Fully automated integrated process for depletion of T cells with or without subsequent purification of NK cells from apheresis using the CliniMACS Prodigy®

Poster ID: 418
contact: angelah@milenyibiotec.de

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

Scan the QR code to visit the Miltenyi Biotec website:

Introduction

Natural killer (NK) cells have shown anti-tumor effect with a low risk of graft-versus-host disease in many clinical studies. NK cells are used in different therapeutic settings including infusion of autologous NK cells, allogeneic NK cells or as donor lymphocyte infusion (DLI) post hematopoietic stem cell transplantation.

The current process to enrich clinical grade NK cells is semi-automated with multiple hands-on steps.
Therefore, we developed an integrated fully automated process enabling either preparation of T cell-depleted DLI or clinical grade purification of NK cells within a single automated process on the CliniMACS Prodigy®.

Methods

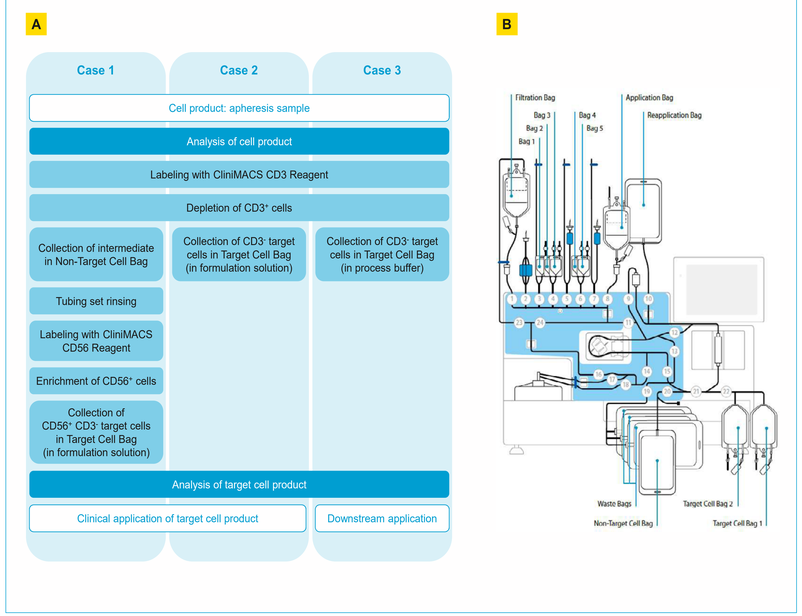
1 Experimental setup and workflow

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® 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 (fig. 2B) 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.
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 Flow cytometry-based quality control, panel overview

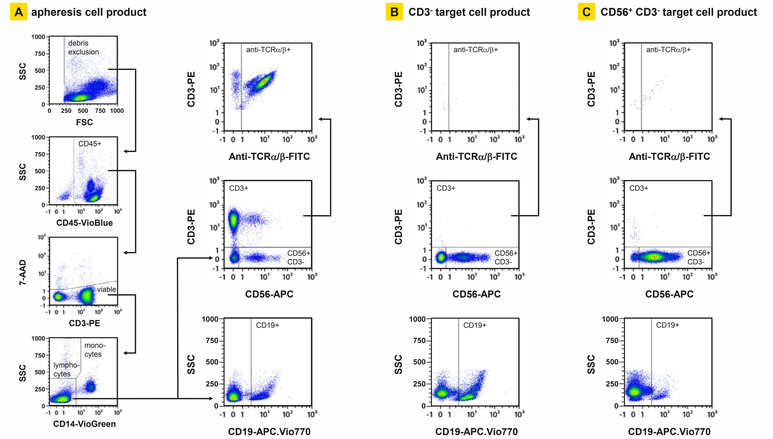
For flow cytometry-based quality control of the separated cells, antibody panels (table 1), corresponding gating strategies as well as automated express mode analysis algorithms have been developed for the MACSQuant® Analyzer 10. These tools enable the convenient and rapid assessment of cell frequencies before and after CD3$^+$ cell depletion as well as after CD56$^+$ cell enrichment (fig. 2 A-C). This ultimately allows to accurately enumerate even of very low numbers of residual unwanted cells.

Table 1

Panel to determine cell count by express mode: "NK_Cell_Count_h_01"			Panel to determine cell frequencies by express mode: "NK_Cell_Composition_h_01"		
Specificity	**Clone**	**Fluorochrome**	**Specificity**	**Clone**	**Fluorochrome**
CD45	REA747	VioBlue®	CD45	REA747	VioBlue®
7-AAD Staining Solution	-	-	CD14	REA599	VioGreen™
			Anti-TCRα/β	REA652	FITC
			CD3	SK7	PE
			CD56	REA196	APC
			CD19	REA675	APC-Vio®770
			7-AAD Staining Solution	-	-

Relevant outcome:
- Cell concentration
- Viability of leukocytes
- Frequency of CD56$^+$ CD3$^-$ cells
- Frequency of CD3$^+$ (TCRα/β$^+$) cells
- Frequency of CD19$^+$ cells

Figure 2



Results

1 Efficient depletion of CD3$^+$ and CD19$^+$ cells, and enrichment of functional NK cells from apheresis product

Inhouse evaluation and verification runs with leukapheresis products (n= 37 in total, n=21 with CD3 depletion, n=16 with CD3 depletion and CD56 enrichment) were performed. After CD3 depletion, mean log depletion of T cells was 4.1. The mean recovery of NK cells was 87.4% with an average NK:T ratio-increase of 11792 fold (data not shown).
After CD3 depletion and CD56 enrichment, mean log depletion of T and B cells was 4.3 and 2.6, respectively.
Purity of NK cells was 88.8% on average with a mean recovery of 44.4%. The majority of non-NK cells after the process were monocytes with a mean frequency of 7.1% (fig. 3).
Functionality of purified NK cells was proven by their ability to lyse AML cells (fig. 4A), to secrete cytokines after co-culture (fig. 4B), and to perform antibody dependent cell-mediated cytotoxicity (ADCC) against Raji cells, using human CD20 antibody (fig. 4C).

Figure 3

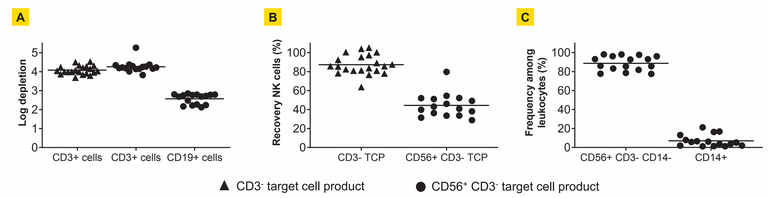
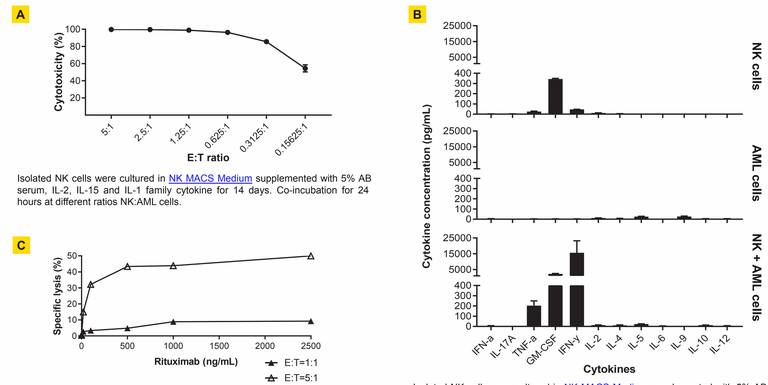


Figure 4



Isolated NK cells were cultured in NK MACS Medium supplemented with 5% AB serum, IL-2, IL-15 and IL-1 family cytokine for 14 days. Co-incubation for 24 hours at different ratios NK:AML cells.

Isolated NK cells were cultured in NK MACS Medium supplemented with 5% AB serum and 500 IU/mL IL-2 overnight and co-incubated with Raji cells for 3 hours.

Isolated NK cells were cultured in NK MACS Medium supplemented with 5% AB serum, IL-2, IL-15 and IL-1 family cytokine for 14 days. MACSplex assay (MACSPlex Cytokine 12 Kit, human) performed after NK cell incubation with or without AML cells (1:1) for 24 hours.

Conclusion

- All application cases of the novel fully automated CliniMACS Prodigy® LP-3-56 Separation process are capable to efficiently deplete CD3$^+$ cells from apheresis product.
- Fully automated combination of depletion of CD3$^+$ cells with subsequent enrichment of CD56$^+$ cells additionally efficiently depletes CD19$^+$ cells, resulting in functional and pure NK cells.
- The entire procedure is performed in a single tubing set (CliniMACS Prodigy® TS 320) and takes between 3.5 - 4.7 h for depletion of CD3$^+$ cells, and 7.6 - 9.8 h for depletion of CD3$^+$ cells with subsequent enrichment of CD56$^+$ cells, requiring minimal hands-on time for process setup and parameter input of about 1 h.
- Functionality of NK cells was shown by their ability to lyse AML cells, to secrete cytokines after co-culture, and to perform antibody dependent cell-mediated cytotoxicity.
- Effective quality control panels and express modes for MACSQuant® flow cytometers enable easy and accurate monitoring of the entire cell manufacturing process.

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