De-escalation of corticosteroids and clonal remission in *UBA1* mutation-driven VEXAS syndrome with 5-azacytidine

VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome has gained significant interest in the medical community both due to the paradigm-shifting genomics first approach elegantly described by Beck *et al.* in 2020,[1] but also because of the florid multi-systemic nature of its presentation. VEXAS classically develops in older men, and presents with fevers, skin changes, relapsing chondritis, pulmonary infiltrates, and vasculitis.[2] Specific hematologic manifestations include significant cytopenias that have a propensity to progress to myelodysplastic syndromes (MDS), plasma cell dyscrasias and high risk of venous thromboembolism (VTE). Detection of disease-defining mutations in *UBA1*, a key player in the ubiquitination pathway that modulates cellular stress and inflammation,[3] is part of the diagnosis of this syndrome.[4] The finding that *UBA1* is found to be mutated in hematopoetic stem cells (HSC) links this pathological mechanism to bone marrow failure.[5]

Owing to the relative recency of its description, and varied presentation to different medical specialties, the diagnosis is often not forthcoming. Additionally, there is no consensus as regards optimal supportive and treatment options with respect to symptom severity and overall prognosis. Inflammatory manifestations of VEXAS are generally corticosteroid sensitive and relapses are seen when weaning steroids, leaving an urgent need to develop steroid-sparing strategies. Multiple modalities, usually reflective of familiarity of the drug by the treating specialty physician, have been reported. Targeting inflammatory mediators such as interleukin-1 (anakinra and canakinumab), interleukin-6 (tocilizumab), and JAK-STAT pathway (ruxolitinib)[6] have been attempted with some success.[7] Mutations in *UBA1*, although originating in HSC, are subsequently lineage-restricted to the myeloid series;[1] therefore, therapy aimed specifically at these aberrant cells is another treatment strategy. By chance, before the description of VEXAS, a cohort of patients labeled as MDS with autoinflammation, who had, therefore, been started on 5-azacytidine, showed improvement in peripheral blood cytopenias and inflammatory symptoms. This, along with previously reported responses in Sweet's syndrome,[8] another autoinflammatory syndrome, provided compelling evidence that 5-azacytidine treatment can be a therapeutic option in VEXAS.[9,10] In a retrospective analysis of a phase II clinical trial of the efficacy of azacytidine in MDS / chronic myelomonocytic leukemia patients with systemic autoimmune and inflammatory disorders, 12 patients treated with 5-azacytidine were found to have VEXAS (*UBA1* positive).[9] Treatment with 5-azacytidine induced a rapid clinical response both in terms of reduction of inflammatory symptoms and steroid independence in around 75% of patients. Other case studies have noted similar responses, and correlation of clinical response to clonal remission lends a degree of confidence to dose tapering and stopping steroids within the first 3 cycles of azacytidine.[11,12]

Since the first description of VEXAS syndrome in late 2020, we have set up a multidisciplinary team to manage this group of patients. Of a cohort of 25 patients referred to our center, we have now treated 11 with 5-azacytidine. None of these patients were treated concurrently with any other immune modulating agents (other than corticosteroids). Of the 11 patients that are being treated with 5-azacytidine, 4 have now completed at least 6 cycles of treatment. We assessed the clinical and biological responses to 5-azacytidine and our prospective longitudinal experience of managing these 4 patients.

Our cohort, representative of those reported in the literature, are older men with multi-system disease; all were on corticosteroids at the time of diagnosis of VEXAS. The median age of our cohort is 71 years (range, 51-79). All 4 patients had MDS with low blasts (MDS-LB), with low-risk Revised International Prognostic Scoring System (IPSS-R) scores, and had severe systemic manifestations of disease. Universally, all experienced skin lesions, weight loss, significant fatigue, and cytopenias. Three out of the 4 were transfusion-dependent. Polychondritis and joint stiffness was common. Thrombotic events (deep vein thrombosis, central retinal vein occlusion, and stroke) were also seen in 2 patients; one was associated with a positive lupus anticoagulant.

In 3 out of 4 patients, 5-azacytidine was started at the patient's local hospital, as per local policy (75 mg/m^2 over 7 days in a 28-day cycle). In one patient, 5-azacytidine was commenced in the context of an intensive care admission with type 1 respiratory failure, thought to be a complication of infection / inflammation. All patients have completed at least 6 cycles of treatment. Treatment was well-tolerated with a low toxicity profile. In spite of delays due to neutrophil recovery, cycles were generally administered in a timely manner. Due to enduring relapse-free remission, we have managed to lengthen the cycle to 6-weekly in one patient, and 8-weekly in another patient, and also reduced the dose of 5-azacytidine. The median duration and number of cycles of 5-azacytidine are 13.5 months (range, 6-38) and 12 (range, 6-31), respectively.

All patients showed a clinical response with respect to

resolution of inflammatory symptoms on no or low-dose steroids within 1-3 cycles; 2 patients were able to completely stop steroids, one was maintained on 2 mg of prednisolone, and one patient required 5 mg of prednisolone. Of the 3 patients who had cytopenias severe enough to require transfusions, transfusion independence was achieved within 3-5 cycles of commencing treatment. This is also commensurate with a reduction in the *UBA1* clone size to very low levels or even undetectable by conventional next-generation sequencing (QiaSeq targeted amplicon and Illumina NextSeq 550, with coverage of all coding regions of *UBA1*). Known clinically significant variants were reported at a variant allele frequency (VAF) of <5% (Figure 1). Emergence of *ASXL1* was noted in one patient after three years, with no change in blood counts or bone marrow morphology (Table 1).

VEXAS is no longer thought of as a rare syndrome. With a population incidence of just over 1:4000 men over the age of 50,[13] many scientific and clinical questions in VEXAS merit urgent attention. The association between inflammation and MDS is not new; however, such overt and multisystem inflammatory manifestation of a disease whose locus is the bone marrow is unique. Whilst the development of bone marrow failure in VEXAS is conceivable, the pathophysiological mechanism that generalizes this to multiple systems is not understood.

Clinically, decisions around which patients need treatment, and the most suitable modality of treatment, need to be assessed. To date, whilst the efficacy of immunomodulatory drugs in managing symptoms in VEXAS has been shown, there is no evidence that these strategies reduce the underlying burden of disease or allow steroids to be completely stopped. Azacytidine not only provides symptomatic relief, but also measurably reduces the *UBA1* clone size burden, suggesting, therefore, it is a suitable addition to a regimen that involves treatment and de-escalated maintenance phases. The consideration of azacytidine as an option also benefits from years of familiarity with administration and management of the toxicity profile in older patients. Moreover, hematology units in smaller hospitals and community settings are usually set up to deliver azacytidine, allowing for VEXAS patients to be treated locally. Thus far,

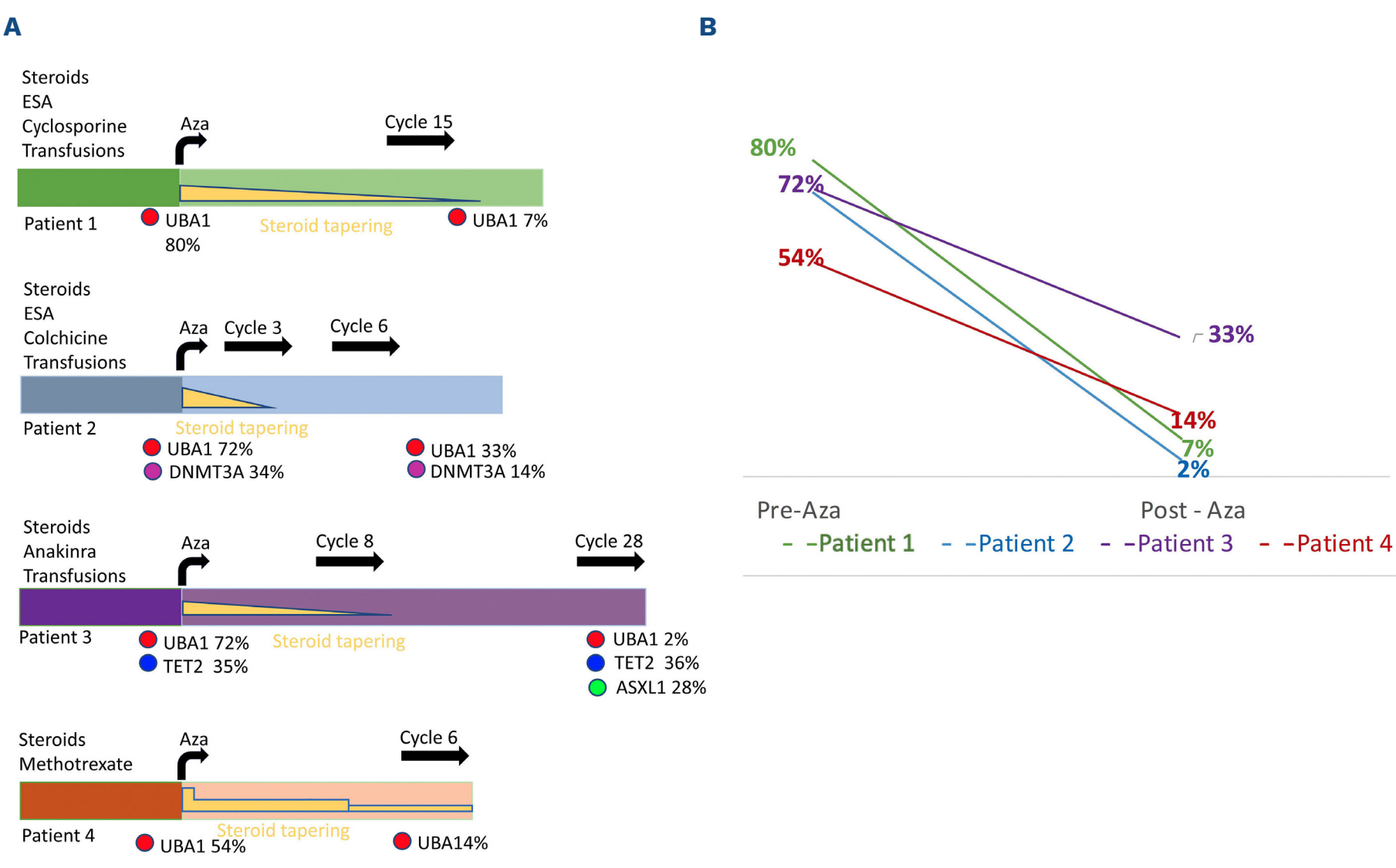

Figure 1. Azacytidine treatment leads to a reduction in *UBA1* clonal burden and reduces steroid dependence in VEXAS syndrome. (A) Schematic showing treatments pre-azacytidine (Aza), steroid weaning in patients started on Aza, and variant allele frequency (VAF) % of *UBA1* and other myeloid mutations. Prior to commencing 5-azacytidine: Patient 1 was on 5 mg of prednisolone which was weaned down to 2 mg; Patient 2 was on high-dose steroids, 60 mg of prednisolone which was weaned and stopped; Patient 3 was on 50 mg of prednisolone which was weaned and stopped; and Patient 4 was on 60 mg of prednisolone which was weaned down to 5 mg. (B) Reduction in UBA1 allele burden after commencing Aza. ESA: erythropoiesis stimulating agents.

Table 1. Baseline clinical features and post-treatment characteristics of 4 patients with VEXAS syndrome.

	Case1	Case 2	Case 3	Case 4
Age in years	68	78	51	74
Presenting features	Skin rash, weight loss, malaise, chondritis, joint pains	Skin rash, weight loss, cellulitis, fevers, CRVO, pneumonitis	Skin rash, weight loss, fatigue, fevers, pneumonitis	Skin rash, weight loss, fatigue, fevers, leg swelling, uveitis, chondritis, DVT, stroke
Pre-treatment full blood count				
WCC x10^9/L	2.0	3.3	0.93	4.1
Hb g/L	79	104	75	96
Plt x10^9/L	64	75	62	86
Neut x10^9/L	1.5	2.7	0.6	1.89
Transfusion dependence	Yes (RBC)	Yes (RBC)	Yes (RBC)	No
Diagnostic details				
WHO 2022 MDS classification	MDS-LB	MDS-LB	MDS-LB	MDS-LB
Cytogenetics	45, X, -Y [8]/46, XY [12]	46 XY [20]	46 XY [20]	46 XY [20]
UBA1 mutation at diagnosis (VAF)	Splice acceptor c.118-1G>C (80%)	pMet41Thr (72%)	Splice Acceptor c.118-1G>C (72%)	pMet41Thr (VAF 54%)
Pre-treatment CH (VAF)	None	*DNMT3A* (34%)	*TET2* (35%)	None
Previous treatments	Cyclosporine, ESA, prednisolone	Colchicine, ESA, prednisolone	Anakinra, prednisolone	Methotrexate, prednisolone
Months on Aza (N of cycles)	19 (15)	8 (8)	38 (31)	6 (6)
UBA1 VAF, months post treatment	7% (PB), 18	32%, 6	No *UBA1* detected at 24 months	14% (BM), 5
Post-treatment CH (VAF)	None	*DNMT3A* (14%)	*TET2* (36%), *ASXL1* (28%) PB	None

Aza: azacytidine; BM: bone marrow; CH: mutations associated with clonal hematopoiesis; CRVO: central retinal vein occlusion; Cyto: baseline cytogenetics at diagnosis; DVT: deep vein thrombosis; ESA: erythropoiesis stimulating agents; Hb: hemoglobin; MDS: myelodysplastic syndromes; MDS-LB: myelodysplastic syndrome-low blasts; Neut: neutrophils; PB: peripheral blood; Plt: platelets; RBC: red blood cells; VAF: variant allele frequency; WCC: white cell count; WHO: World Health Organization. Proportion of metaphases with loss of Y. *DNMT3A*: p.(Arg-882Cys) c. 2644 C>T, *TET2*: p.(Glu1279GlnfsTer85) c.3832_3833dup, *ASXL1*: p.(Val843Ter)c.2527del.

familiar MDS regimens have been utilized; however, further exploration of the timing and patten of response will better inform regimens for VEXAS.

The rapid response seen in our cohort is likely related to reversal / dampening of the VEXAS-related autoinflammation rather than improvement in MDS-related anemia, as responses to hypomethylating agents in low-risk MDS can take more than six months.

The diagnosis of MDS in our cohort was based on morphology, although with the caveat that VEXAS patients can show dysplasia without evidence of MDS (as defined by the WHO/ICC 2022), especially in the absence of excess blasts, ring sideroblasts, MDS-defining cytogenetic abnormality and mutations, which are not related to clonal hemopoiesis. From phenotypic-genotypic correlates we now have some understanding of pathogenicity of *UBA1* mutations that confer severe disease phenotypes, but the contribution of co-evolving myeloid mutations has yet to be elucidated. *TET2* and *DNMT3A* are the most frequently observed myeloid mutation in VEXAS, though they do not appear to directly correlate with disease severity[14] or help to ascertain a diagnosis of MDS. What governs the specific selection of these clones over other high-risk MDS mutations, such as *ASXL1* and *U2AF1*, is not understood.

Since VEXAS is a chronic disease, approaches that reduce hospital visits and treatment burden are a priority. We have effectively spaced out the frequency and reduced the dose of 5-azacytdine, without deleterious effects on symptoms or the *UBA1* clone, but future strategies to stop treatment or replace with oral azacytidine need to be explored. This should be evaluated in future clinical trials with close monitoring of symptoms and the *UBA1* clone to predict relapse of VEXAS. Alternative strategies to eradicate the *UBA1* clone by allogeneic stem cell transplantation is also being evaluated, but is only applicable to a small proportion of younger and fitter patients.[5,15] In addition, rapid response to 5-azacytidine, as is the case for our cohort of patients, if confirmed in a larger cohort might obviate the need for stem cell transplantation, although prospective studies of HSCT are also ongoing.

VEXAS patients in our cohort were not heavily pre-treated with other immunomodulatory agents, like JAK inhibitors, tocilizumab or anakinra, and it is unknown if this would have had a positive impact on the outcome.

Notwithstanding the recent explosion of work on VEXAS, there is an urgent need for consolidating these efforts to prioritise key questions in this disease. Cross-specialty teams to treat VEXAS are currently being set up that will hopefully address this in the near future.

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RT and AK wrote the paper. All authors contributed to data acquisition and the critical review of the manuscript. All authors approved the final version for publication.

Data-sharing statement

Additional data are available upon request to the corresponding author.

References

1. Beck DB, Ferrada MA, Sikora KA, et al. Somatic mutations in UBA1 and severe adult-onset autoinflammatory disease. N Engl J Med. 2020;383(27):2628-2638.
2. Georgin-Lavialle S, Terrier B, Guedon AF, 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):564-574.
3. Beck DB, Werner A, Kastner DL, Aksentijevich I. Disorders of ubiquitylation: unchained inflammation. Nat Rev Rheumatol. 2022;18(8):435-447.
4. Al-Hakim A, Savic S. An update on VEXAS syndrome. Exp Rev Clin Immunol. 2023;19(2):203-215.
5. Gurnari C, Koster L, Baaij L, et al. Allogeneic hematopoietic cell transplantation for VEXAS syndrome: results of a multicenter study of the EBMT. Blood Adv. 2024;8(6):1444-1448.
6. Heiblig M, Ferrada MA, Koster MJ, et al. Ruxolitinib is more effective than other JAK inhibitors to treat VEXAS syndrome: a retrospective multicenter study. Blood. 2022;140(8):927-931.
7. Bourbon E, Heiblig M, Gerfaud Valentin M, et al. Therapeutic options in VEXAS syndrome: insights from a retrospective series. Blood. 2021;137(26):3682-3684.
8. Kulasekararaj AG, Kordasti S, Basu T, Salisbury JR, Mufti GJ, du Vivier AW. Chronic relapsing remitting Sweet syndrome--a harbinger of myelodysplastic syndrome. Br J Haematol. 2015;170(5):649-656.
9. Mekinian A, Zhao LP, Chevret S, et al. A Phase II prospective trial of azacitidine in steroid-dependent or refractory systemic autoimmune/inflammatory disorders and VEXAS syndrome associated with MDS and CMML. Leukemia. 2022;36(11):2739-2742.
10. Comont T, Heiblig M, Riviere E, et al. Azacitidine for patients with Vacuoles, E1 Enzyme, X-linked, Autoinflammatory, Somatic syndrome (VEXAS) and myelodysplastic syndrome: data from the French VEXAS registry. Br J Haematol. 2022;196(4):969-974.
11. Sockel K, Gotze K, Ganster C, et al. VEXAS syndrome: complete molecular remission after hypomethylating therapy. Ann Hematol. 2024;103(3):993-997.
12. Raaijmakers M, Hermans M, Aalbers A, et al. Azacytidine treatment for VEXAS syndrome. Hemasphere. 2021;5(12):e661.
13. David BB, Dale LB, Vandan S, et al. Genomic ascertainment for UBAI variants and VEXAS syndrome: a population-based study. medRxiv. 2022 Aug 1. doi:10.1101/2022.07.27.22277962 [preprint, not peer-reviewed]
14. Patel BA, Gutierrez-Rodrigues F, Kusne Y, et al. Clonal Hematopoiesis in Vexas Syndrome. Blood. 2022;140(Suppl 1):5745-5746.
15. Gurnari C, McLornan DP. Update on VEXAS and role of allogeneic bone marrow transplant: considerations on behalf of the Chronic Malignancies Working Party of the EBMT. Bone Marrow Transplant. 2022;57(11):1642-1648.