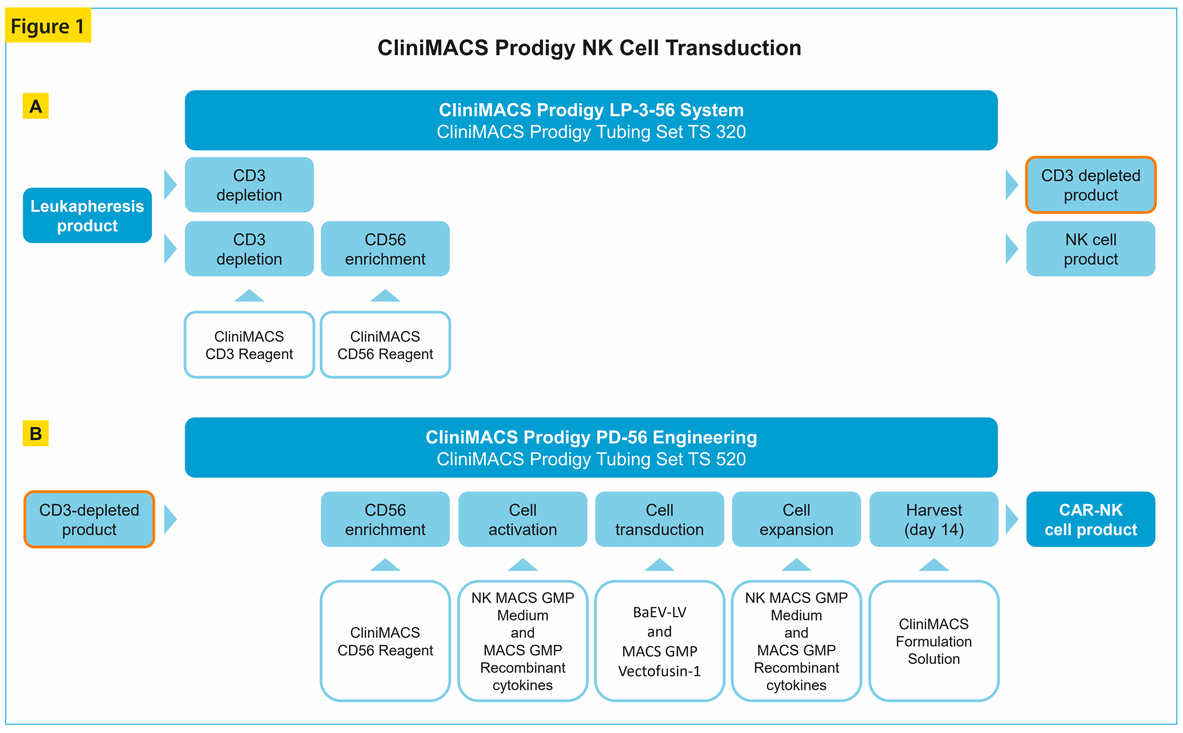
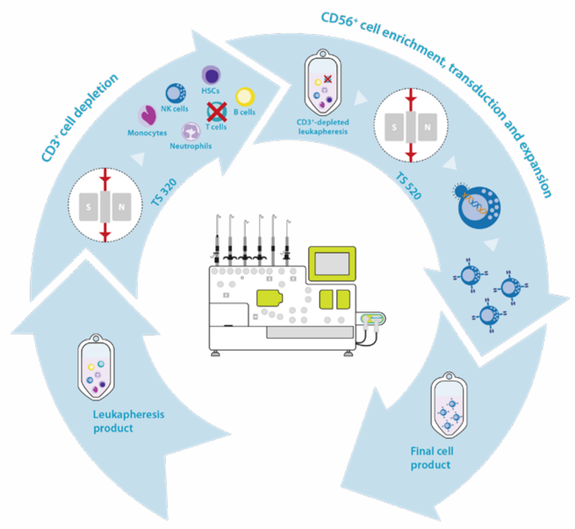


Figure 2



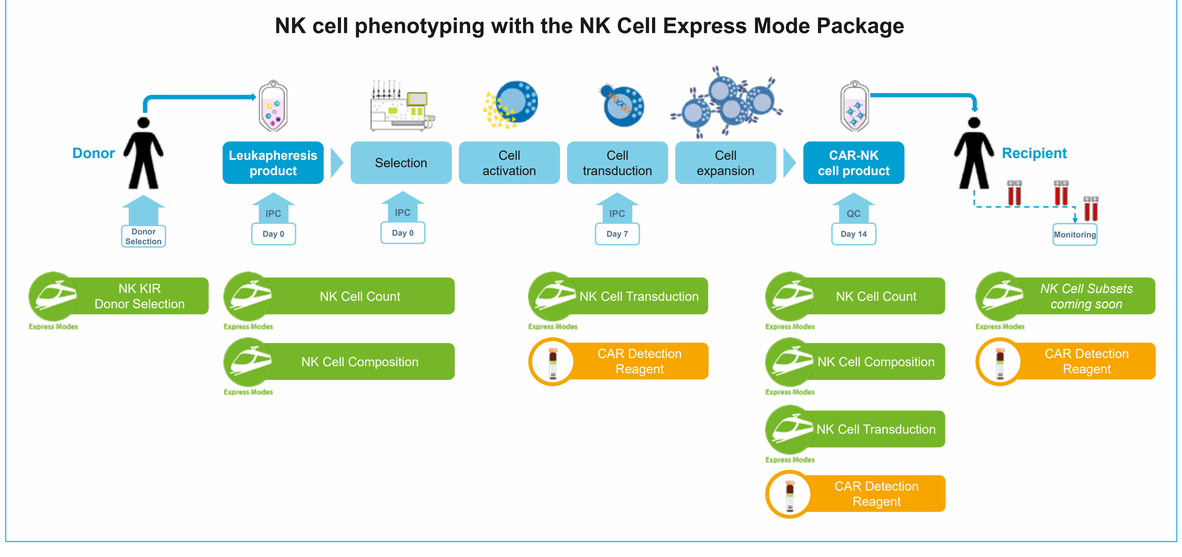
2 Generation and cultivation of CAR-NK cells using CliniMACS Prodigy NK Cell Transduction

Purified NK cells were cultivated with NK MACS® GMP Medium, supplemented with 5% human AB serum, MACS GMP Recombinant Human IL-2, MACS GMP Recombinant Human IL-15, and an additional third cytokine from IL-1 family for activation without the addition of feeder cells. On day 2 of the culture, the transduction was performed using baboon envelope pseudotyped lentiviral vector (BaEV-LV) encoding CAR constructs in combination with MACS GMP Vectofusin®-1 and a 2 h spinoculation. NK cells were washed at day 3 to remove excess vector. NK cells were cultured until day 14 using NK MACS GMP Medium, supplemented with 5% human AB serum, MACS GMP Recombinant Human IL-2 and MACS GMP Recombinant Human IL-15[1].

3 Quality control of target cell product

Various antibody panel were evaluated for the donor selection as well as the phenotypic characterization of the cell product. For automated and standardized data acquisition and subsequent analysis, the NK Cell Express Mode Package was designed to be used with the MACSQuant® Analyzer (fig. 3).

Figure 3

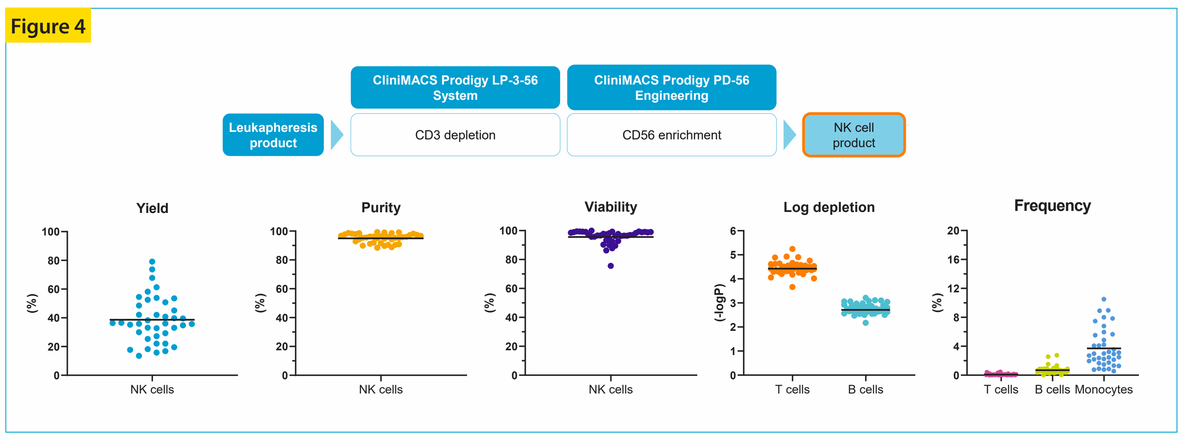


Results

1 NK cell separation

The NK cell recovery is on average of 38.7% after isolation with a high purity (mean 95.0%) and viability (mean 95.7%). An average log depletion of T cells and B cell of 4.5 and 2.8, respectively results in the low frequency of these cells (fig. 4).

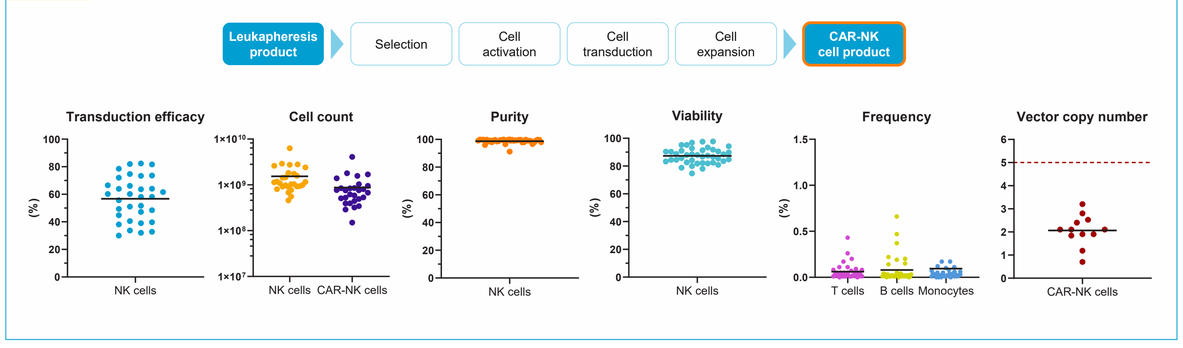
Figure 4



2 Quality assessment of CAR-NK cell product

The cell product was analyzed with the NK Cell Express Mode Package after 14 days of culture.
The mean transduction efficiency was 56.8%, resulting in a mean of 8.7 × 10^8 CAR-NK cells (1.5 × 10^9 total NK cells) with a mean NK cell purity of 98.8% and mean viability of 87.3%. The frequency of remaining CD3$^+$ cells was lower than 0.5% (mean 0.06%). The vector copy number (VCN) was determined with MACS COPY-check Kit, human, resulting in a mean value of 2.1 (fig. 5).

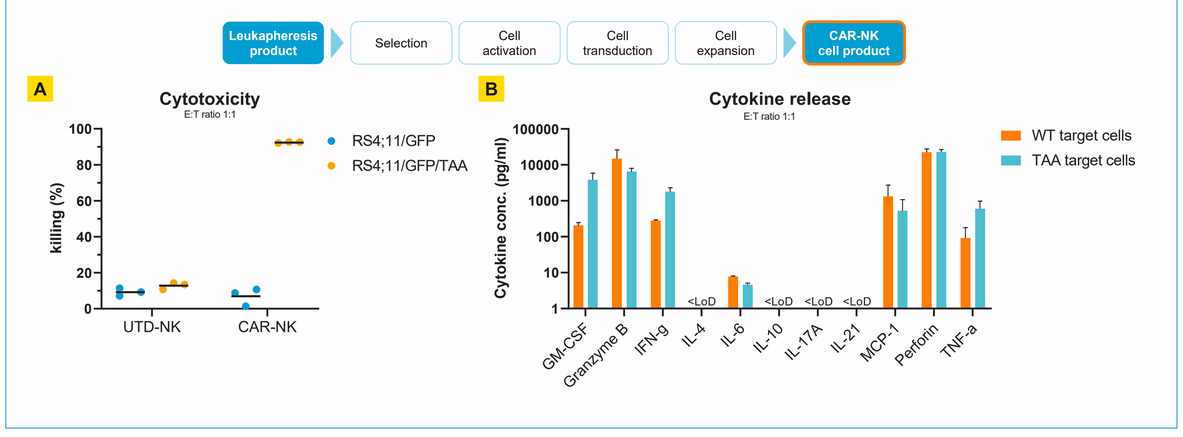
Figure 5



3 Functional assessment of CAR-NK cell product

The cytotoxicity analysis showed that the RS4;11 tumor cells were largely resistant against UTD-NK cells. In contrast, CAR-NK cells could efficiently lyse TAA-expressing tumor cells and meanwhile spared the TAA-negative tumor cells (fig. 6A).
The secretion of proinflammatory cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ) and tumor necrosis factor α (TNFα), was specifically upregulated in CAR-NK cells, whereas secretion of IL-6 was very low and secretion of IL-4, IL-10, IL-17A, and IL-21 secretion could not be detected (fig. 6B).
Taken together, the generation of CAR-NK cells introduced a highly tumor antigen-specific and efficient cytotoxic activity in addition to the innate immunity of the NK cells.

Figure 6



Conclusion

- The integrated fully automated CliniMACS Prodigy LP-3-56 System enables a GMP-compliant purification of NK cells and is approved as medical device application.
- The CliniMACS Prodigy NK Cell Transduction provides a highly automated, efficient, robust and standardized clinical-grade manufacturing platform for the viral engineering of NK cells with high purity and viability.
- A complete IPC/QC solution with automated analysis features enabled by was developed to assess final CAR-NK cell products.
- CAR-NK cells generated by CliniMACS Prodigy NK Cell Transduction show strong and specific antitumor activity against TAA-expressing tumor cells, resulting in enhanced cytotoxicity and proinflammatory cytokine production.

Reference:
[1] Bari, R. *et al.* (2019): A Distinct Subset of Highly Proliferative and Lentiviral Vector (LV)-Transducible NK Cells Define a Readily Engineered Subset for Adoptive Cellular Therapy. Front Immunol. 10:2001.

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