Letter to the editor

First use of thymus transplantation in PAX1 deficiency

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To the Editor:

PAX1 is a paired-box transcription factor which plays an important role in multiple biological processes during embryogenesis, including in the development of the skeletal system and the thymus[1]. Mutations in *PAX1* have recently been identified in syndromic patients with skeletal malformations and immunodeficiency[2]. The patients had been diagnosed with otofaciocervical syndrome type 2 (OTFCS2), a rare, autosomal recessive disorder characterised by external ear anomalies with pathognomic pre-auricular pits and hearing impairment, facial dysmorphism and skeletal anomalies of the vertebrae and pectoral girdle. Additionally, these patients, who presented with severe infections, displayed profound T-cell lymphopaenia. Prior to having a molecular diagnosis, several OTFCS2 patients were treated with haematopoietic stem cell transplantation (HSCT) for undefined $T^-B^+NK^+$ severe combined immunodeficiency (SCID). Despite good donor engraftment, recovery of T-cell immunity failed, and patients remained T-cell lymphopaenic with the presence of memory $CD45R0^+$ T-cells transferred with the graft, but no thymic output, as evidenced by the lack of naïve $CD45RA^+CD31^+$ T-cells and non-detectable T-cell receptor excision circles (TREC) after HSCT[2]. Together with the expression pattern of *PAX1* during thymus organogenesis, this suggested a possible thymic stromal cell defect instead of a primary haematopoietic cell-intrinsic defect. A luciferase reporter assay showed that OTFCS2-causing *PAX1* mutations were associated with reduced transcription of target genes[2]. An altered transcriptional profile was confirmed in thymic epithelial progenitor cells, differentiated from patient-derived induced pluripotent stem cells, specifically for *FOXN1* and other genes known to be upregulated during thymic epithelial cell development[2]. This established congenital athymia as the underlying cause of the $T^-B^+NK^+$ SCID-like phenotype in PAX1-deficient patients.

Congenital athymia is a life-limiting condition which is most commonly associated with complete DiGeorge syndrome (cDGS)[1]. High mortality after HSCT has been reported, in particular when a matched family donor is not available, and patients are at risk of developing severe graft-versus-host disease (GVHD)[1]. In surviving patients, naïve T-cell counts remain low and the T-cell receptor (TCR) repertoire is restricted. Far superior outcomes are achieved after treatment of cDGS with thymus transplantation (TT) using cultured, postnatal thymus tissue[3,4]. Following implantation of lympho-depleted donor tissue into the quadriceps muscles, recipient-derived T-cell precursors repopulate the thymic allograft and undergo thymopoiesis, resulting in good overall survival and better quality T-cell immune reconstitution than after HSCT[3,4]. Immune reconstitution after TT is slow with definite evidence of thymopoiesis seen in peripheral blood from approximately six months after TT and T-cell counts progressively increasing during the first two years[3,4]. While T-cell counts typically remain below the normal ranges for age-matched controls, increased proportions of naïve T-cells with broad TCR

repertoires are maintained over time. TT has also been performed in a few patients who previously underwent HSCT[3]. Whilst donor and recipient are not tissue type matched when TT is performed as first-line treatment, in these cases the donated thymus tissue must be matched with the alleles of the HSCT donor that were mismatched with the recipient. More than 100 patients have been treated with TT to date, including a small number of patients with non-DGS thymic stromal cell defects, such as FOXN1-deficient Nude SCID and genetically undefined $T^-B^+NK^+$ SCID[1,3,4]. We here report the first use of TT in PAX1 deficiency. Two suspected SCID patients, P1 and P2, displayed syndromic features consistent with OTFCS and were referred to the European TT programme at Great Ormond Street Hospital (GOSH)[4] for treatment upon genetic confirmation of their defect. P1 was diagnosed after failed HSCT[2], whereas P2 was diagnosed early after being identified through newborn screening (NBS) for SCID[5].

P1 underwent HSCT for undefined $T^-B^+NK^+$ SCID during her first year of life, using a mismatched unrelated cord blood donor and reduced intensity conditioning[2]. She achieved full donor engraftment, yet reconstitution of T-cell immunity failed[2]. She developed significant complications, including gut GVHD and life-threatening autoimmune haemolytic anaemia (AIHA), which was refractory to treatment with rituximab, azathioprine and mycophenolate mofetil, but stabilised on immunosuppression with ciclosporin and high doses of steroids. *Mycobacterium chimaera* was isolated from her lungs, and she developed chronic norovirus enteropathy. Upon genetic confirmation of PAX1 deficiency, she was diagnosed with congenital athymia and was referred for TT at GOSH at four years old. Steroid dependency made her initially ineligible for TT, together with steroid-induced muscle atrophy and severe failure to thrive. We initiated treatment with sirolimus and successfully weaned her off steroids. Following sufficient muscle gain and upon collection of a partially tissue-type matched thymus, she underwent TT at five years old after receiving antithymocyte globulin (ATG) serotherapy (Genzyme 2mg/kg once daily, 3 doses). Two months after TT, she acutely deteriorated, suffering excessive inflammation of unknown aetiology, including severe AIHA with cold agglutinins, immune-mediated thrombocytopaenia and pulmonary inflammatory disease with pulmonary haemorrhage, necessitating intensive care support. She stabilised after treatment with high dose steroids, rituximab and several cycles of plasma exchange. Shortly thereafter, she required emergency spinal surgery for cord decompression. Five months after TT, having remained stable on sirolimus after successful tapering of steroids to physiological hydrocortisone dose, biopsies were taken at the allograft implantation site. Histopathological assessment showed viable thymic epithelium, but no recovery of thymopoiesis (Figure 1A). She died of line-associated sepsis prior to showing any signs of beginning immune reconstitution 17 months post-TT.

P2 was identified by NBS with absent TRECs[5] and initial immunophenotyping was consistent with $T^-B^+NK^+$ SCID. Through rapid whole-exome-sequencing, homozygous *PAX1* mutations were identified. A diagnosis of OTFCS2 with suspected congenital athymia was made and P2 was referred to GOSH in consideration for TT. While profoundly T-cell lymphopaenic ($CD3^+$ T-cells: 170/µL, $CD4^+$ T-cells: 160/µL, naïve $CD4^+CD45RA^+CD27^+$ T-cells: 50/µL), P2 did not meet the inclusion criteria for treatment with TT[1] ($CD3^+$ T-cells < 50/ µL and absent naïve T-cells; or naïve T-cells <5% of T-cells in patients with oligoclonal T-cell expansions), even though TRECs were persistently undetectable in sorted T-cells (Figure 1B). An initial "watch and wait" approach was agreed. P2 remained stable with supportive care, including antimicrobial prophylaxis and immunoglobulin replacement therapy (IgRT). He did not acquire any infections and did not develop any atypical features. As the lymphopaenia did not improve over time (Figure 1B), TT was performed after ATG serotherapy (Genzyme 2mg/kg once daily, 3 doses). Cyclosporine A was started as post-transplant immunosuppression. A routine biopsy procedure was performed four months after TT. Histopathological assessment confirmed the presence of well-formed thymic tissue with evidence of established thymopoiesis (Figure 1A). At six months after TT, thymic output was demonstrated by increasing T-cell counts and TREC levels (Figure 1B). Ciclosporin and anti-microbial prophylaxis were discontinued. At nine months after TT, P2 presented with a mild petechial rash and was found to be thrombocytopenic (nadir: 2000/µL). Platelet count increased after administration of high dose intravenous immunoglobulin (IVIg). Thrombocytopaenia recurred at 10, 15 and 16 months post-TT, always resolving with IVIg administration. Similar to what has been observed in cDGS patients[3,4], T-cell counts have continued to increase over time, and at last assessment, two years post-TT, P2 had 890/µL $CD3^+$ and 630/µL $CD4^+$ T-cells with 60% naïve $CD4^+$ T-cells. TCR repertoire diversity was significantly improved with a near-normal spectratype analysis showing 16 out of 24 Vβ-families with a Gaussian distribution compared to only 8 pre-TT (- spectratype classification in the GOSH reference laboratory based on the number of Gaussian Vβ-families: normal 17-24, almost normal 11-16, abnormal 0-10). P2 is now 28 months post-TT. He is thriving and has started nursery. IgRT will be discontinued shortly, and he will then undergo childhood immunisation.

In summary, we treated two PAX1-deficient patients with TT. Both patients displayed syndromic features consistent with OTFCS and a $T^-B^+NK^+$ SCID-like phenotype. Congenital athymia was confirmed upon identification of PAX1 deficiency. While one patient was diagnosed shortly after birth in the context of a recently started NBS programme[5], the other patient had previously been treated with HSCT but failed to recover functional T-cell immunity. Second-line "rescue" TT procedures have been performed[1,3], but our experience highlights that this is not always feasible in patients like P1 who develop severe complications after HSCT. Immune reconstitution after TT is slow and clinical stability is crucial for thymopoiesis to successfully establish in the allograft. Inflammatory complications,

including life-threatening immune reconstitution inflammatory syndrome (IRIS), have been observed after TT[1,4], often driven by infections. In P1, relapsing treatment-refractory AIHA in the context of an overall IRIS-like picture required steroid administration shortly after TT, thus inhibiting thymopoiesis. In contrast, P2 benefitted from timely diagnosis and referral for TT as first-line treatment. Autoimmune manifestations, including transient cytopaenias, are relatively common after TT[1,3,4]. Except for mild thrombocytopaenia, which did not recur in the last year, P2 did not have any complications. He remained clinically stable and achieved T-cell immune reconstitution to similar levels reported after TT in cDGS[3,4]. In conclusion, the results in P2 confirm that TT can correct the severe T-cell lymphopenia in athymia associated with PAX1 deficiency.

Figure 1: Thymopoiesis and T-cell immune reconstitution after thymus transplantation:

A. Immunohistochemistry assessment of thymic allografts: At 5 months post-TT in P1: Haematoxylin and eosin (H&E) staining of sections showing two areas of skeletal muscle containing thymic tissue. Immunostaining with cytokeratin 14 (CK14) indicates the epithelial areas. The T-cell compartment is demonstrated by CD3. The lack of detection of CD1a and Ki67 illustrates the absence of thymopoiesis. **At 4 months post-TT in P2:** H&E staining shows one area of thymic tissue surrounded by skeletal muscle. CK14 and CD3 respectively indicate the epithelial and the T-cell compartments. The strong CD1a signal confirms well-established thymopoiesis. Magnification, x4 or x10, is indicated.

B. T-cell immune reconstitution after TT in P2: T-cell and T-cell subset numbers ($x10^6$/L, left Y-axis) and TREC levels (/10^6 T-cells, right Y-axis) over time (in months, X-axis), with time of TT = 0. Naïve $CD4^+$ T-cells express $CD45RA^+CD27^+$ and represent 8, 32, 51, 57, 64 and 60% of $CD4^+$ T-cells respectively at 4, 6, 9, 13, 20 and 25 months post-TT.

Declarations:

Ethical Approval: This work was completed under research ethics approval from London Bloomsbury Research Ethics committee (07/Q0508/43).

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Conflicts of Interest: The authors declare no conflicts of interest.

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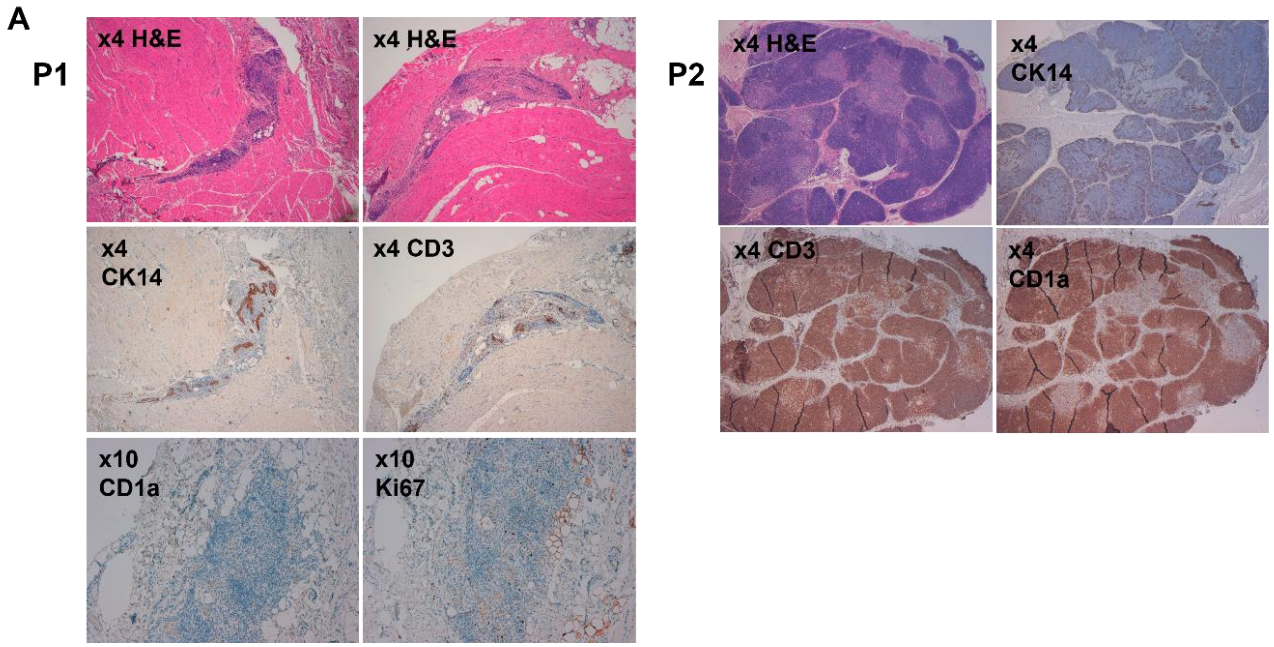
Figure 1: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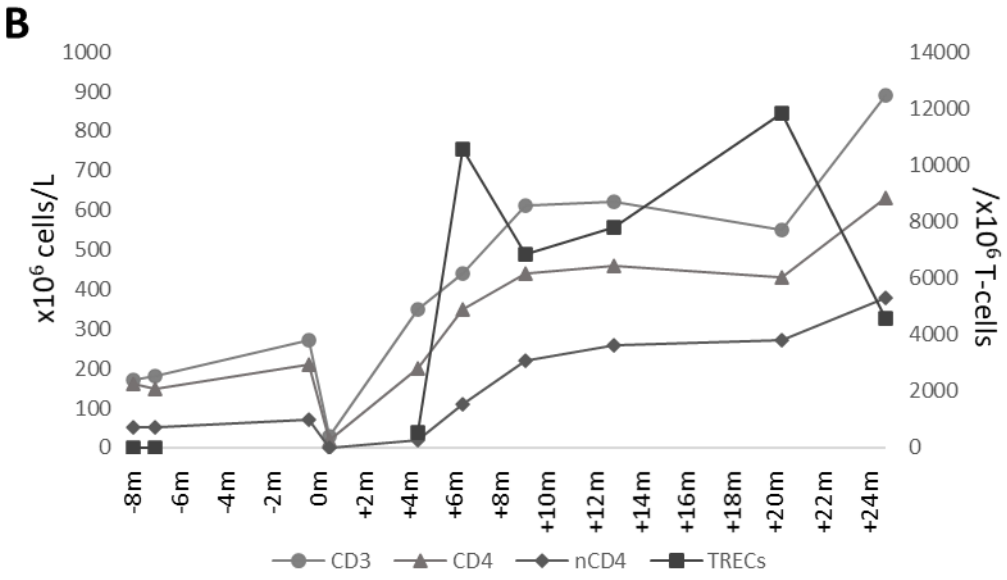
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