Article:
Al-Hakim, A, Poulter, JA, Mahmoud, D et al. (2022) Allogeneic hematopoietic stem cell transplantation for VEXAS syndrome - UK experience. British Journal of Haematology, 199 (5). pp. 777-781. ISSN: 0007-1048

https://doi.org/10.1111/bjh.18488

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY
British Society for Haematology

Allogeneic hematopoietic stem cell transplantation for VEXAS syndrome - UK experience

Journal:	*British Journal of Haematology*
Manuscript ID	BJH-2022-01580.R1
Manuscript Type:	Letter to the Editor
Date Submitted by the Author:	13-Sep-2022
Complete List of Authors:	Al-hakim, Adam; Leeds Teaching Hospitals NHS Trust, Clinical Immunology and Allergy Poulter , James; University of Leeds, Leeds Institute of Medical Research Mahmoud, Dina; Leeds Teaching Hospitals NHS Trust, Clinical Immunology and Allergy Rose, Ailsa; University of Leeds, Leeds Institute of Medical Research Elcombe, Suzzane; Newcastle Upon Tyne Hospitals NHS Foundation Trust, Department of Immunology Lachmann, Helen; University College London, National Amyloidosis Centre Cargo, Catherine; St James's University Hospital, Haematology Duncan, Christopher; Newcastle University, Clinical and Translational Research Institute Bishton, Mark; Nottingham University Hospitals, Haematology Department Bigley, Venetia; Newcastle University, Institute of Cellular Medicine Khan, Anjum; Leeds Teaching Hospitals NHS Trust, Haematology; Savic, Sinisa ; University of Leeds, Leeds Institute of rheumatic and musculoskeletal medicine
Key Words:	CMML, MDS, VEXAS Syndrome, HSCT

Allogeneic hematopoietic stem cell transplantation for VEXAS syndrome - UK experience

Adam Al-Hakim[1*], James A Poulter[2*], Dina Mahmoud[1], Ailsa MS Rose[1], Suzanne Elcombe[3], Helen Lachmann[4], Catherine Cargo[5], Christopher JA Duncan[6,7], Mark Bishton[8,9], Venetia Bigley[6,7], Anjum Khan[10], Sinisa Savic[2,11,12**]

[1]Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds, United Kingdom
[2]Leeds Institute of Medical Research, University of Leeds, Leeds, United Kingdom
[3]Department of Immunology, Newcastle upon Tyne NHS Trust, Newcastle upon Tyne, United Kingdom.
[4] National Amyloidosis Centre Royal Free London NHS Foundation Trust & Division of Medicine University, College London, London United Kingdom.
[5] Haematological Malignancy Diagnostic Service, Leeds Cancer Centre, St James's University Hospital, Leeds, United Kingdom.
[6] Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, United Kingdom
[7] Northern Centre for Bone Marrow Transplantation, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom
[8] Department of haematology, Nottingham university hospitals NHS Trust, United Kingdom
[9] Translational Medical Sciences, University of Nottingham, United Kingdom
[10] Department of Haematology, St James's University Hospital, Leeds, United Kingdom
[11] Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, United Kingdom.
[12] NIHR Leeds Biomedical Research Centre, Leeds Teaching Hospitals NHS Trust, Chapel Allerton Hospital, Leeds, United Kingdom.

* joint first authors

** corresponding author: Sinisa Savic, Leeds Institute of Rheumatic and Musculoskeletal Medicine, Clinical Science Building, Beckett Street, Leeds, LS9 7TF, United Kingdom. S.Savic@leeds.ac.uk

KEYWORDS

VEXAS syndrome
Allogeneic hematopoietic stem cell transplantation
Myelodysplastic syndrome
Chronic myelomonocytic leukaemia

VEXAS (Vacuoles, E1-ligase, X-linked, Auto-inflammatory, Somatic) syndrome, is an acquired, progressive systemic auto-inflammatory disorder with overlapping rheumatological and haematological features(1, 2). It is caused by myeloid-restricted somatic mutations in *UBA1*, the gene which encodes E1 ubiquitin ligase. VEXAS is associated with significant morbidity and reduced life expectancy. Current treatment options are limited to symptomatic control, with corticosteroids being universally effective and JAK inhibitors showing promising results. Allogeneic hematopoietic stem cell transplantation (HSCT) has been proposed as potentially curative option in selected patients. The experience of HSCT in VEXAS is limited to few case reports(3, 4). Here, we describe four additional cases of VEXAS in whom HSCT was performed. We also report on the incidence of undiagnosed VEXAS in patients with MDS and chronic myelomonocytic leukaemia (CMML) who underwent BMT for haematological indications.

Clinical features of four VEXAS patients who underwent HSCT (mean follow up 13.5 months; range 0.4-40 months) are summarised in Table 1 (detailed clinical vignettes provided in supplements). Three patients P1, P2 and P3 were diagnosed with VEXAS prior to transplantation, with the transplant indication being severe and poorly controlled inflammatory illness. Patient 4 was identified retrospectively, after undergoing HSCT for what was thought to be MDS. Patients 1 and 3 were also included in a recent cohort study, although this did not report on their outcomes following HSCT (1, 2).

The average age of disease onset for these patients was 59 years (range 49-64), which is younger than expected for VEXAS, as described in two recent, large cohort studies which demonstrated an average age of 66 and 67 years (2, 5). Patients 1 and 2 had a UBA1 p.Met41Val variant recently found to be associated with a worse outcome (2). Patients 1, 3 and 4 were also transfusion dependent, a feature associated with more severe disease (2). All three patients transplanted for an inflammatory indication had previously failed multiple DMARD's, whilst patient 4 had only received corticosteroids. Outcomes following HSCT were variable. Patient 1 died from sepsis and multiorgan failure in the early post-transplant period (day +11). Patient 2 survived the transplant with good engraftment and full donor chimerism, but recovery was complicated by hemophagocytic lymphohistiocytosis, aseptic encephalitis and Epstein Barr Virus reactivation within 2 months of transplant, and subsequent extensive chronic graft versus host disease requiring systemic treatment, and recurrent bacterial

infections, the sequelae of which have resulted in a Karnofsky performance score of 40. Patient 3 achieved disease control with no molecular evidence of VEXAS, however developed severe post-transplant myelitis resulting in paraplegia, urinary and faecal incontinence, and passed away 11 months post-transplant from infectious complications. Patient 4 is the only one who at the time of writing (40 months post-transplant) remains alive and in good health.

Previous studies have reported VEXAS patients being transplanted in the absence of a prior diagnosis, including one of the patients we report here (P4). We therefore speculated about the incidence of undiagnosed VEXAS cases amongst patients with myeloid disorders who had undergone HSCT. To this end, we studied patients from a single centre, with a diagnosis of MDS or CMML, who were transplanted in the last 10 years, for whom historical pre-HSCT bone marrow DNA samples were available.

We identified 44 patients in total, 34 MDS and 10 CMML. Historical, pre-HSCT bone marrow DNA samples were available for all patients. We performed deep sequencing of the whole *UBA1* gene (for method please see supplements). To date, all pathogenic mutations associated with VEXAS have been confined to exon 3, with Methionine-41 (Met41) being a particularly important site (1, 2). No patients demonstrated any of the known pathogenic mutations in *UBA1*, but 4 patients were found to have rare variants outside exon 3 that have not been previously reported and have unknown functional consequences. The variants identified (and variant allele frequencies) were: c.2554-1G>T (0.5), c.2554-8C>T (0.4), c.2374C>T:p.Gln792Ter (0.14) and c.1321G>A:p.Glu441Ter (0.8). However, none of the patients carrying these variants had any inflammatory symptoms. Three had a diagnosis of MDS, whilst the patient with c.1321G>A, p.Glu441Ter variant was diagnosed with CMML.

A failure to find any typical cases of VEXAS across the entire transplanted cohort was likely predictable. Very few patients had any inflammatory symptoms or disease manifestations classically associated with VEXAS (Table 2). The MDS features were also different. Ferrada et al. reported that MDS was diagnosed in 31% of the total cohort, who were retrospectively found to have VEXAS (2). On review, these patients typically had low risk disease, have a much lower frequency of MDS associated gene mutations and show no evidence of progression to acute myeloid leukaemia (2, 6) consistent with UBA1 mutations being the main driver of cytopenias and bone marrow morphological changes not a distinct MDS. The other haematological manifestations which are almost universal in VEXAS, such as

macrocytic anaemia (close to 100%), were seen in only 32% of patients in our cohort, although other types of anaemia (e.g. microcytic) were more common.

We report variable outcomes of HSCT in our VEXAS case series with 50% (2/4) overall survival but only 25% event free survival at current follow up. There are several potential explanations for this. Patient 1 underwent HSCT as a 'treatment of last resort' for a rapidly progressive, recalcitrant inflammatory disorder. The risk of poor outcome in such circumstances is invariably high, as reflected in his HCT-CI score of 6. Patient 2 was transplanted three years after the onset of symptoms and had received continuous immunosuppressive therapy including corticosteroids during this time. Patient 3 was transplanted almost 10 years after disease onset, during which time he had already developed several complications including renal impairment and steroid toxicity. In addition, he was exposed to several immunosuppressive therapies and had experienced several serious infections. It is unclear to what degree post-transplant complications were influenced by these factors. Nevertheless, the purported benefit of reduced corticosteroid use as a result of better control of his inflammatory illness was significantly offset by his pre-existing, iatrogenic adrenal insufficiency.

What these cases illustrate is the current lack of evidence informing the selection of VEXAS patients who will benefit from HSCT. The tools currently utilised in "classical" MDS and CMML to identify transplant candidates (IPSS, IPSS-R) would not be applicable in patients with VEXAS given the distinct bone marrow pathology and clinical manifestations. The only way to address these issues is through well-designed trials, alongside collection of detailed outcome data for all transplanted patients. An example of such a trial has been proposed by a team from the National Institutes of Health (USA) who are now recruiting patients for a Phase II study of allogeneic HSCT for subjects with VEXAS syndrome (7). An essential design of this trial could be adopted by other transplant centres internationally. This could lead to a larger platform trial to recruit more patients, test more treatment arms and provide results sooner.

Table 1. Clinical characteristics and outcome of HSCT in VEXAS patients

Patient ID	P1	P2	P3	P4
Genetics				
UBA1 mutation	p.Met41Val (c.121A>G)	p.Met41Val (c.121A>G)	p.Met41Thr (c.122T>C)	p.Met41Leu.(c.121A>C)
VAF (5%)	50	24	50	40
Demographics				
Gender	Male	Male	Male	Male
Age of onset	49	64	52	59
Key inflammatory features				
Fever	Yes	Yes	Yes	Yes
Weight loss	Yes	Yes	Yes	No
Skin involvement	Yes (Nodular)	Yes (Nodular)	Yes (Nodular)	Yes (Jessners Infiltrate)
Chondritis	No	No	No	Yes (Ear)
Arthritis	No	No	No	No
Pulmonary infiltrates	Yes	No	Yes	No
PE	No	Yes	No	No
DVT	No	No	No	No
Other	None	Periorbital oedema	Tubular interstitial nephritis	No
Rheumatological diagnosis	None	None	uSAID	Relapsing polychondritis
Haematological disease				
MDS	Yes	No	No	Yes
Myelofibrosis	No	No	No	No
MGUS/MM	No	No	No	No
Transfusion dependent	Yes	No	Yes	Yes
R-IPSS score	3	N/A	N/A	2.5
Laboratory findings				
Macrocytic anaemia	Yes	Yes	Yes	Yes
Thrombocytopenia	Yes	Yes	Yes	No
Neutropenia	No	No	No	No
Lymphopenia	No	Yes	Yes	No
Elevated CRP	Yes	Yes	Yes	Yes
Bone marrow vacuoles	Yes	Yes	Yes	No
Additional genetic aberrations (HTS and cytogenetics)	No	No	No	No
Treatment prior to HSCT				
Total number of previous treatments	4	3	6	1
Type	CS,TOC, Anakinra, BAR, Colchicine	CS, MTX, HCQ	CS, MTX, AZA, MMF, anakinra, TOC, BAR	CS
Complications	None	None	Severe injection site reaction to anakinra, infections (pneumonia, menigitis)	None
Allogeneic HSCT				
Age at transplant	51	67	61	62
Karnofsky performance scale (PS)	70	80	70	90
Seattle HCT-CI score	6	3	3	N/A
Comorbidities	Single kidney with CKD, Deranged liver function, type II diabetes (on oral hypoglycaemics)	TLCO 63%	Moderate pulmonary, mild hepatic impairment, obesity	Chondritis
Conditioning regiment	FLU/BU, Thiotepa	FLU/MEL/CAM	FLU/TREO/CAM	FLU/BU/ATG
Donor	Haplo (son)	MUD	Sibling	MUD
Graft origin	PBSC	PBSC	PBSC	PBSC
GVH prophylaxis	CYC, TAC, MMF	CSA	CAM, CSA & MMF	ATG/CSA
Infectious complications	Salmonella houtenae in blood cultures, pseudomonas in sputum	EBV reactivation	Bacterial, C difficile, SARS-CO-V2	No
Immune complications/ GVHD	N/A	HLH, aseptic encephalitis, GvHD	Metabolic acidosis peri-transplant, myelitis & optic neuropathy	Grade 1 GvHD
Day 100 Donor chimerism CD3	N/A	100%	99%	100 (whole blood)
Day 100 Donor chimerism CD15	N/A	100%	100%	100 (whole blood)
Duration of follow-up post HSCT	11 days	5 months	11 months	40 months
Alive	No	Yes	No	Yes
Cause of death	Sepsis, multiorgan failure, cardiac arrest	N/A	Infection	N/A
Event free survival (EFS)	11 days	5 months	11 months	40 months
GRFS	11 days	4.5 months	11 months	40 months
Current status	Died	Karnofsky 40	Died	Alive in remission

ATG-Anti-thymocyte globulin; AZA-azathioprine; BAR-baricitinib; BU-busulfan; CAM-campath; CKD-chronic kidney disease; CRP-C reactive proteine; CS-cortcosteroids; CSA-ciclosporin; CYC-cyclophosphomide; EBV-Epstein-Barr virus; DVT-deep vein thromosis; FLU-fludarabine; GFRS-GvHD (grade III/IV or chronic GvHD requiring systemic therapy) and relapse free survival; GvHD- graft versus host disease; HCQ-hydroxychloroquine; HCT-CI-Hematopoietic cell transplantation-specific comorbidity index; HLH-hemophagocytic lymphohistiocytosis; HTS-High-throughput sequencing; MDS-myelodysplastic syndrome; MEL-melphalan; MGUS-monoclonal gammopathy of undetermined significance; MM-multiple myeloma; MMF-mycophenolate mofetil; MUD-matched unrelated donor; PBSC-Peripheral blood stem cell; SARS-CO-V2-Severe acute respiratory syndrome coronavirus 2; PE-pulmonary embolus; R-IPSS- Revised International Prognostic Scoring System; TAC-tacrolimus; TLCO-transfer factor for carbon monoxide; TOC-tocilizumab; TREO-treosulfan; uSAID-undiffentiated Systemic Autoinflammatory Disorder; VAF-Variant allele frequency

Table 2. Clinical characteristics of patient cohorts

	Cohort 1 Ferrada et a (n=83)	Cohort 2 Georgin-Lavialle et al (n=116)	Cohort 3 Present study (n=44)
Demographics			
Age of disease onset, median (range)	66 (41-80)	67 (62.5-73)	57 (22-72)
Male sex n (%)	83 (100)	111 (95.7)	25 (58.1)
Clinical Diagnosis			
Relapsing polychondritis n (%)	43 (52)	N/A	0 (0)
Undifferentiated Fever Syndrome n (%)	19 (23)	N/A	0 (0)
Sweets syndrome	18 (22)	N/A	0 (0)
MDS	26 (31)	58 (50)	34 (77)
CMML	0 (0)	0 (0)	10 (23)
Clinical Manifestations n (%)			
Fever	69 (83)	75 (64.6)	0 (0)
Skin involvement	68 (82)	97 (83.6)	6 (14)
Arthritis	48 (58)	33 (28.4)	3 (7)
Pulmonary infiltrates	47 (57)	47 (40.5)	0 (0)
Ear chondritis	45 (54)	37 (31.9)	0 (0)
Unprovoked deep vein thromosis (DVT)	34 (41)	41 (35.3)	0 (0)
Nose chondritis	30 (36)	18 (15.5)	0 (0)
Periorbital edema	25 (30)	10 (8.6)	0 (0)
Hearing loss	24 (29)	N/A	3 (7)
Ocular inflammation	20 (24)	43 (37)	0 (0)
Pulmonary embolism	11 (13)	N/A	0 (0)
Pleural effusion	11 (13)	11 (9.5)	3 (7)
Orchitis	10 (12)	N/A	0 (0)
Airway chondritis	1 (2)	0 (0)	0 (0)
Hematologic Manifestations			
Macrocytic anemia n (%)	81 (97)	N/A	14 (32)
Thrombocytopenia n (%)	40 (83)	N/A	34 (77)
R-IPSS Score* n (%) (MDS patients only)			
Very low risk	9 (39)	N/A	1 (3)
Low risk	12 (52)	N/A	4 (12)
Intermediate risk	0 (0)	N/A	6 (18)
High risk	2 (4)	N/A	16 (47)
Very high risk	0 (0)	N/A	7 (21)
MDS-associated mutations	7/17 (41)	N/A	19/24 (79)

Cohorts 1 and 2, VEXAS patients decribed in Ferrada et all, and Georgin-Lavialle et all respectively. Cohort 3 patients from this study who were transplanted for Myelodysplastic syndrome (MDS) or Chronic myelomonocytic leukemia (CMML) and found not to have VEXAS. R-IPSS- Revised International Prognostic Scoring System

Acknowledgments

Author contributions

SS, AK and JP, conceived and design the study; AA, DM, AMSR, SE, HE, CC, CJAD, MB, VB collected data; JA, AA and SS performed data analysis; SS wrote first draft of manuscript; All authors read, edited and approved manuscript. SS provided funding the study.

Conflict of interest

Authors have no conflict of interest to declare

Funding

SS is supported by the National Institute for Health Research (NIHR) Leeds Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. JAP is supported by a UKRI Future Leaders Fellowship (MR/T02044X/1)

REFERENCES

1. Beck DB, Ferrada MA, Sikora KA, Ombrello AK, Collins JC, Pei W, et al. Somatic Mutations in UBA1 and Severe Adult-Onset Autoinflammatory Disease. N Engl J Med. 2020;383(27):2628-38.
2. Ferrada MA, Savic S, Ospina Cardona D, Collins JC, Alessi H, Gutierrez-Rodrigues F, et al. Translation of cytoplasmic UBA1 contributes to VEXAS syndrome pathogenesis. Blood. 2022.
3. Loschi M, Roux C, Sudaka I, Ferrero-Vacher C, Marceau-Renaut A, Duployez N, et al. Allogeneic stem cell transplantation as a curative therapeutic approach for VEXAS syndrome: a case report. Bone Marrow Transplant. 2022;57(2):315-8.
4. Diarra A, Duployez N, Fournier E, Preudhomme C, Coiteux V, Magro L, et al. Successful allogeneic hematopoietic stem cell transplantation in patients with VEXAS syndrome: a 2-center experience. Blood Adv. 2022;6(3):998-1003.
5. Georgin-Lavialle S, Terrier B, Guedon AF, Heiblig M, Comont T, Lazaro E, et al. Further characterization of clinical and laboratory features in VEXAS syndrome: large-scale analysis of a multicentre case series of 116 French patients. Br J Dermatol. 2022;186(3):564-74.
6. Temple M, Kosmider O. VEXAS Syndrome: A Novelty in MDS Landscape. Diagnostics (Basel). 2022;12(7).
7. https://clinicaltrials.gov/ct2/show/NCT05027945?cond=vexas&draw=2&rank=1

The data that support the findings of this study are available from the corresponding author upon reasonable request.

There are no additional funding bodies to add in the acknowledgments section

Patient 1 (P1)

P1 was a 51-year-old, previously fit and very active male who developed a severe multisystem inflammatory illness 18 months prior to allogeneic haematopoietic stem cell transplantation (HSCT). The disease was characterized by recurrent sterile fevers with elevated inflammatory markers (CRP, ESR), in several instances culminating in multiorgan dysfunction that necessitated ICU management. Accompanying features included fleeting pulmonary infiltrates, neutrophilic dermatosis and pathergy. He also developed profound weight loss, sarcopenia, transfusion-dependent macrocytic normochromic anaemia and intermittent thrombocytopenia (70-90 × 109/l). During the illness he was diagnosed with an incidental transitional renal cell carcinoma requiring nephrectomy and resulting in chronic kidney disease. He had short-lived but ultimately unsustained responses to various immunomodulatory therapies including high-dose prednisolone, anakinra, tocilizumab and baricitinib.

Extensive investigations were undertaken to exclude an infectious or autoimmune cause for the illness including bronchoscopy and bone marrow culture. Computed tomography (CT) scan revealed mild progressive splenomegaly alongside intermittent interstitial pulmonary infiltrates and positron emission tomography (PET) with 2-deoxy-2-[fluorine-18]fluoro-D-glucose (18F-FDG) revealed progressive prominent uptake in the bone marrow and spleen. Bone marrow biopsy showed reduced erythropoiesis and striking erythroid and myeloid precursor vacuolation. Targeted sequencing identified no mutations associated with myeloid malignancy, and chromosome analysis showed a normal male karyotype. Sanger sequencing of *UBA1* identified the Met41Val missense variant at a variant allele fraction of ~0.5. Owing to the treatment resistant and rapidly progressive nature of his disease referral for HSCT was made following recommendation in the National Primary immunodeficiency bone marrow transplant multidisciplinary team meeting.

As there were no matched unrelated donors, the patient received a reduced intensity (Flu/Cy 2Gy TBI), haplo-identical bone marrow transplant with post-transplant cyclophosphamide and ciclosporin/MMF graft versus host disease prophylaxis. The conditioning was well tolerated but fever and diarrhoea developed from the day after donor cells were infused (Day +1). Despite broad spectrum antibiotics the patient required intensive care support for electrolyte derangement and metabolic acidosis from Day +4. Klebsiella and salmonella species were grown from blood cultures, antimicrobial therapy appropriately adjusted and

indwelling lines changed. Following an asystolic cardiac arrest on Day +7, despite maximal intensive support including inotropes, renal and ventilatory support, the patient succumbed to multiorgan failure on Day +11, prior to engraftment.

Patient 2 (P2)

P2 is a previously fit and well male, aged 64yrs, presented with retro-orbital pain and swelling and a peri-orbital erythematous rash, which responded initially to iv corticosteroids and antibiotics. Systemic symptoms developed, including fatigue, fevers, night sweats and weight loss, accompanied by an intermittent nodular erythematous rash, worse on forearms and neck. Inflammatory markers were raised, skin biopsy was consistent with erythema nodosum and positron emission tomography (PET) with 2-deoxy-2-[fluorine-18]fluoro-D-glucose (18F-FDG) was non-diagnostic. Bone marrow examination showed hypercellularity with hyperplastic, left-shifted granulopoiesis, reduced megakaryocytes with micromegakaryocytes, disorganised erythropoiesis and trilineage dysplastic features. Targeted sequencing identified no mutations associated with myeloid malignancy, and chromosome analysis showed a normal male karyotype.

The patient's symptoms stabilised on prednisolone (minimum dose 15mg daily), methotrexate (25mg weekly) and hydroxychloroquine (200mg daily). 8 months after presentation the patient was diagnosed with bilateral pulmonary emboli and received long-term anticoagulation with Rivaroxaban. 2.5yrs after initial presentation, genetic sequencing from a saliva sample revealed UBA1 p.Met41Val somatic mosaicism and repeat bone marrow aspirate, now in the context of a macrocytic anaemia, showed evidence of vacuolation in erythroid and granulocytic precursors.

With ongoing requirement for immunosuppression, increasing anaemia and thrombocytopenia and gradual clinical decline, the patient was considered for bone marrow transplantation, discussed at the National Adult Primary Immunodeficiency Bone Marrow Transplant MDT.

The patient received a reduced intensity, Flu/Mel/Alemtuzumab conditioned transplant with a 10/10 matched unrelated donor. Conditioning was well tolerated and a good graft achieved with engraftment of neutrophils and platelets on post-transplant days 12 and 13 respectively and 100% donor chimerism in myeloid and T cell compartments at three months. However, the patient had a stormy clinical course over the 9 months following transplant with

inflammatory encephalitis and haemophagocytic lymphohistiocytosis (HLH) treated with corticosteroids, anakinra and the JAK inhibitor baracitinib, Epstein Barr Virus (EBV) reactivation treated with rituximab, severe peripheral muscle wasting, severe chronic skin graft versus host disease treated with ciclosporin, corticosteroids and extracorporeal photophoresis, recurrent bacterial chest and urinary infections and hypogammaglobulinaemia (requiring immunoglobulin replacement therapy), with a current Karnofsky performance score of 40.

Patient 3 (P3)

P3 initially presented at the age of 52 with fevers, weight loss, widespread nodular skin rash, bilateral hilar and mediastinal lymphadenopathy and pulmonary infiltrates. He was initially diagnosed with systemic sarcoidosis and treated with corticosteroids, to which he had a good response. Over the next 2 years he remained dependent on corticosteroids for control of his symptoms and failed to respond to multiple traditional synthetic DMARDs. His diagnosis was revised to undifferentiated systemic autoinflammatory disorder and he was treated with anakinra, but shortly after the treatment was initiated, he developed extensive injection site reactions necessitating anakinra discontinuation. Subsequently, he was switched to tocilizumab. He had a partial response and remained dependent on a small dose of prednisolone (5-10mg daily) for symptomatic control. His treatment was complicated by severe pulmonary infections requiring hospital admission. Consequently, tocilizumab was changed to baricitinib, but he remained reliant on prednisolone. Later it was established that he had developed secondary adrenal insufficiency. He was diagnosed with VEXAS 7 years after the initial onset of symptoms. Shortly afterward the diagnosis was established his anaemia progressed and he became transfusion dependent. The indications for his transplant were ongoing, poorly controlled inflammatory illness and progressive bone marrow failure. HSCT was performed 2 years after the diagnosis of VEXAS at the age of 61. His initial recovery after HSCT was relatively straightforward. He had no symptoms of graft versus host disease (GvHD), but shortly after being discharged home, he developed progressive leg weakness and double incontinence, and extensive screening investigations were negative. He was diagnosed with post-transplant myelitis, postulated to relate to a toxic cause, possibly a rare effect of conditioning treatment with fludarabine. Although his day 100 follow-up tests showed almost CD3 and CD15 donor chimerism with no evidence of the original *UBA1* mutation on peripheral blood and bone marrow testing, his haematological graft function was sub-optimal, particularly in the red cell series and he remained red blood cell transfusion dependent. His post-transplant bone marrow was hypocellular and the 9-month CD3 chimerism declined slightly to 91%, CD15 99%. His inflammatory illness remained in complete remission, however he deteriorated progressively from a neurological standpoint, with progressive cord signal abnormalities on MRI spine, complete paraplegia and loss of vision. He did not respond to further attempts at immunomodulation and immunosuppression with steroid pulses and intravenous immunoglobulin, and following multidisciplinary advanced care planning

discussions with his family expressed the wish to be managed at home with palliative care. He passed away from an infective episode 11 months following his transplant.

Patient 4 (P4)

P4 initially presented when 59 years old with fleeting pain in multiple joints, followed by abrupt onset of redness and swelling in his left ear with elevated inflammatory markers and anaemia. Pinna cartilage biopsy confirmed non-specific mild perichondrial chronic inflammation but no features of acute chondritis and the diagnosis of relapsing polychondritis was made. There was an initial excellent clinical response to oral prednisolone although there was symptom recurrence at lower doses. Disease modifying drugs were under consideration, however two years after initial diagnosis he represented with symptoms of progressive anaemia with Hb 49, an MCV of 127 with normal haematinics. Bone marrow biopsy showed modest dyserythropoiesis, megaloblastoid morphology and an increase in granulopoiesis but all with full maturation. There were no ringed sideroblasts, micromegakaryocytes or blasts seen. Cytogenetics and NGS myeloid screen were normal. As there was no response to EPO, and the patient required ongoing two weekly packed cell transfusions, three years after initial presentation the patient received a Flu/Bu/ATG 9/10 DP permissive mismatched MUD. The patient tolerated treatment well, with minimal limited liver and skin GVHD, and had no further recurrence of polychondritis. He is now more than three years post-transplant and remains 100% donor with no active GVHD on 10mg ciclosporin bd.

METHODS

VEXAS patients were identified via two national clinical networks, National Primary immunodeficiency bone marrow transplant group and The HLH Across Speciality Collaboration (https://www.hihasc.org). Clinical data was collected retrospectively. Patients who were transplanted for myelodysplastic syndrome or Chronic myelomonocytic leukaemia indications were identified from a single centre (Leeds Teaching Hospitals NHS Trust). The study was performed in accordance with the local ethical approval for investigation of a suspected haematological disorder (ethical committee approval 16/NE/0105).

Genetic testing was performed by pooled amplicon sequencing of all *UBA1* exons using an adapted method published previously(1). Briefly, primers targeting each exon were designed and tagged with M13 sequences (Supplementary Table 1). Following amplification of each exon individually, all amplicon products from each DNA sample were pooled and purified using a 1:1 ratio of AMPure beads:pooled amplicons. A second round of amplification using 50ng pooled amplicons was then performed to attach indexed sequencing adapters to each sample. Indexed amplicons from all samples were subsequently pooled and an AMPure bead purification performed to create the final sequencing library. 3x 500ng pools were sequenced by GeneWIZ (EZ-Amplicon Seq) to a minimum read depth of 50,000 reads per pool. Resulting fastq files were aligned and variants analysed as previously described(1). Potential variants were validated by PCR and Sanger sequencing of the original DNA samples.

References:

1. Poulter J, Consortium UV, Morgan A, Cargo C, Savic S. A High-Throughput Amplicon Screen for Somatic UBA1 Variants in Cytopenic and Giant Cell Arteritis Cohorts. J Clin Immunol. 2022.

Primer name	Primer sequence (5' to 3')
UBA1_Ex2-3F	TGTAAAACGACGGCCAGTCCCCTCTTTGCTGTAAAATG
UBA1_Ex2-3R	CAGGAAACAGCTATGACCTCATGGCCCAACACATACC
UBA1_Ex4-5F	TGTAAAACGACGGCCAGTAGAGGATAAGGTTGGGTGGG
UBA1_Ex4-5R	CAGGAAACAGCTATGACCTGAATAAACCAGAGATGGGC
UBA1_Ex6-7F	TGTAAAACGACGGCCAGTACTGCCTGAGTCCTCCACAC
UBA1_Ex6-7R	CAGGAAACAGCTATGACCAGGAAGGACTACTGCAGCC
UBA1_Ex8F	TGTAAAACGACGGCCAGTGGCTGCAGTAGTCCTTCCTG
UBA1_Ex8R	CAGGAAACAGCTATGACCGCCAGAACCCAGGAACATATC
UBA1_Ex9-10F	TGTAAAACGACGGCCAGTCCTTATCTTGCAGGGGTTG
UBA1_Ex9-10R	CAGGAAACAGCTATGACCAGAGGCATGGTGAGCCTG
UBA1_Ex11-12F	TGTAAAACGACGGCCAGTGTGTCCACACTCCCTGCC
UBA1_Ex11-12R	CAGGAAACAGCTATGACCAGGGAGGAGGGTTAGAGAGG
UBA1_Ex13F	TGTAAAACGACGGCCAGTCATCATTGGGGACATTTCTG
UBA1_Ex13R	CAGGAAACAGCTATGACCAGGGGTGATGGCCAGAG
UBA1_Ex14F	TGTAAAACGACGGCCAGTGAGTGAGGGGTGATGGGTAG
UBA1_Ex14R	CAGGAAACAGCTATGACCAACAAGAGCGAAACTCCG
UBA1_Ex15F	TGTAAAACGACGGCCAGTCCACCCTGGAACTGCAC
UBA1_Ex15R	CAGGAAACAGCTATGACCCGTGACCACCTCACC
UBA1_Ex16F	TGTAAAACGACGGCCAGTCCTCTCCTGATGTTTCTTTCC
UBA1_Ex16R	CAGGAAACAGCTATGACCTGGCCTCTTTCCCTTC
UBA1_Ex17F	TGTAAAACGACGGCCAGTAATAATGCCTGCGGAAACC
UBA1_Ex17R	CAGGAAACAGCTATGACCGATGCCTTGGCTGGTGG
UBA1_Ex18F	TGTAAAACGACGGCCAGTGTAAGATTGCTCTGGAGCCC
UBA1_Ex18R	CAGGAAACAGCTATGACCTGCAGGCTCCCATTTCAG
UBA1_Ex19-20F	TGTAAAACGACGGCCAGTCGTGAAGATTGTCAGAGAGGC
UBA1_Ex19-20R	CAGGAAACAGCTATGACCGTGGGGAGGCTGGACTAGG
UBA1_Ex21-22F	TGTAAAACGACGGCCAGTATCTCAGACCTTAGCCTGGG
UBA1_Ex21-22R	CAGGAAACAGCTATGACCTCAGGGTCTACAGAGGGG
UBA1_Ex23F	TGTAAAACGACGGCCAGTCCCTCTGTAGACCCTGAGGC
UBA1_Ex23R	CAGGAAACAGCTATGACCGGGTATGACGTGAATCAGAACC
UBA1_Ex24-25F	TGTAAAACGACGGCCAGTGGATGGAGACAGATGGGG
UBA1_Ex24-25R	CAGGAAACAGCTATGACCGACAGCTGGATGATGAGGC
UBA1_Ex26F	TGTAAAACGACGGCCAGTCACTGCCCCTACCTACCTG
UBA1_Ex26R	CAGGAAACAGCTATGACCTGGAAGGGGTGTGGAGAG
M13-F_indexA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGAATATTGTAAAACGACGGCCAGT
M13-F_indexB	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAGGCGTTGTAAAACGACGGCCAGT
M13-F_indexC	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATAGAGTTGTAAAACGACGGCCAGT
M13-F_indexD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCGAGACTGTAAAACGACGGCCAGT
M13-F_indexE	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCTACTTGTAAAACGACGGCCAGT
M13-F_indexF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTCGTCTGTAAAACGACGGCCAGT
M13-F_indexG	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAAGTCTTGTAAAACGACGGCCAGT
M13-F_indexH	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGGACTCTGTAAAACGACGGCCAGT

M13-R_index1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAGCGCTCAGGAAACAGCTATGACC
M13-R_index2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGATATCCAGGAAACAGCTATGACC
M13-R_index3	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCTGCGCAGGAAACAGCTATGACC
M13-R_index4	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACTCATACAGGAAACAGCTATGACC
M13-R_index5	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCTATGTCAGGAAACAGCTATGACC
M13-R_index6	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTATCGCACCAGGAAACAGCTATGACC

Supplementary Table 1 – Primer sequences for amplicon sequencing.