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Depletion of αβ+ T and B Cells Using the CliniMACS Prodigy: Results of 10 Graft-Processing Procedures from Haploidentical Donors

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Abstract
Background: Depletion of TCRαβ+ T cells and B cells with the CliniMACS Plus® has been used for haploidentical hematopoietic stem cell transplantation for a decade. The depletion procedure is time and labour demanding and with variable reported efficiencies. Recently, an automated procedure was launched for the CliniMACS Prodigy® (Miltenyi Biotec) but reported data are scarce. Here, we report the results of the first ten TCRαβ+ and B cell depletion procedures for clinical use performed at our centre. ***Materials and Methods:*** All transplants were from a parent to a child. Collection of peripheral blood stem cells was performed after filgrastim mobilisation by use of the Spectra Optia® (TerumoBCT) set on the MNC program. Because of insufficient hematopoietic stem cell mobilisation, 1 donor received additional plerixafor. ***Results:*** We performed ten uncomplicated processes with the CliniMACS Prodigy. We found the results of the depletion procedures satisfactory with a median log reduction of TCRαβ+ cells of –4.21 (range –3.98 to –4.74), resulting in a median number of TCRαβ+ cells in the depleted product of 28.6 × 10^3/kg recipient weight (range 14.9–69.7 × 10^3/kg). The median CD34 recovery was 83% (range 70–100). To achieve a sufficient number of CD34+ cells, we performed an additional CD34+ enrichment procedure using the CliniMACS Plus for 3 patients. The B cell depletion was slightly less efficient with a median log reduction of –3.72 (range –2.83 to –4.20). ***Conclusion:*** Overall, we found the TCRαβ and CD19 depletion procedure on the CliniMACS Prodigy easy to handle and reliable, providing reproducible good results.

Introduction

Hematopoietic stem cell transplantation (HSCT) with a haploidentical donor is a transplant modality with continued growth [1] and offers an immediately available family donor. For this purpose, a graft depleted of αβ+ T lymphocytes (TCRαβ+) and occasionally also B cells is increasingly used [2, 3]. The removal of TCRαβ+ cells reduces the risk of graft-versus-host disease while maintaining the graft-versus-leukaemia effect of the residual γδ+ T cells and NK cells [4]. The depletion of TCRαβ+ cells, with or without concomitant B cell depletion, has primarily been performed on the CliniMACS Plus® (Miltenyi Biotec, Bergish-Gladbach, Germany), and promising clinical results have been described in several reports [5–8]. Using the CliniMACS Plus device is a time- and labour-demanding procedure that requires trained staff to achieve a satisfying and reproducible result. Re-

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cently, TCRαβ+ and CD19+ B cell depletion was launched for the CliniMACS Prodigy® (Miltenyi Biotec) in an automated and closed system. However, the only available outcome data for the procedure are the "Installation Presentation" delivered by Miltenyi Biotech, which includes internal and external data on a total of thirteen apheresis products, only two of which are from mobilised products. Thus, data on clinically relevant products are needed.

Our centre previously performed TCRαβ+ cell depletion procedures with the CliniMACS Plus, but since 2016 we have used the CliniMACS Prodigy. Here, we report our results and working experiences of the first ten TCRαβ+ and B cell depletion procedures for clinical use that were performed on the CliniMACS Prodigy in our centre between May 2016 and October 2017.

Materials and Methods

All leucapheresis procedures were performed on a Spectra Optia® (TerumoBCT, Leuven, Belgium) using the MNC collection program. All donors were stimulated with filgrastim (Nivestim™ Hospira UK Ltd, Maidenhead, UK; 10 μg/kg) for 5 days where the fifth day was the first day of leucapheresis. All donors were harvested for 2 days on day –1 and 0 relative to the transplantation. The second leucapheresis was performed to ensure sufficient CD34+ cells to obtain engraftment. One donor received additional plerixafor (Mozobil™ Sanofi-Aventis, Denmark; 0.24 mg/kg) due to insufficient G-CSF-induced mobilisation prior to leucapheresis.

All depletion procedures were performed on the CliniMACS Prodigy with the protocol TCRab-CD19 Depletion (software 1.2.0, Miltenyi Biotec) using either the normal- or large-scale procedure. For the normal-scale procedure, the upper limit of cells to be loaded onto the CliniMACS is 40×10^9 total nucleated cells (TNC), 24×10^9 TCRαβ+ cells, and 5×10^9 CD19-positive cells. For the large-scale procedure, the maximum amounts are 80×10^9 TNC, 45×10^9 TCRαβ+ cells, and 10×10^9 B cells.

Preparative steps, such as the connection of buffer bags and setting-up the tubing set were done beforehand. The depletion process was paused after the final labelling and subsequent washing procedure, and the Prodigy was left "to rest" overnight. The process was reassumed at 7:30 a.m. and finished at approximately 2 p.m. the following day.

Samples from the leucapheresis product (start material) and the TCRαβ+ and B cell depleted product (target) were characterised on a Navios Flowcytometer (Beckmann Coulter) with the accompanying software (Cytometer 1.2, Navios). The fluorochromes for labelling were anti-TCRαβ PE, anti-TCRγδ FITC, anti-CD20 PE, anti-CD56 FITC, an APC dump channel with anti-CD14, anti-CD15, anti-CD20, and the FcR-blocking reagent all from Miltenyi Biotec, as well as anti-CD45 ECD, anti-CD3 PC7, and 7AAD from Beckmann Coulter. To determine the number of TCRαβ+ and B cells in the depleted product, we collected 1×10^6 cells per sample and used a sample from the depleted product spiked 1:10 with the non-depleted cells to define the gates. A CD34+ enumeration was performed with the CD34 Stem-Kit (Beckman Coulter) using a lysis-no-wash procedure and a single platform ISHAGE analysis.

The clinical request was a graft with a content of TCRαβ+ cells preferably below 25×10^3 cells/kg and a CD34+ yield above 10×10^6/kg patient weight.

Results

All the transplants were from a parent to a child. The age of the children was a median of 7.0 years (range 0.5–16.4) at HSCT and their median weight was 22.5 kg (range 8.3–66.6). The median donor weight was 72.1 kg (range 51–90) and in 3 cases the weights of the donor and the recipient were almost even.

We performed ten procedures for 9 patients. One child (No. 4) had two transplantations with TCRαβ+ and B cell-depleted grafts because of primary non-engraftment. We performed seven large-scale and three normal-scale procedures. The depletion procedures were initiated after the first leucapheresis in the early afternoon and the mean duration of the processes was 24 h with overnight "rest" (the product is stored within the Prodigy) and re-start the next morning. For the large-scale procedures, the median volume of the start product was 319 mL (range 218–400) and for the normal-scale procedures it was 225 mL (range 177–240).

In the seven products processed with a large-scale depletion, the median number of TNC loaded on to the CliniMACS Prodigy was 71×10^9 (range $52–79 \times 10^9$) with 14.6×10^9 TCRαβ+ cells (range $9.2–24.0 \times 10^9$), 2.0×10^9 B cells (range $0.5–6.5 \times 10^9$), and 612×10^6 CD34+ cells (range $224–1{,}034 \times 10^6$). For the three normal-scale procedures the values were 35×10^9 TNC (range $29–38 \times 10^9$), 8.3×10^9 TCRαβ+ cells (range $6.9–11.0 \times 10^9$), 1×10^9 B cells (range $0.8–1.4 \times 10^9$), and 571×10^6 CD34+ cells (range $251–702 \times 10^6$).

In nine of the ten procedures, the depletion efficiency resulted in a more than 4 log reduction in the number of TCRαβ+ cells, with a median value of TCRαβ+ cells in the depleted product, slightly above the clinical request of 25×10^3/kg (Table 1). Five patients thus only had a fraction of the depleted product for transplantation (Table 1). For the B cells the depletion efficiency was more variable and less efficient (Table 1). In most procedures, the CD34+ recovery was 80% or higher (Table 1), and the median number of CD34+ cells from depleted products was 24.5×10^6/kg (range $6.3–58.7 \times 10^6$/kg). In addition, 3 patients received a CD34+ selected graft containing a median 7.6×10^6 CD34 cells/kg (range $4.6–11.5 \times 10^6$/kg).

Nine of the ten products were engrafted on median day +11 after transplantation (range day +10 to +15). Patient No. 4 did not engraft with the first TCRαβ+ and CD19-depleted graft from the paternal donor, but was re-transplanted 48 days later with a second TCRαβ+ and CD19-depleted graft from the mother and engrafted on day +10. Patient No. 9 was engrafted on day +12, but then rejected the graft and is now scheduled for a re-transplant with the same donor.

Table 1. The results of the ten TCRαβ and CD19 depletion procedures

Patient No.	Weight, kg	Start material					Log depletion		Target cells				% product administered	TCRαβ 10^3/kg administered
		TNC 10^9	CD34 10^6	CD34 10^6/kg	TCRαβ, % of TNC	CD20, % of TNC	TCRαβ	CD20	CD34 10^6/kg	CD34, % recovery	TCRαβ 10^3/kg	CD20 10^3/kg		
1	17.3	61	1,029	59.5	15.1	0.9	–4.15	–3.12	58.7	99	37.0	22.8	33	12.2
2	28.0	79	224	8.0	18.5	1.5	–4.25	–2.83	6.3[c]	79	29.5	62.8	100	29.5
3	27.3	79	1,034	37.9	22.0	2.5	–4.20	–3.87	26.2	70	39.6	9.8	50	19.8
4[a]	17.6	62	773	43.9	20.2	4.2	–4.26	–4.12	34.9	80	39.0	11.1	50	19.5
5	55.0	74	492	8.9	32.4	4.9	–4.20	–3.68	7.1[c]	80	27.6	13.9	100	27.6
4[a]	17.6	38[b]	702	39.9	28.9	3.6	–4.40	–3.94	34.3	86	15.8	9.0	100	15.8
6	66.6	71	548	8.2	23.9	9.2	–4.20	–3.65	7.7[c]	95	16.0	21.6	100	16.0
7	29.4	38[b]	571	19.4	18.2	2.1	–4.20	–4.20	17.0	88	14.9	1.7	100	14.9
8	8.3	29[b]	251	30.2	28.6	2.9	–4.74	–3.76	22.8	75	18.3	17.6	66	12.2
9	17.4	52	612	35.2	22.4	3.7	–3.98	–3.14	36.7	100	69.7	55.7	33	23.2
Median	22.5	62	592	32.7	22.2	3.3	–4.21	–3.72	24.5	83	28.6	15.8	–	17.8
Min.	8.3	29	224	8.0	15.1	0.9	–4.74	–4.20	6.3	70	14.9	1.7	–	12.2
Max.	66.6	79	1,034	59.5	32.4	9.2	–3.98	–2.83	58.7	100	69.7	62.8	–	29.5

[a] The same patient had two products within 2 months from both parents.
[b] Normal scale procedures.
[c] An additional CD34 selected graft was administered.

Discussion

Here, we have presented our single-centre results and experiences from ten procedures of TCRαβ+ and B cell depletion performed using the CliniMACS Prodigy, which allows the procedure to be performed in an automated, GMP-compliant, and closed system. For the TCRαβ+ depletion we achieved a median log depletion of –4.21 (range –3.98 to –4.74), which is less but in the same order of magnitude as reported by the manufacturer Miltenyi Biotech (mean log depletion –4.5; range –3.8 to –5.0). In comparison, for the procedures performed by use of the CliniMACS Plus, Schumm et al. [9] reported a median log depletion of –4.7 (range –3.8 to –5.5) in 102 procedures, and Li Pira et al. [6] reported a mean log depletion of –4.1 (range –3.8 to –5.2) for 200 procedures. Thus, although some groups have reported higher maximum log depletions with the CliniMACS Plus, we found a comparable and satisfying median depletion value and a lower variation on the CliniMACS Prodigy. The procedure thus seems to be robust with reproducible satisfactory results.

The CD19 depletion procedure was less efficient with a median log depletion of –3.8 (range –2.8 to –4.2), which is very similar to data reported by Miltenyi Biotech (mean –3.8; range –2.4 to –5). For the CliniMACS Plus, Schumm et al. [9] reported a log depletion of –4.1 (range –3.0 to –4.7). However, this may be of less clinical importance since several protocols include anti-CD20 (Rituximab) administered post-transplant in order to remove residual B cells from both the graft and the recipient. It is therefore a major disadvantage that the only CE-approved software version of the TCRαβ+ depletion procedure includes a mandatory and costly CD19 depletion.

Apart from a sufficient TCRαβ+ depletion, the recovery of CD34+ cells is most important. We found a median CD34+ recovery of 83% (range 70–100), which is similar to the results from the only two procedures on mobilised products reported from Miltenyi Biotech (72 and 90%, respectively). However, the remaining CD34 recovery data from the company is based on depletion procedures of immobilised products, which is naturally of minor clinical relevance. In addition, although the results demonstrated a mean CD34+ recovery of 88.5%, the reported range was from 47.9 to 182.9%. On the CliniMACS Plus the CD34+ recovery has been reported to vary widely, with a mean value of 73% but a range from 43 to 98% [9]. Of note, in the present report, none of the 3 cases with an insufficient CD34+ dose in the first graft was caused by a low post-depletion CD34+ recovery. In fact, the amount of CD34+/kg in the pre-depleted products was below the requested 10×10^6/kg caused by unfavourable donor/recipient weight ratios and/or insufficient hematopoietic stem cell mobilisation despite the addition of Mozobil. Furthermore, in 1 case the collected product contained a TNC of 120×10^9, which is 33% higher than the upper limit of the large-scale procedure.

With respect to the handling, the automated TCRαβ+ and B cell depletion procedure on the CliniMACS Prodigy replaces several of the manual time- and resource-demanding preparative steps of the CliniMACS Plus procedure,

e.g., platelet wash and the meticulous handling of the product during the antibody labelling. The complex tubing set for the TCRαβ+ depletion procedure is surprisingly feasible to handle and the specifications in the instruction are clear.

The Prodigy is placed in a C cleanroom where the entire process has been performed. A major advantage of this closed system is that no B cleanroom is needed. This influences the overall costs of such procedures as well as the working environment for the staff. It is an advantage with free access to both the front and the back of the Prodigy to ensure access to the bag hangers and the placement of the heavy-to-handle 3-L buffer bags. One bag hanger is, for some reason, shorter than the others, and we had to replace it with a longer one, to prevent kinks that potentially could block the process, and actually did in 1 case. Major drawbacks to the depletion set are the need to add extra waste bags and especially that the final product has to be transferred from the target cell bag to an infusion bag. With respect to the present software version, the time specifications are not correct, which makes it difficult to obtain an overview of the duration of each process step.

We had three emergency stops during the ten procedures, one was caused by a kink as described above, and two were due to air bubbles. All cases were handled in an efficient and timely manner by calls to Miltenyi Technical Support. Very importantly, we did not observe clumps or aggregates in any of the processed products, which was a challenge with the CliniMACS Plus procedure.

We started the process in the afternoon on the first day of apheresis. The overall processing time depends on the choice of either continuous overnight processing (without attending personal) or an overnight storage of the product within the Prodigy after the labelling procedure. We chose the latter for safety reasons and resumed the process the next morning. Thereby, the entire depletion process lasted approximately 22–24 h, not including time used for product evaluation.

Conclusion

Overall, we found the TCRαβ+ and B cell depletion procedure with the CliniMACS Prodigy to be feasible to handle and reliable, with fairly reproducible and satisfactory results. However, the software definitely needs improvement and the mandatory inclusion of CD19 depletion in particular is a drawback that considerably increases the costs of this already expensive procedure.

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Statement of Ethics

The authors have no ethical conflicts to disclose. All donors were parents and guardians and gave their written informed consent.

Disclosure Statement

None of the authors have conflicts of interest to declare.

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Author Contributions

E.H. and A.F.-N. designed the study, analysed the data, and wrote the manuscript. M.I. and C.H. analysed data and wrote the manuscript.

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