Fully automated Natural Killer cell manufacturing platform

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Angela Mekes, Marcus Nitsche, Julia Kostyra, Melissa Quadflieg, Carina Schmitt, Sabine Müller, Julia Hengst, and Nina Möker
Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

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Introduction

Donor-derived natural killer (NK) cells mediate graft-versus-leukemia effects with low risk of inducing alloreactive graft-versus-host-disease and are therefore used for allogeneic cell therapy[1]. As clinical grade purification of NK cells requires two semi-automated separation procedures, including multiple manual hands-on steps, we developed an integrated fully automated process on the CliniMACS Prodigy®. With the goal to improve harmonization of the accompanying analytical strategies, we developed antibody panels and automated gating strategies for the KIR- and NKG2A-based donor selection, as well as the quality control of the target cell product.

Methods

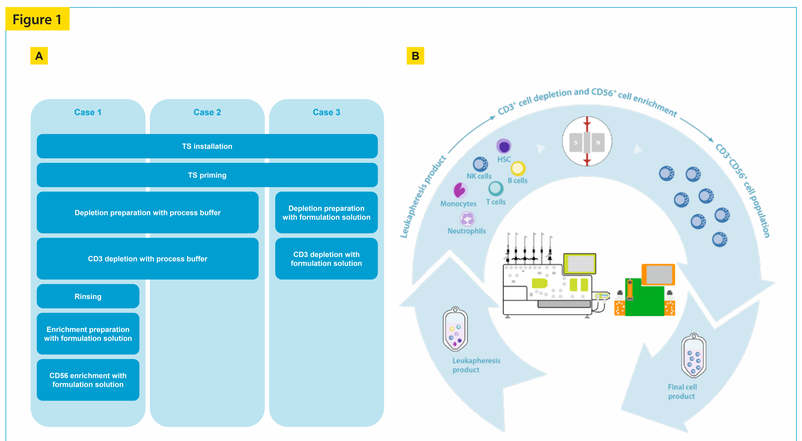
1 Experimental setup and workflow

The novel integrated CliniMACS Prodigy® LP-3-56 System enables a fully automated depletion of $CD3^+$ cells and subsequent enrichment of $CD56^+$ cells in one process in one tubing set.
Depending on the desired separation strategy, the user can choose to perform either:

- depletion of $CD3^+$ cells with subsequent enrichment of $CD56^+$ cells, or
- depletion of $CD3^+$ cells only.

For both strategies target cells are prepared with user-chosen formulation solution (e. g. sodium chloride or NK MACS® GMP Medium).
Additionally, depletion of $CD3^+$ cells can also be performed with process buffer, simplifying downstream applications (e.g. combination with NK cell engineering systems) (fig. 1A).
All separation cases of the CliniMACS Prodigy LP-3-56 System use the CliniMACS Prodigy Tubing Set 320 for an fully automated procedure with integrated labeling and washing steps for both, CD3 and CD56 separation, enabled through an integrated lytic rinsing sequence allowing subsequent CD56 enrichment in the same tubing set (fig.1B).
The new application software effectively depletes 9.6×10^9 $CD3^+$ cells, and enriches 4.5×10^9 $CD56^+$ cells from up to 40×10^9 total white blood cells.

Figure 1



2 Quality control of target cell product

Various antibody panel were evaluated for the donor selection as well as the phenotypic characterization of the purified target cell product. For automated and standardized data acquisition and subsequent analysis, Express Modes were designed to be used with the MACSQuant® Analyzer.
Furthermore, the cells were analyzed with regards to their *in vitro* functionality in co-culture with tumor cells.

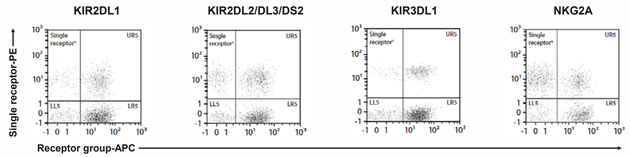
Results

1 Donor selection panel

Antibody panel were developed which allow the simultaneous assessment of the four major inhibitory killer immunoglobulin-like receptors ($KIR2DL1^+$, $KIR2DL2/DL3/DS2^+$, $KIR3DL1^+$) and NKG2A from whole blood.

Each staining cocktail consists of one PE-conjugated antibody and a mixture of the other antibodies conjugated to APC. This enables the analysis of single inhibitory receptor-positive cells (fig. 2).

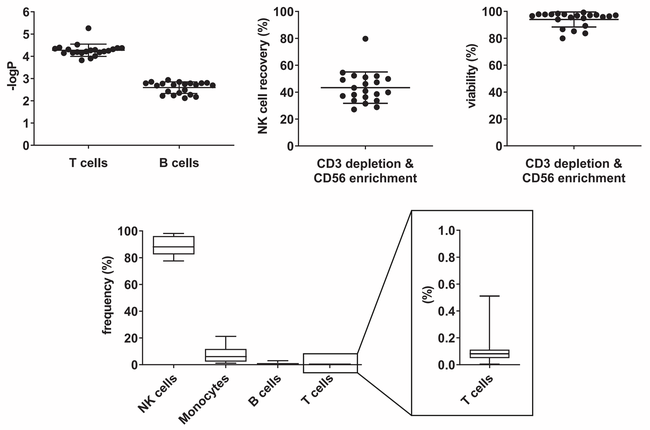
Figure 2



2 Separation performance

Performing the depletion of $CD3^+$ cells with subsequent enrichment of $CD56^+$ cells led to mean log depletion of $CD3^+$ and $CD19^+$ cells of 4.3 and 2.6, respectively. The NK cells were viable (mean 94.4%) and pure (mean 89.3%) with a mean recovery of 43.4%. Most residual non-NK cells were monocytes (mean 7.8%) (fig. 3).

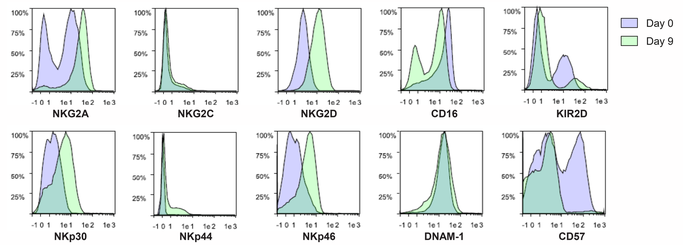
Figure 3



3 Phenotype of purified and expanded NK cells

NK cell phenotype is determined after NK cell separation (Day 0) and expansion (Day 9) by flow cytometric analysis for NK cell activation and maturation markers. The results from a representative experiment are shown in figure 4. Enhanced expression of NKp46, NKp44, NKp30 and NKG2D at day 9 indicated the activated status and cytotoxic potentials of NK cells after expanding with NK MACS GMP Medium supplemented with 5% human AB serum, MACS GMP Recombinant Human IL-2, IL-15 and IL-1 family cytokine. We also observed upregulation of NKG2A and downregulation of CD16 and CD57 on NK cells upon expansion. No substantial changes were found for NKG2C and DNAM-1.

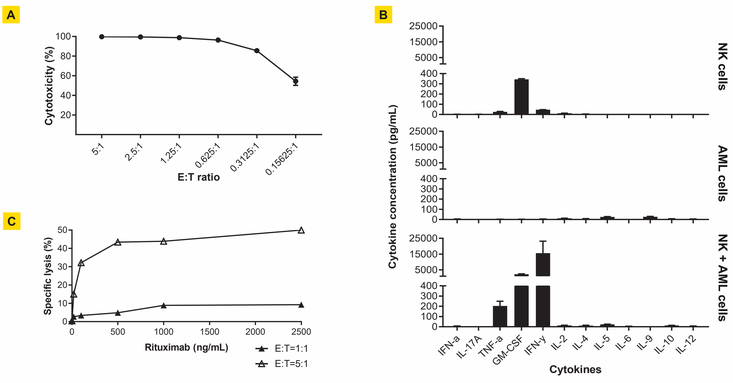
Figure 4



4 Preserved functionality of purified NK cells

The lysis of tumor cells by NK cells was analyzed after co-incubation for 24 hours at different ratios NK:AML cells (fig. 5A).
The secretion of cytokines was analyzed using MACSPlex Cytokine 12 Kit, human after NK cell incubation with or without AML cells (1:1 ratio) for additional 24 hours. The specific activation of NK cells is shown by the increased secretion of TNF-α, GM-CSF, and IFN-γ after co-culture with target cells (fig. 5B).
For both assays, purified NK cells were cultured in NK MACS GMP Medium supplemented with 5% AB serum, MACS GMP Recombinant Human IL-2, IL-15 and IL-1 family cytokine for 14 days.
To demonstrate the ability to perform antibody dependent cell-mediated cytotoxicity, purified NK cell were cultured in NK MACS GMP Medium supplemented with 5% AB serum, and MACS GMP Recombinant Human IL-2 overnight and co-incubated with Raji cells and different concentrations of human CD20 antibody for 3 hours (fig. 5C).

Figure 5



Conclusion

In summary, the integrated fully automated CliniMACS Prodigy LP-3-56 System enables good manufacturing practice (GMP)-compliant purification of NK cells and is approved as medical device application.
In combination with the automated strategies for the inhibitory receptor-based donor selection and the quality control of the target cell product, the CliniMACS Prodigy LP-3-56 System covers the complete workflow for NK cell-based therapeutic applications.

References:
1. Mehta, R.S. *et al.* (2018) *Int J Hematol* 107, 262–270

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