ETV6/FLT3 fusion in a mixed-phenotype acute leukemia arising in lymph nodes in a patient with myeloproliferative neoplasm with eosinophilia

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Abstract The *ETV6/FLT3* fusion gene has been recently reported in association with myeloproliferative neoplasm with eosinophilia (MPN-Eo) and peripheral T cell lymphoma. Favorable clinical response to a targeted FLT3 tyrosine kinase inhibitor (FLT3 TKI) was noted. Here we report a novel phenotype associated with *ETV6/FLT3* rearrangement, in a 38-year-old female with presentation of progressive lymphadenopathy. Lymph node biopsy showed mixed-phenotype acute leukemia (MPAL) with expression of T cell and myeloid markers. Bone marrow morphology was consistent with MPN-Eo and no evidence of MPAL. Karyotype analysis revealed 46, XX, t(12;13)(p13;q12). *ETV6/FLT3* fusion was demonstrated by fluorescence in situ hybridization. This is the first report of *ETV6/FLT3* rearrangement showing a phenotype of extramedullary T/myeloid MPAL arising in the setting of MPN-Eo. We suggest addition of this entity to the WHO category of “myeloid/lymphoid neoplasms with eosinophilia,” particularly given the possibility of clinical response to FLT3 TKI in MPAL, a disease usually associated with poor prognosis.

Keywords *ETV6* · *FLT3* · Mixed-lineage acute leukemia · Myeloproliferative disorders

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Introduction

In the WHO 2008 classification, three diseases presenting as myeloproliferative neoplasm (MPN) with eosinophilia (MPN-Eo) are included in the category “myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* and *FGFR1*.” They all result from a fusion gene encoding an aberrant tyrosine kinase receptor (TKR), making them good candidates for treatment with tyrosine kinase inhibitors (TKI) [1, 2]. The *ETV6/FLT3* fusion gene has been recently reported in association with MPN-Eo [3] and concurrent MPN-Eo and T cell lymphoma [4]. The latter report of two patients suggests responsiveness to *FLT3* TKI [4]. We describe the fourth case of *ETV6/FLT3* rearrangement in the literature and, to our knowledge, the first case showing mixed-phenotype acute leukemia (MPAL).

Clinical history

A 38-year-old woman presented in June 2010 with a several-month history of progressive left cervical and supraclavicular lymphadenopathy. Initial complete blood count showed eosinophilia (hemoglobin 129 g/l, platelets $238 \times 10^9/l$, white blood cell count $11.5 \times 10^9/l$ with the following differential: 56 % neutrophils, 14 % lymphocytes, 6 % monocytes, 20 % eosinophils, and 4 % basophils). After preliminary evaluation with fine needle aspiration, a left supraclavicular lymph node excisional biopsy showed morphology and immunophenotype consistent with MPAL, T/myeloid (see below). A bone marrow aspiration and biopsy showed no evidence of MPAL but were consistent with an unusual MPN with increased eosinophils (see below).

The patient was treated with four cycles of a modified hyper-CVAD protocol (cyclophosphamide, vincristine, doxorubicin [Adriamycin], dexamethasone) [5], with asparaginase

25,000 U/m² added on day 5 of cycle 1, beginning in July 2010, followed by hematopoietic stem cell transplantation (HSCT) in May 2011 with a conditioning regimen consisting of fludarabine, IV busulfan, and 400-Gy total body irradiation. One month post-HSCT, she developed corticosteroid-resistant grade III graft-versus-host disease of the skin and gut, manifested by pancolitis. She was treated with antithymocyte globulin but developed progressive hepatorenal insufficiency and died in late June 2011.

Materials and methods

Five-color flow cytometry was performed using an FC-500 flow cytometer (Beckman Coulter, Miami, FL, USA) for acquisition and analysis. Beckman Coulter monoclonal antibodies CD1a (clone BL6), CD2 (39C1.5), CD3 (UCHT1), CD4 (SFC112T4D11), CD5 (BL1a), CD7 (8H8.1), CD8 (B9.11), CD10 (ALB1), CD11b (Bear1), CD11c (BU15), CD13 (SJ1D1), CD14 (RMO52), CD15 (80H5), CD19 (J3-119), CD20 (B9E9), CD30 (HRS4), CD33 (D3HL60.251), CD34 (581), CD36 (FA6.152), CD45 (J.33), CD56 (N901), CD61 (SZ21), CD64 (22), CD117 (104D2D1), CD123 (SSDCLY107D2), CD235a (11E4B-7-6), and HLA-DR (Immu-357) and DAKO antibodies: sKappa (Polyclonal) and sLambda (Polyclonal) conjugated to the fluorochromes peridinin-chlorophyll protein, fluorescein isothiocyanate, phycoerythrin, and allophycocyanin were used according to the manufacturer's instructions.

For immunohistochemical studies, paraffin-embedded tissue sections were stained using an autostainer (Ventana Medical Systems, Tucson, AZ) with the following antibodies: CD7 (clone CBC.37), Tdt (Polyclonal), MPO (Polyclonal), CD117 (Polyclonal), Lysozyme (EC.3.2.1.17), CD45 (2B11+PD7/26), and CD20 (L26) all from DAKO, Glostrup, Denmark; CD2 (AB75), CD3 (LN10), CD33 (PW544), and CD10 (56C6) (Leica, Richmond, IL, USA); CD5 (SP19) (Ventana Medical Systems, Tucson, AZ); TIA-1 (TIA-1) (Biocare Medical, Concord, CA, USA); CD1a (O10) (Beckman Coulter, Miami, FL, USA); CD56 (MRQ-42) (Cell Marque, Rocklin, CA, USA); and CD57 (NK1) (BD Pharmingen, San Diego, CA, USA).

Fluorescence in situ hybridization (FISH) was performed on interphase cells, 4-μm unstained FFPE sections from the lymph node biopsy. A Vysis LSI ETV6 (TEL) dual-color break-apart rearrangement probe was used to confirm ETV6 rearrangement. Dual-color break-apart FISH was also performed using custom-ordered bacterial artificial chromosome (BAC) probes flanking FLT3 on both the bone marrow and the lymph node tissue. Fluorescently labeled BAC probes were obtained through The Centre for Applied Genomics (TCAG) in Toronto and were chosen based on their location

flanking the FLT3 gene on proximal chromosome 13. BACs RP11-106B15, RP11-795D2, RP11-153M24, and RP11-9D14 were labeled with spectrum orange, and BACs RP11-35M5 and RP11-502P18 were labeled with spectrum green. FISH was performed according to standard procedures [6]. Fluorescence signals were captured after counterstaining with 4'-6-diamidino-2-phenylindole (DAPI) using the Metasystems Imaging System attached to a Zeiss Z2 fluorescence microscope (Zeiss Axio Imager, Göttingen, Germany) equipped with a triple bandpass filter set (DAPI/Green/Orange), dual bandpass filter set (Green/Orange), and single bandpass filters (DAPI, Green, Gold, Aqua, Orange, and Red). A minimum of 100 nonoverlapping interphase nuclei were scored for each probe.

RNA extracted from the bone marrow biopsy was assessed for the presence of FIP1L1-PDGFRα fusion transcripts by real-time quantitative PCR using primers specific for exons 7, 8, 9, and 10 of the FIP1L1 gene and for exon 13 of the PDGFRα gene and detected using a labeled TaqMan probe for the PDGFRα gene. The primer sequences (5' to 3') for FIP1L1 were AAA CAG GAT ACG AAT GGG ACT TG (exon 7), GCA GAG ATC CAA GAT GGC AGA T (exon 7A), CCC TTC CAT CTA CAA AAG CTG AGT (exon 8), ATA TGG GAG GGC CGA ATC A (exon 8a), GGG CAA ATG AGA ACA GCA ACA (exon 9), and CAC TGC TCC ACC TCT GAT TCC A (exon 10). The primer sequence (5' to 3') for PDGFRα was GCA TCT TCA CTG CAA CTT TCA TG (exon 13). The sequence (5' to 3') for the probe was FAM-AAC AGC CTA TGG ATT AAG CCG GTC CCA-TAMRA.

This study was performed in accordance with the University Health Network ethics protocol.

Results

A left supraclavicular lymph node biopsy revealed an infiltrate composed of intermediate to large blastoid cells, with intermixed eosinophils, histiocytes, and small lymphocytes (Fig. 1a). Immunohistochemistry demonstrated a cell population coexpressing CD33 and CD7 (Fig. 1b, c). Some cells in the same area were also positive for TdT (Fig. 1d). Myeloperoxidase and CD117 were detected only in a fraction of cells (Fig. 1e, f). Cells positive for cytoplasmic CD3 were seen in both areas (Fig. 1g). In some areas, the two populations were intermixed, but one or the other appeared to predominate in other areas. Microscopic examination of the fine needle aspirate showed a population of abnormal blastic cells predominantly consisting of medium-size cells with high N/C ratio mixed with small lymphoid cells and large cells with fine chromatin and predominant nucleoli. Many cells presented irregular nuclear contours and a moderate amount of clear cytoplasm. A population strongly positive for CD7,

cytoplasmic CD3, and TdT and coexpressing CD33 but negative for MPO was found by flow cytometry (Fig. 2). The combined marker profile by flow cytometry and immunohistochemistry on these samples indicated a mixed myeloid and T cell lineage which fulfilled WHO 2008 criteria for a diagnosis of MPAL, T/myeloid:

T precursor population: positive for CD 45, CD2, CD5, CD7, CD11c, cytoplasmic CD3, TdT, CD33, and TIA-1
Myeloid population: positive for CD117, CD33, CD7, MPO, and lysozyme

Both cell populations were negative for surface CD3, CD34, CD1a, CD56, CD57, CD4, CD8, CD19, CD10, CD 20, TcR alpha/beta, and TcR delta/gamma.

Blood film showed hypereosinophilia with hypersegmented and partially degranulated aberrant forms, as well as left-shifted neutrophils with hypogranulation and some granulopoietic precursors but no increase of blasts.

A bone marrow aspiration and biopsy showed no evidence of a T precursor population by flow cytometry but were consistent with an unusual MPN with increased eosinophils. The bone marrow biopsy (Fig. 3a) and aspirate showed high cellularity with dysplastic left-shifted granulopoiesis and eosinophilia but no increase of blasts. Aberrant megakaryocytes were confirmed by CD61 immunostaining (Fig. 3b). CD34+ blasts were rare (Fig. 3c). CD117 showed some myeloid precursors and increased numbers of mast cells (Fig. 3d). Few clusters of CD123-positive dendritic cells were noted (Fig. 3e). CD3-positive T cells were loosely scattered (Fig. 3f).

GTG-banded karyotype showed a balanced translocation t(12;13)(p13;q12) as a sole abnormality in 19 of 20 cells. Interphase FISH with the Vysis ETV6 dual-color break-apart

probe set confirmed that *ETV6* was rearranged in 77 % of cells in the lymph node and 76 % of cells in the bone marrow (Fig. 4a, b). Using BAC probes in the *FLT3* (13q12) region, the breakpoint was determined to be centromeric to the telomeric end of the RP11-9D14 probe (AL445262.7) and telomeric to the RP11-795D2 probe (Fig. 4c, d). These findings are consistent with the *FLT3* breakpoints previously defined by PCR by Vu et al. [3] and Walz et al. [4].

FIP1L1-PDGFR fusion transcripts were not detected in the bone marrow sample by RT-PCR, with a lower limit of detection of 1:1,000 to 1:10,000. There was no evidence of a clonal rearrangement of the TCR-beta or TCR-gamma gene regions by PCR.

Discussion

We describe here the first case of *ETV6/FLT3* rearrangement showing a phenotype of MPAL arising in the setting of MPN-Eo. Given the usual poor prognosis of MPAL and lack of effective therapies, the potential role for *FLT3* TKI is compelling, considering the effectiveness of other TKR targeted therapies such as imatinib and sunitinib in MPN that involve gene rearrangements of similar TKRs (*PDGFRA* and *PDGFRB*) [7, 8].

ETV6 (previously named TEL) recurrently fuses with a broad range of genes from many different classes; to date, 28 translocations and 30 partner genes have been identified, commonly found in ALL, acute myeloid leukemia (AML) and other myeloid neoplasms such as myelodysplastic syndrome (MDS), and chronic myelomonocytic leukemia (CMML) [9]. *ETV6* is also a common partner gene in MPN-

Fig. 1 Lymph node showing areas with aberrant immature cells with blast morphology and increased eosinophils (a) (H&E, obj. $\times 40$). Immunohistochemical findings in the lymph node: Most cells in the lymph node are positive for CD33 (b) and CD7 (c). TdT-positive cells are unevenly distributed (d). In some areas, most cells are positive for

myeloperoxidase (e). CD117-positive cells are increased in areas with dominance of myeloperoxidase-positive cells (f). CD3-positive cells (g) are found mainly in TdT-positive areas but also to some extent in the myeloperoxidase-positive areas (obj. $\times 40$)

Fig. 2 Flow cytometry studies were performed on the fine needle aspirate of the lymph node. A population of cells coexpressing CD33, CD7, CD2, CD5, Tdt, and cytoplasmic CD3 was found. The expression of

CD13 and CD11b was variable. Cells were negative for membrane CD3, CD8, and CD4 (not shown)

Fig. 3 Histologic and immunohistochemical findings in bone marrow biopsy: Morphology shows dominance of granulopoiesis with no increase of blasts (a). In some areas, an increase of aberrant CD61+ megakaryocytes was noted (b). CD34+ blasts were not increased (c),

and CD117 staining showed some positive precursors and increased numbers of mast cells (d). In some areas, clusters of CD123-positive dendritic cells were noted (e) while CD3-positive T cells were scarce (f)

Fig. 4 (a, b) Interphase FISH study with LSI ETV6 (TEL) dual-color break-apart rearrangement probe. The normal *ETV6* probed region at 12p13 appears as a fused red and green signal. Translocation rearrangement is demonstrated by separation of the red and green probes. **a** Bone marrow cell showing one green, one red, and one fusion, indicating presence of an *ETV6* rearrangement. **b** Lymph node cells; arrows indicate two cells with one green, one red, and one fusion signal, indicating presence of an *ETV6* rearrangement. **c, d** Using the following BAC

probes flanking the *FLT3* gene: RP11-106B15 and RP11-795D2 (both labeled in spectrum orange), and RP11-35M5 and RP11-502P18 (both labeled in spectrum green), and excluding the BAC probes RP11-153M24 and RP11-9D14 (labeled in spectrum red), produced a clean break-apart of one of the fusion signals (one fusion signal, one red signal, and one green signal) in a **c** bone marrow and **d** lymph node cell, indicating that the breakpoint is telomeric to the RP11-795D2 probe and centromeric to the RP11-35M5 probe

Eo with PDGFRB rearrangement [1]. Sunitinib is an *FLT3* TKI which has recently shown response in two patients with MPN-Eo and *ETV6/FLT3* translocation [4].

MPN-Eo was reported to be associated with the *ETV6/FLT3* fusion gene in two previous studies [3, 4] (Table 1). Vu et al. reported the *FLT3/ETV6* fusion gene for the first time in a 68-year-old female with MPN-Eo and a t(12;13)(p13;q12) [3]. Walz et al. described two patients with the *ETV6/FLT3* fusion gene. One was a 60-year-old male with diagnosis of MPN-Eo in accelerated phase in the bone marrow and findings suggestive of peripheral T cell lymphoma in the lymph node. The immunophenotype of the T cell lymphoma has not been detailed in this report. This patient had a complete and rapid initial response to the *FLT3* TKI sunitinib, including a complete cytogenetic response at 3 months, but eventually progressed with myeloid blast crisis at relapse. The second patient was a 29-year-old male with an initial diagnosis of peripheral T cell lymphoma, which was subsequently revised to T lymphoblastic leukemia/lymphoma at relapse post-therapy, and finally diagnosed with MPN-Eo after

autologous stem cell transplantation [4]. This patient also had an initial complete response of peripheral eosinophilia to sunitinib.

It is interesting to note that both our patient, and the patients described by Walz et al., showed involvement of both myeloid and T cell lineages, either at diagnosis or at different stages of the disease. In some reports of MPN-Eo with abnormalities of PDGFRA, PDGFRB, and FGFR1, development of acute leukemia with T myeloid mixed phenotype or coincidence of MPN and T lymphoblastic lymphoma has been described [10, 11]. These neoplasms are postulated to be disorders of pluripotent stem cells with the capacity to give rise to neoplasms of different lineages. Our case fulfills the criteria for an extramedullary MPAL (bilineage with myeloid/T phenotypes) arising in the setting of MPN-Eo, and the patients described by Walz et al. showed peripheral T cell lymphoma and T lymphoblastic leukemia/lymphoma phenotypes with an underlying or subsequent MPN-Eo [4].

Due to our and previous reports of *ETV6/FLT3* rearrangement associated with MPN-Eo, and the potential response to

Table 1 Comparison of this study with previous similar case reports

Reference	Diagnosis	Age/gender	Primary translocation	Treatment	Karyotype
Our study	LN ^a : T/Myeloid MPAL ^b BM ^c : MPN-Eo ^d	38/female	ETV6-FLT3	Hyper-CVAD ^e , stem cell transplant	46, XX, t(12;13)(p13;q12)[19]/ 46XX[1]
Vu et al. [3]	MPN-Eo ^f	68/female	ETV6-FLT3	IFN-alpha, hydroxyurea, imatinib (no response), chemotherapy (cytarabine, daunorubicin)	46, XX,t(12;13)(p13. 1;q12.3-13)[28]/46,XX[2]
Walz et al. [4]	LN: PTCL ^g BM: MPN-Eo (accelerated phase)	60/male	ETV6-FLT3	Sunitinib (with rapid complete normalization of peripheral blood counts, spleen size, and enlarged lymph nodes and complete cytogenetic response at 3 months), sorafenib, intensive chemotherapy (cytarabine, daunorubicin) CHOP ⁱ chemotherapy, hyper- CVAD, stem cell transplant at relapse, sunitinib (with initial complete response of peripheral eosinophilia)	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]
Walz et al. [4]	LN: PTCL revised to T-ALL ^h BM: MPN-Eo	29/male	ETV6-FLT3		Initially: 46, XY,t(12;13)(p13;q12) [10], later: 46, XY,t(12;13)(p13; q12)[4]/49, idem, +8, +15, +21[5]/ 46, XY[3]

^a Lymph node^b Mixed phenotype acute leukemia^c Bone marrow^d Myeloproliferative neoplasm with eosinophilia^e Cyclophosphamide, vincristine, doxorubicin [adriamycin], dexamethasone^f Myeloproliferative disorder^g Peripheral T cell lymphoma^h T lymphoblastic lymphomaⁱ Cyclophosphamide, doxorubicin [adriamycin], vincristine, prednisone

TKIs such as sunitib, we suggest that this genetic abnormality should be added to the WHO category of myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, and *FGFR1* [1].

Considering that all four patients found to have ETV6/FLT3 gene rearrangement have had a diagnosis of MPN-Eo at some stage of their disease, we suggest that the possibility of this gene rearrangement should be investigated in the differential diagnosis of MPN-Eo, particularly in patients with concurrent mature or precursor lymphoid neoplasms with T cell phenotypes or in patients with T cell neoplasms with bone marrow eosinophilia. This translocation is not subtle by routine G-banding karyotype, but if karyotype fails or is unavailable or to confirm the presence of this translocation, commercial break-apart FISH probes flanking the ETV6 gene can be employed to confirm the presence of an ETV6 gene rearrangement. We are not aware of a commercially available FLT3 break-apart FISH probe, but a custom FISH test such as the one developed in our lab using commercially available labeled BAC clones could be developed in most cytogenetics laboratories, as described in the “[Materials and methods](#)” section. Another possibility, which is potentially cheaper and more amenable to high-throughput workflow, would be a custom PCR/sequencing test based on the previously described breakpoints given by Vu et al. and Walz et al. [3, 4]. However, further evaluation of the variability of fusion breakpoints in such cases is needed to ensure the clinical sensitivity of a PCR-based assay. A FISH test would be more robust in that it would not be affected by minor breakpoint variations; on the other hand, a small cryptic insertion variant (not previously described for this rearrangement) could potentially be missed by FISH, while a PCR/sequencing-based test would be able to detect such a variant.

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Conflict of interest The authors declare that they have no conflict of interest.

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