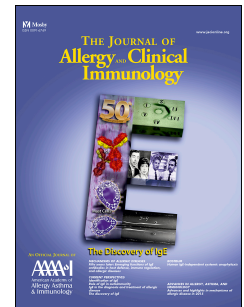


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100% survival after transplantation of 34 Wiskott Aldrich Syndrome patients over 20 years

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To the editor

Outcomes following haematopoietic stem cell transplantation (HSCT) for Wiskott Aldrich syndrome (WAS) have improved over time. Standard myeloablative regimens based on Busulphan (Bu) and Cyclophosphamide (Cyc) conditioning support high levels of donor engraftment, albeit with frequent complications (1-3). We report 100% overall survival in consecutive WAS patients who underwent a variety of transplant procedures at a single centre, including alternative graft sources and reduced conditioning regimens, between 1996-2016. 34 boys received 36 transplants comprising 7 matched sibling donor (MSD) and 29 alternative donor sources. Twenty-one patients had a pre-existing WAS score ≥ 3 , and 16 had a score of 5, indicating autoimmunity or malignancy. Pathogenic WAS gene mutations were confirmed in 25/33 boys and deletions were detected in 8/33; WASp expression was absent in 28/32 patients. Pre-transplant severe infections including CMV pneumonitis (P13, P14), EBV-lymphoproliferative disease (P33), cryptosporidium cholangiopathy (P33), fungal pneumonia (P2, P7, P13) were recorded in 12/34 patients. Pre-transplant autoimmunity was present in 15 patients (44%) and included cytopenias (n=9), vasculitis and/or arthritis (n=2) and autoimmune thrombocytopenic purpura (n=4). All these patients required systemic steroid therapy and 9/15 also received Rituximab; pre-transplant splenectomy was performed in 9 patients for control of autoimmune hemolytic anemia $\pm$ autoimmune thrombocytopenia (P2, P4, P24) or for refractory thrombocytopenia with uncontrolled bleeding episodes (n=6; P8, P19, P26, P29, P32, P33). One patient (P13) had precursor B-ALL and was treated under UKALL 2011 regimen B and C before proceeding to HSCT. Median time from diagnosis to transplant was 27.1 m (range: 4-162m) (table 1).

Twenty-six (76%) patients underwent transplantation before 2 years of age. P33 underwent transplantation after pre-implantation genetic diagnosis (PGD) and selection of a HLA-matched 'savior' sibling. Reduced intensity conditioning (RIC) incorporated Treosulphan (Treo) 42g/m² (n=17), Melphalan (Mel) 140mg/m² (n=7), reduced dose Bu (AUC =55-65 mg/l/hr) (n=2) or Cyc 120 mg/Kg with Methylprednisolone 12mg/kg (n=1) in 27 transplants. The remaining transplants included both Bu 16mg/kg and Cyc 200mg/kg (n=7) or Treo 42g/m²/Flu 150mg/m²/Thiotepa (TT) 10mg/kg (n=2). Serotherapy was used in 80% of the procedures comprising either Alemtuzumab (0.3-1mg/kg (n=25) or rabbit anti-thymocyte globulin (rATG) (n=5; 7.5-25mg/kg). No serotherapy was used in 3 mismatched unrelated donor cord transplants (4). Patients received a median dose of 9.8×10^6 /kg CD34⁺ cells (0.86-62x10⁶cells/kg). Post-transplant prophylaxis against GvHD in 34/36 transplants comprised Cyclosporine A alone or in combination with Methylprednisolone, Mycophenolate mofetil (MMF) or Methotrexate (MTX). Two recipients of haploidentical transplants received no prophylaxis (P14, P28). All patients received prophylaxis with intravenous immunoglobulin (IVIG).

Overall survival at a median follow-up of 63m (range: 6m-172m) was 100%. Viral infection/reactivation was recorded in 15 patients; all within the first 100 days post-transplant, apart from P21 who had a disseminated chicken pox after 18 months (Table 1 and supplementary

file). Of note, CMV viraemia was recorded in 8 cases, including one case of CMV enteritis and five cases of CMV retinitis, all after alternative donor transplants. Ganciclovir/Foscarnet therapy was followed by CMV specific T cell infusions in two boys. Acute GvHD grade II-IV occurred in 9 patients (26%), and these were also all recipients of alternative donor grafts. P32 developed chronic GvHD of the skin and required prolonged steroid therapy. By a median of 4m post-transplant, 7 boys (20%) exhibited immune phenomena (cytopenias (n=5), vasculitis (n=1) and inflammatory gut granulomas (n=1)). Five had suffered pre-transplant autoimmunity and while levels of post-transplant chimerism were not found to be associated with immune dysregulation, ongoing antibody effects from microchimerism of recipient plasma cells cannot be excluded (Figure E1 online repository). Longer-term complications included hemolysis (P12), thrombocytopenia and enteritis (P34) and episodic joint swelling (P32). Two children (both received a RIC conditioning) required post-transplant splenectomy either to control bleeding (P30) or severe immune cytopenias (P12). Platelet counts rose significantly after transplant in both splenectomized ($375 \times 10^9/\text{L}$ vs $46 \times 10^9/\text{L}$) and non-splenectomized ($228 \times 10^9/\text{L}$ vs $25 \times 10^9/\text{L}$) patients. Donor myeloid engraftment $>50\%$ was associated with superior platelet recovery ($375 \times 10^9/\text{L}$) compared to ($163 \times 10^9/\text{L}$) when myeloid chimerism was $<50\%$ ($p < 0.0001$) and one subject (P34) exhibited long term thrombocytopenia below $50 \times 10^9/\text{L}$ (Figure 1). Thirty two patients (94%) engrafted after transplantation, with a median time to neutrophil engraftment of 19 days. Twin brothers (P28, P30) required serial transplant procedures to secure neutrophil engraftment and immune reconstitution (5). In all patients, initial whole blood chimerism by day 30 post-HSCT was 74-100%. At last assessment, T cell engraftment was $>70\%$ in all subjects and myeloid chimerism (CD15) was $>50\%$ in 28 patients, but was low or absent in 6 children (Table E1 online repository, figure 2). All patients achieved CD3 T cell count $>1000/\text{ul}$ by a median of 9 months post-transplant (3-48m) apart from P12 who required prolonged steroid therapy for control of Grade IV skin GvHD. Median time to CD4 recovery ($>300/\text{ul}$) and CD8 recovery ($>500/\text{ul}$) was 6 months and 12 months respectively. Eighty-five percent of the patients (n=29) ceased immunoglobulin replacement therapy at a median of 11 months post-transplant (range 5-63m) and exhibited protective tetanus vaccine responses. On the contrary, response to pneumococcal vaccine was variable with only 12 patients (41%) responding to 7 out of 13 serotypes present in the Prevenar 13 vaccine. In 3/5 patients still receiving immunoglobulin therapy at a median of 60 months, previous Rituximab therapy for EBV viraemia (P33) or recurrent hemolysis (P25 and P12) may have been contributory.

Significant complications were recorded in 12 patients in the first 2 years after transplant, including squamous cell carcinoma (P33), visual loss following CMV retinopathy (P6 and P13), chronic bronchiectasis (P12) and treatment-refractory skin warts (P22). In addition, P34 exhibited an ongoing systemic auto-inflammatory disorder which required long term interleukin-1 blockade. Post-transplant complications were more frequent, though not significantly, for patients with a WAS score of ≥ 3 vs <3 (50% vs 17%) ($p=0.06$). Graft source, patient age, conditioning, did not influence the development of post-transplant complications. Although also

non-significant, patients splenectomized pre-transplant had less reported complications than non-splenectomized patients (23% vs 43%, $p=0.32$) (Figure E2 online repository).

We advocate effective and expedient control of pre-transplant morbidities including autoimmunity, bleeding and infections. In our cohort, 11 boys were splenectomised and although this has been associated with a risk of severe infections (6) and mortality (3), safeguards included long term penicillin prophylaxis and meningococcal/ pneumococcal vaccination. In some cases we used specialized avenues such as PGD for selection of an unaffected HLA-matched sibling donor ($n=1$) and autologous lentiviral gene therapy ($n=3$, data not included in this cohort) as previously described (7). The advantages of the latter include avoiding delays associated with delays in donor searching and obviation of the risk of GvHD. In other patients without matched donors, mismatched or haploidentical transplantation was undertaken as soon as practicable after stabilization. While alternative donor sources have previously been associated with higher rates of post-transplant complications (3) and death (2), the application of reduced intensity regimens supported sustained engraftment with manageable GvHD, and this is consistent with emerging experiences from other non-malignant conditions (8). In this cohort, the 2 patients who received a TCR alpha beta/CD19 depletion haploidentical transplant had reduced toxicity but MAC conditioning to support engraftment. Balashov et al, 2018 demonstrated in a pilot study that the addition of G-CSF/Plerixafor to Treo/Flu/Mel conditioning regimen pre-transplant ensured stable full donor engraftment in WAS patients after TCR alpha beta haploidentical transplants (9). Our cohort will continue to be monitored for long-term effects, including infertility and secondary malignancy.

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197 **Legends for tables and figures:**

198 **Table 1: Patients' characteristics.**

199 **Figure 1: Platelet recovery post-transplant.**

200 **Figure2: Kinetics of donor engraftment.**

201 **Table E1: Outcome of cases with mixed chimerism.**

202 **Figure E1: Effect of donor T and myeloid engraftment and post-transplant autoimmunity.**

203 **Figure E2: Probability of clinical and immunologic complications among 34 WAS patients**
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Table 1: Patients' characteristics

No	WAS score/ WAS protein	WAS mutation	Significant Pre- transplant morbidity	Year of transplant	Age at diagnosis	Age at transplant (months)	Time from Diagnosis to transplan t	Donor/cel l source	Conditioning	GvHD Prophylaxis	Engraftment Day Post-D0 (Neutrophil [†])	GvHD (Grade)	Post-transplant complications	Outcome/ Last Follow Up (months)	Last Chimerism
P1	2/absent	Null mutation exon 4	None	2009	5m	44m	39m	MUD BM	Alemtuzumab/Flu/Treo	CSA/MMF	15	None	None	Off lg 72 m	100%
P2	5/absent	del ex 1,7,12	AIHA (Steroid/Rituximab/ Splenectomy). Fungal Pneumonia	2008	2m	33m	31m	MSD BM	Bu/Cyc	CSA/MTX	21	Grade I (skin)	None	Off lg 68 m	100%
P3Q	2/absent	27bp del exon2/intron 2.2	None	2007	4m	19m	15m	MUD PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF	12	Grade I (skin)	None	Off lg 92 m	100%
P4	5/absent	E133K	AIHA/ITP (Steroids/Rituximab/ Splenectomy) Retinal/CNS haemorrhage (frontal lobectomy)	2007	10m	21m	11m	mMUD (1A) PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF	12	None	None	Off lg 60 m Partial blindness	100%T 50%M 90%B
P5	2/absent	c.100C>T, (R34X)	None	2007	11m	32m	21m	MUD BM	Alemtuzumab/Flu/Treo	CSA	12	None	None	Off lg 98 m.	100%
P6Q	3/absent	c.177delT detectable 10% somatic reversion	None	2006	17m	47m	30m	MUD BM	Alemtuzumab/Flu/Treo	CSA/MMF	10	None	Low grade CMV urine→ No TTT Accidental discovery of old CMV retinitis (school screen;18m post- HSCT)	Off lg Poor vision. 106m	100%
P7Q	5/absent	c.150- 151insT	AIHA (steroids/IVIG/ Rituximab) Severe chicken pox, fungal lung infection, Severe RSV bronchitis	2006	2m	15m	13m	MUD BM	Alemtuzumab/Flu/Treo	CSA	14	None	Early adenoviraemia/ Pneumatoxis intestinalis. Post-transplant splenectomy	Off lg 105m	83%T 0%M
P8	2/absent	c.100C>T(R3 4X)	Refractory thrombocytopenia Pre-HSCT splenectomy	2005	birth-FH	11m	11m	MUD PBSCs	Alemtuzumab/Bu/Cyc	CSA/MMF	17	Grade II (skin/gut)	None	Off lg 105m	80%T 7%M 56%B
P9	3/Absent	R34X	None	2004	14m	22m	8m	MUD BM	Alemtuzumab/Flu/Mel	CSA/MP	12	Grade II (skin)	None	Off lg 96m	100%T 89%M 99%B
P10Q	2/absent	c.469- 473del, and ins18bp in exon 5	None	2004	36m	44m	8m	MSD BM	Alemtuzumab/Bu/Cyc	CSA/MTX	38	None	EBV LPD +MV Atypical Mycobacterium (encephalopathy)	Off lg 133m	100%
P11Q	2/absent	L126P	Severe respiratory infection (MV)	2004	4m	15m	11m	MSD BM	Alemtuzumab/Bu/Cyc	CSA	28	Grade I aGvHD	Adenoviraemia/ pneumonia	Off lg 36m	100%
P12	2/absent	IVS6+2t>c		2004	2m	24m	22m	MUD BM	Alemtuzumab/Flu/Mel	CSA	14	Grade IV skin (steroid refractory)	Adenoviral enteritis/recurren t pneumonia. Recurrent AIHA/ITP/AIN (AB) Post-transplant	On lg (low T and B counts: late effect of prolonged steroid therapy). bronchiectasi s	74%T 0%M

													splenectomy	138m	
P13	5/absent	c777+1 G>A splice site mutation	CMV pneumonitis (MV) ALL Post-chemo fungal disease. AIN (Rituximab)	2013	4m	14m	10m	TCR alpha beta/B celldeplet edhaplo PBSCs	ATG/Flu/Treo/TT	CSA	15	None	CMV viraemia D+3 followed by CMV retinitis after 2m. AIHA, ITP (AB)	Off Ig Drop of vision 21m	100%
P14	5/absent	c777+1 G>A splice site mutation	CMV pneumonitis (no MV)	2014	4m	21m	17m	TCR alpha beta/B cell depleted haplo PBSCs	ATG/Flu/Treo/TT	None	21	Grade II (skin/gut)	CMV viraemia D+7 followed by CMV retinitis after 1m. Atypical ITP	On Ig (low IgM with normal B cell counts). 19m	100%T 94%M
P15Ω	5/low	c.961C>T	AIHA/AIN (Rituximab)	2013	5m	14m	9m	MUD PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF	15	None		Off Ig 21m	100%
P16	5/absent	whole gene del	AIHA/ITP	2014	14m	19m	5m	MUD PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF	21	None		Off Ig 36m	93%T 17%M
P17 Ω	2/Absent	c687del (p.Lys230Arg fsX31)	CMV viraemia	2011	1m	11m	10m	mMUD (1B/1C) cord	Treo/Flu	CSA/MMF	45	Grade II (skin/gut)	Low grade CMV reactivation D+27 followed by detection of retinal scars at 12m post-transplant.	Off Ig 52m	100%
P18	2/low	c.37C>T (PArg13*)	CMV viraemia	2013	3m	14m	11m	MSD BM	rATG/ Bu (RIC)/Flu	CSA/MMF	30	Grade I skin	Paraflu III pneumonitis followed by CMV viraemia	Off Ig 28m	100%
P19Ω	3-4/absent	c687del (p.Lys230Arg fsX31)	CMV viraemia. severe perianal/oral thrush, severe respiratory infection(CPAP). Refractory thrombocytopenia Pre-transplant splenectomy	2012	1m	19m	18m	mMUD 8/10 cord	Treo/Flu	CSA/MMF	17	Grade II skin	CMV retinitis 2m post-transplant	Off Ig Slight drop of vision 44m	100%
P20	2/Absent	stop mutation - c.763del C	None	2013	5m	14m	11m	MUD PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF	19	Grade I skin	None	Off Ig 24m	100%
P21	2/Absent	c.121C>T (p.Arg41X)	RSV bronchitis	2012	3m	17m	14m	MUD PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF	16	None	Adenoviral reactivation Paraflu III pneumonitis Late disseminated varicella 18m post-transplant	24m	100%
P22Ω	2/Absent (FACS)	Exon10c.126 3-1266dup (p.GLY423CYS fsX73)	None	2010	2m	22m	20m	mMUD 9/10 1C cord	Treo/flu	CSA/MMF	20	Grade I skin	Chronic warts for 2 years post-transplant (resolved)	Off Ig 17m	100% T 66% M
P23	5/Low	c.143C>T[pT HR48lle]	AIHA/ITP (Rituximab)	2010	9m	23m	14m	MUD PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF/M P (0.5mg/kg)	19	None	Refractory AIHA/ITP (Rituximab)	Off Ig 63 m	100%T 98%M
P24	5/ Absent	c.100 C>T (R34X)c.100C >T (R34X)	AIHA/anti-HLA (Rituximab)/ Steroids/ Pre-transplant splenectomy	2010	Birth-FH	13m	13m	MUD PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF/M P (0.5mg/kg)	16	Grade I skin		Off Ig 60m	100%
P25Ω	3/Absent	273G>T in intron 2	Chronic periorbital inflammation (no specific organism retrieved)	2010	9m	18m	9m	MUD PBSCs	Alemtuzumab/Flu/Treo	CSA/MMF	17	Grade II (skin/gut)	Recurrent AIHA; positive cold agglutinin (Rituximab)	On Ig (delayed B cell recovery post-	100%

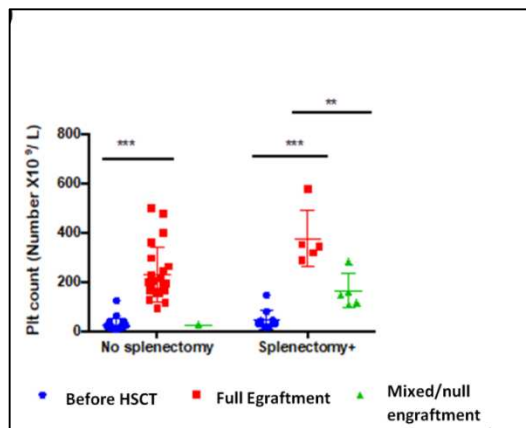
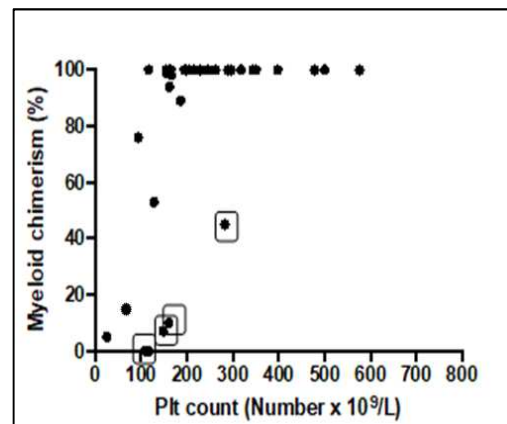
														Rituximab). 63m	
P26	3/NA	IVS7-2a>g	None Refractory thrombocytopenia Pre-transplant splenectomy	1996	NA	36m	NA	MUD BM	Bu/Cyc	CSA/MTX	30	Grade IV skin (steroid dependent)i		Off Ig 172m	>99% (WB)
P27	3/absent	(NA)	CMV viraemia Pre-TX splenectomy	1998	NA	23m	NA	MSD BM	Alemtuzumab/Bu/Cyc	CSA/MTX	28	Grade I skin	CMV enteritis Chronic perineal warts.	Off Ig 6m (No further Follow-up in London).	100%
P28	3/Absent	Q20X	Chronic otitis media Perianal abscesses Repeated cellulitis	1998	7m	1 st TX: 12m. 2 nd : 25m	5m	CD34 + selected Haplo (father) BM CD34 +selected Haplo (father) BM/PBSC s	rATG/Flu/Mel Alemtuzumab 0.6 mg/kg/Cyc 150mg/kg/methylprednis olone 12 mg/kg	CSA None	Failed engraftment D+30 22m	NA None	CMV viraemia Fungal lung	Off Ig 150m	100%
P29	5/Absent	T284C single nucleotide substitution (F84L)	Vasculitis/arthritis Refractory thrombocytopenia Pre-TX splenectomy	2001	NA	144m	NA	MUD BM	Alemtuzumab/Flu/Mel	CSA	21	Grade II skin/gut/live r	RSV pneumonitis EBV viraemia, no PTLD	Off Ig 64m	100%
P30	5/Absent	Q20X	Vasculitis	2001	3m	1st Tx:144m 2 nd Tx: 150m	141m	MSD BM (P28) Combine d MSD/ CD34+sel ected haplo(fat her) PBSCs	Alemtuzumab/Flu/Mel rATG/Bu/Cyc	CSA CSA	PB (3m) 10% donorT cells,0% donorM 14	NA Grade I skin	Vasculitis controlled by steroid therapy	Off Ig 149 m	100%
P31Ω	5/Low	c.271C>T	ITP/Vasculitis (Steroids/Rituximab/ IVIg)	2012	5m	55m	50m	MSD BM	Bu (RIC)/Flu	CSA/MTX	20	Grade I skin	None	Off Ig 36 m	100%
P32	5/Absent	T284C single nucleotide substitution (F84L)	Arthritis Recurrent Candida infection Refractory thrombocytopenia Pre-transplant splenectomy	2000	9m	108m	99m	MUD BM	Alemtuzumab /Flu/Mel	CSA/MP	23	None	Intermittent joint swelling (on/off Ibuprofen)	Off Ig 81 m	74% T 0%M
P33Ω	5/NA	G405fsX495	AI colitis with severe failure to thrive. Localised cutaneous EBV LPD of the thigh Cryptosporidium cholangiopathy Refractory thrombocytopenia Pre-transplant splenectomy Recurrent Molluscum/skin warts	2001	42m	204m	162m	MUD BM	Alemtuzumab/Flu/Mel	CSA	31	None	SCC at gastrostomy site at 3m post- Transplant. Cryptosporidium enteritis EBV viraemia (Rituximab)	On Ig 60 m (Post- Rituximab; normal B, low serum IgM).	100%

P34Ω	5/Absent 4% By flow	premature stop codon exon 10	Neonatal enteocolitis. Lymphoid hyperplasia Carrier of FMF gene Autoinflammatory phenomenon. Coomb's positive (steroids/Anakinra)	2013	4m	9m	8m	MUD PBSCs	Alemtuzumab/Treo/Flu	CSA/MMF	19	Grade I skin	Recurrent auto- inflammatory phenomenon with poor donor myeloid engraftment (DLI X3 started 6 months post- transplant). Gut malrotation with Ladd operation at 22 months post- transplant. Patchy granulomas of the gut at 24 months post-transplant (Sulfasalazine)	On Ig 44 m	
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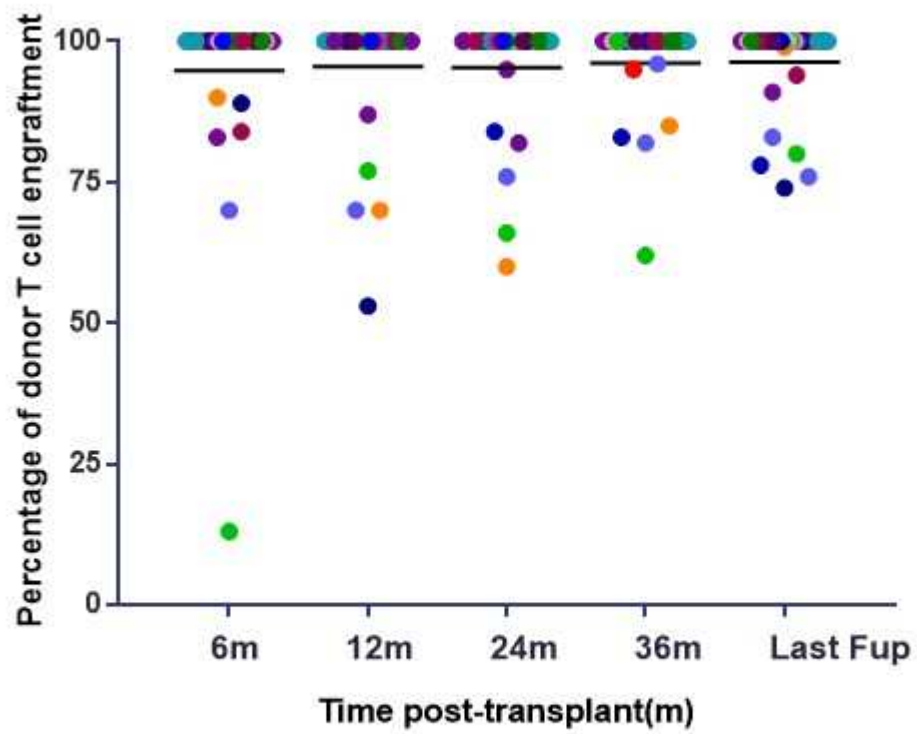
Abbreviations: Bu:Busulfan,Cyc:Cyclophosphamide ,rATG-rabbit ATG, Treo:Treosulfan, TT:Thiotepa, Mel:Melphalan, MP::methylprednisolone, BP: peripheral blood, T: T cell, M: myeloid,CMV:Cytomegalovirus, Flu:Fludarabine, GvHD- Graft versus Host Disease, , mMUD- mismatched unrelated donor, AIN:autoimmune neutropenia, AIHA::Autoimmune haemolyticanaemia, AI:autoimmune, MSD:matched sibling donor, MUD:matched unrelated donor, Haplo:haploidentical transplant, mMUD:mismatched unrelated donor, BM:bonemarrow, PBSCs:peripheral blood stem cells , MV-mechanical ventilation, LPD-lymphoproliferative disease. DLI-donor lymphocyte infusion, SCC:squamous cell carcinoma, Haplo:haploidentical, RIC: reduced intensity conditioning.FH: family history.

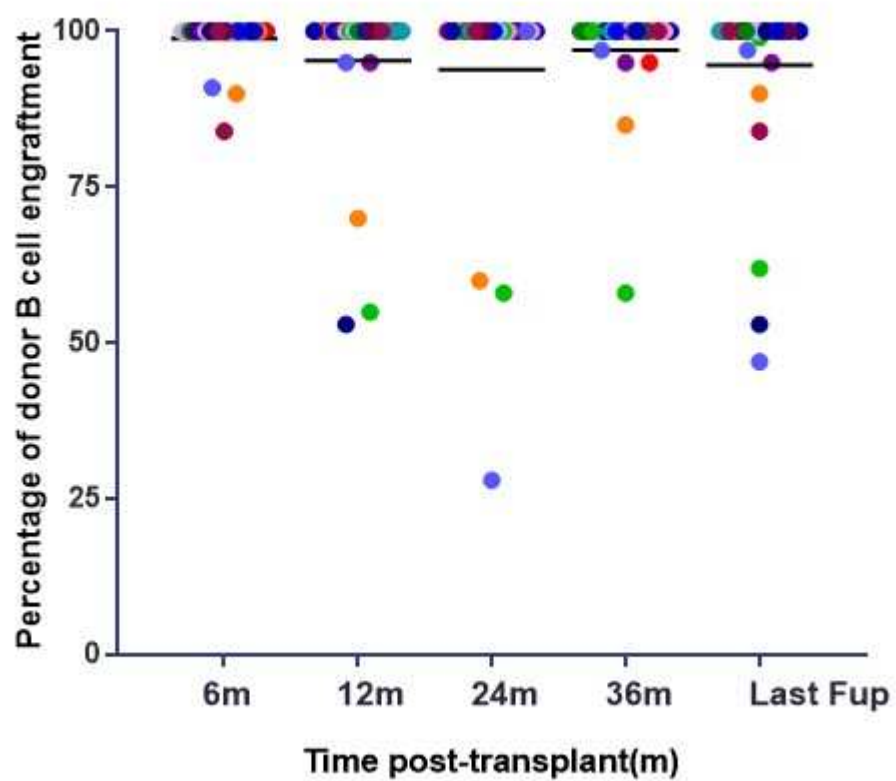
Ω Denotes patients who had novel mutations. P17 and P19 are brothers who share the same novel mutation.

† Neutrophil engraftment: ANC >0.5x10⁹/L for 3 consecutive days. P26-P28received their transplant beforethe year 2000.

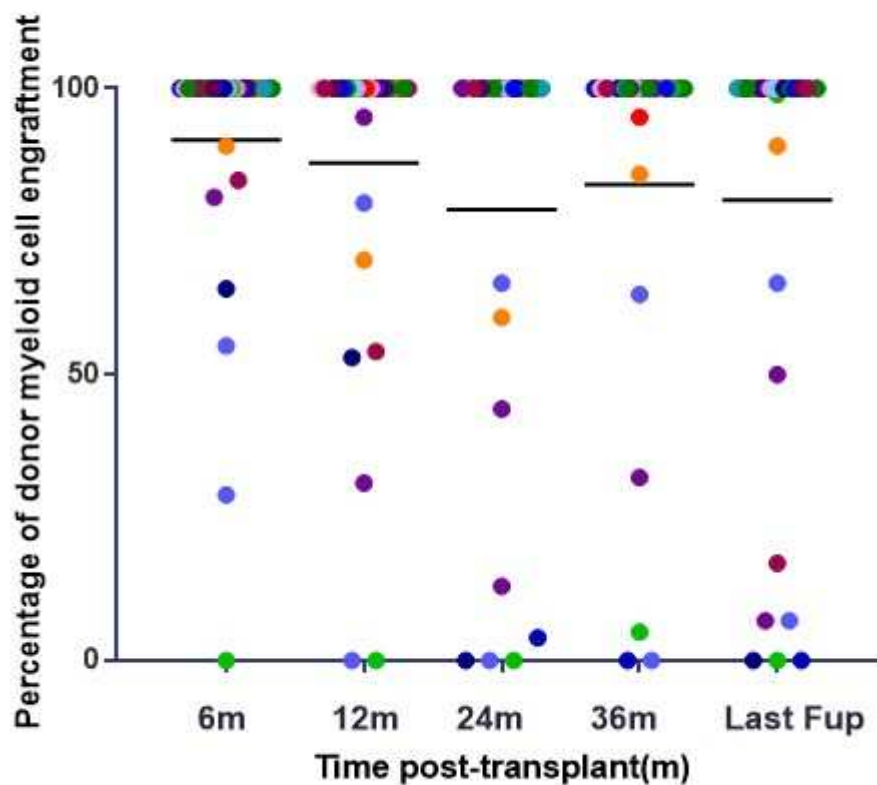
Figure 1: Platelet recovery post-transplant**1a)****1b)**

In figure 1b: Boxes represent patients who had donor myeloid engraftment below 50% and were splenectomized.





2c) Donor myeloid cell engraftment



Abbreviations: Fup: follow-up.

P8 had a drop in donor T cell engraftment to 13% at 6 months post-transplant this was followed by a sustained higher donor T cell engraftment above 70% from 1 year post-transplant till last follow-up.

Supplementary File

Methods:

Mutation analysis and WASp expression

Mutation analysis was performed by PCR-based amplification of genomic DNA using primers spanning the WASp gene. WASp expression was analyzed using western blot) or flow cytometry (FACs) (12).100µl of peripheral blood was collected in EDTA, fixed and permeabilized using Caltag fix buffer and permeability Buffers A and B according to manufactures protocol. 1 ul of anti- WASp or IgG2a isotype control (both BD Biosciences) was added, cells were incubated for 30 minutes at room temperature and then washed with PBS + 5% fetal calf serum (both Invitrogen) . Then, 1 ul biotinylated goat anti-mouse IgG2a (Southern Biotech) was added, cells were incubated for 15 minutes, washed and then 1 ul of Streptavidin-APC (BD Biosciences) was added. Cells were further incubated for 15 minutes, washed, and fixed (CellFix BD Biosciences). 10,000 lymphocyte events were acquired on a FACs Calibur and analyzed using CellQuest software (BD Biosciences). Lymphocytes were gated, and WASp expression was compared to the IgG2a control. Normal values were defined as 80% or more WASp expression above the IgG2a. Values below 5% was considered as absent WASp expression.

Disease severity:

Disease severity before transplantation was expressed as a score of 1 to 5 according to Ochs and Thrasher, 2006 and Ochs et al, 2009

References:

1. Ochs HD, Thrasher AJ. The Wiskott-Aldrich syndrome. J Allergy Clin Immunol. 2006; 117: 725-738.
2. Ochs HD, Filipovich, AH, Veys P, Cowan MJ, and Kapoor N. Wiskott-Aldrich syndrome: diagnosis, clinical and laboratory manifestations, and treatment. Biol. Blood Marrow Transplant. 2009; 15, 84–90.

Chimerism analysis:

Lineage specific chimerism was assessed using polymerase chain reaction amplification of specific polymorphic DNA sequences (short tandem repeats) in circulating lymphoid and myeloid cells.

Statistical Analysis:

Mean WAS scores and platelet counts were compared using a Student t-test. Kaplan-Meier survival curves were plotted and log-rank P values were determined for differences in rate of complications. Spearman rank correlation coefficient used to test the association between myeloid/lymphoid donor engraftment and platelet count. Graphprism 7 was used to plot the

kinetics of donor T, donor B and donor myeloid cell engraftment at different time points post-transplant. Significance was considered at $P < 0.05$.

Ethical approval:

All patients provided transplant consent according to UK paediatric BMT group regulations.

Table E1: Outcome of cases with mixed chimerism

<u>ID</u>	<u>WAS Score</u>	<u>Transplant</u>	<u>Post-HSCT complications</u>	<u>Outcome/Follow-up</u>
P7	5	MUD,BM Alemtuzumab/Flu/Treo	Post-HSCT adenoviraemia Pneumatosis intestinalis Post-HSCT splenectomy (low PLT at 6m)	Off Ig. PIT=160 x10 ⁹ /L. Donor M: 0%. 83% T 105m
P8	2 Pre-HSCT splenectomy	MUD,PBSCs Alemtuzumab/Bu/Cyc	aGVHD grade II skin/gut	Off Ig. PIT=148x10 ⁹ /L. Donor Myeloid 7%80% T 105m
P12	2	MUD,BM Alemtuzumab/Flu/Mel	Grade IV skin aGVHD Adenoviral enteritis Recurrent AIHA/ITP/AIN Post-HSCT splenectomy	On Ig therapy (low B cell number) Bronchiectasis. PIT=116x10 ⁹ /L. Donor M: 0%. 74% T 138m
P16	5	MUD, PBSCs Alemtuzumab/Flu/Treo	On/off epistaxis	Off Ig. PIT=78 X10 ⁹ /L Donor myeloid 17%, 93% T 36m

P32	5	MUD, BM	Limited cGvHD	Off Ig. PIT=109x10 ⁹ /L.
	Pre-HSCT splenectomy	Alemtuzumab/Flu/Mel		Donor M: 0%. T:74%
				60m
P34	5	MUD, PBSCs	Recurrent vasculitis/autoinflammatory rash	On Ig therapy. PIT: 25x10 ⁹ /L.
		Alemtuzumab/Flu/Treo	Patchy gut granulomas	Donor M: 7%. 91% T
				44m

Abbreviations: M: myeloid engraftment. PIT: platelets, Flu: Fludarabine, Treo: Treosulphan, Mel: Melphalan, FMF: familial Mediterranean fever, MUD: matched unrelated donor, BM: bone marrow, PBMCs: peripheral mononuclear stem cells, Ig: immunoglobulin, m: months, cGvHD: chronic GvHD.

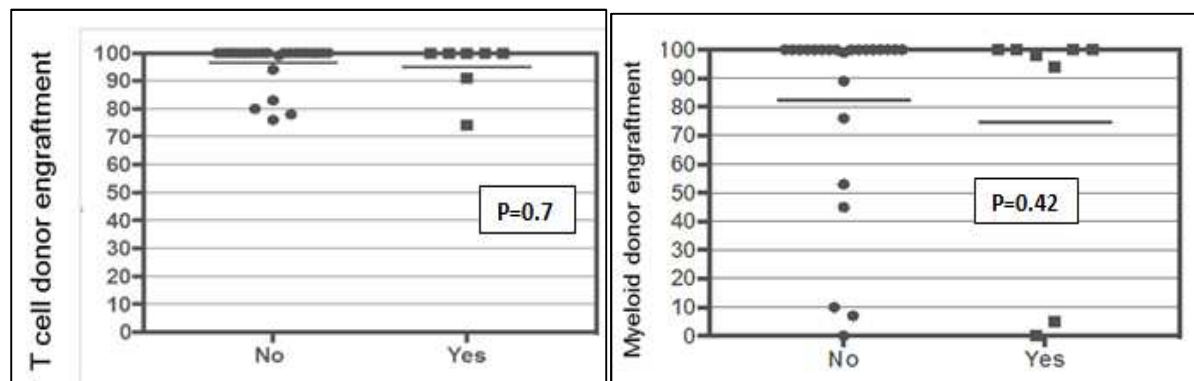
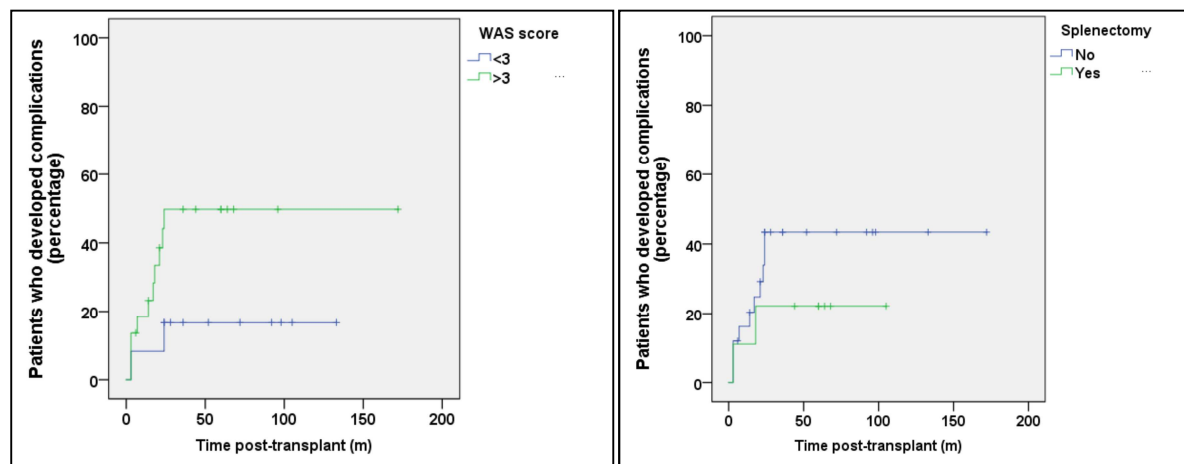
Figure E1: Effect of donor T and myeloid engraftment and post-transplant autoimmunity

Figure E2: Probability of clinical and immunologic complications among 34 WAS patients

Complications included post-transplant autoimmunity, need for a second intervention, chronic GvHD, post-transplant splenectomy, reduced vision (post-CMV retinitis), bronchiectasis and secondary malignancy.