CLINICAL CELLULAR THERAPY

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In Vitro Expanded Umbilical Cord Blood T Cells Used for Donor Lymphocyte Infusions after Umbilical Cord Blood Transplantation

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Umbilical cord blood (UCB) is an alternative graft source for hematopoietic stem cell transplantation and has been shown to yield results comparable to transplantation with other stem cell sources. Donor lymphocyte infusion (DLI) is an effective treatment for relapsed hematological malignancies after hematopoietic stem cell transplantation. However, DLI is not available after UCB transplantation.

In this study, in vitro cultured T-cells from the UCB graft were explored as an alternative to conventional DLI. The main aim was to study the safety of treatment with the cultured UCB T-cells, as cell products prepared in this particular manner has not been used before. We also wanted to study potential benefits of the treatment.

The cultured UCB T cells (UCB DLI) were given to four patients with mixed chimerism (n = 2), minimal residual disease (n = 1) and graft failure (n = 1). No adverse reactions were seen at transfusion. Graft-versus-host disease (GVHD) is a known risk in conventional DLI treatment. We thus carefully assessed the included patients for signs of GVHD. Three of the patients did not show any signs of GVHD after treatment with UCB DLI. However, GVHD could not be wholly excluded in the last patient. The symptoms were, however, not consistently in temporal association with the treatment, and the patient also had a severe adenovirus infection that could explain the symptoms. In the patient with minimal residual disease, the malignant cell clone was detectable shortly before infusion but undetectable at treatment and for 3 months after infusion. In one patient with mixed chimerism, the percentage of recipient cells decreased in temporal association with UCB DLI treatment.

In summary, we saw no certain adverse effects of treatment with UCB DLI. Events that could indicate possible benefits were seen but with no certain causal association with the treatment.

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Immunological Effects of Decidual Stromal Cell Treatment in Patients with Severe Chronic Graft-Versus-Host Disease

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Introduction: Decidual stromal cells (DSCs) isolated from fetal membranes of term placentas are easily expanded and highly immunosuppressive *in vitro*. DSCs have a high expression of integrins that are of importance for homing to damaged tissue. In the present study, we introduce DSCs as a cellular therapy for chronic Graft-versus-Host Disease (cGvHD).

Patients and Methods: Three patients (1 (ALL), 2 (AML), and 3 (CML)) with severe extensive cGvHD were treated with DSCs (1-2.8 x 10^6 cells/kg). Patients 1 and 2 received two infusions and patient 3 received one dose. One third of DSCs administered to patient 1 and 2 were labelled with ^{111}Indium and the *in vivo*-distribution was tracked for 48h. Blood samples were obtained before and up to 4-10 weeks after the first infusion. Samples were analyzed by flow cytometry and luminex.

Results: All patients had cGVHD of skin, liver and obstructive bronchiolitis. Patients 1 and 2 are regarded as partial responders (PR) and patient 3 as a non-responder (NR). Response was evaluated according to the NIH guidelines for diagnosing cGvHD.

Patients receiving ^{111}In-DSCs showed the same distribution pattern of the isotope over time. The isotope was initially located in the lungs, followed by dissemination to liver and spleen.

The flow cytometry and luminex data are presented as the median frequency of data from all time points for each patient. Patient 3 had high frequencies of HLA-DR$^+$ cells within the CD3$^+$CD4$^+$ cell population (Th) (median 72.9%, range 72.7-73.3%). The corresponding proportions in patients 1 and 2 were 21.5% (17.6-21.9) and 36.5% (25.8-50-8), respectively. Among CD3$^+$CD8$^+$cells (Tc), the frequency of HLA-DR-expression was 33.6% (30.9-37.5), 60.5% (56.7-68.1) and 80.6% (70.8-83.8) for patient 1, 2, and 3, respectively.

The percentage of Th-cells with a naïve (CD45RA$^+$CCR7$^+$) phenotype was 4.8% (3.6-6.3) in patient 3, but 24.4% (4.3-24.4) and 25.1% (11.2-26.3) in patient 1 and 2, respectively. The proportion of terminally differentiated (CD45RA$^-$CCR7$^+$) Th-cells was 2.3% (2.1-2.6), 7.4% (2.4-8.7) and 12.7% (10.9-23.2) in patients 1, 2, and 3, respectively.

The frequency of Tregs (CD4$^+$CD25^{high}CD127$^{low/-}$) was 11.5% (8.63-15.9) for patient 3, whereas they were 6.4% (4.8-6.5) and 3.3% (2.5-4.8) for patient 1 and 2, respectively. Patient 3 had the highest proportion Th-cells with a Th17 (CD45RA$^-$CXCR3$^-$CCR4$^+$CCR6$^+$), Th1/Th17 (CD45RA$^-$CXCR3$^+$CCR4$^-$CCR6$^+$) and Th2 phenotype (CD45RA$^-$CCR4$^+$CXCR3$^-$CCR6$^-$). Patient 3 also had the highest median plasma concentrations of IL-17, IL-4 and IFN-γ.

Discussion: DSCs are safe to infuse with no adverse effects. We determined how stromal cells are distributed *in vivo* following infusion in a GvHD setting. The data also support that the non-responder had a more activated/exhausted immune system than the partial responders. This study may provide a basis for further controlled investigations into use of DSCs as a treatment for severe cGvHD.

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Increased Collection Efficiency of Spectra OPTIA Reduces the Blood Volume Processed to Acquire Targeted CD34+ Dose

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Peripheral blood progenitor cell collection (PBPC) is a routine procedure in auto-HCT recipient and allo-HCT donors. Spectra Optia is a next generation apheresis platform following Cobe Spectra. Increasing the efficiency of PBPC

Table 1
Comparison of MNC collections on Cobe Spectra and Spectra Optia.

	Autologous			Allogeneic		
	Cobe Spectra (n=71)	Spectra Optia (n=26)	p	Cobe Spectra (n=21)	Spectra Optia (n=8)	p
Patient weight (kg)	89.2 [76.2;99.8][1]	86.1 [63.4;112.8]	0.88[2]	82.9 [67.1;89.7]	81.4 [76.1;115.8]	0.55
Pre WBC (x10^6/mL)	48.5 [33.0;70.3]	60.2 [51.6;71.1]	0.16	61.2 [51.2;69.1]	43.9 [43.1;52.3]	**0.030**
Pre CD34 (cells/ul)	75.0 [42.0;140.0]	104.0 [42.0;147.0]	0.28	114.0 [69.0;147.0]	65.5 [40.0;217.5]	0.42
W B processed (L)	27.4 [23.5;30.8]	16.8 [14.6;18.7]	**<0.001**	22.0 [19.3;27.2]	16.3 [15.2;18.2]	**<0.001**
Product Hct (%)	2.1 [1.7;3.4]	1.3 [1.0;2.1]	**<0.001**	2.4 [1.9;3.1]	1.2 [1.1;1.5]	**<0.001**
CD34+ (x10^6/kg)	6.2 [3.2;11.2]	10.2 [4.4;15.4]	0.059	8.1 [6.2;9.7]	6.8 [3.7;12.0]	0.92
CD34 CE2 (%)	31.2 [21.5;38.5]	50.1 [42.3;54.7]	**<0.001**	24.4 [19.6;29.3]	44.3 [37.8;50.6]	**<0.001**
MNC CE2 (%)	34.3 [25.2;49.2]	73.7 [50.6;81.2]	**<0.001**	33.2 [27.0;47.2]	65.1 [61.5;82.6]	**<0.001**

[1] Results are depicted as median [IQR].
[2] p values were calculated with Mann-Whitney U test.

collections is a necessary evolution to reduce the volume of processed blood while acquiring the targeted CD34+ cell dose. Retrospective analysis of mononuclear cell (MNC) collections was performed in 61 auto-HCT candidates and 28 donors for allo-HCT using Cobe Spectra or Spectra Optia. The target CD34+ cell dose was 10x10^6/kg for multiple myeloma and 5-7x10^6/kg for lymphomas. For healthy allo-HCT donors the target depended on the recipient diagnosis. Collection efficiency was evaluated as [total number CD34+ cells in product/(peripheral blood CD34+ cell count x blood volume processed)]. Pre-apheresis WBC and CD34+ measurements were not significantly different. The implementation of Spectra Optia in auto-HCT recipients increased the CD34+ CE2 from 31.2% to 50.1% compared to Cobe Spectra, and from 24.4% to 41.3% in allo-HCT donors. This translated in significantly lower volumes of blood processed to obtain an equal CD34+ dose (Table I). In addition, MNC CE2 significantly increased and median Hct in the product decreased on Spectra Optia, indicating a higher purity of the collected product. Moreover, we witnessed an increase of the CD34+ collection efficiency on Spectra Optia in years 2014 versus 2013, indicating a learning curve was inherent to the implementation. Our study shows that the integration of MNC collections on Spectra Optia greatly increased efficiency of CD34+ cell collection in comparison to the Cobe Spectra. Consequently, lower blood volumes were processed to obtain equal to better CD34+ cell dose. These results invite to assess further the impact of implementation of Spectra Optia on the number of collection days.

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Administration of LMP-Specific Cytotoxic T-Lymphocytes to Patients with Relapsed EBV-Positive Lymphoma Post Allogeneic Stem Cell Transplant

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Epstein Barr virus (EBV) associated tumors in the immunocompetent host express the type II latency antigens LMP1 and LMP2 which can serve as potential targets for immunotherapy. For many patients with chronic active EBV (CAEBV) and/or relapsed EBV+ Non Hodgkin Lymphoma (NHL) or Hodgkin Lymphoma (HL) the only potential curative option is allogeneic hematopoietic stem cell transplant (allo-HSCT). However, relapse rates remain high (>50%) and the risk of inducing graft versus host disease (GvHD) after DLI limits the broad application of this approach. We hypothesized that the administration of donor-derived cytotoxic T lymphocytes (CTLs) directed to LMP1/2 (LMP-CTL) would rapidly restore LMP-specific T-cell immunity and prevent relapse in these high-risk patients without increasing the incidence of GvHD. LMP-CTLs were expanded using donor monocytes and EBV transformed lymphoblastoid cell lines, transduced with an adenoviral vector encoding either LMP2 (n=4) or ΔLMP1 and LMP2 (n=11). Characterization of CTL lines at cryopreservation revealed CD4+ and CD8+ cells which were predominantly effector and effector memory phenotype. CTL lines had LMP2 (+/-LMP1) specific activity as determined by ELISPOT and cytotoxicity assays. Fifteen patients (n=5 HL, n=4 B-cell CAEBV/NHL, n=6 T-cell CAEBV/NHL) received 2 infusions of CTLs (dose range: 0.4-3x10e8/m^2) approximately 100 days post HSCT. No immediate toxicities related to CTL infusions were observed. Infusion of LMP-CTLs resulted in a maximum 2.5 fold increase in the frequency of LMPs precursors as detected by IFNγ eliSPOT assay. No patient developed de novo GVHD post CTLs. GVHD was seen in 4 patients with a pre-existing history (1 patient with DLBCL experienced grade II skin GvHD controlled with steroids, 2 patients (1 NK-T and 1 HL) showed signs of grade I GvHD promptly controlled with steroids. One patient with DLBCL developed grade III GvHD after weaning immune suppression and subsequently developed sepsis and died 10 months after an HLA matched-unrelated HSCT). 13 patients were in remission at the time of CTL infusion and 12/13 of these patients remained in remission for a median of 30 months post CTLs (range 6-90 months). One patient with NK/T cell NHL relapsed 6 months post CTLs and died of progressive disease. Two patients (1HL and 1 T-cell CAEBV) received CTLs for relapsed disease after HSCT and both died of disease. Disease progression in the patient with HL, was likely due to immune-escape since tumor biopsies obtained post CTL were EBV-negative. In conclusion, donor-derived LMP-CTLs seem to be well tolerated in high-risk patients with CAEBV and/or EBV+ lymphoma post HSCT. Furthermore, the use of donor-derived tumor-directed T-cells as adjuvant therapy post HSCT may restore LMP-specific T-cell immunity and prevent relapse in this high-risk patient population.