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

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contact: sabinekow@miltenyibiotec.de

Melanie Sohmen, Sabine Müller, Julia Kostyra, Angela Mekes, Marcus Nitsche, Juliane Stuth, Martha Elia Luevano Salinas, Nadine Nickel, and Nina Möker
Miltenyi Biotec B. V. & Co. KG, Bergisch Gladbach, Germany

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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 germ-line 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 have low risk in inducing graft-versus-host-disease as well as a reduced risk of life-threatening cytokine storms during therapy. Both properties make NK cells ideal for allogeneic application. To enhance the anti-tumor specificity and efficiency, NK cells can be further modified by chimeric antigen receptors (CARs). Promising clinical outcome of CAR-engineered NK cells has been recently reported for patients with relapsed or refractory B cell malignancies.

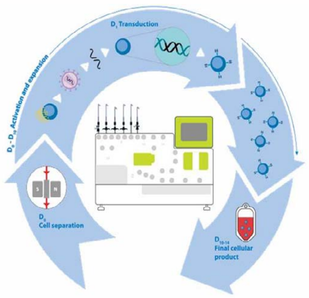
Methods

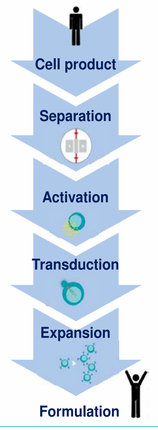
1 Leukapheresis derived NK cell culture using CliniMACS Prodigy®

NK cells can be derived from leukapheresis using the CliniMACS Prodigy® System. The first step in this CliniMACS Prodigy® Natural Killer cell Engineering (NKE) System takes place in CliniMACS Prodigy® TS 320 using CliniMACS Prodigy® LP-3-56 System to deplete T cells. After successfully depleting T cells, resulting in less than 0.1 % $CD3^+$ cells, the $CD56^+$ NK cells are enriched using CliniMACS Prodigy® TS 520 within the CliniMACS Prodigy® PD-56 Engineering System. Subsequent to NK cell cytokine based activation the NK cell transduction is performed at culture day 2. The CAR^+/-NK cells are cultivated and harvested at culture day 14 (fig. 1).

Figure 1

CliniMACS Prodigy Natural Killer cell Engineering System





Leukapheresis maximum 36 hours after harvest

CD3 depletion (CliniMACS Prodigy LP-3-56 System) + CD56 enrichment (CliniMACS Prodigy PD-56 System)

MACS GMP Recombinant Human IL-2, IL-15, and IL-1 family in NK MACS GMP Medium + 5% AB serum

BAEV Vector + Vectofusin-1 + 2h spinocculation, next day vector removal

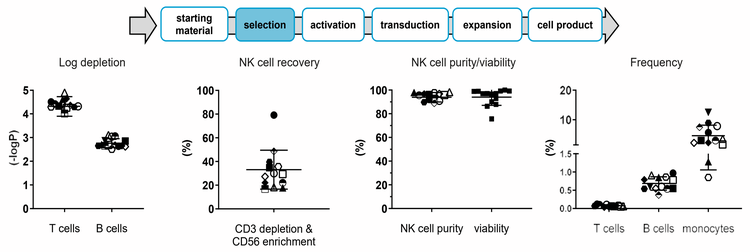
MACS GMP Recombinant Human IL-2, and IL-15 in NK MACS GMP Medium + 5 % AB serum

Harvest in formulation solution

Results

2 NK isolation using CliniMACS Prodigy® NKE System

Figure 2

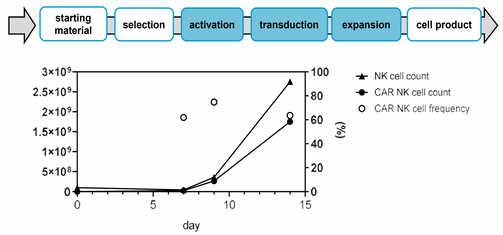


The CliniMACS Prodigy® LP-3-56 System enables a fully automated, two-step NK cell separation in a single tubing set (CliniMACS Prodigy® TS 320). The process is designed in distinct blocks, enabling the use of CD3 depletion as a stand-alone process, as it is used in the CliniMACS Prodigy® NKE System.

The CliniMACS Prodigy® PD-56 Engineering System enables a fully automated NK cell separation with subsequent culture. Starting with CD3 pre-depleted material derived from CliniMACS Prodigy® LP-3-56 System, the $CD56^+$ enrichment, using CliniMACS Prodigy® PD-56 Engineering System, results in a highly pure (93 %) and viable (93 %) NK cell product (fig. 2 NK cell purity/viability). The recovery of NK cells in mean is about 33 % (fig. 2 NK cell recovery). An average log depletion of T cells of 4.3 and B cells of 2.7 (fig. 2 Log depletion) results in a low frequency of contaminating T and B cells as well as monocytes (fig. 2 Frequency).

3 NK cell culture and CAR generation in CliniMACS Prodigy® NKE System

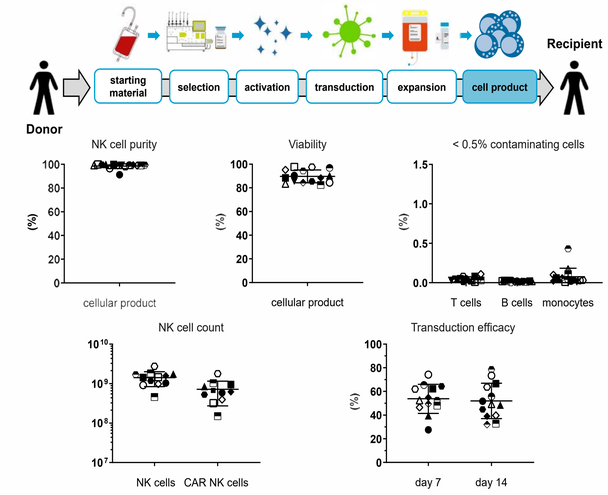
Figure 3



NK cells derived from CD3 depletion and CD56 enrichment are activated at day 0 with MACS GMP Recombinant Human IL-2, MACS GMP Recombinant Human IL-15 and a 3rd cytokine from IL-1 family. The transduction process using baboon-enveloped lentiviral vector takes place at day 2 of culture in the presence of MACS GMP Vectofusin-1® and a subsequent spinocculation for 2h. A cell wash at day 3 removes excess lentiviral vector as well as the 3rd cytokine. The CAR^+/- NK cell culture is continued until day 14 using MACS GMP Recombinant Human IL-2 and IL-15 in NK MACS GMP Medium with 5 % AB serum. Figure 3 exemplifies a CAR^+/- NK growth curve resulting in $2.8x10^9$ NK cells with a transduction efficiency of 64 % ($1.8x10^8$ CAR-NK cells) at day 14 without using feeder cells for expansion.

4 Quality assessment of final CAR-NK cell product

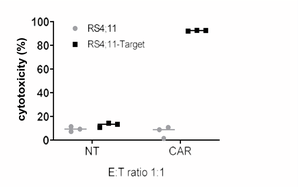
Figure 4



The transduction of NK cells in a GMP-compliant system resulted in a highly pure (>99 %) and viable (90 %) NK cell product with low $CD3^+$ cell content (mean 0.09 %). On average the expansion resulted in $1.19x10^9$ NK cells including $5.7x10^8$ CAR-NK cells. The transduction efficacy during culture is high (mean 50 %) and stable compared between day 7 and 14.

5 Functional assesment of final CAR-NK cell product

Figure 5



To test the in-vitro killing capability, the generated CAR^+/- NK cells were challenged using a resistant tumor cell line. After 24 h and an E:T ratio of 1:1 no killing of the tumor cell line using not-transduced (NT) NK cells was observed, the tumor model therefore works well. The transduced CAR-NK cells show no cytotoxicity in the absence of the target on the tumor cells, but show CAR-mediated NK cytotoxicity in the presence of the target. Apart from the big safety aspect of the CAR-NK cells they show a high killing capability only in the presence of the target.

Conclusion

- Development of novel process for automated NK purification, transduction and cultivation in a closed GMP compatible system.
- High level of automation enables standardized, consistent and operator-independent genetic engineering of NK cells.
- NK cell transduction using baboon-enveloped LV results in high and stable transduction efficacy.
- After 14 days of culture, the generated CAR-NK cells show high purity and viability as well as in-vitro functionality.

To get more information contact Martha Elia Luevano Salinas: marthal@miltenyibiotec.de