

Letter to the Editor

Hematopoietic stem cell transplantation for *CTLA4* deficiency

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 al¹ identified 7 patients from 4 families with lymphoproliferation, organ infiltration, autoimmune cytopenias, and B-cell abnormalities. Schubert et al² identified 14 patients from 6 families, of whom 11 had enteropathy and 10 hypogammaglobulinemia; 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. There are no published reports of hematopoietic stem cell transplantation (HSCT) for this disorder. We report 8 patients with *CTLA4* haploinsufficiency who have undergone HSCT in 3 pediatric centers: the Great North Children's Hospital, Newcastle upon Tyne, United Kingdom (4 patients), Royal Manchester Children's Hospital, Manchester, United Kingdom (1 patient), and the University of Washington and Seattle Children's Hospital, United States (3 patients).

The diagnosis was made retrospectively in 7 patients who underwent HSCT for life-threatening, treatment-resistant immune dysregulation and in 1 patient prospectively. Clinical and laboratory features are summarized in Table I. Novel heterozygous variants in *CTLA4* were predicted to be deleterious in all cases (Table II⁴⁻⁹), confirmed by functional testing in a recombinant system for the missense variants identified in patients 1 to 5 (Fig 1; see this article's Methods section in the Online Repository at www.jacionline.org). 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 because of ongoing autoimmunity and risk of lymphoma because his father had complex autoimmune disease and died after autologous HSCT for non-Hodgkin 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 hemolytic anemia and thrombocytopenia from the age of 4 years and developed enteropathy and bronchiectasis. She had severe adverse 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 years. 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 before HSCT. His fulminant diarrhea responded to a combination

of prednisolone, sirolimus, and belatacept, and his Hodgkin disease was successfully treated with 3 cycles of chemotherapy before transplantation. Patient 5 had brittle diabetes from the age of 2 years with severe enteropathy requiring total parenteral nutrition (TPN), cytopenias necessitating splenectomy and cholecystectomy, recurrent deep vein thrombosis, bronchiectasis, vitiligo, and alopecia and severe adverse effects from steroid therapy. He was refractory to treatment including alemtuzumab, infliximab, and adalimumab, and his mother died because of a gastrointestinal lymphoma. Patient 6 had trilineage cytopenias, enteropathy with pancreatic insufficiency since age 7 years 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 before transplant, and all except patients 3 and 4 had high-dose intravenous immunoglobulin and rituximab as immunomodulatory therapy. Patient 4 had replacement intravenous immunoglobulin because of his hypogammaglobulinemia but no rituximab. Consent for HSCT and genetic workup was obtained according to local center and the European Society for Blood and Marrow Transplantation guidelines. All received well-matched unrelated donor grafts after 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 before transplant. Transplant characteristics are summarized in Table III.

Neutrophil engraftment (first day of neutrophils $>0.5 \times 10^9/L$) ranged from day +13 to day +21 and platelets were greater than $50 \times 10^9/L$ between day +13 and day +15 post-HSCT. Patients 1 and 8 have stable mixed donor chimerism of 85% or more in all cell lineages, and patients 3, 4, 6, and 7 have 100% donor chimerism. Six of the 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 these patients had 100% donor chimerism. Patient 1 had cytomegalovirus reactivation early post-HSCT and autoimmune hemolytic anemia 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 medications. Patient 4 had a relapse of inflammatory colitis with 10 to 20 stools per 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 posttransplant. 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, 4 of 8 patients experienced GvHD despite having well-matched donors and receiving alemtuzumab in 3 out of 4 cases. The high levels of inflammation in which these patients enter the HSCT process may promote the

TABLE I. Patients' characteristics

Patient/ sex	Heterozygous change in CTLA-4	Lymphocyte subsets* (cells/ μ L) (normal range)	Immunoglobulins (g/L) (normal range)	Age at onset (y) Clinical features	Family history
1/M	c.518G>A p.G173E	14 y CD3 1325 (800-3500) CD4 771 (400-1200) Naive CD4 225 CD8 531 (200-1200) Naive CD8 305 CD3CD25 10% CD3DR 16% NK 59 (70-1200) CD19 296 (200-600)	14 y IgG 10.9 (3.8-15.2) IgA 0.30 (0.64-2.58) IgM 0.76 (0.43-1.9) Preimmunoglobulin therapy Pre-RTX	1.5 y ● Autoimmune pancytopenia ● Recurrent abdominal pain ● Arthritis	Father: Immune dysregulation Cytopenias Lymphoma
2/M	c.529T>G p.Y177D	13 y CD3 934 (800-3500) CD4 436 (400-1200) Naive CD4 nil CD8 371 (200-1200) Naive CD8 nil CD3CD25 11% CD3DR 38% NK 140 (70-1200) CD19 2700 (200-600)	13 y IgG 13.5 (3.8-15.2) IgA 0.86 (0.64-2.97) IgM 1.06 (0.43-1.9) Preimmunoglobulin therapy Pre-RTX	10 y ● ITP and autoimmune neutropenia ● Reactive lymphoid hyperplasia—lymph nodes, lung, frontal lobe brain	Nil
3/F	c.437G>T p.G146V	7 y CD3 738 (800-3500) CD4 284 (400-1200) Naive CD4 nil CD8 339 (200-1200) Naive CD8 nil CD3CD25 15% CD3DR 31% NK 160 (70-1200) CD19 0 (200-600)	7 y IgG 3.54 (3.8-15.2) IgA 0.41 (0.64-2.58) IgM 0.21 (0.43-1.9) Preimmunoglobulin therapy No RTX	5 y ● Autoimmune cytopenias ● Enteropathy ● Bronchiectasis	Mother: Enteropathy Evan's syndrome Brother: Patient no. 4
4/M	c.437G>T p.G146V	16 y CD3 190 (622-2402) CD4 124 (24-406) CD8 49 (500-1500) NK 11 (109-897) CD19 9 (120-645)	16 y IgG 4.92 (6.0-16.0) IgA 1.33 (0.8-2.8) IgM 0.24 (0.5-2.0) Preimmunoglobulin therapy No RTX	16 y ● Enteropathy ● Hodgkin disease (mixed cellularity) treated with Euronet PHL-C1 Hodgkin lymphoma 2007 protocol, received 3 courses of ABVD	Mother: Enteropathy Evan's syndrome Sister: Patient no. 3
5/M	c.410C>G p.P137R	18 y CD3 2842 (690-2540) CD4 2350 (410-1590) Naive CD4 682 CD8 492 (190-1140) Naive CD8 455 CD3CD25 21% CD3DR 15% NK 114 (90-590) CD19 0 (90-660)	18 y IgG 8.84 (5.8-15.4) IgA 0.62 (0.64-2.07) IgM 1.49 (0.24-1.9) On immunoglobulin therapy Post-RTX	2 y ● Autoimmune cytopenias ● Enteropathy—PN dependent for 5 y ● 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 y 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 y IgG 7.94 (6.0-15.8) IgA 0.40 (0.38-2.00) IgM 0.61 (0.35-2.52) Preimmunoglobulin therapy Pre-RTX	7 y ● 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

(Continued)

TABLE I. (Continued)

Patient/ sex	Heterozygous change in CTLA-4	Lymphocyte subsets* (cells/ μ L) (normal range)	Immunoglobulins (g/L) (normal range)	Age at onset (y) Clinical features	Family history
7/F	c.567+6T>G p.D153Afs*21 (Splicing)	28 y CD3 622 (700-2100) CD4 496 (300-1400) CD8 112 (200-900) CD3DR 11% NK 56 (0-771) CD19 0 (100-500)	28 y IgG 0.93 (5.4-16.8) IgA 0.68 (0.74-2.61) IgM 1.51 (0.40-1.95) Preimmunoglobulin therapy Pre-RTX	1-2 y ● ITP & autoimmune hemolytic anemia ● Enteropathy/lymphocytic colitis ● Hypocalcemia, vitamin D deficiency, osteoporosis ● Interstitial lung disease ● Juvenile rheumatoid arthritis ● Eczema	Father: ITP Sister: Patient no. 8
8/F	c.567+6T>G p.D153Afs*21 (Splicing)	26 y CD3 648 (700-2100) CD4 464 (300-1400) Naive CD4 33 CD8 160 (200-900) Naive CD8 54 CD3DR 23% NK 48 (0-771) CD19 88 (100-500)	26 y IgG 4.9 (5.4-16.8) IgA 0.27 (0.74-2.61) IgM 0.84 (0.40-1.95) Preimmunoglobulin therapy Pre-RTX	23 y ● 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 no. 7

ABVD, Adriamycin (Doxorubicin), bleomycin, vinblastine, dacarbazine; *naive CD4*, CD3⁺CD4⁺CD27⁺CD45RA⁺; *naive CD8*, CD3+CD4–CD27+CD45RA+; *Euronet PHL-C1*, Euronet pediatric Hodgkin lymphoma-C1; *F*, female; *IDDM*, insulin-dependent diabetes mellitus; *ITP*, idiopathic thrombocytopenic purpura; *M*, male; *NK*, natural killer; *PN*, parenteral nutrition; *RTX*, rituximab.

*Lymphocyte subset results at first visit to specialized immunology center.

TABLE II. Variants

Patient/ sex	Heterozygous change in CTLA-4	Known mutation*	SIFT ⁴ prediction	PROVEAN ⁵ prediction	MutationTaster ⁶ prediction	PolyPhen-2 ⁷ prediction	PON-P2 ⁸	CADD ⁹ 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	NA	NA	NA	NA	NA	NA	Transmembrane and intracellular domains

F, Female; *M*, male; *NA*, not applicable due to large deletion/frameshift created by aberrant mRNA splicing of exon 4 to exon 2 (exon 3 is skipped).

*According to ESP6500, cg69, dbSNP, 1000G, and ExAC databases.

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 the 8 patients had complete resolution of severe enteropathy and cytopenias after HSCT; however, diabetes is irreversible, highlighting the importance of early recognition and treatment. Improved outcome after HSCT for autoimmune diseases¹⁰ and for children with other nonmalignant disorders after reduced intensity conditioning^{11,12} 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.¹³ These were tried with probable benefit in the only patient to receive a molecular diagnosis before HSCT, but did not alter the indication for transplant, which was his non-Hodgkin lymphoma. *CTLA4*

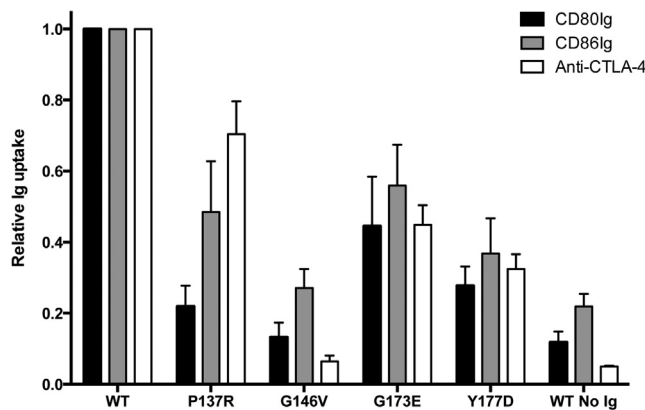


FIG 1. Mutations in CTLA-4 affect ligand uptake. Chinese hamster ovary cells expressing WT or mutant forms of CTLA-4 were cultured in the presence of CD80-immunoglobulin, CD86-immunoglobulin, or an anti-CTLA-4 antibody. Cells were analyzed for their ability to uptake ligand or antibody at 37°C relative to total cellular CTLA-4 expression. Each mutant was then normalized to CTLA-4 WT-expressing cells. *Ig*, Immunoglobulin; *WT*, wild-type.

haploinsufficiency shows a variable phenotype, and further studies are needed to guide treatment selection including which patients could benefit from *CTLA4*-ligand-targeted immunomodulation versus HSCT, optimal timing of HSCT, and long-term outcome post-HSCT.

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TABLE III. Transplant characteristics

Patient/ sex	Age at HSCT (y)	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 y
2/M	15	2008	Alem, Flu, Mel	PBSC 10/10	MMF/CSP	Acute grade IV gut	100%	Died 4 mo (GvHD)
3/F	10	2005	Alem, Flu, Mel	BM 10/10	CSP	None Off immune suppression	100%	Alive and well 10.2 y
4/M	17	2015	Alem, Flu, Treo, Thio	BM 10/10	MMF/CSP	Flare of autoimmune colitis D+2 to +10. Treated with methylprednisolone and belatacept. Remains on CSP alone	100%	Alive and well, discharged from hospital 3.5 mo
5/M	20	2008	Alem, Flu, Mel	PBSC 10/10	MMF/CSP	Acute grade II skin resolved Immune suppression stopped	100%	Died 2.5 y (DKA)
6/M	17	2013	Flu, TBI	PBSC 10/10	MMF/CSP	Acute grade III skin and gut resolved Chronic oral and ocular GvHD. Continues on sirolimus and physiologic prednisone	CD3+ 100% CD19+ 100% CD56+ 100% CD33+ 100%	Alive and well 2.0 y
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 y
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 mo

Alem, Alemtuzumab total dose 1.0 mg/kg; BM, bone marrow; CSP, cyclosporine; DKA, diabetic ketoacidosis; F, female; Flu, fludarabine total dose 150 mg/m²; M, male; Mel, melphalan total dose 140 mg/m²; MTX, methotrexate; PBSC, peripheral blood stem cell; rATG, rabbit antithymocyte globulin total dose 6.0 mg/kg; TAC, tacrolimus; TBI, total body irradiation total dose 4 Gy; Thio, thiotepa total dose 10 mg/kg; Treo, treosulfan total dose 42 g/m².

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METHODS

Sequence analysis of the CTLA4 cDNA

To evaluate mRNA splicing in patients 7 and 8, mRNA was isolated from PBMCs using the Aurum Total RNA Mini Kit (Bio-Rad, Hercules, Calif). cDNA was prepared using the Omniscript RT Kit (Qiagen, Hilden, Germany). The *CTLA4* cDNA was amplified by PCR using the following primers: Forward 5'-GTCCTTGATTCTGTGTGGGTTC-3', Reverse 5'-GGGCTGTGCCATTCCTAAC-3'. The amplified cDNA was sequenced bidirectionally by dye-terminator chemistry using BigDye v3.1 (Applied Biosystems, Carlsbad, Calif).

CTLA-4 ligand uptake assay

To determine ligand uptake, Chinese hamster ovary cells expressing wild-type or mutant forms of CTLA-4 were cultured in Dulbecco modified

Eagle medium (Invitrogen, Carlsbad, Calif) complete culture media containing 1 μ g/mL CD80-immunoglobulin (R&D Systems, Minneapolis, Minn), CD86-immunoglobulin (R&D Systems), or N-terminal humanized anti-CTLA-4 mAb for 90 minutes at 37°C. Cells were then fixed in 4% paraformaldehyde and permeabilized in 0.1% PBS-saponin solution. Cells were stained with directly conjugated C-terminal anti-CTLA-4 antibody (C-19, Santa Cruz Biotechnology, Dallas, Tex; conjugated to Alexa647) to control for expression level and with rabbit antihuman IgG-phycoerythrin (SouthernBiotech, Birmingham, Ala) to detect uptake of fusion protein. After washes, all stained cells were analyzed on a BD LSR II flow cytometer using BD FACSDiva Software (BD Biosciences, Franklin Lakes, NJ). Data analysis was performed using FlowJo software (TreeStar, Portland, Ore).