

Novel automatic application for enrichment of CD34⁺ cells from apheresis products using the CliniMACS Prodigy[®]

Poster code: A010

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Introduction

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for many hematologic malignant and non-malignant diseases, both in allogeneic transplantation and in the context of gene therapy. Lately, the stem cell boost has also emerged as a potent treatment modality not only in the classic HSCT field

but also as a measure to overcome cytopenias in the context of CAR-T cell therapy^{1–5}. To this end, the number of the transplantable CD34⁺ cells as well as the purity of the graft are critical parameters for these therapeutic approaches.



Metho

Experimental setup and workflow

Based on MACS Technology the new LP-34-(320) Enrichment application enables the automatic manufacturing of a highly pure CD34-enriched stem cell product which is simultaneously passively depleted of unwanted cells, e.g., T and B cells. The scalable process is sufficient for the enrichment of up to 1.2×10⁹ CD34⁺ cells from up to 120x10⁹ total WBC. Starting volumes can range of 50–600 mL. Process time from start of cell loading until final elution of target cells is between 3.0 to 5.5

hours (Fig. 1).

Central enhancements of the new application, which utilizes the CliniMACS Prodigy TS 320 with a large volume chamber (Fig. 2A), include a newly developed platelet removal step, a WBC concentration-adjusted separation and the possibility of product release at a requested timepoint because the process allows storage for up to 16 hours (Fig. 2B).





Results

Results of inhouse evaluation and verification runs (n = 24) using mobilized leukapheresis products are shown in Fig. 5A & Fig. 5B. A mean depletion of 4.7 log for T cells (range 4.2 – 5.4), 3.2 log for B cells (range 2.0 – 3.8), and 2.8 log (range 2.0 – 3.7) for platelets was achieved in the CD34-enriched product independent of the process scale (NS = 18, LS = 6). The viability of CD34⁺ cells in the target product was higher than 99%. A mean purity of the CD34-enriched target products of $90.9\% \pm 4.0\%$ (range 82.7% – 97.0%) was obtained, while the yield was in av-

erage 65.2% ± 6.9% (range 57.4% - 79.5%). Of note, although the yield was comparable to the respective values of $65.4\% \pm$ 15.4% published in the 10-year data collection of CliniMACS Plus CD34 separations (Panch et. al., 2019), the Prodigy presented lower standard deviation, suggesting that CD34 enrichment runs on the CliniMACS Prodigy produce more predictable results. Mean WBC recovery of the processed leukapheresis products was 85.4% (range 73.7% - 101.5%) (data not shown).





Flow cytometry based quality control

A comprehensive flow cytometric QC protocol has been developed for analysis of starting and target cell products which combines a convenient and rapid assessment of cell concentrations, cell frequencies, and viability of CD34⁺ cells and leukocytes as well as the accurate detection of potential contaminants, such as residual T and B cells, and a classical immunophenotyping.

The antibody panels and the corresponding gating strategies for the MACSQuant[®] Analyzer 10 and 16 are shown in Fig. 3A & 3B. In detail, based on the ISHAGE guidelines for CD34⁺ cell deCell Panel (Fig. 3A) facilitates the determination of the cell concentration, the viability, as well as the frequency of stem cells and leukocytes.

Employing the gating strategy for the Immune Cell Composition Panel (Fig. 3B) a determination of residual T and B cells as well as a detailed presentation about the graft composition is provided.

Fig. 4 illustrates the analysis of CD34⁺, CD34⁺/CD133⁺ (using the Stem Cell Panel), CD3⁺, and CD19⁺ cells (using the Immune Composition Panel) after LP-34-(320) Enrichment as described in Fig.

Court/mL

9.52e5 9.4e5 9.12e5 1.32e4 1.2e4 1.15e4 1.15e4 8.57e3



Fig. 5C presents the results of the total inhouse runs (including runs performed during development, evaluation and verification) on the CliniMACS Plus Instrument (Plus, n = 17) and on the CliniMACS Prodigy device (Prodigy, n = 38) in regard of the obtained purity and the yield of the CD34⁺ cells in the target product. A mean purity of 91.3% ± 5.8% (range 76.5% – 96.9%) for the Plus and 89.8% ± 6.3% (range 64.8% – 97.0%) for the Prodigy was achieved. A mean yield of the CD34⁺ cells of $61.3\% \pm$ 13.4% (range 33.8% – 79.0%) for the Plus and 64.1% ± 10.3% (range 44.6% – 84.3%) for the Prodigy was obtained. In regard

of the purity and the yield the Plus and the Prodigy systems deliver comparable results. Although the yield was comparable between the Plus and the Prodigy, the Prodigy presented lower standard deviation, suggesting that CD34 enrichment runs on the CliniMACS Prodigy produce more predictable results. Additional equivalency between the Plus and Prodigy was demonstrated by splitpack runs (n = 10), which resulted in a Plus / Prodigy ratio for the yield of 0.97 and the purity of 1.02 (data not shown).

Conclusions

- The CliniMACS Prodigy LP-34-(320) System using the TS 320 and the novel automated LP-34-(320) Enrichment process is
- In regard to the purity and yield, comparability to the existing CliniMACS Plus system was shown. Additionally, in

termination by flow cytometry the gating strategy of the Stem 3A & 3B.

Figure 3A



capable to label and enrich CD34⁺ cells and to deplete T cells, B cells and platelets efficiently from apheresis products delivering a ready to use stem cell product requesting minimal hands on time.

- The duration of a complete run is between 3.0 5.5 hours depending on the cell numbers (excluding the optional in-process storage of up to 16 hours and process set-up).
- In the experiments shown, a mean depletion of 4.7 log for T cells (range 4.2 – 5.4), 3.2 log for B cells (range 2.0 – 3.8), and 2.8 log for platelets (range 2.0 – 3.7) was achieved. The viability of the enriched CD34⁺ cells in the target product was above 99%, the mean purity was 90.9% (range 82.7%) – 97.0%) and the mean CD34 yield was 65.2% (range 57.4%) - 79.5%).

parallel split pack runs, equivalency of the CliniMACS Plus system versus CliniMACS Prodigy system was demonstrated.

- Effective quality control panels for MACS Quant[®] Flow Cytometers enable easy and accurate analysis of the starting and target cell products.
- Important next steps are the submission to an European notified body of the CliniMACS Prodigy LP-34-(320) System for approval as medical device application (CE). An equivalent version of the CE process will be released under a GMP label as HSC Enrichment process (release scheduled by end of 2024).

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