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**Allogeneic hematopoietic stem cell transplantation for  
VEXAS syndrome  
- UK experience**

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Key Words:	CMML, MDS, VEXAS Syndrome, HSCT

# Allogeneic hematopoietic stem cell transplantation for VEXAS syndrome - UK experience

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## KEYWORDS

VEXAS syndrome

Allogeneic hematopoietic stem cell transplantation

Myelodysplastic syndrome

Chronic myelomonocytic leukaemia

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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

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3 infections, the sequelae of which have resulted in a Karnofsky performance score of 40.  
4  
5 Patient 3 achieved disease control with no molecular evidence of VEXAS, however developed  
6  
7 severe post-transplant myelitis resulting in paraplegia, urinary and faecal incontinence, and  
8  
9 passed away 11 months post-transplant from infectious complications. Patient 4 is the only  
10  
11 one who at the time of writing (40 months post-transplant) remains alive and in good health.

12  
13 Previous studies have reported VEXAS patients being transplanted in the absence of a  
14  
15 prior diagnosis, including one of the patients we report here (P4). We therefore speculated  
16  
17 about the incidence of undiagnosed VEXAS cases amongst patients with myeloid disorders  
18  
19 who had undergone HSCT. To this end, we studied patients from a single centre, with a  
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21 diagnosis of MDS or CMML, who were transplanted in the last 10 years, for whom historical  
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23 pre-HSCT bone marrow DNA samples were available.

24  
25 We identified 44 patients in total, 34 MDS and 10 CMML. Historical, pre-HSCT bone  
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27 marrow DNA samples were available for all patients. We performed deep sequencing of the  
28  
29 whole *UBA1* gene (for method please see supplements). To date, all pathogenic mutations  
30  
31 associated with VEXAS have been confined to exon 3, with Methionine-41 (Met41) being a  
32  
33 particularly important site (1, 2). No patients demonstrated any of the known pathogenic  
34  
35 mutations in *UBA1*, but 4 patients were found to have rare variants outside exon 3 that have  
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37 not been previously reported and have unknown functional consequences. The variants  
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39 identified (and variant allele frequencies) were: c.2554-1G>T (0.5), c.2554-8C>T (0.4),  
40  
41 c.2374C>T:p.Gln792Ter (0.14) and c.1321G>A:p.Glu441Ter (0.8). However, none of the  
42  
43 patients carrying these variants had any inflammatory symptoms. Three had a diagnosis of  
44  
45 MDS, whilst the patient with c.1321G>A, p.Glu441Ter variant was diagnosed with CMML.

46  
47 A failure to find any typical cases of VEXAS across the entire transplanted cohort was  
48  
49 likely predictable. Very few patients had any inflammatory symptoms or disease  
50  
51 manifestations classically associated with VEXAS (Table 2). The MDS features were also  
52  
53 different. Ferrada et al. reported that MDS was diagnosed in 31% of the total cohort, who  
54  
55 were retrospectively found to have VEXAS (2). On review, these patients typically had low risk  
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57 disease, have a much lower frequency of MDS associated gene mutations and show no  
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59 evidence of progression to acute myeloid leukaemia (2, 6) consistent with *UBA1* mutations  
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61 being the main driver of cytopenias and bone marrow morphological changes not a distinct  
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63 MDS. The other haematological manifestations which are almost universal in VEXAS, such as

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3 macrocytic anaemia (close to 100%), were seen in only 32% of patients in our cohort, although  
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5 other types of anaemia (e.g. microcytic) were more common.  
6

7 We report variable outcomes of HSCT in our VEXAS case series with 50% (2/4) overall  
8  
9 survival but only 25% event free survival at current follow up. There are several potential  
10  
11 explanations for this. Patient 1 underwent HSCT as a 'treatment of last resort' for a rapidly  
12  
13 progressive, recalcitrant inflammatory disorder. The risk of poor outcome in such  
14  
15 circumstances is invariably high, as reflected in his HCT-CI score of 6. Patient 2 was  
16  
17 transplanted three years after the onset of symptoms and had received continuous  
18  
19 immunosuppressive therapy including corticosteroids during this time. Patient 3 was  
20  
21 transplanted almost 10 years after disease onset, during which time he had already  
22  
23 developed several complications including renal impairment and steroid toxicity. In addition,  
24  
25 he was exposed to several immunosuppressive therapies and had experienced several serious  
26  
27 infections. It is unclear to what degree post-transplant complications were influenced by  
28  
29 these factors. Nevertheless, the purported benefit of reduced corticosteroid use as a result  
30  
31 of better control of his inflammatory illness was significantly offset by his pre-existing,  
32  
33 iatrogenic adrenal insufficiency.

34 What these cases illustrate is the current lack of evidence informing the selection of  
35  
36 VEXAS patients who will benefit from HSCT. The tools currently utilised in "classical" MDS  
37  
38 and CMML to identify transplant candidates (IPSS, IPSS-R) would not be applicable in patients  
39  
40 with VEXAS given the distinct bone marrow pathology and clinical manifestations. The only  
41  
42 way to address these issues is through well-designed trials, alongside collection of detailed  
43  
44 outcome data for all transplanted patients. An example of such a trial has been proposed by  
45  
46 a team from the National Institutes of Health (USA) who are now recruiting patients for a  
47  
48 Phase II study of allogeneic HSCT for subjects with VEXAS syndrome (7). An essential design  
49  
50 of this trial could be adopted by other transplant centres internationally. This could lead to a  
51  
52 larger platform trial to recruit more patients, test more treatment arms and provide results  
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54 sooner.  
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Patient ID	P1	P2	P3	P4
<b>Genetics</b>				
UBA1 mutation	p.Met41Val (c.121A>G)	p.Met41Val (c.121A>G)	p.Met41Thr (c.122T>C)	p.Met41Leu.(c.121A>C)
VAF (%)	50	24	50	40
<b>Demographics</b>				
Gender	Male	Male	Male	Male
Age of onset	49	64	52	59
<b>Key inflammatory features</b>				
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
<b>Haematological disease</b>				
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
<b>Laboratory findings</b>				
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
<b>Treatment prior to HSCT</b>				
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, meningitis)	None
<b>Allogeneic HSCT</b>				
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 regimen	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-corticosteroids; CSA-ciclosporin; CYC-cyclophosphamide; EBV-Epstein-Barr virus; DVT-deep vein thrombosis; 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-undifferentiated Systemic Autoinflammatory Disorder; VAF-Variant allele frequency

**Table 2. Clinical characteristics of patient cohorts**

	Cohort 1 Ferrada et al (n=83)	Cohort 2 Georgin- Lavialle et al (n=116)	Cohort 3 Present study (n=44)
<b>Demographics</b>			
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)
<b>Clinical Diagnosis</b>			
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)
<b>Clinical Manifestations n (%)</b>			
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 thrombosis (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)
<b>Hematologic Manifestations</b>			
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 described in Ferrada et al, and Georgin-Lavialle et al 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			

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### 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

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3 The data that support the findings of this study are available from the corresponding author upon  
4 reasonable request.  
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For Peer Review

### 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 ( $70-90 \times 10^9/l$ ). 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 unsustainable 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

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2  
3 indwelling lines changed. Following an asystolic cardiac arrest on Day +7, despite maximal  
4 intensive support including inotropes, renal and ventilatory support, the patient succumbed  
5 to multiorgan failure on Day +11, prior to engraftment.  
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## 10 **Patient 2 (P2)**

11 P2 is a previously fit and well male, aged 64yrs, presented with retro-orbital pain and swelling  
12 and a peri-orbital erythematous rash, which responded initially to iv corticosteroids and  
13 antibiotics. Systemic symptoms developed, including fatigue, fevers, night sweats and weight  
14 loss, accompanied by an intermittent nodular erythematous rash, worse on forearms and  
15 neck. Inflammatory markers were raised, skin biopsy was consistent with erythema nodosum  
16 and positron emission tomography (PET) with 2-deoxy-2-[fluorine-18]fluoro-D-glucose (18F-  
17 FDG) was non-diagnostic. Bone marrow examination showed hypercellularity with  
18 hyperplastic, left-shifted granulopoiesis, reduced megakaryocytes with  
19 micromegakaryocytes, disorganised erythropoiesis and trilineage dysplastic features.  
20 Targeted sequencing identified no mutations associated with myeloid malignancy, and  
21 chromosome analysis showed a normal male karyotype.  
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32 The patient's symptoms stabilised on prednisolone (minimum dose 15mg daily),  
33 methotrexate (25mg weekly) and hydroxychloroquine (200mg daily). 8 months after  
34 presentation the patient was diagnosed with bilateral pulmonary emboli and received long-  
35 term anticoagulation with Rivaroxaban. 2.5yrs after initial presentation, genetic sequencing  
36 from a saliva sample revealed UBA1 p.Met41Val somatic mosaicism and repeat bone marrow  
37 aspirate, now in the context of a macrocytic anaemia, showed evidence of vacuolation in  
38 erythroid and granulocytic precursors.  
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45 With ongoing requirement for immunosuppression, increasing anaemia and  
46 thrombocytopenia and gradual clinical decline, the patient was considered for bone marrow  
47 transplantation, discussed at the National Adult Primary Immunodeficiency Bone Marrow  
48 Transplant MDT.  
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52 The patient received a reduced intensity, Flu/Mel/Alemtuzumab conditioned transplant with  
53 a 10/10 matched unrelated donor. Conditioning was well tolerated and a good graft achieved  
54 with engraftment of neutrophils and platelets on post-transplant days 12 and 13 respectively  
55 and 100% donor chimerism in myeloid and T cell compartments at three months. However,  
56 the patient had a stormy clinical course over the 9 months following transplant with  
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3 inflammatory encephalitis and haemophagocytic lymphohistiocytosis (HLH) treated with  
4 corticosteroids, anakinra and the JAK inhibitor baracitinib, Epstein Barr Virus (EBV)  
5 reactivation treated with rituximab, severe peripheral muscle wasting, severe chronic skin  
6 graft versus host disease treated with ciclosporin, corticosteroids and extracorporeal  
7 photopheresis, recurrent bacterial chest and urinary infections and  
8 hypogammaglobulinaemia (requiring immunoglobulin replacement therapy), with a current  
9 Karnofsky performance score of 40.  
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**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

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3 discussions with his family expressed the wish to be managed at home with palliative care.  
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5 He passed away from an infective episode 11 months following his transplant.  
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#### 10 **Patient 4 (P4)**

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

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Primer name	Primer sequence (5' to 3')
UBA1_Ex2-3F	TGAAAAACGACGGCCAGTCCCCTCTTTGCTGTAAAATG
UBA1_Ex2-3R	CAGGAAACAGCTATGACCTCATGGCCCAACACATACC
UBA1_Ex4-5F	TGAAAAACGACGGCCAGTAGAGGATAAGGTTGGGTGGG
UBA1_Ex4-5R	CAGGAAACAGCTATGACCTGAATAAACCCAGAGATGGGC
UBA1_Ex6-7F	TGAAAAACGACGGCCAGTACTGCCTGAGTCCTCCACAC
UBA1_Ex6-7R	CAGGAAACAGCTATGACCAGGAAGGACTACTGCAGCC
UBA1_Ex8F	TGAAAAACGACGGCCAGTGGCTGCAGTAGTCCTTCCTG
UBA1_Ex8R	CAGGAAACAGCTATGACCGCCAGAACCAGGAACATATC
UBA1_Ex9-10F	TGAAAAACGACGGCCAGTCCTTATCTTGCAGGGGTTG
UBA1_Ex9-10R	CAGGAAACAGCTATGACCAGAGGCATGGTGAGCCTG
UBA1_Ex11-12F	TGAAAAACGACGGCCAGTGTGTCCCACTCCCTGCC
UBA1_Ex11-12R	CAGGAAACAGCTATGACCAGGGAGGAGGGTTAGAGAGG
UBA1_Ex13F	TGAAAAACGACGGCCAGTCATCATTGGGGACATTTCTG
UBA1_Ex13R	CAGGAAACAGCTATGACCAGGGGTGATGGCCAGAG
UBA1_Ex14F	TGAAAAACGACGGCCAGTGAGTGAGGGGTGATGGGTAG
UBA1_Ex14R	CAGGAAACAGCTATGACCAACAAGAGCGAAACTCCG
UBA1_Ex15F	TGAAAAACGACGGCCAGTCCACCCTGGAAGTGCAC
UBA1_Ex15R	CAGGAAACAGCTATGACCCGTGACCACCTCACC
UBA1_Ex16F	TGAAAAACGACGGCCAGTCCTCTCCTGATGTTTCTTTCC
UBA1_Ex16R	CAGGAAACAGCTATGACCTGGCCTCTTTCCCTTC
UBA1_Ex17F	TGAAAAACGACGGCCAGTAATAATGCCTGCGGAAACC
UBA1_Ex17R	CAGGAAACAGCTATGACCGATGCCTTGGCTGGTGG
UBA1_Ex18F	TGAAAAACGACGGCCAGTGTAAGATTGCTCTGGAGCCC
UBA1_Ex18R	CAGGAAACAGCTATGACCTGCAGGCTCCCATTTCAG
UBA1_Ex19-20F	TGAAAAACGACGGCCAGTCGTGAAGATTGTCAGAGAGGC
UBA1_Ex19-20R	CAGGAAACAGCTATGACCGTGGGGAGGCTGGACTAGG
UBA1_Ex21-22F	TGAAAAACGACGGCCAGTATCTCAGACCTTAGCCTGGG
UBA1_Ex21-22R	CAGGAAACAGCTATGACCTCAGGGTCTACAGAGGGG
UBA1_Ex23F	TGAAAAACGACGGCCAGTCCCTCTGTAGACCCTGAGGC
UBA1_Ex23R	CAGGAAACAGCTATGACCGGGTATGACGTGAATCAGAACC
UBA1_Ex24-25F	TGAAAAACGACGGCCAGTGGATGGAGACAGATGGGG
UBA1_Ex24-25R	CAGGAAACAGCTATGACCGACAGCTGGATGATGAGGC
UBA1_Ex26F	TGAAAAACGACGGCCAGTCACTGCCCTACCTACCTG
UBA1_Ex26R	CAGGAAACAGCTATGACCTGGAAGGGGTGTGGAGAG
M13-F_indexA	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGAATATTGAAAAACGACGGCCAGT
M13-F_indexB	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAGGCGTTGAAAAACGACGGCCAGT
M13-F_indexC	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATAGAGTTGAAAAACGACGGCCAGT
M13-F_indexD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCAGACTGTAAAAACGACGGCCAGT
M13-F_indexE	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCTACTTGTAAAAACGACGGCCAGT
M13-F_indexF	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTCTGTGTAAAAACGACGGCCAGT
M13-F_indexG	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAAGTCTTGTAAAAACGACGGCCAGT
M13-F_indexH	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGGACTCTGTAAAAACGACGGCCAGT

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

**Supplementary Table 1 – Primer sequences for amplicon sequencing.**

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