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Immunomagnetic selective donor-derived CD4⁺CCR7⁺ T cell depletion procedure for peripheral blood stem cells graft.

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ABSTRACT

Purpose of the study

While acute graft-versus-host-disease (GVHD) is a T cell-mediated disease caused by alloreactive donor T cells, we and others have highlighted that patients who received higher proportion of donor CD4⁺ naïve and central memory T cells expressing the chemokine receptor 7 (CCR7) more often developed acute GVHD than those who did not. Consequently, we then investigated *in vitro* the impact of selective CD4⁺ CCR7⁺ T cell depletion on immune reactions and showed that such a depletion reduced alloreactivity without altering acquired anti-infectious reactions.

In order to translate these findings to clinic, we now **developed a compliant procedure for a selective** reduction of the CD4⁺ naïve and central memory T cell subset relevant to peripheral blood stem cell (PBSC) allografts.

Patients and methods

We performed a two-step immunomagnetic depletion of CD4⁺ CCR7⁺ T cells from ten G-CSF-mobilized PBSC apheresis samples.

Results

A median of 89% (82-94%) of CD4⁺ CCR7⁺ T cells could be depleted. This allowed a marked reduction of the alloreactive immune response against allogenic dendritic cells compared with unmanipulated cells. The preservation of CD34⁺ cell number and the hematopoietic progenitor function were controlled. Functional tests showed that the selection procedure did not interfere with the capacity of pathogen-specific T cells to produce interferon-gamma in response to **certain viral pathogens.**

Conclusion

Our results pave the way to a feasible procedure that can be used in patients undergoing allo-hematopoietic cell transplantation and particularly for improving haploidentical transplant results **by controlling GVHD, the main immune complication.**

Keywords: Naïve T cells, T-Lymphocyte Subsets, Graft-versus-host disease, Graft engineering, Selective T cell depletion

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is still considered as the best curative option for hematological disorders with high-risk features or patients with relapsed disease [1,2]. However, while contributing to potential curative therapy through the graft-versus-leukemia effect (GVL), alloreactivity of T cell-replete grafts increases the risk of severe graft-versus-host-disease (GVHD), especially in haplo-identical settings [3,4]. Prophylactic immunosuppressive treatment can partially control proliferative alloreactive T cells *in vivo* [5,6]. Alternatively, *ex vivo* extensive T cell depletion of HLA-mismatched allografts proved effective at preventing GVHD, but led to delayed post-transplant immune reconstitution, thereby aggravating the risk of infections and possibly decreasing GVL effects [7,8].

Unmanipulated grafts contain both naïve and memory T cells. Most T cells that cause GVHD reside within the naïve T-cell population that can be identified by CD45RA expression, whereas memory T cells can transfer immunity against common pathogens encountered after transplantation [9-11]. Clinical grade immunomagnetic procedures for the selective depletion of the CD45RA⁺ T-cell subset have been established [12,13]. Extensive depletion of CD45RA⁺ T-cell from the graft has been reported to reduce the incidence and/or the severity of GVHD while transferring protective immunity in HLA-matched allograft recipients [14] both after haplo-HCT [15] and in HLA-mismatched pediatric allograft recipients [16].

Not only T cell dose but also T cell subset composition can affect major outcomes after allogeneic HCT (allo-HCT). In addition to CD45RA⁺ naïve T cells, some memory T cells may also contribute to the alloresponse, particularly those within the CD4⁺ central memory subset characterized by the expression of the CC-chemokine receptor 7 (CCR7) and L-selectin (CD62L) [17–20]. Accordingly, prospective studies indicate that a threshold cell dose of CD4⁺ CCR7⁺ T cells or CD4⁺ CD62L⁺ T cells in the allograft is associated with the risk of severe acute GVHD [21–23], whereas partial selective depletion of donor-derived CD4⁺ CCR7⁺ T cells did not alter *in vitro* responses of allo-HCT recipients to common viral antigens [24].

Considering preservation of CD8⁺ T cells would significantly contribute to anti-infective and anti-tumoral immunological effects [20,25,26], we developed a two-step immunomagnetic depletion procedure to partially reduce the proportion of naïve and central memory CD4⁺ T

cells while preventing any loss in other lymphocyte subsets including effector memory CD4⁺ T cells. Our objective is to set up a feasible procedure that can be used in patients undergoing allo-HCT and particularly for haplo-identical transplant **in order to control alloreactivity resulting from this type of mismatched transplantation.**

MATERIALS AND METHODS

The study was approved by the Lille university hospital board and conducted according to the declaration of Helsinki (NCT03280290). All donors have given their informed consent.

Apheresis products

Ten Peripheral blood Stem cell (PBSC) apheresis samples of 1.0 to 2.9 mL (median 1.8 mL) were analyzed. For each specimen, related recipient weight, volume of PBSC bag, and serological status of donor were known. Donor HLA status was determined by using high resolution class I and II molecular DNA typing.

Two-step positive and negative selection for depleting CD4⁺CCR7⁺ T cells

A two-step cell selection procedure was carried out on PBSC apheresis specimens using the AutoMACS Pro instrument and appropriate immunomagnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). A starting number of 1×10^8 viable mononuclear cells was used (PRE-SORT sample). Before each of the two sorting steps, the starting cells were resuspended in 5 mL of AutoMACS[®] Running Buffer (Miltenyi Biotec), then filtered (30 μ m filters, Miltenyi Biotec) and centrifuged (300 x g, 10 min). Figure 1 presents a flow diagram of the sequential cell selection procedure.

In the first step, CD8⁺ and CD19⁺ cells were purified from the starting PBSC apheresis specimens: the PRE-SORT cell suspension was incubated for 15 minutes at +4°C with both CD8 and CD19 microbeads according to manufacturer's instructions and loaded onto the AutoMACS Pro instrument. The "Possel-s" program was used for selection. The positive fraction from this selection was stored at +4°C until completion of the next sorting step, which was performed on the negative fraction.

In the second step, the CD8⁺ and CD19⁺-depleted fraction was submitted to CCR7⁺ cell depletion by successive incubation at +4°C with a monoclonal antibody against CCR7 (CD197 (CCR7)-Biotin human, Miltenyi Biotec) for 10 minutes and then with anti-Biotin

microbeads (Miltenyi Biotec) for 15 minutes, before the selection with the AutoMACS Pro instrument and the “Possel” program. **The whole sorting procedure lasts approximately 2 hours.**

Finally, the positive fraction from the first selection and the negative fraction from the second selection were pooled for subsequent analysis of final product’s cell composition and assessment of functional immune responses (POST-SORT mix).

Multi-parametric flow cytometric analysis of cell products

T-cell subsets, B cells, NK cells, monocytes, and hematopoietic stem cells were identified comparatively in the starting PBSC apheresis products (PRE-SORT) and the CD4⁺ CCR7⁺ depleted final products (POST-SORT). Cells were stained for 15 minutes at room temperature using optimal concentrations of the following fluorochrome-conjugated monoclonal antibodies: CD14-FITC, CD45-Ko, CD4-PacB, CD8-APC, CD19-Pc5.5, CD16-PE, CD56-Pe, CD4-Ko, CD45RA-FITC, CCR7-PE, CD34-Pc7, and CD3-Pc7 (all from Beckman Coulter, Villepinte, France). Flow-Count fluorospheres (Beckman Coulter) were loaded and acquired simultaneously along with the cells to determine the absolute cell counts, using a NaviosTM flow cytometer (Beckman Coulter).

Hematopoietic progenitor cell culture

To compare the PRE-SORT and POST-SORT products, we determined the numbers of colony forming units for granulocytes/macrophages (CFU-GM) and burst forming units-erythroid (BFU-E) after fourteen days of culture at 36.5°C and 5% CO₂, each initiated with 250 CD34⁺ cells. CFU-GM assays were run in triplicate in MethoCultTM H84535 (Stemcell Technologies, Vancouver, Canada). BFU-E assays were run in duplicate in MB medium prepared from MethoCultTM H4100 (Stemcell Technologies), Iscove’s Modified Dulbecco’s medium (ThermoFisher scientific, Waltham, USA), BIT 9500 (Stemcell Technologies), Fetal Bovine Serum for Human Myeloid Colony-forming Cells (13%, Stemcell Technologies), recombinant human IL-3 (10 ng/mL, Active Bioscience, Hamburg, Germany) and Erythropoietin at 400 UI/mL (Epoetin alfa 4000, Eprex[®], Janssen, Johnson and Johnson, Issy les Moulineaux, France).

One-way Mixed Lymphocyte Dendritic Cell (MLDC) Reactions

Monocytes were purified from total peripheral blood mononuclear cells from healthy adult volunteers by negative selection (EasySep Human Monocyte Enrichment Kit, Stemcell Technologies) and differentiated into monocyte-derived dendritic cells by culture in Cellgro medium (CellGenix, Freiburg im Breisgau, Germany) supplemented with IL-4 and GM-CSF (Premium grade, Miltenyi Biotec). After five days, recombinant IL-4, GM-CSF, TNF- α , IL-6, IL-1 β (Premium grade, Miltenyi Biotec) and prostaglandin E2 (Sigma-Aldrich) were added for two more days to complete maturation. The resultant mature dendritic cells were used as stimulus (1×10^4 cells per well) in co-culture with 2×10^5 PRE-SORT or POST-SORT responder cells in 96-well U-bottomed plates (Falcon, Durham, USA). After 6 days, cells were harvested for ELISPOT assay.

Functional pathogen-specific T cell response to viral antigens

PRE-SORT and POST-SORT cells were plated in triplicate at 2×10^5 per well in RPMI medium (Gibco by ThermoFisher Scientific) in 96-well U-bottomed microplates (Falcon) and stimulated for 6 days at 37°C with the following viral peptides, each at 1 μ g/ml per well: AdV (peptivator-AdV hexon), CMV (peptivator-CMVpp65 and peptivator CMV IE-1 mixed in equal proportions) and EBV (peptivator-EBV), all from Miltenyi Biotec. HTLV-1 Tax 11-19 (Bachem, Bubendorf, Switzerland) served as a negative control. After six days of culture at 37°C and 5% CO₂, cells were harvested for ELISPOT assay.

ELISPOT assay for interferon- γ production (IFN- γ ELISPOT)

Harvested cells were resuspended in AIM-V medium (Gibco, ThermoFisher Scientific) and returned to culture for an additional 20 hour in immunospot plates coated with a monoclonal antibody to IFN- γ (T-Spot TB, Oxford Immunotec, Abingdon, UK). In order to measure the response to viral antigens, viral peptides were added at 1 μ g/ml per well. IFN- γ spots were enumerated with TB Scan software (Oxford Immunotec).

Statistical analysis

Results are given as median (range). Where appropriate, paired PRE-SORT and POST-SORT data were compared by using the Wilcoxon signed-rank test performed with Prism Software (GraphPad). A *P* value of <0.05 was considered as significant.

RESULTS

Validation of the two-step CD4⁺ CCR7⁺ T cell partial depletion procedure

Viability of the ten G-CSF-mobilized PBSC apheresis products used in this study was consistently high, with a median of 98% (95-100%) viable cells. The efficacy of the two-step immunomagnetic depletion was assessed by immunophenotypic analysis. There was a wide variation in the initial number of CD4⁺ CCR7⁺ T cells among the PRE-SORT samples, between 53 and 266 x10⁶ CD4⁺ CCR7⁺ cells/kg (Table 1). After mixing the CD8⁺ and CD19⁺ fraction from the first selection step and the CCR7-depleted fraction from the second step (POST-SORT), 3 to 23 x10⁶ residual CD4⁺ CCR7⁺ cells/kg were recovered (Figure 2), *i.e.*, a median reduction of 89% (82-94%). The resulting reduction in CD4⁺ naïve and CD4⁺ central memory T cells was approximately equivalent (median of 91% and 86%, respectively).

As shown in Table 1, most of the initial CD8⁺ T cells were recovered, with a median of 20% loss in the POST-SORT mix, and the CD8⁺CCR7⁺ and CD8⁺CCR7^{neg} subsets were similarly preserved by the selection procedure (17% and 28% median loss, respectively). The POST-SORT mix retained a median proportion of 50% memory T cells in the CD4⁺ subset (*vs* 17% in PRE-SORT mix) and 33% memory T cells in the CD8⁺ subset (*vs* 34% in PRE-SORT mix). CD19⁺ B cells and CD3^{neg} CD56⁺ and/or CD16⁺ NK cells were likewise preserved (10% median loss: 2-27% for B cells and 1-33% for NK cells).

Preservation of CD34⁺ cells and hematopoietic progenitor function

All ten POST-SORT products contained more than the target dose of 3 x 10⁶ viable CD34⁺ cells/kg (Table 1). The early differentiation capacity of these cells was assessed by enumerating colonies obtained after culture of the PRE-SORT and POST-SORT products (Figure 3). There was no statistically significant difference before and after the selection steps, whether comparing CFU-GM colonies (median of 50 *vs.* 47) or BFU-E colonies (40 *vs.* 38).

Reduced alloreactive response against allogeneic dendritic cells

Monocyte-derived DCs coming from an unrelated volunteer with at least 9/10 HLA mismatches with the PBSC donors were used as stimulus in mixed lymphocyte reactions. A

marked reduction of IFN- γ secretion was highlighted in the co-cultures of CD4⁺ CCR7⁺ cell depletion products (Figure 4), with only 59 (0-86) spots/200,000 responder cells as compared with 224 (23-35,486) spots/200,000 responder cells before the selection (P <0.01).

Preservation of functional pathogen-specific T cells

PRE-SORT and POST-SORT cells were stimulated comparatively with a panel of viral peptides to evaluate their immune reactivity (Figure 5). Intense IFN- γ secretion was detected with the PBSC specimens from all six CMV seropositive donors, with a median of 364 (227-559) IFN- γ spots per 200,000 cells before and 514 (389-600) spots/200,000 cells after the selection. The EBV specific response was likewise well-preserved in the selection products from six EBV-seropositive donors with a median of 43 (16-63) IFN- γ spots per 200,000 cells before and 91 (47-181) spots/200,000 cells after the selection. Stimulation with an adenovirus (AdV) peptide served as positive control, with responses preserved in the selection products from all 10 donors: median 121 (13-244) spots/200,000 cells vs. 78 (24-352) spots/200,000 cells in unmanipulated PBSCs.

DISCUSSION

Engineering the graft to optimize the immune cell content may improve GVL effect and immunologic reconstitution without generating more GVHD. To this end, we have developed and validated a two-step immunomagnetic depletion procedure that targets the naïve and central memory CD4⁺ T cell subsets, which are thought to be more alloreactive. Many publications from our team and others [21–23] already validated the positive effect of CD4⁺ CCR7⁺ partial depletion on GVHD rate and/or severity. Selective CD4⁺ T cell depletion is a critical point as others failed to show a positive effect on GVHD when excessive concomitant depletion of other immunocompetent cell populations occurred [12,14].

Our final objective was to perform this procedure on cliniMACS that is usually restricted to high volume samples. Because of both ethical and financial concerns, we were limited to develop this method on small volumes. Interestingly, AutoMACS-to-cliniMACS transition has been successfully completed by other teams [27]. This makes us strongly believe that the use of an automatic sorting process on AutoMACS Pro in this study can be easily transferred to cliniMACS Pro System implemented in clinical hematopoietic cell processing laboratories.

In preliminary experiments, we tested a first step of positive selection with CD4 microbeads, but most magnetic beads failed to come off after the selection, thereby preventing sorting according to a second marker. Magnetic particles that can be enzymatically released are available, but they are not licensed for clinical use. Therefore, CD8 and CD19 microbeads were used to collect CD8⁺ and CD19⁺ cells in the positive fraction, whereas the CD8⁺ and CD19⁺-depleted fraction, enriched with CD4⁺ T cells, was submitted to CCR7⁺ cell depletion. At the end of this procedure, the positive fraction from the first step and the negative fraction from the second step were pooled and analyzed.

Cells retained high viability (>95%) over cell processing. Not using CD45RA for the second selection step allowed preserving the minor CD45RA⁺ subset among CD34⁺ stem cells [28]. With only 27% loss within CD34⁺ cells, the resulting cell mix consistently met the target cell doses of 3 x 10⁶ viable CD34⁺ cells/kg, and functional assays verified the preservation of CD34⁺ cell hematopoietic progenitor function. CD56⁺ and/or CD16⁺ NK cells were likewise preserved, which would allow taking advantage of the beneficial effect of haplo-identical NK cell anti-tumoral reactivity [29]. Use of CCR7 immunobeads instead of CD62L to partially deplete naïve and central memory T cells was chosen, because L-selectin is known to undergo proteolytic cleavage by metalloproteases released during G-CSF mobilization of PBSC [30].

Selection procedure allowed to reproducibly deplete more than 80% of the CCR7⁺ CD4⁺ T cells, whatever the initial proportion of CCR7⁺ cells in the graft specimen. The present sorting strategy is therefore applicable to all potential donors, knowing that the proportion of CD4⁺ naïve T cells varies with donor age [31]. This depletion of naïve and central memory CD4⁺ T cells is far less than the four log and more depletion achieved by published CD45RA⁺ cell depletion procedures [12,13,15]. As expected, this reduction in the proportion of CD4⁺ CCR7⁺ T cells was enough to markedly decrease the alloreactive response against fully mismatched allogeneic dendritic cells, as evidenced by a significant decline in IFN- γ production, a pro-inflammatory cytokine known to play a central role in GVHD [32,33].

The CD4⁺ CCR7^{neg} effector memory cells as well as the CD8⁺ naïve and effector cells were relatively preserved, with conservation of the relative proportions of the four subsets within the CD8⁺ T cell compartment. Functional tests also indicated that the selection procedure did not interfere with their capacity to produce IFN- γ in response to Adenovirus, EBV and CMV, pathogens which are known to significantly contribute to morbidity and mortality associated

with HSCT. Although not tested, preserving 10 to 20% of naïve CD4⁺ T cells might also help maintaining primary responses to pathogens or tumor antigens.

In conclusion, this study demonstrates the feasibility of selective reduction of CD4⁺ CCR7⁺ T cells from an allograft by a two-step immunomagnetic depletion procedure, which contrasts with alternative forms of *ex vivo* depletion of alloreactive cells while preserving CD8⁺ and NK cell populations for post-transplant immune reconstitution. Although by not depleting all alloreactive T cells, the present cell selection procedure requires much less time than *in vitro* stimulation of PBSC products followed by depletion of activated alloreactive T cells [34,35]. We are currently working on a first-in-man phase two study to investigate the safety and efficacy of our procedure for the prevention of acute GVHD especially in haplo-SCT setting.

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AUTORSHIP

Contribution

Designed research, performed research: PV, ML

Analyzed and interpreted data: PV, SR, JT, JD, ML

Manuscript writing: All authors

Final approval of manuscript: All authors

Conflict of Interest

All these authors PV, SR, JT, JD, ML have nothing to declare

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Table 1. Cellular composition of PBSC apheresis products before (PRE-SORT) and after (POST-SORT mix) the two-step CD4⁺CCR7⁺ cell selection procedure.

Cell subset	PRE-SORT (Cells x 10 ⁶ /kg) Median, range	POST-SORT (Cells x 10 ⁶ /kg) Median, range	Proportion of loss in POST-SORT fraction Median (%), range (%)
Total nucleated cells	553 (374-964)	298 (208-419)	46 (43-66)
Immunocompetent cells			
CD3 ⁺ T cells	190 (97-368)	82 (42-99)	58 (50-74)
CD3 ⁺ CD4 ⁺ T cells	115 (66-292)	24 (13-45)	79 (69-85)
CD4 ⁺ CCR7 ⁺ T cells	97 (53-266)	10 (3-23)	89 (82-94)
CD4 ⁺ T _N cells	60 (33-168)	6 (2-10)	91 (83-96)
CD4 ⁺ T _{CM} cells	29 (15-98)	4 (1-15)	86 (79-92)
CD4 ⁺ CCR7 ^{neg} T cells	19 (12-40)	12 (8-32)	31 (11-47)
CD4 ⁺ T _{EM} cells	18 (10-31)	12 (7-27)	28 (9-45)
CD4 ⁺ T _{EMRA} cells	1 (0-10)	1 (0-5)	39 (17-44)
CD3 ⁺ CD8 ⁺ T cells	65 (31-92)	48 (24-75)	20 (14-31)
CD8 ⁺ CCR7 ⁺ T cells	26 (11-70)	21 (10-59)	17 (4-28)
CD8 ⁺ T _N cells	23 (10-69)	19 (9-58)	16 (4-27)
CD8 ⁺ T _{CM} cells	2 (1-6)	2 (1-4)	28 (2-34)
CD8 ⁺ CCR7 ^{neg} T cells	22 (17-65)	16 (9-50)	28 (21-47)
CD8 ⁺ T _{EM} cells	9 (5-18)	6 (4-14)	25 (16-36)
CD8 ⁺ T _{EMRA} cells	13 (5-47)	9 (4-36)	28 (12-36)
CD19 ⁺ B cells	43 (27-130)	41 (27-107)	10 (2-27)
CD3 ⁺ CD56 ⁺ and/or CD16 ⁺ NK cells	13 (4-28)	11 (4-22)	10 (1-33)
CD14 ⁺ monocytes	253 (42-412)	140 (25-239)	43 (37-53)
CD34 ⁺ cells	5,5 (4,5-9,8)	4 (3,4-6,9)	27 (16-40)

T_N naive T cells; T_{CM} central memory T cells; T_{EM} effector memory T cells; T_{EMRA} CD45RA⁺ effector memory T cells.

Legends of figures

Figure 1. Two-step immunomagnetic selection procedure. Procedure involving a positive selection of CD8⁺ and CD19⁺ cells from PBSC apheresis specimens (PRE-SORT), and then CCR7 deletion from the negative fraction. The two selection products were then pooled and referred to as POST-SORT to evaluate the cellular composition and functional responses of the mix.

Figure 2. Selective reduction of CD4⁺ CCR7⁺ T cells by the two step immunomagnetic procedure. (A) Flow cytometry plots showing the proportion of CD4⁺CCR7⁺ T cells in a representative PBSC apheresis product before (PRE-SORT) and after (POST-SORT mix) the selection. Percentages of the live-gated population for each quadrant are included. (B) CD4⁺ CCR7⁺ cell content (adjusted to 10⁶ cells/kg) in the initial PBSC product (PRE-SORT) and after mixing the two final selection fractions (POST-SORT).

Figure 3. Hematopoietic progenitors before and after the two-step selection procedure. (A) CFU-GM colonies in the initial PBSC apheresis product (PRE-SORT) and in the pooled selection products (POST-SORT) (B) BFU-E colonies in the PRE-SORT and POST-SORT products. Resultant colonies were enumerated after 14 days of culture initiated with 5 x 10⁵ CD34⁺ cells/mL. Bars represent the medians. *NS*: not significant.

Figure 4. One-way mixed lymphocyte dendritic cell reactions to compare the alloreactive response before and after the two-step selection procedure. (A) IFN- γ ELISPOT wells from representative co-cultures of PRE-SORT and POST-SORT products from the same donor. (B) IFN- γ responses in the ten PRE-SORT and POST-SORT products after six days of stimulation by allogeneic dendritic cells. Bars represent median values. **indicates $P < 0.01$.

Figure 5. ELISPOT assays evaluating pathogen-specific IFN- γ secretion in PRE-SORT and POST-SORT products stimulated with a panel of viral peptides. (A) IFN- γ ELISPOT wells comparing responses of representative PRE-SORT and POST-SORT selection products from CMV,

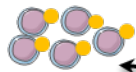
EBV, and Adenovirus (ADV) seropositive donors, respectively. HTLV1 antigen served as a negative control. (B) IFN- γ response of all ten PRE-SORT (circles) and POST-SORT selection products (squares) after six days of stimulation with two CMV peptides. (C) IFN- γ responses in seven PRE-SORT (circles) and POST-SORT selection products (squares) after six days of stimulation by an EBV peptide. As shown, there were no responses with cells from the seronegative donors.



PBSC apheresis sample
(PRE-SORT)

1st step: Double CD8-CD19 selection

CD8⁺ and CD19⁺ cells



2nd step: CCR7 selection

CCR7^{neg} cells



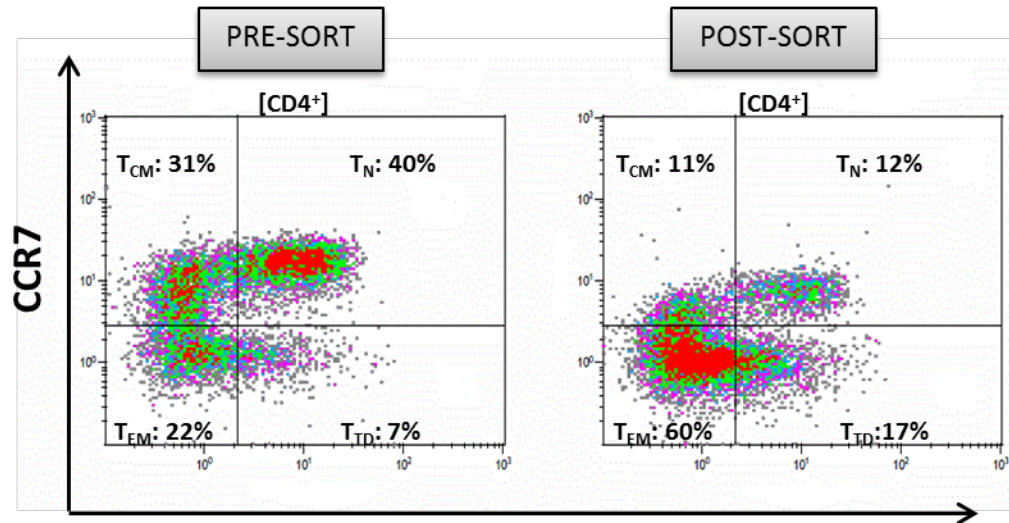
CCR7⁺ cells



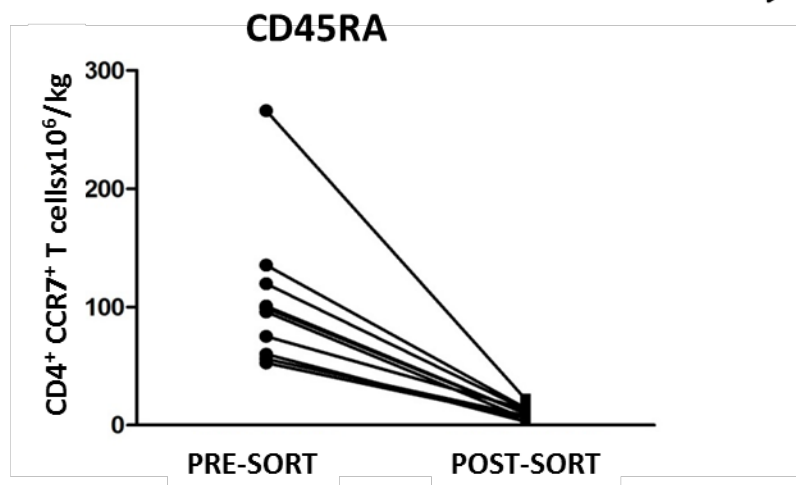
PBSC apheresis sample
CD4⁺ CCR7⁺ depleted
(POST-SORT)



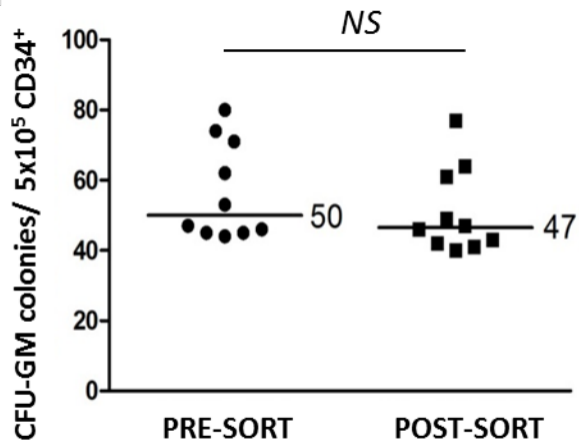
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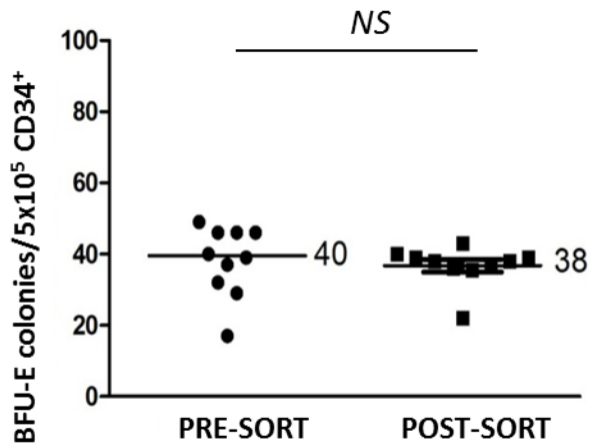
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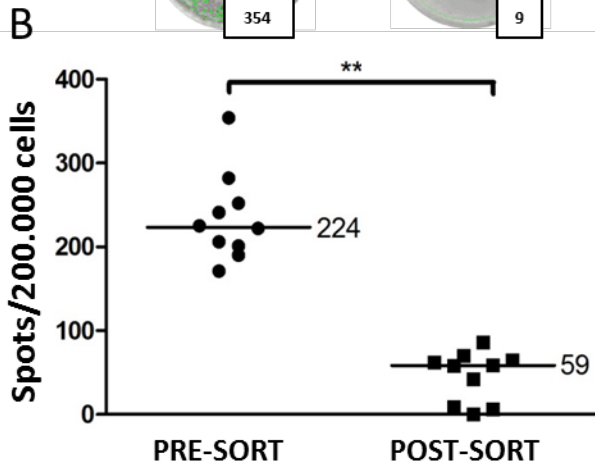
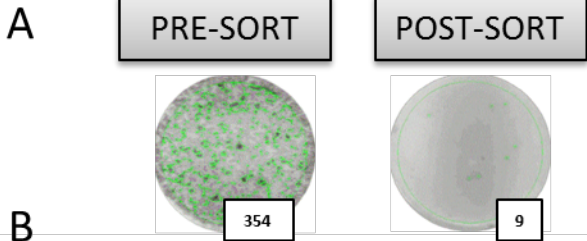


A



B





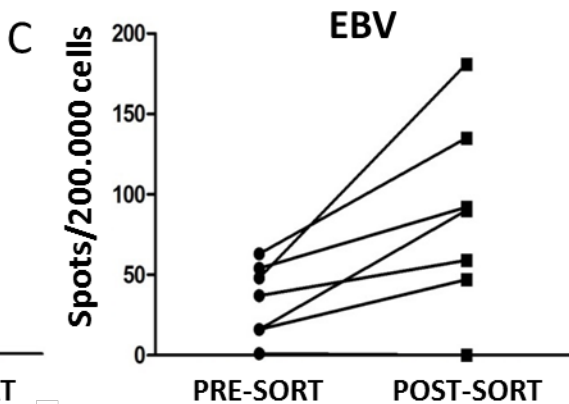
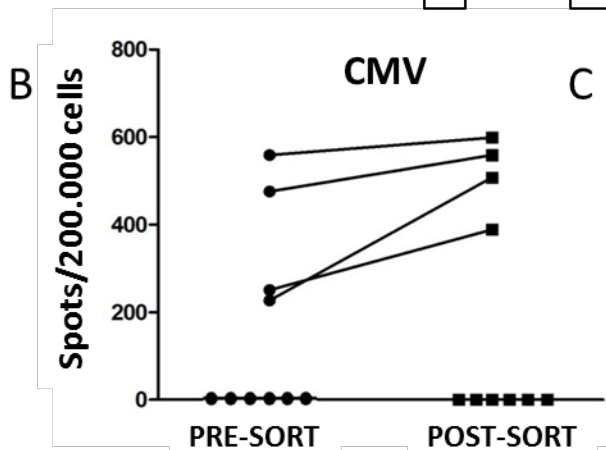
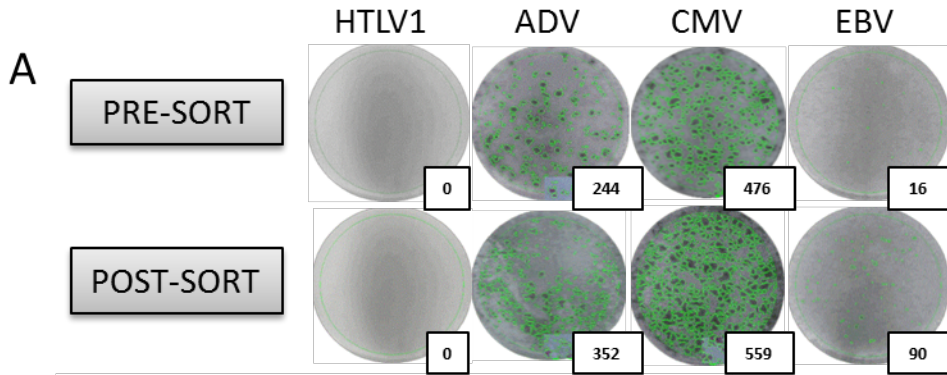


Table 1. Cellular composition of PBSC apheresis products before (PRE-SORT) and after (POST-SORT mix) the two-step CD4⁺CCR7⁺ cell selection procedure.

Cell subset	PRE-SORT, cells x 10 ⁶ /kg (Median , range)	POST-SORT ,cells x 10 ⁶ /kg (Median, range)	Proportion of loss in POST-SORT fraction, percent (Median, range)
Total nucleated cells	553 (374-964)	298 (208-419)	46 (43-66)
Immunocompetent cells			
CD3⁺ T cells	190 (97-368)	82 (42-99)	58 (50-74)
CD3⁺ CD4⁺ T cells	115 (66-292)	24 (13-45)	79 (69-85)
CD4⁺ CCR7⁺ T cells	97 (53-266)	10 (3-23)	89 (82-94)
CD4⁺ T_N cells	60 (33-168)	6 (2-10)	91 (83-96)
CD4⁺ T_{CM} cells	29 (15-98)	4 (1-15)	86 (79-92)
CD4⁺ CCR7^{neg} T cells	19 (12-40)	12 (8-32)	31 (11-47)
CD4⁺ T_{EM} cells	18 (10-31)	12 (7-27)	28 (9-45)
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