Haematopoietic stem cell transplantation for CTLA4 deficiency

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Capsule summary

Mutations in Cytotoxic T lymphocyte antigen 4 cause an immune dysregulation syndrome with disrupted T and B cell homeostasis. We report 8 patients treated by haematopoietic stem cell transplantation, 6 survived with resolution of symptoms.

Keywords

CTLA4, haematopoietic stem cell transplantation (HSCT), total parenteral nutrition (TPN)

Word count 1139

To the Editor

Pathogenic mutations in Cytotoxic T lymphocyte antigen 4 (*CTLA4*) behave in an autosomal dominant manner with incomplete penetrance, resulting in a complex immune dysregulation syndrome with disrupted T and B cell homeostasis[1-3]. Kuehn et al. identified 7 patients from 4 families with lymphoproliferation, organ infiltration, autoimmune cytopenias and B cell abnormalities[1]. Schubert et al. identified 14 patients from 6 families, of whom 11 had enteropathy and 10 hypogammaglobulinaemia; other manifestations included granulomatous lymphocytic interstitial lung disease, respiratory infections, organ infiltration, cytopenias, lymphadenopathy, skin diseases, autoimmune thyroiditis, arthritis and one case of solid cancer[2] . There are no published reports of haematopoietic stem cell transplantation (HSCT) for this disorder. We report 8 patients with *CTLA4* haploinsufficiency who have undergone HSCT in 3 paediatric centres: the Great North Children's Hospital, Newcastle upon Tyne, UK (4 patients), Royal Manchester Children's Hospital, Manchester, UK (1 patient) and the University of Washington and Seattle Children's Hospital, USA (3 patients).

The diagnosis was made retrospectively in seven patients who underwent HSCT for life-threatening, treatment-resistant immune dysregulation and in one patient prospectively. Clinical and laboratory features are summarised (Table 1). Novel heterozygous variants in *CTLA4* were predicted to be deleterious in all cases (Table 2), confirmed by functional testing in a recombinant system for the missense variants identified in patients 1-5 (Figure 1 and supplementary methods). Patient 6 has a different amino acid substitution at the same residue as patient 5 which was not tested separately. Sequencing of *CTLA4* cDNA confirmed that the mutation identified in patients 7 and 8 led to skipping of exon 3 with splicing of exon 4 to exon 2 leading to a frameshift and premature termination, deleting the transmembrane and intracellular domains of *CTLA4* and abrogating protein expression (data not shown).

Patient 1 had arthritis, neutropenia and thrombocytopenia, lymphadenopathy and abdominal pain. This patient was offered HSCT due to ongoing autoimmunity and risk of lymphoma as his father had complex autoimmune disease and died following autologous HSCT for non-Hodgkin's lymphoma. Patient 2 had thrombocytopenia, associated bleeding, neutropenia and lymphoid hyperplasia in lungs, lymph nodes and brain, refractory to immunomodulatory therapy. Patient 3 had autoimmune

haemolytic anaemia and thrombocytopenia from the age of 4 and developed enteropathy and bronchiectasis. She had severe side effects from steroid therapy. Her mother was also affected with cytopenias, hypothyroidism and eczema. Patient 4 (sibling to patient 3) was well until he presented with inflammatory colitis and Hodgkin lymphoma (inguinal and para-aortic region) at age 16. Because of his sibling's history, *CTLA4* haploinsufficiency was confirmed by both genetic and protein level testing, the only patient in this cohort to have an identified mutation prior to HSCT. His fulminant diarrhoea responded to a combination of prednisolone, sirolimus and Belatacept and his Hodgkin Disease was successfully treated with three cycles of chemotherapy prior to transplantation. Patient 5 had brittle diabetes from the age of 2 with severe enteropathy requiring parenteral nutrition (TPN), cytopenias necessitating splenectomy and cholecystectomy, recurrent deep vein thrombosis, bronchiectasis, vitiligo and alopecia and severe side effects from steroid therapy. He was refractory to treatment including Alemtuzumab, Infliximab and Adalimumab and his mother died due to a gastrointestinal lymphoma. Patient 6 had trilineage cytopenias, enteropathy with pancreatic insufficiency since age 7 requiring TPN, and diabetes. In addition he had recurrent infections including pulmonary aspergillosis. Patient 7 had enteropathy, cytopenias, and juvenile idiopathic arthritis beginning in childhood.

All 8 patients received steroids and a calcineurin inhibitor prior to transplant, and all except patients 3 and 4 had high dose IVIg and rituximab as immunomodulatory therapy. Patient 4 had replacement IVIg because of his hypogammaglobulinemia but no rituximab. Consent for HSCT and genetic work-up was obtained according to local centre and EBMT guidelines. All received well-matched unrelated donor grafts following reduced intensity conditioning. Five patients (1, 2, 5, 6, and 8) had peripheral blood HSC grafts and received cyclosporine and mycophenolate mofetil (MMF) for graft versus host disease (GvHD) prophylaxis. Three (3, 4, and 7) received bone marrow HSC grafts and had cyclosporine alone, cyclosporine and MMF, or methotrexate and tacrolimus.. Patient 6 had prednisolone, sirolimus and Belatacept until 8 days prior to transplant. Transplant characteristics are summarised in Table 3.

Neutrophil engraftment (1st day of Neutrophils greater than 0.5 x 10^9/l) ranged from D+13 to D+21 and platelets were greater than 50 x10^9/l between D+13 and Day+15 post HSCT. Patients 1 and 8

have stable mixed donor chimerism of ≥85% in all cell lineages and patients 3, 4, 6, and 7 have 100% donor chimerism. Six of 8 patients are alive and well. Patient 2 died with transplant-related mortality of severe acute gut GvHD. Patient 5 did well post HSCT, became TPN-independent after 5 months, but unfortunately died from diabetic ketoacidosis 2.5 years post HSCT. Both of these patients had 100% donor chimerism. Patient 1 had CMV reactivation early post HSCT and autoimmune haemolytic anaemia 6 months post HSCT, which responded to steroids; he is now off all medication. Patient 3 had an uncomplicated transplant course and is also off all medication. Patient 4 had a relapse of inflammatory colitis with 10 - 20 stools/day on day 2 after transplant but this was controlled by day 10 with steroids and Belatacept which have both been discontinued. He is now well 14 weeks post-transplant. Patient 6 remains on sirolimus for oral and ocular chronic GvHD which have resolved. Patient 7 had chronic oral and skin GvHD and she is now off all immune suppression. Patient 8 has had no GvHD but continues on MMF and cyclosporine for GvHD prophylaxis 4 months after transplant. In summary 5 of 8 patients experienced GvHD despite having well-matched donors and receiving Alemtuzumab in 2. The high levels of inflammation in which these patients enter the HSCT process may promote the development of alloreactivity and so future patients are likely to benefit from either enhanced pre-HSCT immunosuppression, or more aggressive post-HSCT GvHD prophylaxis. Seven of 8 patients had complete resolution of severe enteropathy and cytopenias following HSCT, however diabetes is irreversible, highlighting the importance of early recognition and treatment. Improved outcome after HSCT for autoimmune diseases[4] and for children with other non-malignant disorders following reduced intensity conditioning[5,6] makes HSCT an attractive option for severe cases and our series suggests a similar transplant related mortality (1 of 8 patients) to that for other immune disorders. Other therapeutic options proposed for *CTLA4* deficient patients include soluble *CTLA4* fusion proteins (abatacept and belatacept), which bind to CD80 and CD86 and inhibit immune activation[7]. These were tried with probable benefit in the only patient to receive a molecular diagnosis prior to HSCT, but did not alter the indication for transplant which was his non-Hodgkin's lymphoma. *CTLA4* haploinsufficiency shows a variable phenotype and further studies are needed to guide treatment selection including which patients could benefit from *CTLA4*-ligand-targeted immunomodulation vs. HSCT, optimal timing of HSCT and long-term outcome post-HSCT.

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Acknowledgements

Funding from the following grants contributed to this work: NIH R13 AI094943, NIH U54 AI082973, and NIH P01 HL122173. One of the U.S. patients was enrolled on a clinical trial supported in part by NIH grant P01 HL122173, as well as research funding from Medac, GmbH (Hamburg, Germany). In addition, Medac, GmbH provided Treosulfan for the study in the U.S.

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Figure 1

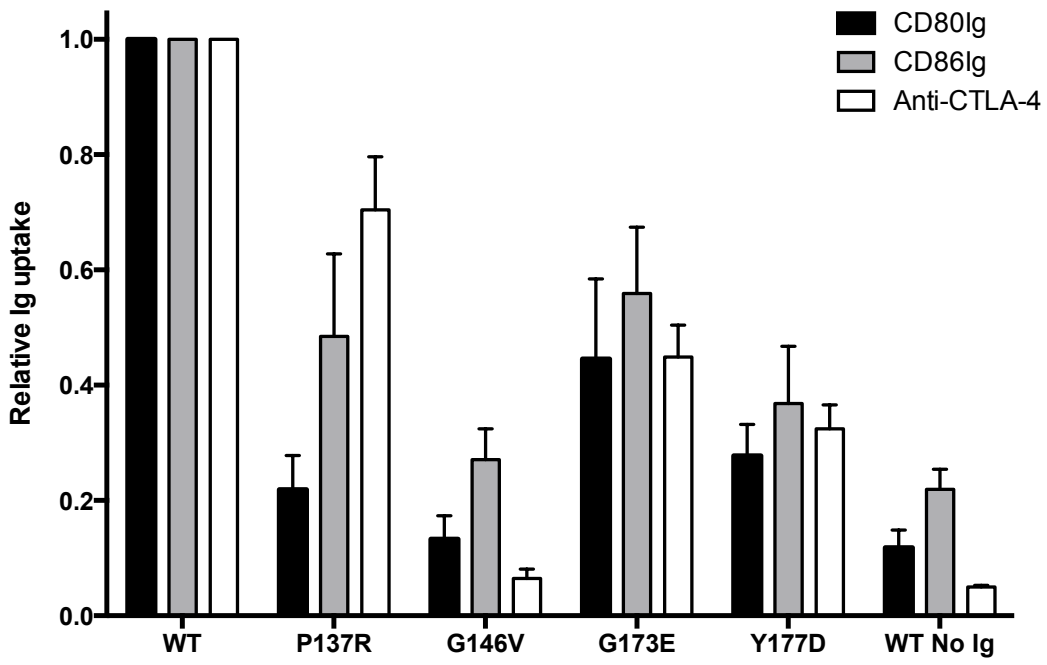

Figure 1 - Mutations in CTLA-4 affect ligand uptake. CHO cells expressing WT or mutant forms of CTLA-4 were cultured in the presence of CD80-Ig, CD86-Ig or an anti-CTLA-4 antibody. Cells were analysed for their ability to uptake ligand or antibody at 37°C relative to total cellular CTLA-4 expression. Each mutant was then normalised to CTLA-4 WT expressing cells.

Table 1 - Patient Characteristics

Patient/ Gender	Heterozygous change in CTLA-4	Lymphocyte subsets* cells/uL (Normal range)	Immunoglobulins g/L (Normal range)	Age at onset Clinical features	Family history
1 M	c.518G>A p.G173E	14 years CD3 1325 (800-3500) CD4 771 (400-1200) Naïve CD4 225 CD8 531 (200-1200) Naïve CD8 305 CD3CD25 10% CD3DR 16% NK 59 (70-1200) CD19 296 (200-600)	14 years IgG 10.9 (3.8-15.2) IgA 0.30 (0.64-2.58) IgM 0.76 (0.43-1.9) Pre Ig Pre RTX	1.5 years • Autoimmune pancytopenia • Recurrent abdominal pain • Arthritis	Father: Immune dysregulation Cytopenias Lymphoma
2 M	c.529T>G p.Y177D	13 years CD3 934 (800-3500) CD4 436 (400-1200) Naïve CD4 nil CD8 371 (200-1200) Naïve CD8 nil CD3CD25 11% CD3DR 38% NK 140 (70-1200) CD19 2700 (200-600)	13 years IgG 13.5 (3.8-15.2) IgA 0.86 (0.64-2.97) IgM 1.06 (0.43-1.9) Pre Ig Pre RTX	10 years • ITP and autoimmune neutropenia • Reactive lymphoid hyperplasia - lymph nodes, lung, frontal lobe brain	nil
3 F	c.437G>T p.G146V	7 years CD3 738 (800-3500) CD4 284 (400-1200) Naïve CD4 nil CD8 339 (200-1200) Naïve CD8 nil	7 years IgG 3.54 (3.8-15.2) IgA 0.41 (0.64-2.58) IgM 0.21 (0.43-1.9) Pre Ig	5 years • Autoimmune cytopenias • Enteropathy • Bronchiectasis	Mother: Enteropathy Evan's syndrome Brother: Patient #4

		CD3CD25 15% CD3DR 31% NK 160 (70-1200) CD19 0 (200-600)	No RTX		
4 M	c.437G>T p.G146V	16 years CD3 190 (622-2402) CD4 124 (24-406) CD8 49 (500-1500) NK 11 (109-897) CD19 9 (120-645)	16 years IgG 4.92 (6.0-16.0) IgA 1.33 (0.8-2.8) IgM 0.24 (0.5-2.0) Pre Ig No RTX	16 years • Enteropathy • Hodgkin Disease (mixed cellularity) treated with Euronet PHL-C1 Hodgkin's Lymphoma 2007 protocol, received 3 courses of ABVD	Mother: Enteropathy Evan's syndrome Sister: Patient #3
5 M	c.410C>G p.P137R	18 years CD3 2842 (690-2540) CD4 2350 (410-1590) Naïve CD4 682 CD8 492 (190-1140) Naïve CD8 455 CD3CD25 21% CD3DR 15% NK 114 (90-590) CD19 0 (90-660)	18 years IgG 8.84 (5.8-15.4) IgA 0.62 (0.64-2.07) IgM 1.49 (0.24-1.9) On Ig Post RTX	2 years • Autoimmune cytopenias • Enteropathy –PN dependent for 5 years • IDDM • Exocrine pancreatic insufficiency • Bronchiectasis • Recurrent deep vein thromboses	Mother: Lymphoma Brother: Autoimmune gut disease Sister: Arthritis Autoimmune thyroiditis
6 M	c.410C>T p.P137L	13 years CD3 592 (800-3500) CD4 402 (400-2100) CD8 171 (200-1200) CD3CD25 6% CD3DR 35% NK 100 (0-771) CD19 271 (200-600)	13 years IgG 7.94 (6.0-15.8) IgA 0.40 (0.38-2.00) IgM 0.61 (0.35-2.52) Pre Ig Pre RTX	7 years • Autoimmune cytopenias • Enteropathy • Exocrine pancreatic insufficiency • IDDM • Recurrent infections: Sinusitis & Streptococcal pharyngitis. Pulmonary Aspergillosis. • Renal insufficiency	Father: Hashimoto thyroiditis Mother: Autoimmune thyroiditis Maternal Grandmother: Persistent diarrhea
7 F	c.567+6T>G	28 years	28 years	1-2 years	Father:

	p.D153Afs*21 (Splicing)	CD3 622 (700-2100) CD4 496 (300-1400) CD8 112 (200-900) CD3DR 11% NK 56 (0-771) CD19 0 (100-500)	IgG 0.93 (5.4-16.8) IgA 0.68 (0.74-2.61) IgM 1.51 (0.40-1.95) Pre Ig Pre Rtx	• ITP & Autoimmune hemolytic anemia • Enteropathy/lymphocytic colitis • Hypocalcemia, Vit D deficiency, Osteoporosis • Interstitial lung disease • Juvenille rheumatoid arthritis • Eczema	ITP Sister: Patient #8
8 F	c.567+6T>G p.D153Afs*21 (Splicing)	26 years CD3 648 (700-2100 CD4 464 (300-1400) Naïve CD4 33 CD8 160 (200-900) Naïve CD8 54 CD3DR 23% NK 48 (0-771) CD19 88 (100-500)	26 years IgG 4.9 (5.4-16.8) IgA 0.27 (0.74-2.61) IgM 0.84 (0.40-1.95) Pre Ig Pre Rtx	23 years • Interstitial Lung Disease (“Nodular Lymphoid Hyperplasia”) • Transverse myelitis • Recurrent white matter and brainstem lesions with oligoclonal bands and elevated IgG index • Arthritis	Father: ITP Sister: Patient #7

Abbreviations: ITP=Idiopathic thrombocytopenic purpura, IDDM= Insulin dependent diabetes mellitus, PN= parenteral nutrition, naïve CD4 = CD3+CD4+CD27+CD45RA+, naïve CD8 = CD3+CD4-CD27+CD45RA+, Ig = immunoglobulin therapy, RTX = Rituximab, ABVD: Adriamcyin (Doxorubicin), Bleomycin, Vinblastine, Dacarbazine

*Lymphocyte subset results at first visit to specialized immunology center

Table 2 - Variants

Patient/ Gender	Heterozygous change in CTLA-4	Known mutation*	SIFT prediction	PROVEAN prediction	Mutation Taster prediction	PolyPhen-2 prediction	PON-P2	CADD score	Affected domain
1/M	c.518G>A p.G173E	No	Damaging	Neutral	Disease causing	Probably damaging (score: 0.978)	Unknown (score: 0.369)	1.955	Transmembrane domain
2/M	c.529T>G p.Y177D	No	Damaging	Deleterious	Disease causing	Probably damaging (score: 0.998)	Unknown (score: 0.722)	4.201	Transmembrane domain
3/F, 4/M	c.437G>T p.G146V	No	Damaging	Deleterious	Disease causing	Probably damaging (score: 1.000)	Pathogenic (score: 0.872)	2.791	Ligand-binding domain
5/M	c.410C>G p.P137R	No	Damaging	Deleterious	Disease causing	Probably damaging (score: 1.000)	Pathogenic (score: 0.869)	2.664	Ligand-binding domain
6/M	c.410C>T p.P137L	No	Damaging	Deleterious	Disease causing	Probably damaging (score: 1.000)	Pathogenic (score: 0.804)	3.058	Ligand-binding domain
7/F, 8/F	c.567+6T>G p.D153Afs*21	No	N/A	N/A	N/A	N/A	N/A	N/A	Transmembrane & Intracellular domains

*According to ESP6500, cg69, dbSNP, 1000G and ExAC databases.
N/A = Not applicable due to large deletion/frameshift created by aberrant mRNA splicing of exon 4 to exon 2 (exon 3 is skipped).

Table 3 - Transplant characteristics

Patient/ Gender	Age at HSCT (Years)	Year of HSCT	Conditioning	Donor source/ HLA matching	GVHD Prophylaxis	GvHD	Chimerism	Outcome/ Follow up
1 M	16	2010	Alem, Flu, Treo	PBSC 10/10	MMF/CSP	None Off immune suppression	CD3+ 90% CD19+ 95% CD15+ 96%	Alive and well 4.75 years
2 M	15	2008	Alem, Flu, Mel	PBSC 10/10	MMF/CSP	Acute Grade IV gut	100%	Died 4 months (GvHD)
3 F	10	2005	Alem, Flu, Mel	BM 10/10	CSP	None Off immune suppression	100%	Alive and well 10.2 years
4 M	17	2015	Alem, Flu, Treo, Thio	BM 10/10	MMF/CSP	Flare of autoimmune colitis D+2 - +10. Treated with methylpredisolone and belatacept. Remains on CSP alone	100%	Alive and well, discharged from hospital 3.5 months
5 M	20	2008	Alem, Flu, Mel	PBSC 10/10	MMF/CSP	Acute Grade II skin resolved. Immune suppression stopped	100%	Died 2.5 years (DKA)
6 M	17	2013	Flu, TBI	PBSC 10/10	MMF/CSP	Acute Grade III skin and gut resolved Chronic Oral and ocular GvHD.	CD3+ 100% CD19+ 100% CD56+ 100% CD33+ 100%	Alive and well 2.0 years

						Continues on sirolimus and physiologic prednisone		
7 F	30	2011	rATG, Flu, Treo	BM 10/10	MTX/TAC	Acute Grade II skin and gut GVHD resolved Chronic oral and skin GVHD resolved. Off immune suppression	CD3+ 100% CD33+ 100%	Alive and well 4 years
8 F	32	2015	Flu, TBI	PBSC 10/10	MMF/CSP	None. Continues on tapering doses of MMF/CSP.	CD3+ 85% CD56+ 100% CD33+ 100%	Alive and well 4 months

Abbreviations: Alem = Alemtuzumab total dose 1.0mg/kg, Flu = Fludarabine total dose 150mg/m^2, Mel = Melphalan total dose 140mg/m^2, rATG = rabbit anti-thymocyte globulin total dose 6.0 mg/kg, Thio - Thiotepa total dose 10mg/kg, TBI = total body irradiation total dose 4 Gy , Treo = Treosulfan total dose 42g/m^2, MTX = Methotrexate, CSP = Cyclosporine, TAC = Tacrolimus, MMF = Mycophenolate Mofetil, PBSC = peripheral blood stem cells, BM = bone marrow, GvHD=Graft versus host disease, DKA = diabetic ketoacidosis