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Preliminary Evaluation of a Highly Automated Instrument for the Selection of CD34+ Cells from Mobilized Peripheral Blood Stem Cell Concentrates

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Abstract

Background—Cell selection is an important part of manufacturing cellular therapies. A new highly automated instrument, the CliniMACS Prodigy, was evaluated for the selection of CD34+ cells from mobilized peripheral blood stem cell (PBSC) concentrates using monoclonal antibodies conjugated to para-magnetic particles.

Methods—PBSCs were collected by apheresis from 36 healthy subjects given G-CSF or G-CSF plus plerixafor. CD34+ cells from 11 PBSC concentrates were isolated with the automated CliniMACS Prodigy and 25 with the semi-automated CliniMACS Plus Instrument.

Results—The proportion of CD34+ cells in the selected products obtained with the two instruments was similar; 93.6±2.6% for the automated and 95.7±3.3% for the semi-automated instrument ($p > 0.05$). The recovery of CD34+ cells from PBSC concentrates was less for the automated than the semi-automated instrument (51.4±8.2% versus 65.1±15.7%; $p=0.019$). The selected products from both instruments contained few and similar quantities of platelets and red blood cells. The depletion of CD3+ cells was less with the automated instrument (4.34±0.2 log

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Potential Competing Interests

One of the authors, KL, is an employee of the company that sells the instruments tested and the reagents and supplies used in this study.

depletion versus 5.20 ± 0.35 log depletion; $p < 1 \times 10^{-6}$). Removal of platelets from PBSC concentrates by washing was associated with better CD34+ cell recovery. We explored the reasons for lower CD34+ cell recovery by the Prodigy and found that the non-selected cells for the Prodigy contained more platelets than those for the CliniMACS Plus.

Conclusions—CD34+ cells can be effectively selected from mobilized PBSC concentrates with the CliniMAC Prodigy, but the recovery of CD34+ cells and depletion of CD3+ cells was lower than with the semi-automated CliniMACS Plus Instrument.

Keywords

CD34+ cells; hematopoietic stem cells; cell selection

INTRODUCTION

The selection or depletion of specific cell types from leukocyte rich products collected by apheresis is an important step in the manufacture of many cellular and gene therapies. The selection of CD4+ cells from peripheral blood mononuclear cell (PBMC) concentrates collected by apheresis is the first step in the production of T Rapamycin cells which are being used to prevent disease relapse following allogeneic transplantation involving HLA-compatible siblings¹. For several clinical applications natural killer (NK) cells are being isolated from PBMC concentrates by CD3+ cell depletion or CD3+ cell depletion followed by CD56+ cell selection²⁻⁵. The most common use of cell selection is the isolation of CD34+ cells from granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cell (PBSC) concentrates for allogeneic hematopoietic stem cell (HSC) transplantation⁶⁻⁸ and for autologous gene therapy⁹. For allogeneic HSC transplantation CD34+ cells are selected to obtain a graft that has low levels of CD3+ cells in order to reduce the risk of graft-versus-host disease (GVHD) to the recipient^{8, 10}. For autologous gene therapy CD34+ cells are selected so that only the hematopoietic progenitor cells are transduced which reduces the quantity of vector required for effective transduction.

One of the most commonly used methods for cell selection and depletion makes use of a sterile closed system which includes monoclonal antibodies conjugated to magnetic particles^{11,12}. After the conjugated antibodies are incubated with cells in suspension, unbound antibodies are removed by washing and the targeted cells are isolated with a magnetic cell separator. An instrument is available for the clinical scale selection or depletion of cells from PBMC or PBSC concentrates, the CliniMACS Plus Instrument. This instrument allows for Good Manufacturing Practices (GMP)-compliant processing of cells using GMP grade antibody and sterile disposable closed system kits^{12,13}. The use of the CliniMACS Plus Instrument allows for the effective isolation of many cell types, but it is only partially automated and several manual steps are required for cell selection or depletion. The starting cellular material must be washed to remove platelets and to suspend the leukocytes in the appropriate buffered solution. The conjugated antibodies must also be added manually. Finally, the cells must be washed to remove unbound antibody before the cells are selected by the CliniMACS Plus Instrument.

A more automated closed system instrument, the CliniMACS Prodigy, is now available for clinical scale GMP cell selection and depletion. This instrument uses the same clinical grade conjugated monoclonal antibodies as the CliniMACS Plus System and has a sterile closed system disposable kit. In addition, this new instrument has an integrated centrifuge chamber and fluid pumps which allows for automated cell washing and concentration. The centrifuge chamber also services for incubation of cell product with antibody. As a result, all steps including the washing, antibody addition and incubation steps are automated.

We evaluated the CliniMACS Prodigy for the selection of CD34+ cells from mobilized PBSC concentrates collected from healthy subjects. The performance of the CliniMACS Prodigy was compared to that of the CliniMACS Plus Instrument for the selection of CD34+ cells from similar mobilized PBSC concentrates.

MATERIALS AND METHODS

Study Design

CD34+ cells were selected with the CliniMACS Prodigy (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) from mobilized PBSC concentrates collected from healthy subjects from January 2014 through December 2014. The results of the selection of CD34+ cells with the CliniMACS Prodigy were compared with the selection of CD34+ cells by the CliniMACS Plus Instrument (Miltenyi Biotec GmbH) from PBSC concentrates collected from January 2013 through December 2014. All selected CD34+ cells were used for laboratory research. The studies were approved by institutional review boards from intramural programs of the NIH, Bethesda, Maryland, USA and all participants signed informed consents.

Mobilization and Collection of CD34+ Cells

Hematopoietic stem cells (HSCs) were mobilized from healthy subjects by the administration of G-CSF (10 µg/kg/day subcutaneously, Neupogen, Amgen, Thousand Oaks, CA, USA) daily for 5 days; some subjects received G-CSF (10 µg/kg/day) daily for 5 days plus one dose of subcutaneous plerixafor (0.24 mg/kg, Mozobil, Sanofi-Aventis, Bridgewater, NJ) the day before the collection. For all subjects enrolled in both mobilization protocols PBSC concentrates were collected using a blood cell separator (Cobe Spectra, Terumo BCT, Lakewood, CO, USA) and 10 to 15 liters of blood was processed.

CD34+ Cell Selection

Processing of the PBSC concentrates using the CliniMACS Prodigy or the CliniMACS Plus Instrument began within 3 hours of the collection. When PBSC concentrates were isolated with the CliniMACS Plus Instrument, the PBSC concentrates were washed to reduce the number of platelets by diluting the PBSC concentrates to 500 mL using CliniMACS PBS/EDTA buffer (Miltenyi Biotec GmbH) with 0.5% human serum albumin (HSA) and centrifuging at 419 Relative Centrifugal Force (RCF) using a floor model centrifuge (Sorvall RC 3BP+ Centrifuge, Thermo Scientific, Frederick, MD, USA) for 10.5 minutes. The pelleted cells were resuspended in 500 mL of PBS/EDTA buffer plus 0.5% HSA. This process was repeated twice and after the final wash the cells were resuspended in 95 mL of

PBS/EDTA buffer with 0.5% HSA. The washed cells were incubated with intravenous immunoglobulin (IVIG) at 1.5 mg/mL and CD34 antibody (CD34 Reagent, Miltenyi Biotec Inc. Auburn, CA) for 30 minutes, at room while gently rotating. At the end of the incubation period the cells were washed again twice and loaded on to the CliniMACS Plus instrument. Selection was performed using the LP-34 enrichment program and a Prodigy 310 tubing set. For CD34+ cell selection with the CliniMACS Prodigy instrument, the PBSC concentrate was loaded into the instrument and cell washing and selection was performed using the default settings. The reagents used included PBS/EDTA buffer with 0.5% HSA, intravenous immunoglobulin (IVIG) at 0.75 mg/mL and CD34 antibody (CD34 Reagent, Miltenyi Biotec Inc. Auburn, CA)

Cell Analysis

All cell counts were performed with an automated cell counter (Cell-Dyn 3700, Abbott Diagnostics, Abbott Park, IL, USA). Flow cytometry analysis was performed using CD34-PE, CD45-FITC, CD3-PE-Cy7 and CD19-APC monoclonal antibodies all from BD Biosciences, San Jose, CA, USA). For analysis of the apheresis bag 100,000 events were acquired and for the selected cells 20,000 events were acquired on a FACScanto cytometer (BD Bioscience). The data were analyzed using a FACSDiva (BD Bioscience)

Statistical Analysis

Data are represented as the mean plus/minus 1 standard deviation (SD). The differences between groups were compared using 2-tailed T tests (Excel, Microsoft, Redmond, WA) and $p < 0.05$ was considered as significant.

RESULTS

PBSC Concentrates

CD34+ cells were selected from 11 PBSC concentrates with the CliniMACS Prodigy and 25 with the CliniMACS Plus Instrument. All PBSC products were collected from healthy subjects. All 11 PBSC concentrates processed with the CliniMACS Prodigy were from subjects given GSF and for the CliniMACS Plus 22 PBSC concentrates were from subjects given G-CSF and 3 from healthy subjects given G-CSF plus plerixafor. The composition of the PBSC concentrates from the two groups was similar including the percent and quantity of CD34+ cells (Table 1).

Selection of CD34+ Cells

The 11 CD34+ cell enriched products selected by the CliniMACS Prodigy contained $364 \pm 179 \times 10^6$ WBCs and $342 \pm 117 \times 10^6$ CD34+ cells and very few platelets and RBCs (Table 2). When the cells selected with the CliniMACS Prodigy were compared with those isolated with the CliniMACS Plus, the total number of WBCs, CD34+ cells, RBCs and platelets did not differ (Table 2). The proportion of leukocytes expressing CD34 antigen was high after selection by both the CliniMACS Prodigy and CliniMACS Plus Instruments and the results were similar for the two instruments (Table 2 and Figure 1, Panel A), however, the proportion of CD34+ cells recovered from the PBSC concentrates was lower with the CliniMACS Prodigy ($51.4 \pm 8.2\%$ versus $65.1 \pm 15.7\%$; $p=0.012$) (Figure 1, Panel B).

CD3+ and CD19+ Cell Depletion

There were also differences in the quantities of contaminating leukocytes in the products. Those selected with the CliniMACS Prodigy contained significantly more CD3+ T cells ($1.13 \pm 0.61 \times 10^6$ versus $0.17 \pm 0.17 \times 10^6$, $p < 1 \times 10^{-3}$) and CD19+ B cells ($5.20 \pm 4.24 \times 10^6$ versus $1.18 \pm 0.84 \times 10^6$, $p < 1 \times 10^{-3}$) than those selected by the CliniMACS Plus. The proportion of CD3+ and CD19+ cell depleted from the starting PBSC concentrates was also lower with the CliniMACS Prodigy (Figure 2). For CliniMACS Prodigy the depletion of CD3+ cells was 4.34 ± 0.21 log compared to 5.19 ± 0.35 log for the CliniMACS Plus ($p < 0.001$) and the CD19+ cell depletion was 3.03 ± 0.28 log compared to 3.62 ± 0.30 log for the CliniMACS Plus ($p < 0.001$).

Platelets and CD34+ Cell Recovery

The presence of greater quantities of platelets in PBSC concentrates at the time of addition of CD34 antibody has been reported to reduce the efficiency of CD34+ selection by the CliniMACS Plus¹⁴. The procedures used to select CD34+ cells with both the CliniMACS Prodigy and the CliniMACS Plus Instrument involves washing to reduce the levels of contaminating platelets. To determine if the degree of platelet removal differed among the two instruments, we compared the quantities of platelets among the cells in the negative fraction bag or unselected cell fraction from the CliniMACS Prodigy and CliniMACS Plus Instrument. We found that the cells that were not selected by the CliniMACS Prodigy contained more platelets and RBCs than those that were not selected by the CliniMACS Plus Instrument, but there was no difference in the quantities of WBCs among the negative cell fractions from the two instruments (Table 3).

When the quantity of platelets in the negative cell fraction and CD34+ cell recovery for each of the 11 PBSC concentrates processed with the CliniMACS Prodigy was compared, we found that the CD34+ cell recovery dropped as the quantity of platelets in the negative cell fraction increased (Figure 3, Panel A). For the CliniMACS Plus Instrument, there was no difference in the CD34+ cell recovery among products with high and low quantities of platelets among the negative cell fraction (Figure 3, Panel B). There was no relationship between the quantity of platelets in the negative cell fraction and depletion of CD3+ cells and CD19+ cells by the CliniMACS Prodigy (data not shown).

DISCUSSION

We compared the performance of the CliniMACS Prodigy and the CliniMACS Plus for selecting CD34+ cells from mobilized PBSC concentrates and found that the purity of the CD34+ cells and the quantities of contaminating platelets and RBCs was similar among the products selected with the two instruments. However, there were differences in the recovery of target cell population and the depletion of CD3+ and CD19+ cells. These results are similar to those of Spohn and colleagues who also assessed the CliniMACS Prodigy for the selection of CD34+ cells¹⁵.

The mean proportion of CD34+ cells in the CliniMACS Prodigy and CliniMACS Plus selected products was similar in our study and in that of Spohn; 93.6% versus 93.9%

respectively for the CliniMACS Prodigy and 95.7% versus 95.9% for the CliniMACS Plus¹⁵. Cho and colleagues found that the mean proportion of CD34+ cells in the CliniMAC Plus selected products was 95.8%¹⁶, Dvorak and colleagues found the median proportion of cells expressing CD34+ antigen in CliniMAC Plus selected products was 97% (range: 87%-99%)¹⁷, and for the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) the median was 96.7% (61.5%-99.8%)¹⁸.

In our study the mean recovery of CD34+ cells from the mobilized PBSC concentrates by the CliniMACS Prodigy was $51.4 \pm 8.2\%$ compared to $65.1 \pm 15.7\%$ with the CliniMACS Plus. Spohn obtained a great CD34+ cell recovery with the CliniMACS Prodigy, $73.6 \pm 13.2\%$ ¹⁵. The CD34+ cell recovery by the CliniMACS Prodigy in our study was also slightly less than reported for the CliniMACS Plus by other groups. Dvorak¹⁷ found that the median recovery of CD34+ cells selected with the CliniMACS Plus from G-CSF-mobilized PBSC products was 60% (44% to 77%)¹⁷; and the BMT CTN found the mean CD34+ cell recovery was $66.1\% \pm 20.3\%$ ¹⁸.

The quantity of CD3+ cells in the CliniMACS Prodigy selected products was greater than in the CliniMACS Plus selected products in both our study and that of Spohn¹⁵. However, the mean log depletion of CD3 cells we obtained with the CliniMACS Plus, 5.20 ± 0.35 log, was slightly greater than the mean values of 4.7, 4.8 and 5.1 reported by others using the CliniMACS Plus^{14,18}. Since many clinical hematopoietic stem cell transplant programs add some peripheral blood lymphocytes back to the selected CD34+ cells in order prevent graft failure⁸, it is not likely that this difference in the quantities of T cells in the CliniMACS Prodigy and CliniMACS Plus selected products will be clinically important. For gene therapy protocols that make use of autologous CD34+ cells selected from mobilized PBSCs, the slight increase of CD3+ cells in the CliniMACS Prodigy selected products is also unlikely to affect clinical outcome.

We also found that the CliniMACS Prodigy selected products contained greater quantities of CD19+ cells than the CliniMACS Plus. The mean log depletion of CD19+ cells was 3.03 ± 0.28 for the Prodigy compared to 3.62 ± 0.30 for the CliniMACS Plus. Using the CliniMACS Plus Del Fante and colleagues have found a very similar level of CD19+ cells depletion; 3.5 to 3.6 log-depletion¹⁴. This slight difference in depletion of CD19+ cells by the 2 instruments is not likely to result in meaningful differences in clinical outcomes of hematopoietic stem cell transplant or gene therapy clinical trials.

We cannot exclude the possibility that the differences noted were due, at least in part, to difference in the starting material. The results of selecting CD34+ cells from G-CSF mobilized PBSC concentrates with the CliniMACS Prodigy System were compared, in part, to historic data obtained with the CliniMACS Plus Instrument. A better comparison would have involved splitting the PBSC concentrates and processing half on each concentrate on each instruments or the randomly assigning the processing of an entire unit to one of the two instruments.

Both the CliniMACS Prodigy and the CliniMACS Plus used the same CD34 antibody reagent, so the differences in CD34+ cell recovery were not due to antibody source. It is

likely that differences in the quantities of platelets in the PBSC products at the time of antibody sensitization contributed to the reduction of CD34+ cell recovery with the CliniMACS Prodigy. It is known that the presence of large quantities of platelets among leukocytes at the time of antibody sensitization can reduce CD34+ cell recovery possibly by interfering with antibody binding to CD34+ cells¹⁹. The PBSC concentrates that were processed with the CliniMACS Prodigy and CliniMACS Plus initially contained similar quantities of platelets and PBSC concentrates processed by both instruments were washed to reduce platelet levels. We did not measure product platelet counts after the washing, but we did measure the quantity of platelets among the negative cell fraction. We found that the CliniMACS Prodigy negative cell fraction contained more platelets than the CliniMACS Plus negative cell fraction indicating that the removal of platelets by the CliniMACS Prodigy by automated washing was less effective than that of the manual washing used with that CliniMACS Plus processing. Others have found that adding additional PBSC concentrate washing steps further reduced the number of platelets and improved the CD34+ cell recovery by the CliniMACS Plus¹⁴. It is possible that improving the platelet removal by the CliniMACS Prodigy by changing the instruments wash steps could improve CD34+ cell recovery.

In conclusion, the highly automated Prodigy instrument was effective at selecting CD34+ cells from mobilized PBSCs but the composition of the selected cells differed slightly from those selected with the CliniMACS Plus. The CD34+ cell recovery was slightly lower and the selected cells contained more CD3+ and CD19+ cells with the CliniMACS Prodigy. These procedures are some of the first to be performed with the CliniMACS Prodigy and it is expected that its performance will likely improve as more experience is obtained and the processing procedure is optimized. It is possible that increased washing could improve the CD34+ cell recovery with the CliniMACS Prodigy.

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Figure 1.

Comparison of the purity and recovery of CD34+ cells in 11 CliniMACS Prodigy and 25 CliniMACS Plus Instrument selected products. The CD34+ cells were selected from mobilized PBSC concentrates. The proportion of CD34+ among the selected leukocytes are shown in panel A and the proportion of CD34+ cells that was recovered from the PBSC concentrates are shown in panel B. For each panel the results of processing with the CliniMACS Prodigy CD34+ cells are shown on the right and with the CliniMACS Plus Instrument on the left. Each symbol represents one selected product.

Figure 2.

Comparison of the depletion of CD3+ and CD19+ cells from mobilized PBSC concentrates by the CliniMACS Prodigy and the CliniMACS Plus Instrument for each of the 11 products selected by the CliniMACS Prodigy and the 25 products selected by the CliniMACS Plus Instrument. The log depletion of CD3+ cells is shown in panel A and the log depletion of CD19+ cells is shown in panel B. For each panel the results of processing with the CliniMACS Prodigy CD34+ cells are shown on the right and with the CliniMACS Plus Instrument on the left. Each symbol represents one selected product.

Figure 3.

Effect of the quantities of platelets among negative or unselected cell fractions on CD34+ cell recovery. The CD34+ cells were selected from mobilized PBSC concentrates. The CD34+ cell recovery and the quantity of platelets among the negative cell fraction for each of the 11 products processed with the CliniMACS Prodigy are shown in Panel A. For each of the 25 products processed with the CliniMACS Plus Instrument the CD34+ cell recovery and quantity of platelets among the negative cell fraction are shown in panel B. Each symbol represents one selected product.

Table 1

Comparison of PBSC concentrates processed on the CliniMACS Prodigy and CliniMACS Plus cell selection instruments

	CliniMACS Prodigy (n=11)	CliniMACS Plus* (n=25)
Volume (mL)	275 ± 42	251 ± 41
WBC Count (×10⁶/mL)	300 ± 42	333 ± 118
Total WBC (×10⁹)	81.5 ± 13.0	80.4 ± 23.6
Neutrophils (%)	38 ± 13	41 ± 20
Lymphocytes (%)	30 ± 8	30 ± 10
Monocytes (%)	25 ± 6	24 ± 10
Platelets (×10⁶/mL)	2804 ± 653	2813 ± 592
Total Platelets (×10⁹)	751 ± 134	692 ± 130
RBCs (×10⁹/mL)	1.39 ± 0.36	1.48 ± 0.35
Total RBCs (×10⁹)	378 ± 107	368 ± 99
CD34+ cells (%)	0.84 ± 0.45	0.82 ± 0.41
CD34+ cells (×10⁶)	697 ± 420	657 ± 371

* When CliniMACS Prodigy and CliniMACS Plus samples were compared, $p > 0.05$ for all factors

Table 2

Comparison of CliniMACS Prodigy and CliniMACS Plus CD34+ cell selected products

	CliniMACS Prodigy (n=11)	CliniMACS Plus* (n=25)
Volume (mL)	69 ± 16	73 ± 15
WBC Count (10⁶/mL)	5.40 ± 3.04	5.75 ± 2.98
Total WBCs (10⁶)	364 ± 179	445 ± 260
Viable WBCs (%)	98 ± 1	98 ± 2
Platelets (10⁶/mL)	11 ± 16	7 ± 9
Total Platelets (10⁶)	605 ± 722	425 ± 540
RBCs (10⁹/mL)	0.0040 ± 0.0120	0.0006 ± 0.0018
Total RBCs (10⁹)	0.1949 ± 0.5897	0.0330 ± 0.0861
CD34+ Cells (%)	93.6 ± 2.6	95.7 ± 3.3
Total CD34+ Cells (×10⁶)	342 ± 177	429 ± 262

* When CliniMACS Prodigy and CliniMACS Plus selected cells were compared, $p > 0.05$ for all factors

Table 3

Comparison of the composition of the unselected cell fractions for the CliniMACS Prodigy and CliniMACS Plus Instruments

	CliniMACS Prodigy (n=11)	CliniMACS Plus [†] (n=20)
Volume (mL)	393 ± 17	396 ± 64
WBC Count (10⁶/mL)	182 ± 30	152 ± 87
Total WBC (10⁹)	71.5 ± 12.3	69.8 ± 20.8
Platelets (10⁶/mL)	527 ± 112	238 ± 92 [*]
Total Platelets(10⁹)	207.1 ± 44.5	91.6 ± 58.1 ^{\$}
RBCs (10⁹/mL)	0.60 ± 0.15	0.40 ± 0.22 [#]
Total RBCs (10⁹)	236.1 ± 62.6	122.3 ± 92.7 [*]

^{*} p < 1×10⁻³

^{\$} p < 1×10⁻⁵

[#] p < 0.02

[†] Data was available from 20 of the 25 CliniMACS Plus selected products