Title: 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 34 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 ± 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/m^2 (n=17), Melphalan (Mel) 140mg/m^2 (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/m^2/Flu 150mg/m^2/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 9.8x10^6/kg $CD34^+$ cells (0.86-62x10^6cells/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 ($375x10^9$/L vs$46x10^9$/L) and non-splenectomized ($228x10^9$/L vs $25x10^9$/L) patients. Donor myeloid engraftment >50% was associated with superior platelet recovery ($375x10^9$/L) compared to ($163x10^9$/L) when myeloid chimerism was <50% ($p < 0.0001$) and one subject (P34) exhibited long term thrombocytopenia below $50x10^9$/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/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/ul) and CD8 recovery (>500/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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Legends for tables and figures:

Table 1: Patients' characteristics.
Figure 1: Platelet recovery post-transplant.
Figure2: Kinetics of donor engraftment.
Table E1: Outcome of cases with mixed chimerism.
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