ZBTB44–FLT3 fusion in a patient with a myeloproliferative neoplasm

Zinc finger and BTB/POZ domain-containing proteins (ZBTB) are a group of transcription repressors featuring an N-terminal protein-interacting BTB/POZ domain and C-terminal DNA binding zinc fingers. The ZBTB family is heavily involved in both normal and malignant haematopoiesis; for example, when *BCL6 (ZBTB27)* is juxtaposed to the enhancers of immunoglobulin genes in mature B-cell lymphoma, *BCL6* is upregulated, leading to blockage of B-cell apoptosis in the germinal centre.[1] *ZBTB16 (PLZF)* is rearranged with *RARA*, resulting in the interruption of retinoic acid signaling in a subgroup of acute promyelocytic leukaemia (APL).[2]

ZBTB44 is universally expressed; however, its cellular function is not known, nor is its role in tumourigenesis. We discovered a *ZBTB44* rearrangement in a myeloproliferative neoplasm with eosinophilia, which was fused to fms-like tyrosine kinase 3 (*FLT3*), a major regulator of haematopoiesis. FLT3 is expressed in haematopoietic stem cells and myeloid, lymphoid and dendritic progenitor cells, and synergizes with other growth factor-signaling pathways in bone marrow to stimulate the proliferation and differentiation of multiple haematopoietic lineages. The phenotype of our patient, as detailed below, is reminiscent of a group of unique tumours characterized by receptor tyrosine kinase (RTK) rearrangements, that is, myeloid/lymphoid neoplasms with *PDGFRA, PDGFRB, FGFR1 or PCM1–JAK2* rearrangement, respectively,[3] according to the WHO 2016 classification.

A 64-year-old male presented with fatigue and weight loss for three months. Physical examination found a enlarged spleen (6 cm below the left costal margin) but was negative for adenopathy, bruise/bleed and hepatomegaly. Peripheral blood counts demonstrated leukocytosis and thrombocytopenia with white blood cells (WBC) 128·68 × 10^9/l, haemoglobin (HGB) 137 g/l and platelet count 36 × 10^9/l. A differential count found 35·6% neutrophils, 14·2% lymphocytes, 13·3% monocytes, 3% basophils, 1% myelocytes and 32·6% eosinophils (absolute eosinophil count 43 × 10^9/l). Bone marrow aspiration smears showed marked granulocytic proliferation, with a myeloid-to erythroid ratio of 34·8:1 (Fig 1A). Myeloblasts and promyelocytes were not increased. Eosinophils were significantly increased, accounting for 31% of all nucleated cells, with some of them showing sparse granulation and cytoplasmic vacuolation. Erythroid and lymphoid elements were markedly proportionally decreased with no significant dysplasia. Megakaryocytes were not seen. Flow cytometry showed 0·1% of $CD34^+$ cells and increased eosinophils. The patient was diagnosed with myeloproliferative neoplasm, unclassifiable (MPN-U). A targeted DNA next-generation sequencing (NGS) panel for 121 leukaemia-related genes was negative for any mutations. Karyotype analysis from unstimulated bone marrow showed a balanced translocation between chromosomes 11 and 13 as the sole change: 46,XY,t(11;13)(q24;q12) (Fig 1B). A fluorescence *in-situ* hybridization (FISH) assays showed *KMT2A* remained on the derivative chromosome 11, suggesting an 11q breakpoint telomeric to the *KMT2A* (Fig 1C). An RNA NGS panel targeting 30 genes commonly rearranged in myeloid tumours was negative for any rearrangements. A mate-pair DNA NGS[4] capable of detecting genome-wide gene rearrangement showed both *FLT3* and *ZBTB44* sequences in the same circulated amplicon. *ZBTB44* and *FLT3* are located at 11q24 and 13q12, respectively, consistent with the breakpoints defined by karyotype. A reverse transcription polymerase chain reaction (RT-PCR) was then performed to amplify the fusion transcript of the *ZBTB44–FLT3*(Table SI, Table SII). As shown in Fig 1D, Sanger sequencing of the PCR products showed that the first two exons of *ZBTB44*, minus the last nucleotide of exon 2, was fused to the 3' *FLT3* starting in the middle of exon 14, and the reading frame remained intact after gene fusion. The expected fusion protein contains an intact BTB/POZ domain and the FLT3 tyrosine kinase domain (TKD) (Fig 1E,F). The reciprocal product of the translocation, that is the *FLT3–ZBTB44*, was also expressed. Two *FLT3–ZBTB44* transcripts were detected due to alternative *FLT3* splicing; however, both were reading frame-shifted and are assumed functionally inactive. The BTB/POZ domain of the ZBFB family is capable of homodimerization and heterodimerization,[5,6] and because a common activation mechanism of the rearranged RTKs involves ligand-independent dimerization induced by their fusion partners, the ZBTB44–FLT3 fusion protein is likely constitutively activated by ZBTB44 BTB/POZ-induced dimerization, and cross-phosphorylation by the FLT3 tyrosine kinase. The patient has been treated with hydroxycarbamide at 1 g orally daily and WBC is controlled under 10 × 10^9/l for six months now.

A total of 15 cases with *FLT3* rearrangement is summarized in Table I. These tumours occur mostly in the middle-aged population with a median age of 46 and a male predominance of 2:1. Most patients present with MPN and/or T-lymphoblastic leukaemia/lymphoma (T-ALL); however, chronic myelomonocytic leukaemia (CMML) and juvenile

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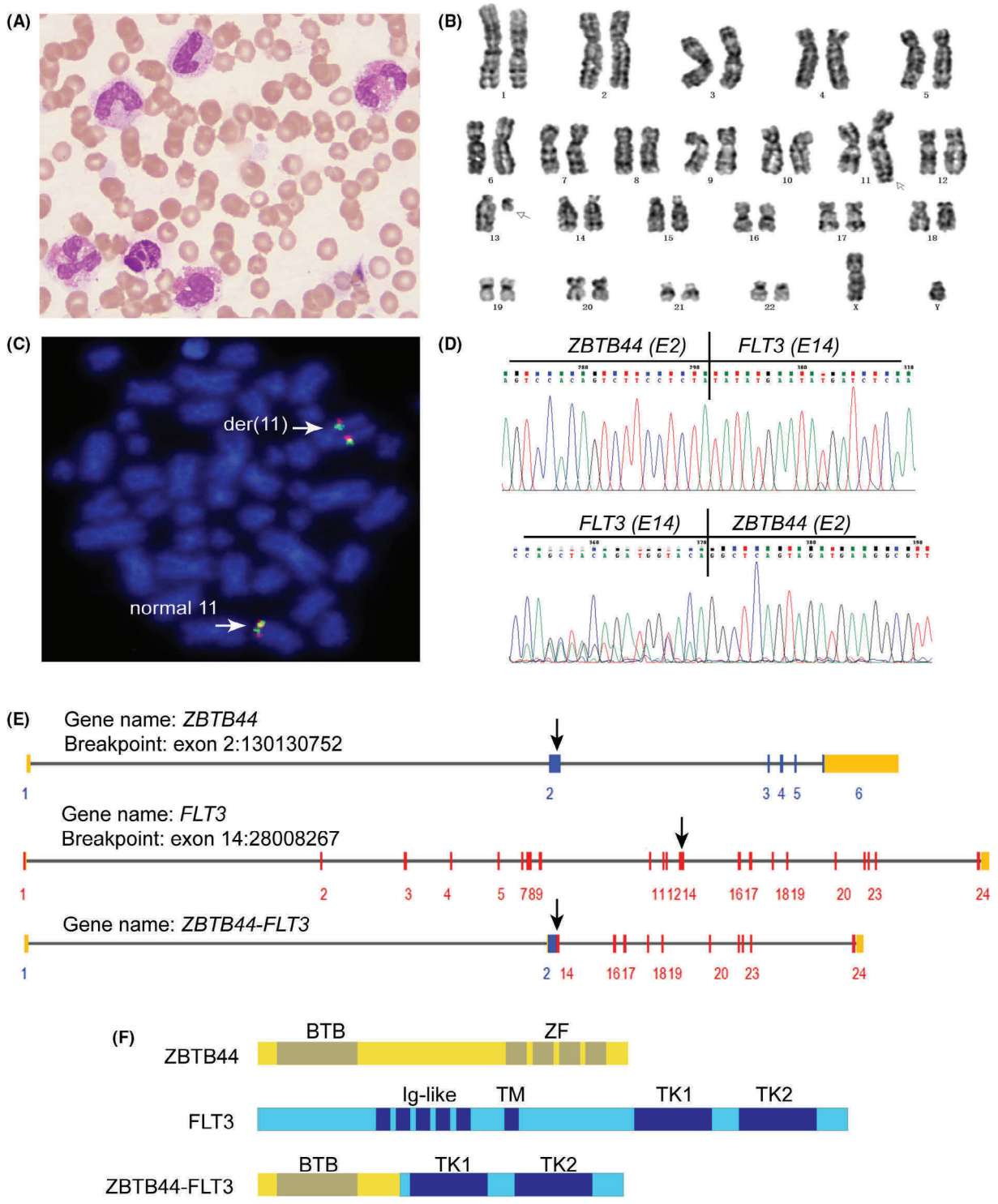

Fig 1. MPN with *ZBTB44-FLT3* rearrangement. (A) Wright stain of bone marrow aspirate smears showed marked granulocytic proliferation with eosinophilia. (B) Chromosome analysis of the bone marrow aspirate showed a balanced translocation between chromosomes 11 and 13 as the sole change. (C) Fluorescence *in-situ* hybridization (FISH) on abnormal metaphase showed the *KMT2A* remaining on the derivative chromosome 11, consistent with a breakpoint telomeric to *KMT2A*. (D) Sanger sequencing of the reverse transcriptase-polymerase chain reaction (RT-PCR) products, both *ZBTB44–FLT3* (top) and its reciprocal *FLT3–ZBTB44* (bottom), confirmed the fusion between *ZBTB44* exon 2 and *FLT3* exon 14. (E) Genomic structure of *ZBTB44* and *FLT3* and the location of the breakpoints. (F) Functional motifs of ZBTB44, FLT3 and ZBTB44-FLT3 fusion. BTB: broad-complex, tramtrack and bric a brac, also known as the POZ domain; ZF, zinc fingers; IG-like, immunoglobulin-like loops; TM, transmembrane domain; TK, tyrosine kinase domain. [Colour figure can be viewed at wileyonlinelibrary.com]

Table I. Clinical, histological and genetic features of 15 cases of myeloid/lymphoid neoplasms with *FLT3* rearrangement.

Age/sex	Karyotype	Fusion with exon#	Other genes mutated	Histology	EOS	References (DOI)
33/M	46,XY,t(12;13)(p13;q12)	*ETV6* (E6)–*FLT3* (E14)	N/A	MPN,T-ALL	yes	10.1038/leu.2012.333
40/F	46,XX,t(12;13)(p13;q12)	*ETV6* (E5)–*FLT3* (E15)	None (53 genes)	MPN	yes	10.1038/leu.2014.168
49/M	46,XY,t(12;13)(p13;q12)[20]	*ETV6* (E6)–*FLT3* (E14)	None (35 genes)	CMML	yes	10.1101/mcs.a003079
60/M	46,XY,del(9)(q22),der(12)t(12;13)(p13;q14)t(9;13)(q34;q22),der(13)t(12;13)(p13;q14)[4]/46,XY[2]	*ETV6* (E4)–*FLT3* (E14)	N/A	MPN-eo,PTCL	yes	10.1182/blood-2011-03-343426
29/M	46,XY,t(12;13)(p13;q12)[10]	*ETV6* (E5)–*FLT3* (E14)	N/A	T-ALL,MPN-eo	yes	10.1182/blood-2011-03-343426
38/F	46,XX,t(12;13)(p13;q12)[19]	*ETV6–FLT3*	N/A	MPN-eo,MPAL	yes	10.1007/s12308- 014-0203-6
68/F	46,XX,t(12;13)(p13.1;q12)[28]/46,XX[2]	*ETV6* (E5)–*FLT3* (E14)	N/A	MPN-eo	yes	10.1038/sj.leu.2404266
47/M	46,XY,t(13;17)(q12;q12)[20]	*MYO18A* (E28)–*FLT3* (E14)	None (35 genes)	Atypical CML	no	10.1101/mcs.a003079
62/M	46,XY,t(13;14)(q12;q32)[18]	*TRIP11* (E16)–*FLT3* (E14)	KITD816V	MPN,SM, T-ALL	yes	10.1016/j.cancergen.2017.05.001
20 w/M	45,XY,-7,t(13;14)(q12;q32)	*CCDC88C* (E23)–*FLT3* (E14)	None (480 genes)	JMML	no	10.1038/s41375-019-0549-y
71/F	46,XX,t(3;13)(q13;q12)	*GOLGB1* (E14)–*FLT3* (E14)	N/A	MPN-eo,T-ALL	yes	10.1038/leu.2016.304
32/F	46XX,t(2;13;2;21)(p13;q12;q33;q11.2)	*SPTBN1* (E3)–*FLT3* (E13)	N/A	MPN	yes	10.1016/j.exphem.2007.07.002
48/F	46,XX (cryptic 8 Mb inversion at 13q12)	*ZMYM2* (E20)–*FLT3* (E14)	None (28 genes)	MPN-eo	yes	10.1038/leu.2017.240
47/M	46,XY (cryptic 8 Mb inversion at 13q12)	*ZMYM2* (E20)–*FLT3* (E14)	None (28 genes)	MPN-eo	yes	10.1038/leu.2017.240
64/M	46,XY,t(11;13)(q24;q12)([20]	*ZBTB44–FLT3*	None (118 genes)	MPN	yes	this study

myelomonocytic leukaemia (JMML) were reported in one case each. Thirteen of the 15 cases had eosinophilia. Interestingly, none of these cases showed an abnormal subclone in addition to the *FLT3* rearrangements by karyotype analysis. All cases had *FLT3* rearrangement as the sole change except for a JMML with additional monosomy 7 and an MPN with additional del(9q). Seven of these cases had DNA NGS assays of a varying number of targeted genes. None of them found additional gene mutations except for a *KIT* D816V in a patient with concurrent MPN and systemic mastocytosis. These results suggest a dominant role of *FLT3* rearrangement in the tumourigenesis of this group of tumours.

FLT3 activation by internal tandem duplication (FLT3-ITD) or TKD mutations is found in 25–30% of acute myeloid leukaemia (AML) and 35% of the early T-cell precursor lymphoblastic leukaemia (ETP-ALL). FLT3-ITD causes constitutional kinase activation by disrupting the inhibitory mechanism of the juxtamembrane domain (JMD), which binds negatively charged plasma membrane with its basic amino residues to block kinase accessibility.[7] TKD mutations led to kinase activation by changing the conformation of the activation loop to open the kinase catalytic pocket.[8] An interesting question is why aberrant FLT3 activation by various mechanisms causes different tumours. Both FLT3-ITD and TKD-mutated FLT3 contain an intact transmembrane domain (TD) and are expected to remain cell membrane-bound. However, an FLT3-rearranged protein lost its TD and is likely located within cells. The different cellular locations of the altered FLT3 may activate different oncogenic signaling pathways, contributing to the development of different tumours. Also, AML or ETP-ALL often has other mutations in addition to the activated FLT3, for example, *FLT3-ITD* is often seen in APL with *RARA* rearrangement. On the other hand, no other mutations are seen in myeloid/

lymphoid neoplasms with *FLT3* rearrangement. The additional difference likely involves the timing of when mutations occur. FLT3 rearrangement likely occurs in haematopoietic stem cells and thus can lead to both myeloid tumours and/or lymphoid tumours. The FLT3-ITD and TKD-mutated FLT3 likely occur after haematopoietic cells committed to myeloid or lymphoid differentiation.

FLT3-rearranged tumours are generally very aggressive. The FLT3 inhibitors sunitinib and sorafenib induced rapid response in several patients with *FLT3* rearrangements, although the diseases eventually relapsed.[9,10] It will be interesting to see how these patients respond to newer-generation FLT3 inhibitors such as midostaurin and gilteritinib, which are more specific and more powerful compared to earlier inhibitors. Hydroxycarbamide, a ribonucleotide reductase inhibitor, induced complete haematological and cytogenetic responses in two patients with *FLT3* rearrangement, although both cases relapsed after one year.[11,12] Our patient also responds well to hydroxycarbamide and is under close monitoring of the peripheral blood count. Currently, bone marrow transplantation remains the standard of care for long-term survival in this group of deadly diseases.

In summary, we described a novel *ZBTB44–FLT3* fusion in an MPN with eosinophilia. Together with the other 14 published cases, we concluded that *FLT3* fusion is a dominant driver in this group of similar diseases. The association of *FLT3* rearrangements with eosinophilic neoplasms supports the inclusion of *FLT3* rearrangements in the current WHO subgroup of myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA, PDGFRB* or *FGFR1* or with *PCM1-JAK2*.

Conflict of interest

The authors declare that they have no conflict of interest.

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Supporting Information

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

Table SI. Primers used for *ZBTB44–FLT3* fusion confirmation. Base-stacking calculation is used for melting temperature (T_m) estimation.

Table SII. Primers used for reciprocal *FLT3–ZBTB44* fusion confirmation. Base-stacking calculation is used for melting temperature (T_m) estimation.

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