NK cell engineering enabling fully automated separation, cultivation, and genetic modification of NK cells

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Introduction

Chimeric antigen receptors (CARs) have been used to direct autologous T cells to hematopoietic cancers with promising clinical results. The use of allogeneic T cells however comes with the risk of inducing graft-versus-host disease (GVHD). In this regard, CAR-expressing natural killer (CAR-NK) cells provide potential advantages: NK cells can be used in an allogeneic setting as they pose a low risk of GVHD. Moreover, besides the CAR-guided antitumor action, NK cells exert their activity through their native receptors, potentially preventing cancer cell antigen escape.

Ex vivo modification of NK cells to induce CAR expression requires isolation, activation, transduction, and expansion steps. To simplify procedures and widen the applicability for future clinical therapies, we are developing automated complex cell manufacturing processes in a closed system.

Methods

1 CD3$^-$CD56$^+$ NK cell isolation in the CliniMACS Prodigy®

We developed a fully automated procedure for the isolation of NK cells from leukapheresis products, the CliniMACS Prodigy CD3$^+$ cell depletion / CD56$^+$ cell enrichment process, enabling a two-step separation in a single tubing set (CliniMACS Prodigy TS 310, fig. 1). The process is designed in distinct blocks, enabling the use of both separation steps individually. NKCE: natural killer cell engineering (see results section 3).

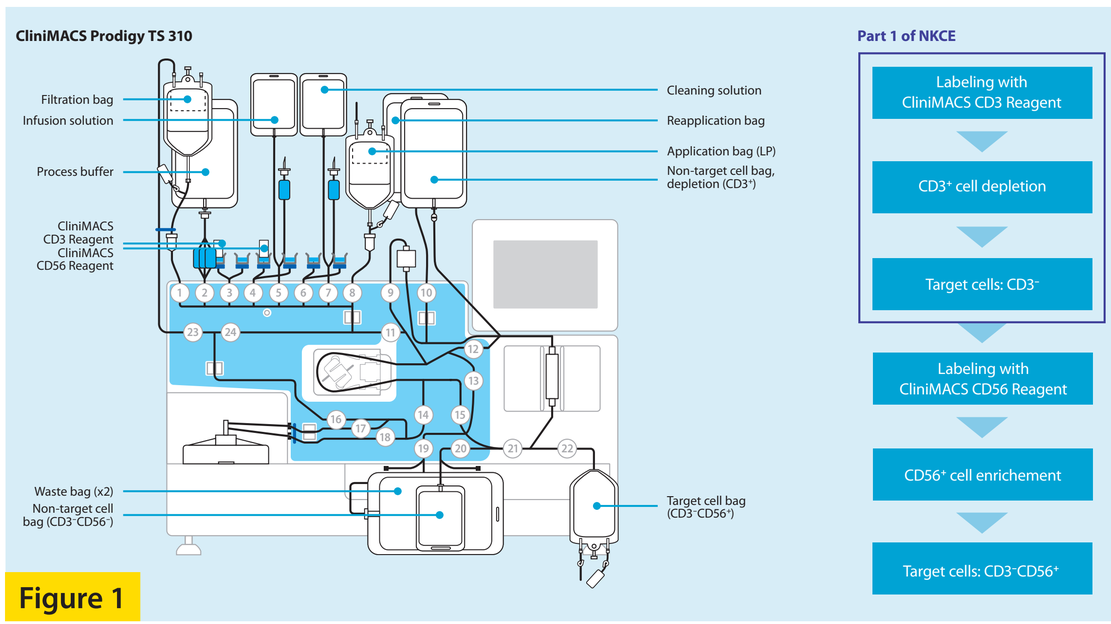

Figure 1

2 Enrichment of CD56$^+$ cells and cultivation of NK cells in the CliniMACS Prodigy®

For cell cultivation and transduction, we developed the CliniMACS Prodigy CD56$^+$ cell enrichment and cultivation process providing fully automated CD56$^+$ cell enrichment and subsequent expansion of NK cells. The entire process takes place in a single tubing set (CliniMACS Prodigy TS 520, fig. 2)).

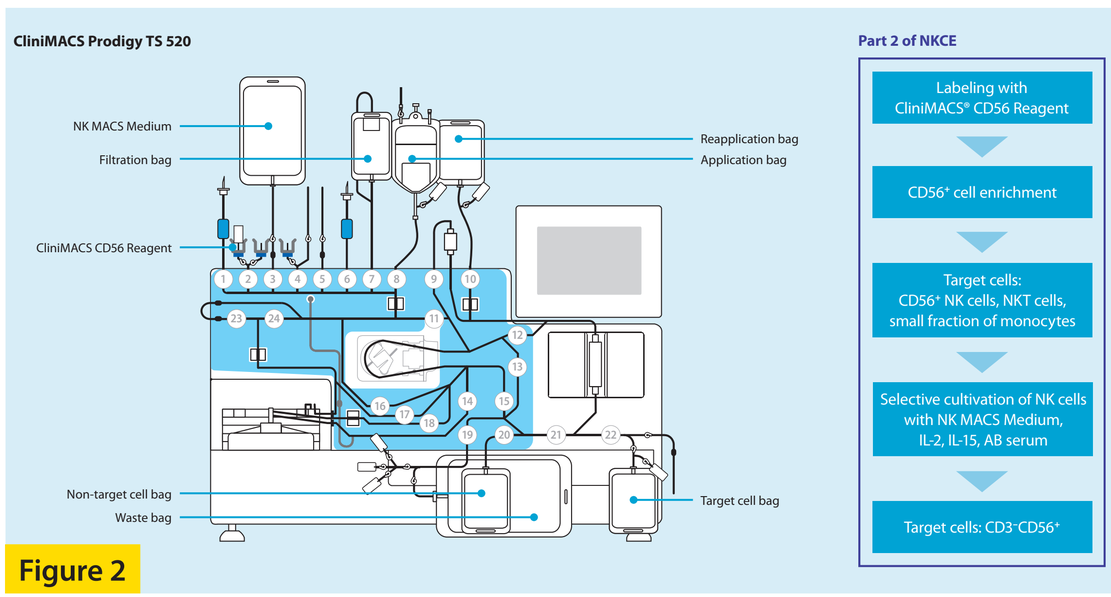

Figure 2

2 Selective expansion of cytotoxic NK cells in NK MACS® GMP Medium

NK cells were expanded from PBMCs (n = 3) in the presence of 5% AB serum, 500 IU/mL IL-2, and 140 U/mL IL-15 for 14 days. Expansion rates were high for both NK MACS Medium (for research use) and NK MACS GMP Medium (fig. 4A). Both media supported selective NK cell growth, which resulted in a mean purity of approximately 80% NK cells after 14 days of culture (fig. 4B). Starting NK cell frequency in PBMCs amounted to 15% on average. Expanded NK cells showed high cytotoxicity against K-562 cells at different effector-to-target cell ratios after 14 days of culture in both NK MACS Medium (research use) and NK MACS GMP Medium (fig. 4C).

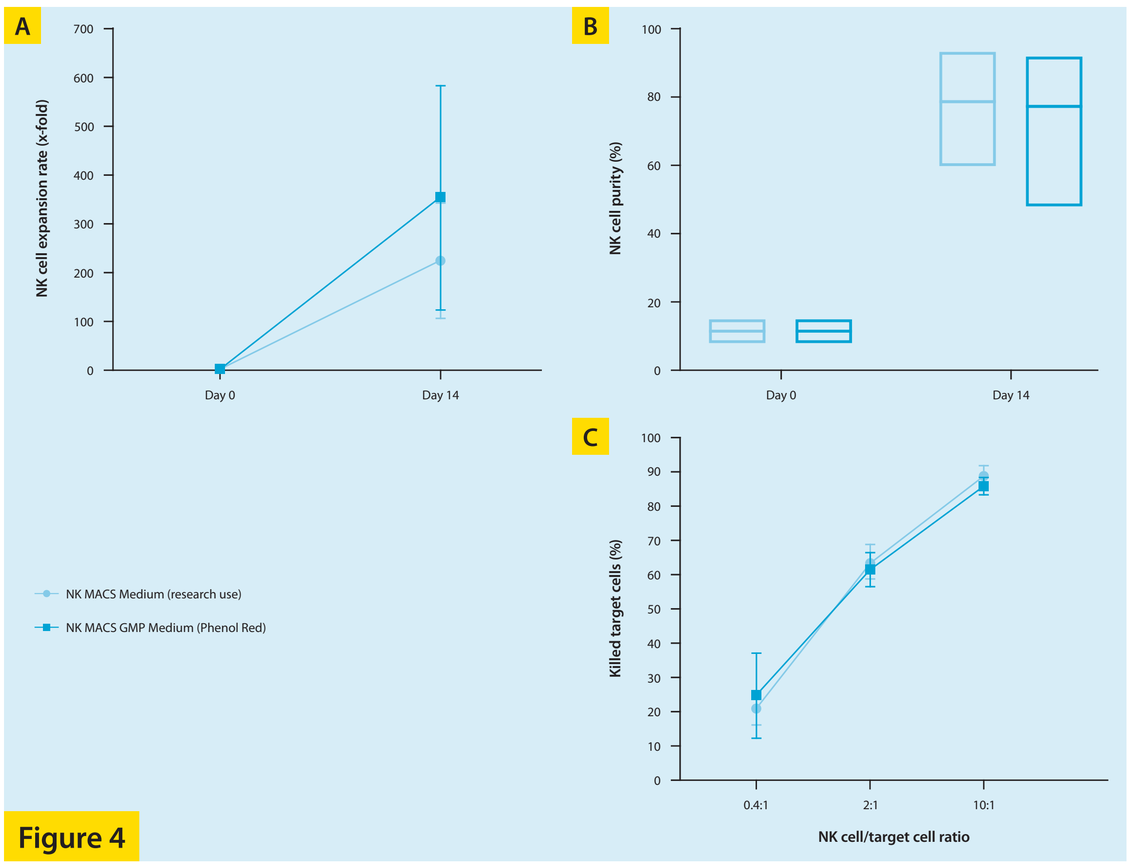

Figure 4

3 CD3$^-$CD56$^+$ NK cell isolation and cultivation generates highly pure NK cells providing the option of genetic engineering

To provide highly pure genetically engineered NK cells, a process for natural killer cell engineering (NKCE) is currently being developed. In a first approach, CD3$^+$ cell depletion will be performed in a separate process (fig. 1, part 1 of NKCE). The resulting cell product, which is depleted of CD3$^+$ cells, will be the starting material for automated CD56$^+$ cell enrichment, transduction, and expansion (fig. 2, part 2 of NKCE). Figure 5A exemplifies the results for cell separation and cultivation of a sample from one donor. After separation, an average NK cell purity of 97% was achieved with a T cell depletion of 4.4–4.9 log (NK:T cell ratio of 1,949–5,591). Figure 5B shows the NK cell expansion over time, resulting in an average NK cell number of 2.4×10 with a purity of >99.8% (fig. 5A). This process represents the basis for a fully automated platform enabling genetic engineering of NK cells.

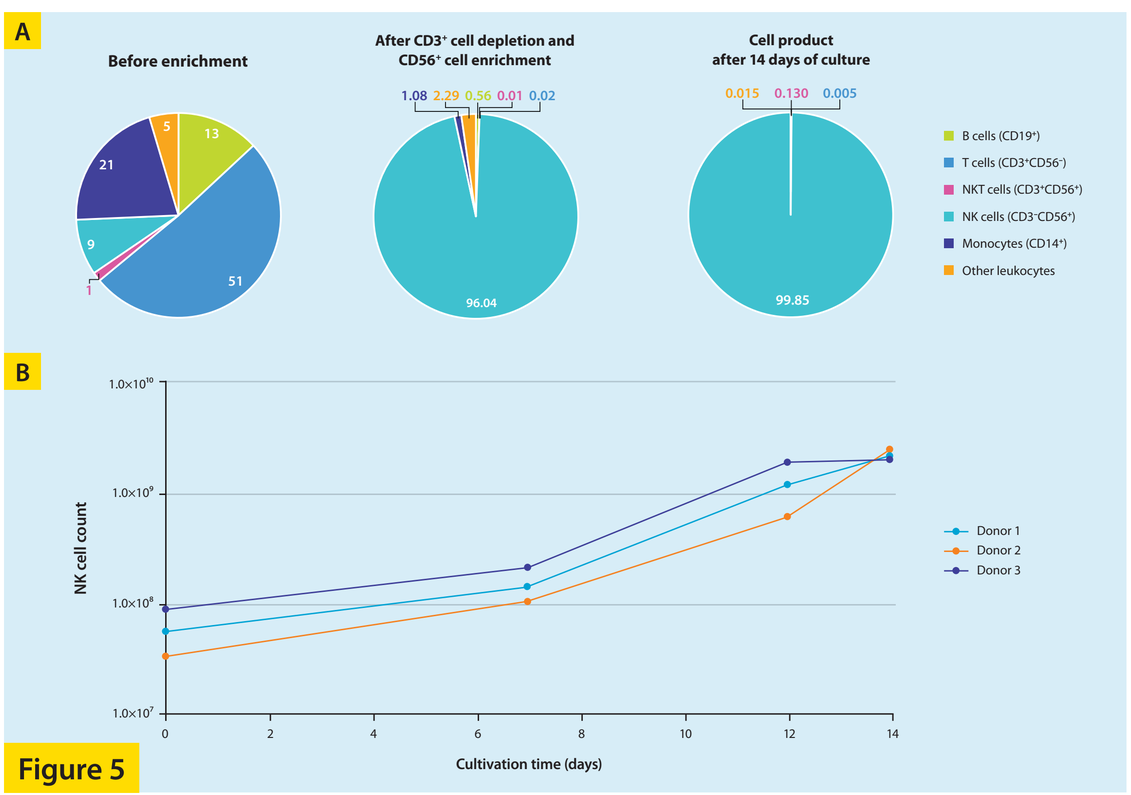

Figure 5

Results

1 CD3$^-$CD56$^+$ NK cell isolation in the CliniMACS Prodigy®

The CliniMACS Prodigy CD3$^+$ cell depletion / CD56$^+$ cell enrichment process resulted in a 4 log (average) depletion of T cells (fig. 3A) and a 98% purity of CD3$^-$CD56$^+$ cells. The resulting NK cell product contained about 2×10^8 NK cells (fig. 3B). NK cell recovery was in the range of 60% (fig. 3C) and the NK:T cell ratio amounted to 1,400 on average (fig. 3D).

Figure 3

Conclusion

- CD3$^-$CD56$^+$ NK cells can be enriched to high purity in the CliniMACS Prodigy.
- NK MACS GMP Medium enables high NK cell expansion rates for clinical settings.
- NK cells can be expanded at high purity (>99%) in the CliniMACS Prodigy.
- These fully automated processes provide the basis for natural killer cell engineering in a closed system. This will simplify CAR NK cell manufacturing procedures and therefore widen the applicability in future clinical applications.