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SHORT REPORT

Persistence of *UBTF* tandem duplications in remission in acute myeloid leukaemia

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Abstract

UBTF tandem duplications are recurrent in adult and paediatric acute myeloid leukaemia and have been reported to be associated with a poor prognosis. Co-mutations in *WT1* and *FLT3* are common while morphological dysplasia is frequent. The role of UBTF-TDs in leukemogenesis is yet to be elucidated; however they have been proposed as early initiating events, making them attractive for assessment of MRD and a potential therapeutic target. We present two cases where the *UBTF*-TD was observed in remission and discuss the implications of these findings in the clinicobiological understanding of this emerging entity.

KEYWORDS
acute leukaemia, cancer genetics, molecular

Tandem duplications in exon 13 of upstream binding transcription factor (*UBTF*-TDs) (NM_014233.3) are recurrent in acute myeloid leukaemia (AML) and define a subtype with specific clinical and biological features. Umeda *et al.* recently reported *UBTF*-TDs in approximately 10% of paediatric patients with relapsed AML [1]. Duployez et al. subsequently demonstrated a frequency of approximately 3% in adult patients with similar clinicogenetic features [2]. *UBTF*-TDs are associated with myelodysplasia, normal karyotype or trisomy 8, and somatic variants in *WT1* and *FLT3*. The presence of *UBTF*-TD in AML is associated with a poor prognosis with high rates of early relapse. Given the relatively few cases described, our understanding of the full clinicopathological spectrum of this entity is still emerging. Herein, we describe the novel observation of two patients with *UBTF*-TD AML where the *UBTF*-TD was present in remission with no measurable residual disease (MRD) identified by multiparametric flow cytometry and discuss the implications of these observations.

The first patient was a 72-year-old female who presented with pancytopenia and circulating blasts. Subsequent bone marrow biopsy demonstrated AML with marked dysmegakaryopoiesis. Cytogenetic testing demonstrated a normal karyotype while a *FLT3*-ITD was

identified by capillary electrophoresis with an allelic ratio of 0.03. Targeted next generation sequencing (NGS) demonstrated an *IDH2* NM_002168.2:c.419G > A; p.(Arg140Gln) variant at 45% variant allele frequency (VAF) while no WT1 variants were identified. Induction with 7+3 (cytarabine and idarubicin) with midostaurin was complicated by clinical tumour lysis syndrome and severe lobar pneumonia. Disease assessment post induction demonstrated morphological complete remission with undetectable MRD by flow cytometry (sensitivity 10^{-4}). Targeted NGS performed in remission demonstrated persistence of the *IDH2* p.(Arg140Gln) at 43% VAF suggestive of its presence in a preleukemic clone. Consolidation was delayed due to emergent bowel surgery required for severe diverticulitis and subsequent poor performance status. Morphological relapse occurred after 3 months in remission. Given the presence of the *IDH2* p.(Arg140Gln), treatment with enasidenib was initiated. She achieved complete remission on enasidenib monotherapy with NGS testing in remission showing a reduction of the *IDH2* VAF to 2%. She remained in remission on enasidenib for a further 24 months but then relapsed with re-emergence of the *IDH2* variant while the previous *FLT3*-ITD was undetectable. The patient was treated with supportive care and died in the weeks following relapse. We performed fragment length analysis of *UBTF* exon 13 using fluorescent labelled primers on the diagnostic sample and identified a 54-base pair insertion (Figure S1). NGS subsequently confirmed the insertion to be NM_014233.4:c.1335_1336ins[CCC;1285_1335] (Figure S2). Serial testing on stored samples throughout the patient's disease course demonstrated that the *UBTF*-TD was detectable in flow MRD-negative remission post induction (Figure 1A). Interestingly, the *UBTF*-TD remained at a similar VAF until enasidenib was commenced at which point the *UBTF*-TD became undetectable (along with a marked reduction in the VAF of the *IDH2* variant). These observations are suggestive of the presence of a preleukemic clone harbouring both *IDH2* and *UBTF*-TD, which responded to enasidenib treatment. The low level *FLT3*-ITD detected at diagnosis was not detected at the subsequent relapses. Immunohistochemistry with *UBTF* antibody was performed and demonstrated a moderate reduction in nucleolar and nuclear staining when compared to a patient with *NPM1*-mutated AML without *UBTF*-TD control where UBTF staining intensity was increased across the nucleus (Figures S3).

The second case was an 8-year-old female who presented with fever, pancytopenia and circulating blasts. Bone marrow biopsy demonstrated myelodysplastic syndrome with excess blasts 2 (MDS-EB2). Karyotype was normal by conventional cytogenetics. Fragment analysis identified a *FLT3*-ITD with an allelic ratio of 0.03 while a targeted NGS panel identified two *WT1* variants with VAFs of 41% and 2%, respectively. The patient progressed to AML after 2 months, associated with an increase in *FLT3*-ITD allelic ratio to 0.22, at which time she was treated with induction chemotherapy (mitoxantrone [12 mg/m^2 × 4], cytarabine [100 mg/m^2 × 20] and gemtuzumab ozogamicin [3 mg/m^2 × 1]). Morphological complete remission was achieved after induction cycle 1, with undetectable MRD by flow cytometry (sensitivity 10^{-4}) and undetectable *FLT3*-ITD by capillary electrophoresis. The patient received a second cycle of induction chemotherapy with fludarabine (30 mg/m^2 × 5), cytarabine (2 g/m^2 × 5) and idarubicin (8 mg/m^2 × 3) before proceeding to matched sibling donor allogeneic stem cell transplantation, with busulfan (3.8 mg/kg × 4), fludarabine (37.5 mg/m^2 × 4) and thiotepa (5 mg/kg × 2) conditioning. Bone marrow assessment prior to transplant demonstrated ongoing flow MRD negative complete remission. The patient received sorafenib maintenance therapy post-transplant, and at 1 year follow-up remains alive and disease-free with no detectable MRD by flow cytometry. Again, we retrospectively analysed for *UBTF*-TD on stored samples from time of MDS diagnosis, progression to AML, post induction cycle 1 and cycle 2. A 69-base pair insertion in *UBTF* was identified (NM_014233.4:c.1329_1330ins[TT;1292_1329;TA;1305_1329]) that persisted post both induction cycles despite being in morphological remission and negative MRD by flow cytometry, again suggestive of the presence of the *UBTF*-TD in a preleukemic clone (Figure 1B).

UBTF is a nucleolar protein and is an essential factor for RNA polymerase I (Pol I) transcription of rRNA genes (rDNA) within the nucleoli. It is a key component of the Pol I pre-initiation complex and is also enriched at highly active polymerase II-transcribed genes across the genome [3]. *UBTF*-TDs are recurrent in paediatric AML and while rarer in adult AML they appear to have similar clinical and genetic features. AML with *UBTF*-TD is associated with normal karyotype or trisomy 8, variants in *WT1* and *FLT3* and an inferior prognosis [1, 2]. *UBTF* encodes for the Pol I transcription factor UBF, a member of the HMG-box DNA-binding protein family which plays a critical role in rRNA transcription [4, 5]. Increased levels of Pol I transcription are a common feature in haematological malignancies [3, 6]. UBTF functions by decondensing rDNA chromatin to establish a euchromatic state and mediates the recruitment of Pol I to rDNA promotor regions. Depletion of UBTF leads to silencing of rRNA genes due to chromatin repression [7].

Analysis of *UBTF* genomic abnormalities to date has predominantly been in diagnostic or relapsed tumour samples where *UBTD*-TD has been identified as a putative driver mutation due to its mutual exclusivity with other recognised drivers of leukemogenesis. Furthermore, in vitro data have established that *UBTF*-TDs are sufficient in themselves to promote myeloid expansion [1]. However, our two cases demonstrate that *UBTF*-TD may exist in a preleukemic state. As seen in our cases at diagnosis, the bone marrow morphology of AML with *UBTF*-TD frequently demonstrates trilineage dysplasia in the absence of molecular lesions typically associated with MDS. Indeed, Duployez et al. reported that the majority of cases of adult AML with *UBTF*-TD demonstrate dysplasia in the erythroid or megakaryocytic lineages [2]. This trilineage dysplasia is supportive of *UBTF*-TD occurring outside of the leukemic clone. While *FLT3*-ITD can co-occur with *UBTF*-TD in founding clones, it is well recognised that it is an unstable marker that is often subclonal and can be lost at progression of disease [8]. As demonstrated by these cases, *UBTF*-TD can persist in the absence of *FLT3*-ITD including at relapse, suggesting their cooperation is not required for leukemogenesis.

While the prognostic relevance of this novel subgroup is emerging, the recognition of *UBTF*-TD as a cooperative driver mutation identifies it as a potential MRD marker. UBTF genomic abnormalities duplicate a portion of the HMG4 domain, most commonly as a result of an

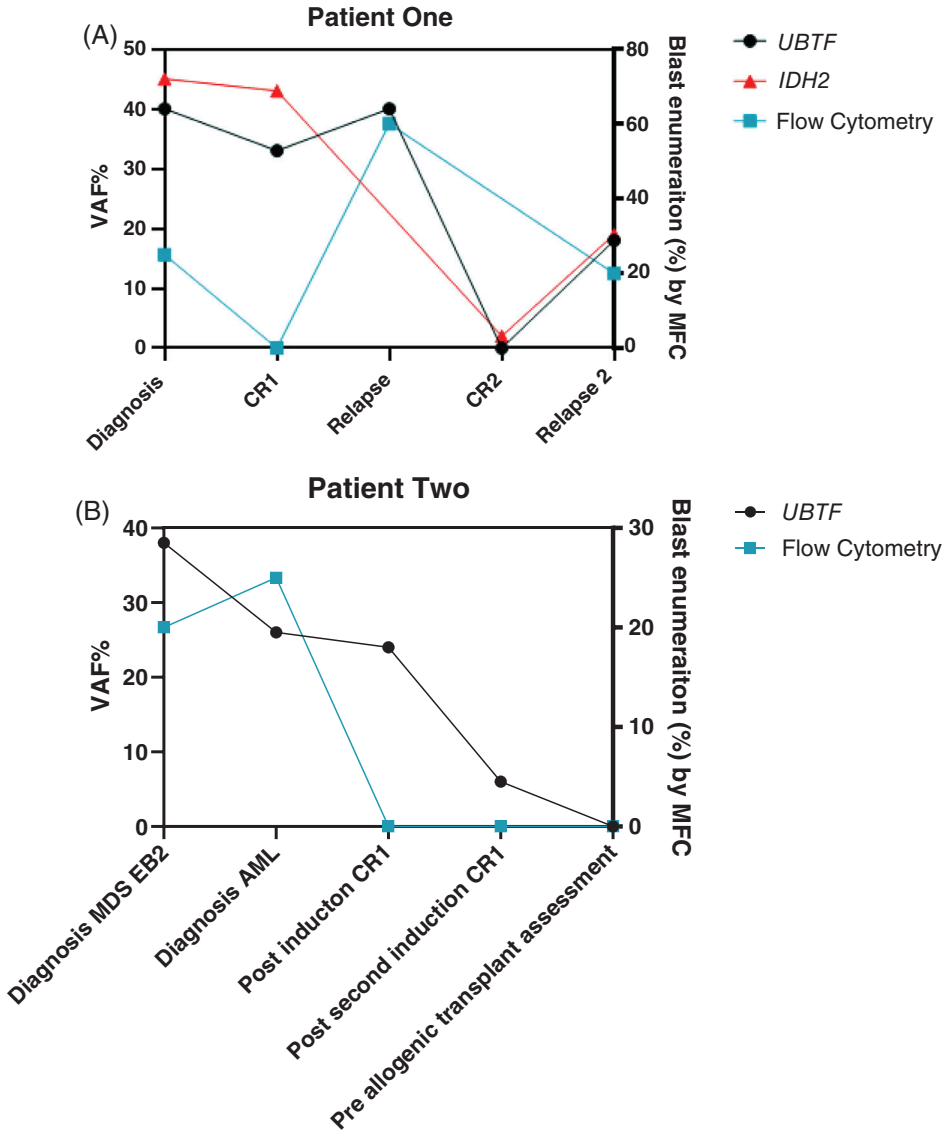

FIGURE 1 (A) Assessment of *UBTF* variant allele frequency at key clinical timepoints in patient one. VAF, variant allele frequency; CR1, first complete remission; CR2, second complete remission; MFC, multiparametric flow cytometry. (B) Assessment of *UBTF* variant allele frequency at key clinical timepoints in patient two.

in-frame insertion at the 3′ end of exon 13 or in-frame duplications of exon 13. These characteristics may make *UBTF*-TD amenable to NGS-based MRD approaches [1, 2, 9–11]. Furthermore, *UBTF*-TD is often present at high VAF and remains stable during disease progression, suggesting it arises as an early event in leukemogenesis [2]. However, our observation of *UBTF*-TD in remission highlights that further characterisation is required to determine its suitability as a reliable marker of residual disease. The role of *UBTF*-TD in clonal haematopoiesis and progression to MDS/AML is currently unclear. Transcriptional studies have demonstrated high levels of UBTF expression in UBTF-TD AML however UBTF expression is often high in cases of AML [1]. Immunohistochemistry performed in patient one is suggestive of a relative decrease in UBTF protein expression compared to the transcriptionally similar *NPM1* mutated AML; however further studies are required to elucidate the consequences of *UBTF*-TD on the function and cellular localisation of UBTF.

Therapeutically, inhibition of the Pol I pathway in *UBTF*-TD AML may be an attractive target as *UBTF* expression is high in *UBTF*-TD AML [1]. Inhibition of Pol I transcription in phase I trials in haematological malignancies has only led to modest responses, however *UBTF*-TD AML mechanistically represents a potentially promising subgroup for further investigation [12, 13]. Furthermore, AML with *UBTF*-TD shares a similar transcriptional profile with AML with mutated *NPM1*, *KMT2A*-PTD and *DEK::NUP214*, all of which are characterised by *HOXB5-9* expression [1]. Menin is an oncogenic cofactor in *KMT2A* rearranged (*KMT2A*-r) that leads to *HOX* and *MEIS1* leukaemogenic transcription. While in *NPM1* mutated AML, *KMT2A* is an oncogenic regulator and menin disruption leads to repression of *HOX* gene expression [14–16]. Clinical trials of menin inhibitors in *KMT2A*-r and *NPM1* mutated AML are ongoing with promising preclinical and phase I data, making understanding the relationship between MRD and preleukemic states imperative [17, 18].

In summary, *UBTF*-TD AML is an important emerging leukemic entity, which has a distinct molecular profile with a poor prognosis that supports the clinical relevance of identifying this high-risk subtype. Our description of *UBTF*-TDs occurring in flow-MRD negative remission, suggestive of it occurring in a pre-leukemic clone adds to our understanding of the biology of this genomic lesion and its role as both a potential marker of MRD and a therapeutic target.

AUTHOR CONTRIBUTIONS

SH performed the experiments, analysed the data and wrote the paper. PN, CW, DB, GR and TN performed the experiments, analysed the data and reviewed the paper. MA, SK, MM, IT and ES reviewed the paper. PB conceived the experiments, analysed the data and reviewed the paper.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

ETHICS STATEMENT

This study was conducted under local institutional ethics approval (Human Research Ethics Committee, Peter MacCallum Cancer Centre). No material was reproduced from other sources.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

PATIENT CONSENT STATEMENT

The authors have confirmed patient consent statement is not needed for this submission.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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