Developing a GMP-compliant, automated process to generate CAR NK cells in a closed system for clinical use

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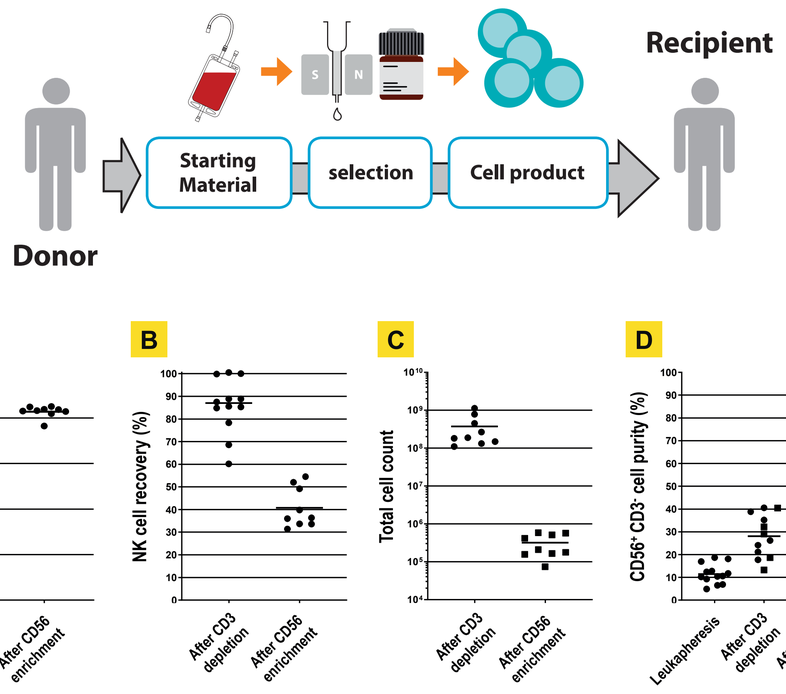
Introduction

Major advances have been made in harnessing natural killer (NK) cells in cancer immunotherapy in recent years. Regulated by their germline-encoded activating and inhibitory receptors, NK cells can recognize and eliminate tumor cells rapidly without prior sensitization. Clinical evidence has shown that donor-derived NK cells pose a low risk of inducing graft-versus-host-disease (GVHD), thus making them ideal candidates for allogeneic transplantation[1].
NK cells and T cells share similar killing machineries. Accordingly, NK cells can also be redirected by chimeric antigen receptors (CARs) against tumor cells. To simplify the manufacturing procedure and facilitate the clinical application of CAR NK cells, here we developed a highly efficient process to generate CAR NK cells under good manufacturing practice (GMP)-compliant conditions in a closed system using the CliniMACS Prodigy® Platform. The process covers the complete procedure of NK cell manufacturing, including cell separation, activation, gene modification, and expansion/cultivation.

Methods and results

1 The CliniMACS Prodigy® LP-3-56-System for NK cell isolation

Figure 1

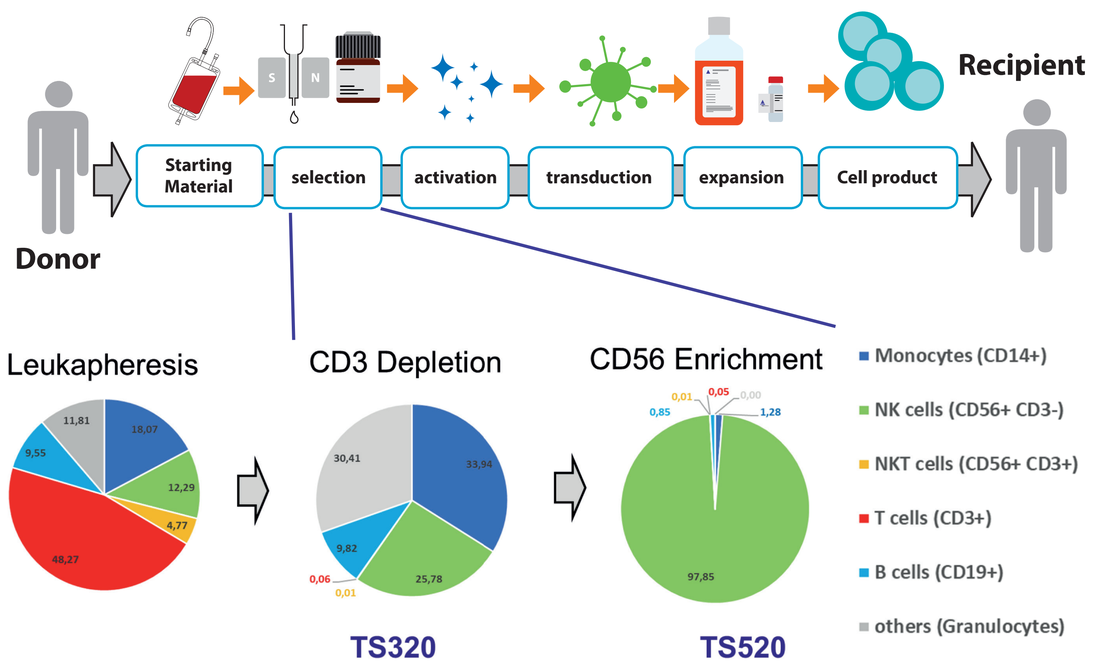


The CliniMACS Prodigy® LP-3-56 System enables fully automated, two-step isolation of NK cells from fresh leukapheresis samples in a single tubing set (CliniMACS Prodigy® TS 320). The process comprises distinct blocks: i) $CD3^+$ cell depletion, which can also be used as a stand-alone process, and ii) $CD56^+$ cell enrichment.

The CliniMACS Prodigy® LP-3-56 System resulted in a 3.8 log depletion of $CD3^+$ cells (Fig. 1A), and an 87% NK cell recovery on average after $CD3^+$ cell depletion (Fig. 1B). Subsequent $CD56^+$ cell enrichment resulted in a 4.1 log depletion of $CD3^+$ cells (Fig. 1A), and a 41% NK cell recovery on average (Fig. 1B). The resulting NK cell product contained about 2.7×10^8 NK cells (Fig. 1C) with 98% purity on average (Fig. 1D).

2 The natural killer cell engineering (NKCE) process on the CliniMACS Prodigy®

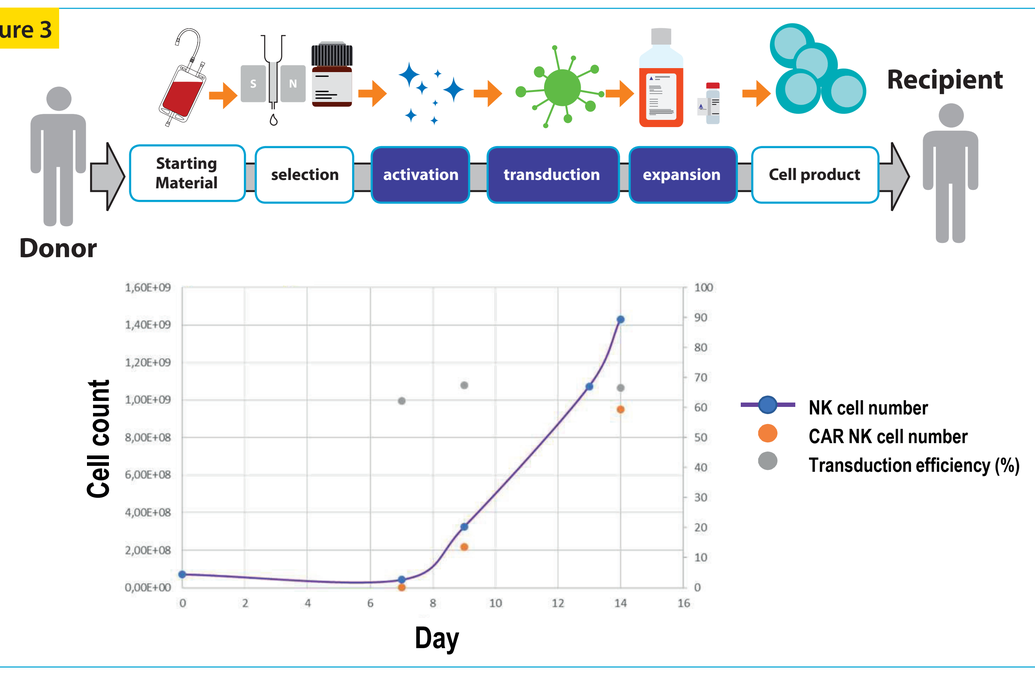
Figure 2



For the generation of genetically engineered NK cells we developed the NKCE process. It consists of two separate steps: i) $CD3^+$ cell depletion using the CliniMACS Prodigy® LP-3-56 system and TS 320, and ii) $CD56^+$ cell enrichment and manipulation using the CliniMACS Prodigy® PD-56-engineering system and TS 520. After $CD3^+$ cell depletion and $CD56^+$ cell enrichment an average NK cell purity of 93% and an average T cell log depletion of 4.3 was achieved (n = 7).

3 CAR NK cell generation and expansion using the NKCE process

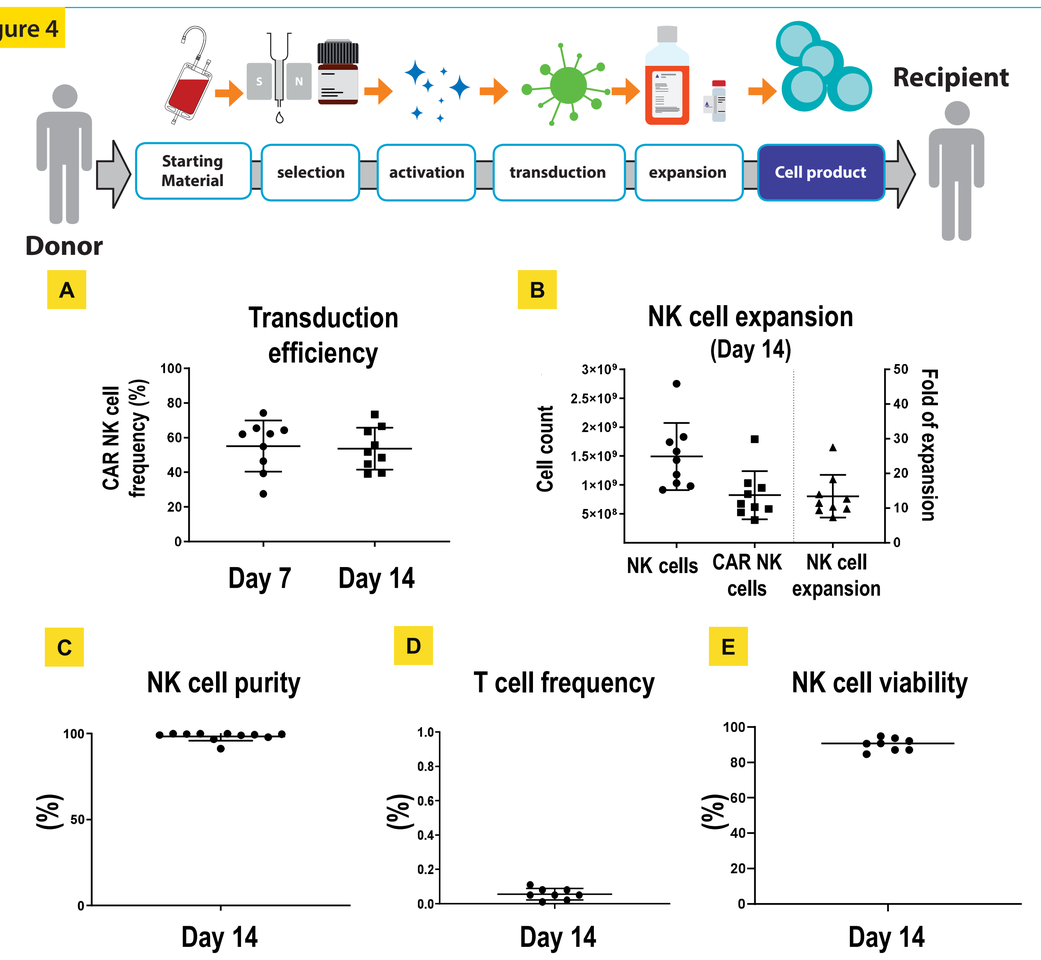
Figure 3



NK cells, obtained by the separation steps of the NKCE process, were activated on day 0 with IL-2, IL-15, and a cytokine from the IL-1 family. The transduction process using baboon-enveloped lentiviral vector took place on day 2 of the culture. For the expansion phase from day 3 to 14 only IL-2 and IL-15 were added. Cells were cultured in the absence of feeder cells. Figure 3 exemplifies a typical (CAR) NK growth curve resulting in 1.4×10^9 NK cells with a transduction efficiency of 66% (9.5×10^8 CAR NK cells) on day 14.

4 Quality assessment of final CAR NK cell product

Figure 4



The transduction of NK cells in a GMP-compliant system resulted in a stable (day 7 compared to day 14) and high transduction efficiency of 45% at the end of the culture (median; n = 9). On average the expansion resulted in 1.19×10^9 NK cells including 5.7×10^8 CAR NK cells. The culture of transduced NK cells resulted in a cellular product with high NK cell purity (>99%), low $CD3^+$ cell content (mean 0.09%), and a good viability with an average of 90% (n = 8).

5 Conclusion

We developed a novel process for automated NK cell purification, transduction, and cultivation in a closed GMP-compliant system. The high level of automation enables standardized, consistent, and operator-independent genetic engineering of NK cells for future clinical applications.

Reference

1. Leung W. Infusions of Allogeneic Natural Killer Cells as Cancer Therapy. *Clin Cancer Res* 2014;20:3390-3400.