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Received: March 22, 2024.

Accepted: June 3, 2024.

Citation: Roochi Trikha, Kar Lok Kong, James Galloway, Tanya N. Basu, Lynn Quek, Jamie Wilson, Louise Gamble, Henna Wong, Steven Best, and Austin Kulasekararaj. De-escalation of corticosteroids and clonal remission in *UBA1* mutation-driven VEXAS syndrome with 5-azacytidine. *Haematologica*. 2024 June 13. doi: 10.3324/haematol.2024.285519 [Epub ahead of print]

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

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Acknowledgment

We thank the co-authors for collection of data and for critically reviewing the manuscript.

Author contribution

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

Data Sharing agreement:

Additional data are available upon request to the corresponding author.

Disclosures

No conflicts of interests to disclose for all authors

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 described elegantly by Beck et al in 2020¹, but also because of florid multi-systemic nature of its presentation. VEXAS classically develops in older men and presents with fevers, skin changes, relapsing chondritis, pulmonary infiltrates and vasculitis². Specific hematological 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 inflammation³, is diagnostic of this syndrome⁴. The finding that *UBA1* is found to be mutated in hematopoietic stem cells (HSCs), links this pathological mechanism to bone marrow failure.

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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 success⁷. Mutations in *UBA1* though originate in HSCs, are subsequently lineage restricted to the myeloid series¹, therefore therapy aimed specifically at these aberrant cells is another treatment strategy. Serendipitously, before the description of VEXAS, a cohort of patients labelled as MDS with autoinflammation and therefore commenced on 5-azacytidine showed improvement in peripheral blood cytopenias and inflammatory symptoms. This, along with previous reported responses in Sweet's syndrome⁸, 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 2 clinical trial of the efficacy of Azacytidine in MDS/CMML 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 three quarters of patients. Other case studies have noted similar responses, and correlation of clinical response to clonal remission lends a degree of confidence in dose tapering and stopping steroids within the first three 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 centre, 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, four have now completed at least six cycles of treatment. We assessed the clinical and biological responses to 5-azacytidine and our prospective longitudinal experience of managing these four patients.

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

In 3 out of 4 patients, 5-azacytidine was commenced at the patient's local hospital, as per local policy (75mg/m² over 7 days in a 28 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 low toxicity profile. Notwithstanding 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, 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 – two 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 three 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 reported at a VAF of <5%). Emergence of *ASXL1* was noted in one patient after 3 years, with no change in blood counts or bone marrow morphology.

VEXAS is no longer thought of as a rare syndrome. With a population incidence of just over 1:4000 men over the age of 50¹³, 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 needs 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 ability to completely stop steroids. Azacytidine not only provides symptomatic relief but also measurably reduces the *UBA1* clone size burden,

therefore intuitively feels amenable to a regimen that involves treatment and de-escalated maintenance phases. Azacytidine as an option also benefits from years of familiarity with administration and toxicity profile in older patients. Moreover, haematology units in smaller hospitals and community setting are usually set up to deliver Azacytidine, allowing for VEXAS patients to be treated locally. Thus far, familiar MDS regimens have been utilized, however more work around the timing and pattern 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 of MDS related anaemia, as responses to hypomethylating agents in low risk MDS can take more than 6 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 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 there does not appear to be a direct correlate with disease severity¹⁴ or helps to ascertain a diagnosis of MDS. What governs the specific selection of these clones over other the high-risk MDS mutations such as *ASXL1* and *U2AF1* is not understood.

VEXAS being 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-azacytidine, without deleterious effects on symptoms or *UBA1* clone, but future strategies to stop treatment or replace with oral azacytidine require exploring. This should be evaluated in future clinical trials with close monitoring of symptoms and *UBA1* clone to predict relapse of VEXAS. Alternate 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 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 positively impacted on the outcome.

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

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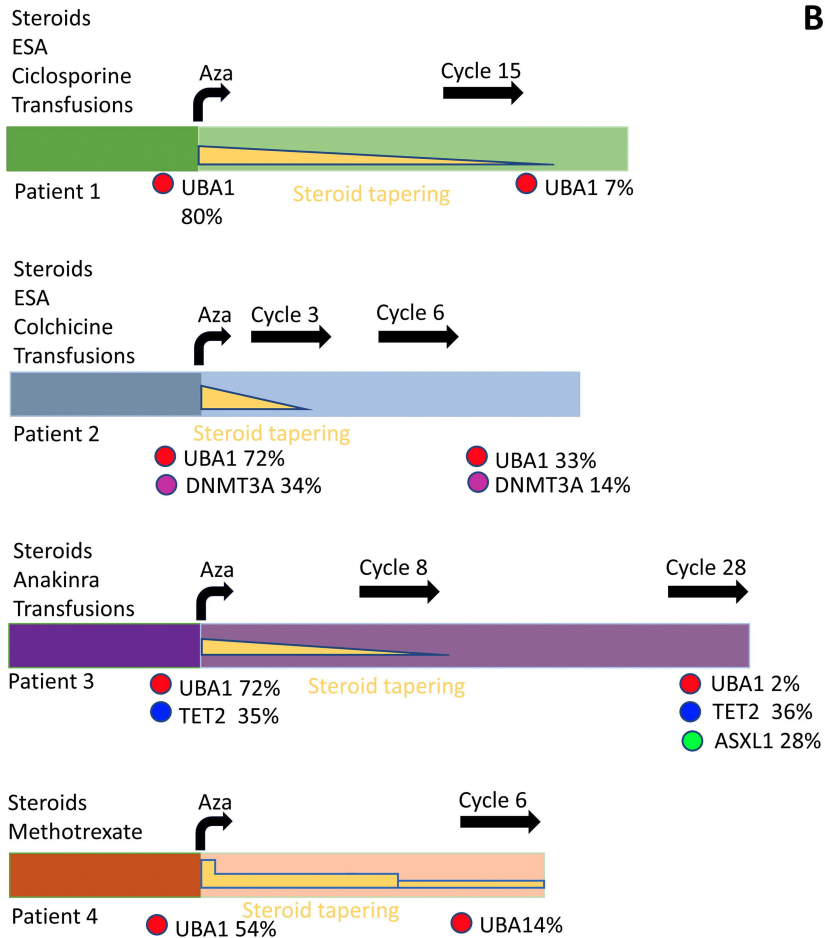
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Table 1: Baseline clinical features and post treatment characteristics of 4 patients with VEXAS syndrome

	Case 1	Case 2	Case 3	Case 4
Age	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 2.0 X10 ⁹ /L Hb 79 g/L Plt 64 X10 ⁹ /L Neut 1.5 X10 ⁹ /L	WCC 3.3 X10 ⁹ /L Hb 104 g/L Plt 75 X10 ⁹ /L Neut 2.7 X10 ⁹ /L	WCC 0.93 X10 ⁹ /L Hb 75 g/L Plt 62 X10 ⁹ /L Neut 0.6 X10 ⁹ /L	WCC 4.1 X10 ⁹ /L Hb 96 g/L Plt 86 X10 ⁹ /L Neut 1.89 X10 ⁹ /L
Transfusion dependence	Yes (Red cells)	Yes (Red cells)	Yes (Red cells)	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]
- <i>UBA1</i> mutation (VAF) at diagnosis	Splice acceptor c.118-1G>C (80%)	pMet41Thr (72%)	Splice Acceptor c.118-1G>C (72%)	pMet41Thr (VAF 54%)
- CH Mutations (pre-treatment) (VAF)	Nil	<i>DNMT3A</i> (VAF 34%)	<i>TET2</i> (VAF 35%)	Nil
Previous treatments	Cyclosporine, ESA, Prednisolone	Colchicine, ESA, Prednisolone	Anakinra, Prednisolone	Methotrexate, Prednisolone
Months (No of cycles) on Aza	19 months (15 cycles)	8 months (8 cycles)	38 months (31 cycles)	6 months (6 cycles)
<i>UBA1</i> VAF- Months Post treatment	VAF 7% (PB) – 18 months	VAF 32 % - 6 months	No <i>UBA1</i> detected at 24 months	VAF 14% (BM) – 5 months
CH Mutations (post-treatment)	nil	<i>DNMT3A</i> (VAF 14%)	<i>TET2</i> (VAF 36%), <i>ASXL1</i> (VAF 28%) PB	nil

VAF: Variant Allele Frequency, TD: transfusion dependence; White cell count (WCC), Haemoglobin (Hb), Platelets (Plt), Neutrophils (Neut), Cyto: Baseline cytogenetics (at diagnosis), CH: Mutations associated with Clonal hematopoiesis. CRVO: Central retinal vein occlusion, DVT: Deep vein thrombosis, MDS-LB- Myelodysplastic syndrome-low blasts. Proportion of metaphases with loss of Y. *DNMT3A*:p.(Arg882Cys) c. 2644 C>T, *TET2*: p.(Glu1279GlnfsTer85) c.3832_3833dup, *ASXL1*: p.(Val843Ter)c.2527del.

Figure 1: Azacytidine treatment leads to reduction in *UBA1* clonal burden and reduces steroid dependence in VEXAS syndrome. Panel A: Schematic showing treatments pre-Azacytidine, steroid weaning in patients started on Azacytidine 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, Patient 4 was on 60 mg of Prednisolone which was weaned down to 5 mg. **Panel B:** Reduction in *UBA1* allele burden after commencing Azacytidine. ESA: Erythropoiesis Stimulating Agents.

A**B**