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HEMATOPOIETIC STEM CELLS

Automated CD34+ cell isolation of peripheral blood stem cell apheresis product

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Abstract

Background aims. Immunomagnetic enrichment of CD34+ hematopoietic "stem" cells (HSCs) using paramagnetic nano-bead coupled CD34 antibody and immunomagnetic extraction with the CliniMACS plus system is the standard approach to generating T-cell-depleted stem cell grafts. Their clinical beneficence in selected indications is established. Even though CD34+ selected grafts are typically given in the context of a severely immunosuppressive conditioning with anti-thymocyte globulin or similar, the degree of T-cell depletion appears to affect clinical outcomes and thus in addition to CD34 cell recovery, the degree of T-cell depletion critically describes process quality. An automatic immunomagnetic cell processing system, CliniMACS Prodigy, including a protocol for fully automatic CD34+ cell selection from apheresis products, was recently developed. We performed a formal process validation to support submission of the protocol for CE release, a prerequisite for clinical use of Prodigy CD34+ products. *Methods.* Granulocyte-colony stimulating factor—mobilized healthy-donor apheresis products were subjected to CD34+ cell selection using Prodigy with clinical reagents and consumables and advanced beta versions of the CD34 selection software. Target and non-target cells were enumerated using sensitive flow cytometry platforms. *Results.* Nine successful clinical-scale CD34+ cell selections were performed. Beyond setup, no operator intervention was required. Prodigy recovered $74 \pm 13\%$ of target cells with a viability of $99.9 \pm 0.05\%$. Per $5 \times 10E6$ CD34+ cells, which we consider a per-kilogram dose of HSCs, products contained $17 \pm 3 \times 10E3$ T cells and $78 \pm 22 \times 10E3$ B cells. *Conclusions.* The process for CD34 selection with Prodigy is robust and labor-saving but not time-saving. Compared with clinical CD34+ selected products concurrently generated with the predecessor technology, product properties, importantly including CD34+ cell recovery and T-cell contents, were not significantly different. The automatic system is suitable for routine clinical application.

Key Words: *allogeneic, automation, cell therapy, clean room, CliniMACS, CD34, good manufacturing practice, haplo-identical, immunomagnetic, naked haplo, Prodigy, stem cell transplantation*

Introduction

CD34+ selected allogeneic stem cell grafts are used for patients at very high risk of severe graft-versus-host disease because of poor human leukocyte antigen matching (specifically in the haplo-identical setting), patients intolerant to immunosuppressants or those with non-malignant diseases who will not benefit from immunological graft-versus-leukemia effects [1—5]. CD34+ selected autologous grafts are being used for stem cell support for autoimmune disease and some pediatric solid tumors [6—11]. Manufacturing of CD34+ selected grafts with the CliniMACS Plus system is approved in many countries [12]. The selection package currently consists of a semi-automatic immunomagnetic selection/depletion device, CliniMACS Plus, and cognate

selection software, as well as nanobead-coupled anti-body (AB) and a single-use tubing system with magnetic column. The protocol consists of successive platelet depletion, AB incubation, magnetic selection and column elution/formulation. The T-cell depletion achieved with this method is so profound that very sensitive flow cytometry protocols are required for residual T-cell enumeration. Although semi-manual and complex, the process is of sufficient robustness to consistently generate clinical products for transplantation. Labor consumption is considerable, almost 4 hours, more than 3 hours of which require the presence of two operators. Recently, a protocol was introduced for the automatic cell manipulator CliniMACS Prodigy, which shares important components with CliniMACS Plus and in principle performs the same protocol as CliniMACS Plus, but fully automatically. This protocol was validated with research mobilized apheresis products under full-scale clinical conditions to generate data for submission for regulatory approval/CE marking. Data from this validation are presented and compared against data from CliniMACS Plus products, which were concurrently generated by the same operators and the quality of which was assessed with the same flow cytometry platform.

Methods

Donors and cells

Granulocyte-colony stimulating factor−mobilized apheresis products were collected from healthy volunteers who had undergone apheresis for unrelated donor stem cell donation [13] and had agreed to extend the apheresis for the purposes of this validation. Written informed donor consent was collected. The study was performed in agreement with the Helsinki declaration with ethics committee approval (ethics committee of Johann Wolfgang Goethe University Medical Center, Frankfurt, protocol #486/13). Aphereses were performed with standard Terumo mononuclear cell apheresis equipment as previously described [14,15], not to exceed a total apheresis duration (clinical graft plus validation product) of 300 min. Target hematocrit was <4 mg/dL. Apheresis products containing $4.75 \pm 0.5 \times 10E10$ (mean $\pm$ SEM; range: $2.3-7.2 \times 10E10$) white blood cells with $4.8 \pm 0.6 \times 10E8$ (mean $\pm$ SEM; range: $2.3-7.6 \times 10E8$) CD34+ cells and $38 \pm 5\%$ (mean $\times$ SEM; range: 7−51%) neutrophil content were used. Apheresis collections and selections were done in the second quarter of 2014.

Hemocytometry

Leukocyte concentrations in starting population, non-target population and target population were determined using the Sysmex XT1800 automatic hemocytometer for plausibility control against the single-platform flow cytometry assays. Flow cytometry was performed with FACSCalibur and LSRFortessa (Becton-Dickinson). Cells were stained with 7-AAD viability dye (BD Biosciences) and the following antibodies (all from BD Biosciences unless otherwise noted): anti-CD45-FITC (2D1)/anti-CD34-PE (8G12) (BD Stem Cell Reagent), anti-CD14-V450 (MφP9), anti-CD3-APC (SK7), anti-CD4-AmCyan (SK3), anti-CD8-APC-Vio770 (BW135/80, Miltenyi Biotec), anti-CD20-APC-eFluor780 (2H7, eBioscience) and anti-CD56-PE-Cy7 (CMSSB, eBioscience). *In-vitro* diagnostic-grade ABs were used where possible. Three platforms each were tested on apheresis product, positive and negative fraction (the commercial single-platform stem cell enumeration (SCE) kit; BD Biosciences), our clinical routine single-platform residual T-cell detection panel and a second residual cell identification panel designed for extended characterization of non-CD34+ cells for the purpose of these studies. The first two platforms were formally validated, are described in standard operating procedures and were performed in accordance with those procedures; the residual T-cell detection panel, in addition to containing BD counting beads for single-platform T-cell enumeration, includes CD34 and CD45 AB and ISHAGE (International Society for Hematology and Graft Engineering, now ISCT, International Society for Cellular Therapy)-conforming gating for bona fide CD34+ cells, to allow for additional cross-validation/plausibility control against the robust and simple three-color SCE protocol [16]. The extended research panel, including additional quantification of CD20 and CD56 and ISHAGE-conforming gating for CD34+ cell enumeration, was not formally validated to GMP level, but results were similarly compared against the two other, formally validated panels. Given the demonstrable precision of CD34+ cell enumeration, whenever the CD34+ cell count with any of the residual cell panels differed from the count with SCE by $\pm10\%$, the measurement had to be repeated. Unless otherwise indicated, all cell concentrations, frequencies or numbers refer to 7AAD-negative (viable) cells only.

Selection reagents and consumables

The CliniMACS Prodigy device [17,18], Prodigy TS310 tubing sets, CliniMACS CD34 Reagent (2 vials) and CliniMACS PBS/EDTA buffer were received from Miltenyi Biotec. NaCl 0.9%, H_2O injectionem and human serum albumin (HSA) were from Baxter.