722. CLINICAL ALLOGENEIC TRANSPLANTATION: ACUTE AND CHRONIC GVHD, IMMUNE RECONSTITUTION: POSTER II | DECEMBER 07, 2017

Cord Blood Regulatory T Cells for Prevention of Graft Versus Host Disease

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Abstract

Background: Regulatory T-cells (Tregs) are a T-cell subset capable of suppressing effector T-cells (Teffs) and, when given at the time of allogeneic hematopoietic stem cell transplantation (Allo-SCT), have been shown to prevent graft v. host disease (GVHD). We previously demonstrated that cord blood (CB) Tregs can be ex vivo expanded to clinically meaningful doses and prevent GVHD in a xenogenic mouse model. The efficacy of the ex vivo expanded CB Tregs in preventing GVHD was markedly improved with fucosylation, which adds fucose resulting in creation of a siayl-Lewis X moiety found on P-selectin ligand, thereby facilitating trafficking through the endothelium into inflammatory sites. We hypothesized that adoptive therapy with fucosylated Tregs could prevent GVHD even when given at suboptimal Tregs:Teffs ratios. We conducted a Phase I clinical trial to evaluate the clinical safety of fucosylated CB Treg infusion for GVHD prophylaxis (ww.clinicaltrials.gov NCT02423915). We now report on 5 patients who received

GVHD prophylaxis with 3rdparty, ex-vivo expanded CB Tregs at a dose level of 1.0 x10e6 cells/ kg (2 unfucosylated and 3 fucosylated CB Treg products).

Patients and methods: A 3rd party CB unit with at least 3/6 HLA matching to the recipient was selected. Treg isolation and expansion was performed as shown previously. When indicated, expanded CB Tregs were incubated with substrate (GDP β-fucose) and fucosyltrasferase-VI (FT6) enzyme (TZ101, Targazyme, Carlsbad, CA) for 30 minutes, washed and infused on day -1. The first 2 patients underwent double cord transplant with Flu/Cy/TBI reduced intensity conditioning and received unfucosylated CB Tregs at a dose of 1 x 10e6/kg. The subsequent 3 patients underwent peripheral blood (PB) matched unrelated donor (MUD) transplant with Fludarabine/ Melphalan myeloablative conditioining and received fucosylated CB Tregs at a dose of 1 x 10e6/kg. All patients received additional pharmacologic GVHD prophylaxis with sirolimus and mycophenolate mofetil. All patients received their designated CB Treg dose. The median proportion of $CD4^{+}25^{+}CD127^{-}$ in the infused Treg product was 96% (94-98%). Expanded CB Treg product showed suppression of T cell proliferation using CFSE assay. For the first 2 patients, the infused graft Teffs dose was at least 12 times more than the CB Tregs and for the subsequent 3 patients, the dose of infused Teffs dose was at least 132 times more than that of the infused fucosylated CB Tregs. No infusion toxicities were observed.

Results: All patients receiving fucosylated and non-fucosylated CB Tregs followed by PB MUD transplants exhibited infectious high fevers up to 40 C accompanied by an erythematous rash starting on post-transplant day +5 that lasted for 5-7 days and resolved after steroid administration (IV methylprednisolone 1mg/kg) for 24-96 hours. Skin biopsies were consistent with engraftment syndrome and not GVHD. All 5 patients engrafted at a median of 13 days. The three patients who received fucosylated CB Tregs and one of two patients receiving untreated CB Tregs developed ≥ grade II acute GVHD at a median of day +22 which had completely resolved by day 130 post-transplant. One patient had late onset aGVHD. No cGVHD was documented in any patient. One recipient of non-fucosylated Tregs died on day +40 due to unrelated causes (intracranial bleeding). No GVHD was observed in this patient. PB Flow cytometric analysis revealed an increase in inflammatory T-cell subsets in the first week post-transplant with a concurrent bimodal increase in the Treg populations on day -1 and day +7 (figure 1A). NK and DC but not B cell subsets were significantly increased. A consistent increase in the cell populations secreting IFN#, IL-4 and IL-17 was observed in the post-transplant period and a bi-modal increase in the IL-10 secreting cells was observed on Day -1 and Day +7 consistent with the circulating Treg cells (figure 1B).

Conclusions: Our group is the first to show safety of infusing 3rd party, ex-vivo expanded and fucosylated CB Tregs in a PB MUD transplant setting. At a dose level of 1.0 x 10e6 /kg, 3rd party CB Tregs were administered without acute infusional toxicity and no negative impact on engraftment. We are now enrolling additional patients with unfucosylated Tregs in a PB MUD transplant setting to investigate the mechanisms underlying this phenomenon.

Table 1: Patient characteristics and outcomes

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (yrs)	59	58	51	65	34
Gender	Female	Male	Male	Male	Male
Diagnosis	Acute Myeloid Leukemia	Mycosis Fungoides	Multiple Myeloma	Myeloid sarcoma	Acute Myeloid Leukemia
Conditioning regimen*	Flu/Cy/ TBI	Flu/Cy/ TBI	Flu/Mel140	Flu/Mel140	Flu/Mel140
Type of transplant	Double cord	Double cord	PB MUD	PB MUD	PB MUD
CB Treg dose (1x10e6 cells/ kg)	1.2	1.19	1.1	1.2	1.1
Fucosylated	No	No	Yes	Yes	Yes
Donor total nucleated cell dose (1x10e6 cells/ kg)	CB#1: 41.27	CB#1: 21.88	1486.25	635.21	2008.61
Teff dose (1x 10e6 cells/ kg)	CB#1: 19.04 CB#2: 9.65	CB#1: 6.74 CB#2: 8.03	391.7	158.41	371.19
Ratio of Treg: Teff	1:24	1:12	1:356	1:132	1:337
Infusion reaction	No	No	No	No	No
Engraftment syndrome#	No	No	Yes	Yes	Yes
Time to Engraftment (days)**	16	17	10	13	11
D30 donor Chimerism (%)	100	62	100	100	100
Acute GVHD: could be omitted as you show GVHD grade next line	Yes	No	Yes	Yes	Yes
aGVHD grade	II	0	II	IV	II
aGVHD site	Gastro-Intestinal	N/A	Liver	Skin	Skin
Time to aGVHD (days)**	25	N/A	19	40	19
aGVHD resolution D 100	Yes	N/A	Yes	No	No
Time to aGVHD resolution (days)**	69	N/A	40	130	124
GVHD Recurrence	Yes	N/A	No	No	No
Time to GVHD Recurrence (days)**	322	N/A	No recurrence	No recurrence	No recurrence
Alive	Yes	No	Yes	Yes	Yes
Last Follow up (days)**	535	40	361	292	249
Disease Relapse	No	No	No	No	No
Disease Response	CR	N/A	CR	CR	CR

*Flu/Cy/TBI: Fludarabine 40 mg/ M2 IV (D-8 to D-5), cyclophosphamide 50 mg/kg plus Mesna D-6, Total body radiation 2Gy D-4; Flu/Mel140: Fludarabine 40 mg/ M2 IV (D-5 to D-2); Melphalan 140 mg/ M2 IV D-2

** All days calculated from the day of transplant

N/A: not applicable

Figure 1. Peripheral blood immune reconstitution of patients receiving CB Tregs. 1A. Absolute number of circulating cell populations in the peripheral blood. I). Regulatory T cells. II). Effector T cells. III). Natural Killer cells. **1B.** Percentage gated on live cells from patient samples. I). Interferon gamma secreting cells (TH1 subset); II). Interleukin-4 (IL-4) (TH2 subset); III). Interleukin-17 (IL-17) (TH17 subset) and IV) Interleukin-10 (IL-10) (Treg subset)

Figure 1A

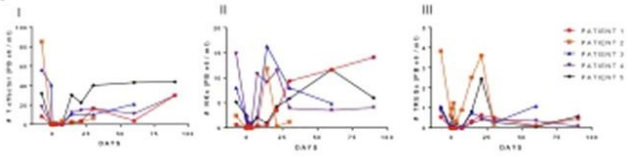
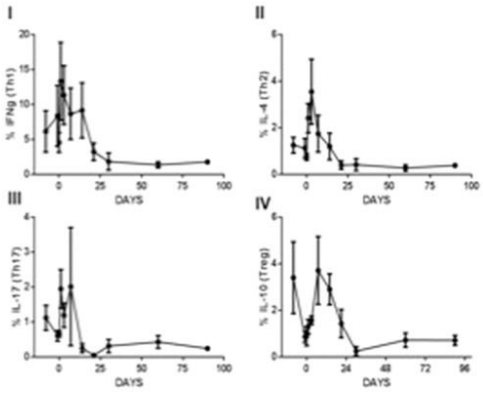


Figure 1B



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Author notes

*Asterisk with author names denotes non-ASH members.