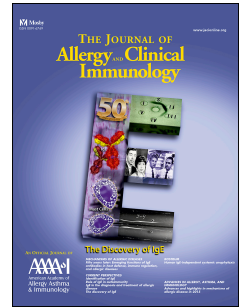# Accepted Manuscript

Umbilical cord blood transplantation without in vivo T-cell depletion for children with MHC class II deficiency

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Capsule summary: An alternative approach for transplanting MHC class II patients is described using transfer of donor T cells during cord blood transplantation to promote rapid T cell engraftment and viral clearance.

Tables and Figure Legends:

Table 1: Patients characteristics and post-UCBT complications.

Figure1: Longitudinal distribution of donor engraftment.

Figure 2: CD4+ T cell recovery post-UCBT.

Table E1: Status of the graft and immunity at last assessment post-UCBT.

Figure E1: Data on myeloid and T cell donor engraftment for P4 and P5.

To the editor:

Without haematopoietic stem cell transplant (HSCT), patients with MHC class II generally accumulate morbidities and have poor long term survival (1). After transplant, immunity usually improves, although some patients continue to require immunoglobulin replacement therapy, and MHC class II antigen presentation in epithelial tissue may still be defective. Despite advances in HSCT, transplanting patients with MHC class II deficiency is particularly challenging in the absence of HLA matched family donors, with suboptimal outcomes in comparison to other combined immune defects. One important issue is the use of serotherapy and other T cell depletion strategies when using unrelated or mismatched donors (2,3). We have previously reported that children who underwent umbilical cord blood transplantation (UCBT) without serotherapy for malignant and non-malignant diseases achieved early T cell recovery with a median time to achieve CD4+T cells $\geq$ 300 of 30 days due to the expansion of adoptively transferred post-thymic T cells (4).We now report the application of such T replete cord grafts in 6 Arabian subjects with MHC class II deficiency Median age at presentation was 4 months (0.25-5). Active infections at the time of referral, included CMV viraemia , Pneumocystis jiroveci, Echovirus meningitis , respiratory syncytial pneumonitis . Diarrhoea and failure to thrive with chronic Norovirus II excretion affected 5 patients. One patient presented with severe gastro-oesophageal reflux (P3) and in 2 patients there was histological confirmation of severe eosinophilic enteritis with/without perianal fistulas (P1, P5). MHC II expression was confirmed absent by flow cytometry on B cells and activated T cells in all patients and variable hypogammaglobinemia was observed. RFXANK mutations were confirmed in four subjects. At a median of 25.5 months (12-69), patients underwent either a 10/10 HLA matched or one single antigen mismatched UCBT. Transplant conditioning included (total) Treosulphan (Treo) 42g/m^2 and Fludarabine (Flu) 150 mg/m^2.

The median nucleated cell dose was $12x10^7$/kg with CD34 cell $2.1x10^5$ and median CD3 cell dose of $7.7x10^6$. All patients received Ciclosporin/Mycophenolate Mofetil as a graft versus host disease (GvHD) prophylaxis. All patients engrafted neutrophils by a median of 26.5 days (15-30), platelets by 37 days (35-101) and all exhibited donor chimerism >90% by 28 days. This was followed by stable donor myeloid and T cell chimerism > 60% in all except P4 (Figure 1, Figure E1 in online repository). Within a month of transplant median CD3$^+$T cell counts were >300 cells/µl and were > 500 cells/µl by 6-12 months post-transplant in 5/6 patients with evident thymopoeisis. CD4$^+$T cells predominated (Figure 2) and by six months post-transplant, 5/6 patients had a CD4 counts >300 cells/ul. P5 reached CD4 levels of 300/ul by 1 year post-UCBT following prolonged steroid therapy for GvHD. Most Vβ families were polyclonal with normal spectratype at 1 month post-UCBT in P1, P2, P4, P6.All patients had CD19 B cells ≥ 50 cells/ul at 3 months post-UCBT ; 4/6 had CD19 ≥100 cells/ul with a median of 560cells/µl .Although IgM and IgA normalized in P1-P4 between 3-8 months post-UCBT, P1,3,5,6 remained on immmunoglobulin replacement. P2 had documented vaccine responses against tetanus and pneumococcal serotypes (Table E1 in online repository). P5 developed autoimmune thrombocytopenia and pulmonary haemorrahge in the presence of HHV6 pneumonitis and received 4 doses of Rituximab; 375 mg/m2 together with ganciclovir therapy (Virus related complications included CMV/HHV6 and adenovirus reactivation in 2 patients which were successfully managed with antiviral therapy. Four patients were able to clear Norovirus II between 3 and 6 months' post-transplant. Echo meningoencepahlitis was diagnosed pre-transplant in P4 and by 4 months had been cleared from CSF. Four patients developed aGvHD grade I-II disease which responded to topical or systemic steroids and one patient suffered Grade III skin /gut aGvHD requiring prolonged steroid therapy and infliximab for 42 months post-HSCT (P5). P4 developed engraftment syndrome with protracted fevers at the time of neutrophil engraftment, which resolved with systemic steroid

therapy. P6 developed limited skin cGvHD; responsive to systemic steroids. Four patients had post-transplant enteropathy, 2 with prolonged dependency on parenteral nutrition (P3, P5). Interestingly, these same patients had suffered severe enteropathy pre-transplant. Although there was no histological evidence of aGvHD of the gut (in P1 and P3), they responded to systemic steroids and regular infliximab therapy. All patients were alive at last follow-up. Table 1 summarizes other complications after HSCT, which of note included prolonged B cell aplasia post-Rituximab for severe autoimmune cytopenias in P5. P4 had pre-existing hydrocephalus and suffered an unexplained neurological deterioration with acute loss of vision &hearing at 6 months post-transplant during the period of immune reconstitution as well as autoimmune haemolytic anaemia (AIHA) which responded to methylprednisolone therapy.

Previous reports of cord transplants for MHC class II deficiency described *in vivo* T cell depletion in 4/5 transplants (2,3, 5-7). Disseminated viral infections and severe bacterial sepsis were noted, and one patient died of chemotherapy-related toxicity. Limited cGvHD was reported in one case and two patients had mixed chimerism, one suffered of AIHA. We found that Treo/Flu based conditioning without serotherapy was sufficient to support donor engraftment and allow for rapid CD4$^+$T cell recovery. Expansion of donor derived post-thymic CD4$^+$T cells is particularly relevant in MHC class II deficiency where maturation and thymic selection of CD4+ T cells is impaired. In murine experiments, it has been shown that donor derived thymocytes expressing MHC class II can facilitate CD4+ T cell selection in the thymus (8). A major consequence of using T replete donations is an increased risk of GvHD and this was problematic in two of our subjects, and may be a particular concern in the context of pre-existing enteropathy and poor host immunity against viruses. Low dose Alemtuzumab was shown by Lane et al, 2014 (9) to allow early immune reconstitution post-

UCBT in a group of PID subjects, although in the absence of pharmacokinetic data, further extrapolation is limited. Similarly, pharmacokinetic studies of ATG may allow dose targeted host T cell depletion while preserving donor derived T cell engraftment that appears critical in treating MHC class II deficiency.

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Table 1: Patients characteristics and post-UCBT complications

ID	Presenting age (m) Sex	Age at UCBT (m)	Donor match	Pre-UCBT morbidities	Active problems at time of UCBT	Post-UCBT viral infection	aGVHD	cGVHD	Other complications
P1	3.5/F	38	10/10	Disseminated BCGiosis ICU admission with MV. Enterobacter Cystitis Failure to thrive	Noro II (stool). Inflammatory enteritis; TPN dependent Reduced lung volume bilaterally and subcarinal/left lower lobe calcification(old BCGiosis)	Cleared Norovirus II	Grade I (skin). .	No	Post-transplant (D+61) eosinophilic colitis responded to Prednisolone / Sulfasalzine / Infliximab / CSA
P2	5/M	20	9/10	Severe Respiratory infection (unknown pathogen) ICU without MV. Failure to thrive	Noro II (stool). Chronic Diarrhoea	Cleared	GradeII(skin/Gut) Responded to Budesonide therapy	No	NA
P3	4/M	17	9/10	PCP pneumonitis ICU with MV CMV vireamia (ganciclovir) RSV pneumonitis. Failure to thrive Delayed developmental milestones	Noro II (stool). Severe GORD Eosinophilic gastroenteritis.	Cleared Norovirus II Adenovirus reactivation With no disease D+22 (Ganciclovir/Foscarnet)	GradeII (skin) .	No	Post-transplant gastritis/colitis 1 year post-UCBT responded to Prednisolone / Sulfasalazine / CSA
P4	0.25/F	30	10/10	Positive FH Neonatal ICU without MV (neonatal jaundice/respiratory distress) Bronchopneumonia (Possibly TB) Streptococcus parasanguinous septicaemia	Echo (CNS) Noro II (stool) Persistent Candidaemia Intermittent febrile episodes	Cleared Echo/Norovirus II	None	No	Engraftment syndrome. Unexplained neurological phenomenon at 6m (blindness and deafness as a sequel). AIHA at 26m post-UCBT.

				Candidaemia Hydrocephalus with oropharyngeal dysfunction/hypertonia/developmental delay Failure to thrive					
P5	4/M	34	9/10	Positive FH Severe respiratory infection ICU without MV (unknown pathogen) Failure to thrive	Noro II (stool). Eosinophilic pan-enteritis/anal ulcerations TPN dependent Lower lobe bronchiectasis	HHV6 pneumonitis (responded to Ganciclovir). CMV reactivation (cleared by Ganciclovir) Chronic Norovirus II	Grade III(skin/gut)	cGVHD of skin / gut responded toPrednisolone / CSA / Infliximab	Autoimmune thrombocytopenia / pulmonary haemorrhage 1year post-UCBT (prednisolone / rituximab). Prolonged B cell aplasia post-Rituximab therapy
P6	0.5/M	12	9/10	Severe respiratory infection ICU without MV (unknown pathogen) Recurrent Oral thrush	None	None	Grade II (skin)	cGVHD of skin (limited) Responded to prednisolone	NA

M:male, F: female, MV: mechanical ventilation, TPN: total parental nutrition, ICU: intensive care unit, NA: not applicable. m: months. RSV: respiratory syncytial virus, CMV:cytomegalovirus, CSA: Ciclosporin.

Figure 1: Longitudinal distribution of donor engraftment

Figure 2: CD4+ T cell recovery post-UCBT

All patients had CD4+ counts above 300 at 6 months post-UCBT apart from P5.
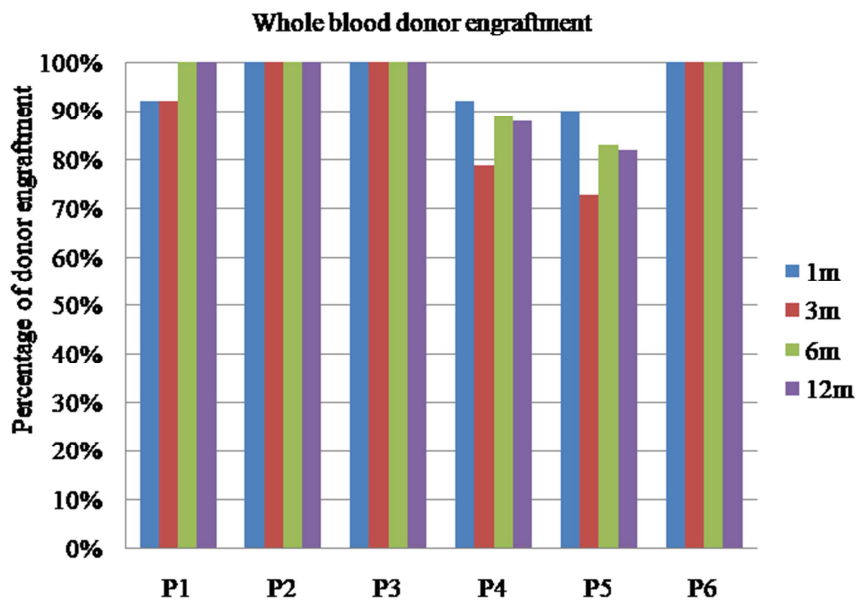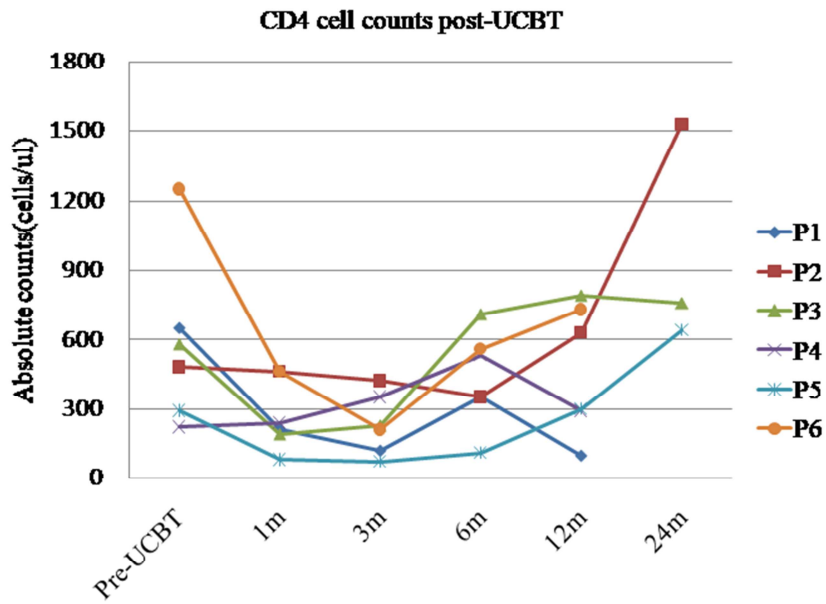
Figure 1: Longitudinal distribution of donor engraftment

Whole blood donor engraftment

Figure 2: CD4+ T cell recovery post-UCBT

CD4 cell counts post-UCBT

All patients had CD4+ counts above 300 at 6 months post-UCBT apart from P5.

Table E1: Status of the graft and immunity at last assessment post-UCBT

ID	Age at last assessment(y) Last Follow up (m)	Latest Engraftment	B cell counts (cells/ul) Ig levels	IVIG therapy vaccine response	T cell Counts (cells/ul)	Molecular spectratyping TRECS PHA
P1	4y 10/12 12m	WB:100%	140 (200-2100) Normal IgM/IgA	On NA	CD3:120 (900-4500) CD4:100(500-2400) CD8:10(300-1600) Naïve CD4:50 (430-1500) Naïve CD8:NA Memory CD4: NA Memory CD8:NA	Normal Spectratype 4970
P2	3y,6/12 24m	WB:100%	750 (200-2100) Normal IgM/IgA	Off Tetanus response: normal Response to pneumococcus: 8/13	CD3: 2540(900-4500) CD4:1530(500-2400) CD8:820(300-1600) Naïve CD4:1040 (430-1500) Naïve CD8:610 (380-1100) Memory CD4:460 (220-660) Memory CD8:190 (90-440)	NA 16636
P3	4y, 6/12 36m	WB:100%	460 (200-2100) Normal IgM/IgA	On NA	CD3: 1230(900-4500) CD4:480(500-2400) CD8:640(300-1600) Naïve CD4:530 (430-1500) Naïve CD8:470 (380-1100) Memory CD4:300 (220-660) Memory CD8:10(90-440)	NA 5272 (at 24m)
P4*	3y,4/12 28 m	WB: 42% Split engraftment: NA	320 (200-2100) Normal IgA / IgM	Off Awaiting vaccination NA	CD3:940(900-4500) CD4:620(500-2400) CD8:350(300-1600) Naïve CD4:80 (430-1500) Naïve CD8:130 (380-1100) Memory CD4:450 (220-660) Memory CD8: 220 (90-440)	NA 1480 NA
P5	8y, 9/12 69m	CD15: 72% CD3:61%	10 (200-1600) Absent IgM/IgG	On NA	CD3: 2660(700-4200) CD4: 1170(300-2000) CD8: 850(300-1800) Naïve CD4: 400 9320-1000) Naïve CD8:420 9310-900) Memory CD4:740 (230-630) Memory CD8:240 (70-390)	Normal spectratype 24340 NA
P6	2y,3/12 19m	WB:100%	580 (200-2100) Normal IgA / IgM	On NA	CD3: 2420(900-4500) CD4: 1520(500-2400) CD8: 830(300-1600) Naïve CD4:805 (430-1500) Naïve CD8:590(380-1100) Memory CD4:623 (220-660) Memory CD8:149(90-440)	NA Normal

P4 bloods were taken at 26 months post-UCBT. NA: not applicable, Ig: immunoglobulin

Figure E1: Data on myeloid and T cell donor engraftment for P4 and P5

Figure E1: Data on myeloid and T cell donor engraftment for P4 and P5

Donor T and Myeloid engraftment