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ETV6/FLT3 fusion gene detected in a patient with T-cell lymphoblastic lymphoma

CAP TODAY *and the Association for Molecular Pathology have teamed up to bring molecular case reports to* CAP TODAY *readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Washington University School of Medicine in St. Louis and Mayo Clinic, Rochester, Minn. If you would like to submit a case report, please send an email to the AMP at* amp@amp.org. *For more information about the AMP and all previously published case reports, visit* www.amp.org.

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Genetic alterations of the gene *FLT3*, especially internal tandem duplications in the juxtamembrane domain and point mutations in the tyrosine kinase domain, are commonly seen in patients with newly diagnosed myeloid leukemias. However, chromosome rearrangements involving the *FLT3* gene are extremely rare in hematologic malignancies. The *FLT3* gene has only a few known partner genes, including the gene *ETV6*, which encodes a transcriptional repressor. *ETV6* has a wide variety of translocation partner genes, several of which are tyrosine kinase genes. When *ETV6* is fused to a tyrosine kinase gene, the N-terminal helix-loop-helix domain of *ETV6* functions as a homodimerization motif that activates the tyrosine kinase domain of its partner gene. *ETV6/FLT3* fusions are extremely rare and have been reported in a small number of cases with myeloid/lymphoid mixed neoplasms in association with eosinophilia.

We present a patient with T-lymphoblastic lymphoma-associated eosinophilia. Chromosome analysis revealed translocation t(12;13)(p13;q12) in the bone marrow specimen. The follow-up mate-pair sequencing analysis refined the translocation breakpoints and identified the *ETV6/FLT3* gene fusion, which is potentially targetable by *FLT3*-specific tyrosine kinase inhibitors for treatment.

Case. A 22-year-old male patient presented to the clinic with rapid enlargement of the cervical lymph nodes. A complete blood count showed a normal white blood cell count with marked eosinophilia and slightly elevated absolute monocyte count. Computed tomography scan of the neck showed significant bilateral cervical lymphadenopathy at all anatomical levels. An excisional biopsy of a right enlarged deep cervical node was obtained. The lymph node specimen was extensively involved by T lymphoblastic lymphoma histologically characterized by immature-appearing CD3+ lymphoid cells with coexpression of Tdt, CD1a, CD2, CD4, CD5, and CD7. A significant subset of these cells showed coexpression of CD4 and CD8. A bone marrow aspirate/biopsy was obtained for histopathological evaluation. There was limited morphologic or immunophenotypic evidence of T-cell malignancy, with only scattered CD3+ T cells noticed in this bone marrow specimen. Instead, an increase of immature B cells were noticed in this bone marrow specimen. The patient was subsequently started on chemotherapy.

A follow-up positron emission tomography (PET)-CT scan at three months post-treatment initiation showed interval resolution of hypermetabolic activity. However, a later restaging PET-CT detected a widespread increase in fluorodeoxyglucose uptake involving multiple lymph node groups, and also an overall increased uptake in the bone marrow. Concurrent CBC also showed recurring eosinophilia indicative of disease relapse. A core needle axillary lymph node biopsy and a bone marrow aspirate/biopsy were then obtained and showed histopathologic features of disease persistence/relapse.

Chromosome analysis performed at diagnosis on bone marrow aspirate revealed a translocation involving chromosomes 12 and 13, with break-

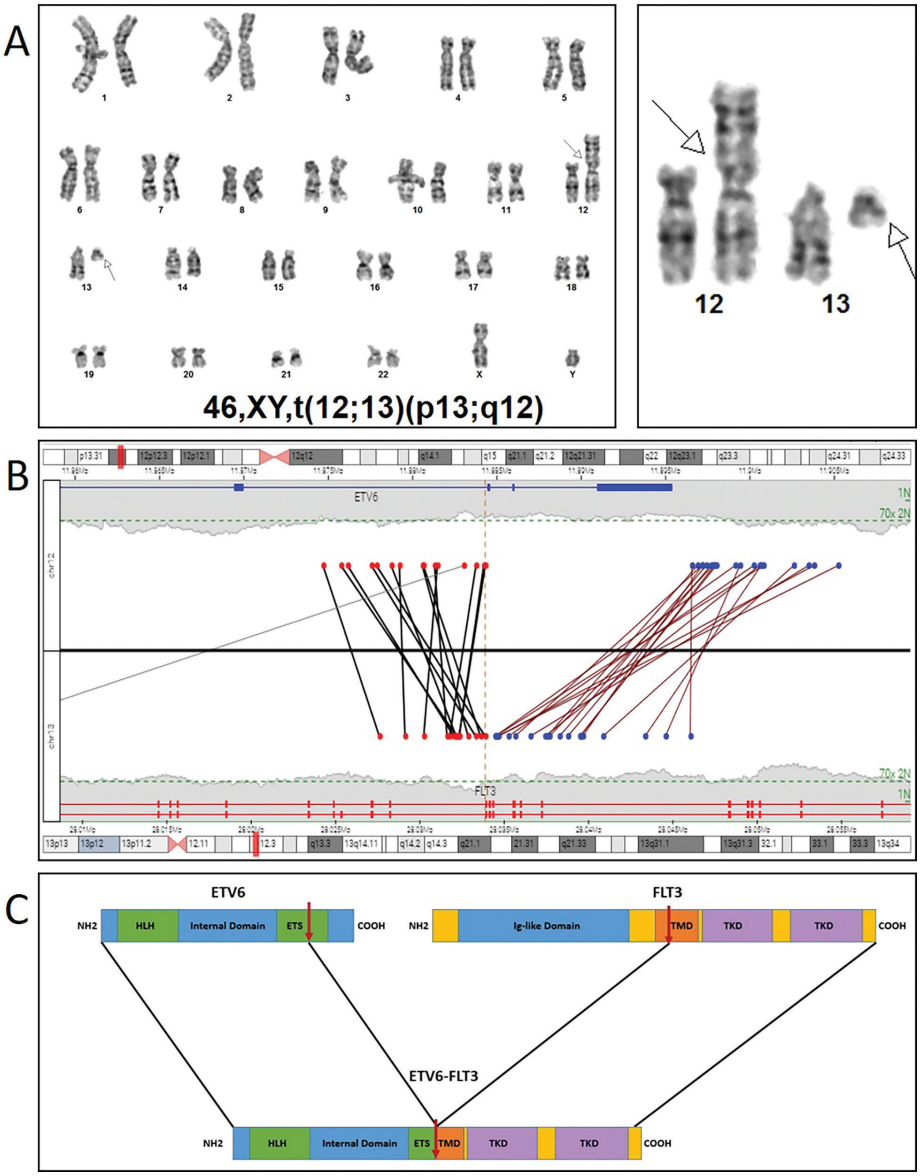

Fig. 1: Results of cytogenetic and NGS testing on bone marrow. **(A)** Chromosome analysis revealed a clonal translocation between chromosomes 12 and 13 with breakpoints at 12p13 and 13q12. **(B)** MPseq junction plot demonstrating a balanced translocation between *ETV6* (exons 1–6, transcript NM_001987) and 3' *FLT3* (exons 14–24, transcript NM_004119). **(C)** Schematic representation of *ETV6-FLT3* fusion genes and predicted oncoprotein structure. The predicted chimeric protein contains the 5' end of *ETV6* (including the helix-loop-helix domain, internal domain, and part of the ETS domain) and the 3' end of *FLT3* (including the transmembrane domain [TMD] and tyrosine kinase domain [TKD]). Breakpoints are indicated by red arrows.

points at 12p13 and 13q12 (**Fig. 1A**) in five of 20 metaphases. The same translocation was identified at six months post-chemotherapy initiation, detected in 14 of 20 metaphases. Fluorescence in situ hybridization analysis using probe panels targeting common cytogenetic abnormalities in T-cell and B-cell neoplasms were negative: no aneuploidies of chromosomes 4, 10, or 17; no rearrangements of *BCR/ABL1*, *MLL*, *IGH*, *ETV6/RUNX1*, *E2A*, *TRA/D*, or *TRB*; and no *CDKN2A* deletion were detected.

To further characterize this t(12;13), post-chemotherapy bone marrow aspirate specimen was sent to the Mayo Clinic clinical genomics laboratory for DNA-based next-generation sequencing mate-pair sequencing (MPseq) for breakpoint detection and characterization. The t(12;13) translocation was confirmed by MPseq with breakpoints located within the *ETV6* and the *FLT3* genes on chromosomes 12 and 13, respectively. This rearrangement is predicted to create an in-frame *ETV6/FLT3* fusion gene consisting of 5′ *ETV6* (exons 1–6, transcript NM_001987) and 3′ *FLT3* (exons 14–24, transcript NM_004119), seq[GRCh38]t(12;13)(12qter>12p13.2(11,896,468)::13q12.2(28,034,123)->13qter;12pter->12p13.2(11,884,677)::13q12.2(28,034,219)->13pter) (**Fig. 1B**). The fused 5′ end of *ETV6* encodes the helix-loop-helix domain (exons 3 and 4), the internal domain (exon 5), and part of the ETS domain (exons 6–8), whereas the fused 3′ end of *FLT3* includes the transmembrane domain (TMD) and the tyrosine kinase domain (TKD) (**Fig. 1C**).

Discussion. *ETV6/FLT3* fusions are very rare in hematologic malignancies, with only eight cases reported to date since 2006 (**Table 1,** page 3). This gene fusion is oncogenic and has been shown to induce interleukin-3-independent transformation of Ba/F3 murine hematopoietic cells in vitro and development of myeloproliferative neoplasms phenotype in transgenic mice.[1,2] Structural rearrangements involving *ETV6* are common in leukemias with more than 30 characterized partner genes.[3] Several of these identified *ETV6* partners encode tyrosine kinases, which are homo-dimerized when fused to the functional helix-loop-helix domain of *ETV6*, resulting in ligand-independent constitutive activation of the partner kinase domain receptors. Furthermore, *ETV6* fusions could also contribute to leukemogenesis by modifying the original functions of fused transcription factors, including the loss of transcriptional repression mediated by wild-type *ETV6*.[3,4]

Rearrangements of *ETV6* with other tyrosine kinase encoding genes such as *ABL1* and *FGFR1* are among described drivers of clonal eosinophilia.[5-7] With the growing list of recurrent rearrangements associated with eosinophilia, rearrangements involving *PDGFRA/B* and *FGFR1*, or the *PCM1-JAK2* rearrangement,

are now recognized by the 2016 WHO classification of hematopoietic tumors as part of the diagnostic criteria for myeloid/lymphoid neoplasms with eosinophilia.[8] The importance of the timely identification of these rearrangements lies in the potential response to tyrosine kinase inhibitor therapy. Although TKI monotherapy or with chemotherapy does not typically achieve long-term disease control, its early use for disease cytoreduction allows bridging to allogeneic stem cell transplant and seems to be associated with better outcomes.[9,10]

In this case, the *FLT3*-specific TKI, midostaurin, was initiated as an adjuvant to hyper-CVAD chemotherapy after the identification of *ETV6/FLT3* fusions. Following completion of chemotherapy, PET-CT scan showed resolution of hypermetabolic activity, and bone marrow biopsy was negative for disease involvement. The patient then proceeded to haploidentical bone marrow transplant. He continues to be in remission to date, over two years post-transplant. Although the genetic finding of an *ETV6/FLT3* fusion may lead to effective treatment using *FLT3*-specific TKIs, it is still unclear how this rearrangement correlates with the patient's hematopathologic findings.

In all previously reported cases (summarized in Table 1), the t(12;13)(12p13;13q12) rearrangements were not cryptic and were evident by karyotyping. Although these breakpoints are molecularly heterogeneous, the presence of eosinophilia, which is a constant feature (along with T-cell lymphoproliferative phenotypes or less specifically myeloid proliferative neoplasm), should raise

Table 1. Clinical data on case reports with *ETV6/FLT3* fusion

Literature	Age/ gender	Diagnosis	Eosinophilia %WBC	Genetic testing	Breakpoints	Response to TKI	Outcome
Current case	22/M	T-cell lymphoblastic lymphoma (T-LBL)	30.9%	Karyotyping, MPseq	In-frame fusion between *ETV6* exon 6 and *FLT3* exon 14	Negative PET-CT and bone marrow biopsy following midostaurin adjuvant therapy	Remission following haploidentical stem cell transplant
Vu, et al. 2006[2]	68/F	Myeloproliferative neoplasm (MPN)	54%	Karyotype, FISH, RT-PCR, RACE-PCR, nested PCR, sequencing	In-frame fusion between *ETV6* exon 5 and *FLT3* exon 14	Poor response to high-dose imatinib	Progression to blast phase. Death 1 month after induction chemotherapy.
Walz, et al. 2011[12]	60/M	Bone marrow biopsy: MPN in accelerated phase Lymph node biopsy: consistent with peripheral T-cell lymphoma (PTCL)	24%	Karyotype, FISH, RT-PCR, RACE-PCR, sequencing	In-frame fusion between *ETV6* exon 4 and *FLT3* exon 14	Sunitinib: complete cytogenetic response followed by relapse after 6 months	Rapid increase in myeloid blasts after 6 months. Pancytopenia following sunitinib reinitiation and acquisition of $FLT3^{N841K}$ resistance mutation. Death due to pancytopenia and infection.
	29/M	PTCL, later modified to T-cell lymphoblastic lymphoma	22%	Karyotype, FISH, RT-PCR, RACE-PCR, sequencing	In-frame fusion between *ETV6* exon 5 and *FLT3* exon 14	Sunitinib: transient response with resolved eosinophilia followed by relapse	Death after starting sunitinib
Falchi, et al. 2014[14]	40/F	Eosinophilia-associated MPN and myelofibrosis (MF-2) unclassified	12%	Karyotype, FISH, RACE-PCR, RT-PCR, nested PCR	In-frame fusion between *ETV6* exon 5 and *FLT3* exon 15	Sorafenib: complete hematologic response and partial cytogenetic response	Reported complete morphologic and cytogenetic remission following haplo-identical allogeneic stem cell transplant
Chonabayashi, et al. 2014[10]	33/M	T-LBL and eosinophilia-associated MPN	18%	Karyotype, FISH, RT-PCR	In-frame fusion between *ETV6* exon 6 and *FLT3* exon 14	NA	Reported complete molecular remission for up to 6 years following match allogeneic stem cell transplant
Hosseini, et al. 2014[15]	20/F	Extramedullary T-cell and mixed phenotype acute leukemia with eosinophilia-associated MPN	20%	Karyotype, FISH, RT-PCR	In-frame fusion between *ETV6* exon 5 and *FLT3* exon 14	NA	Allogeneic stem cell transplant complicated by severe graft-versus-host disease and death
Zhang, et al. 2018[16]	49/F	Chronic myelomonocytic leukemia	Present	Karyotype, FISH, RT-PCR, sequencing	In-frame fusion between *ETV6* exon 6 and *FLT3* exon 14	NA	Reported remission >43 months following allogeneic stem cell transplant

suspicion of an *ETV6/FLT3* rearrangement.[5,11] T-cell phenotypes associated with this rearrangement seem to commonly resemble T-cell lymphoblastic lymphoma, as reported in this case and other previous reports.[10,12] Employing an *ETV6* break-apart FISH probe could be useful to focus testing on identifying partner-fused genes. Subsequent testing using conventional FISH probes or nested PCR primers could also be pursued. However, this strategy would entail clinical validations of multiple gene-specific fluorescent probes or primer sets. Testing workups to determine eligibility for TKIs based on gene rearrangements can hence become extensive and ultimately uninformative in some cases.[13] With the availability and reduced cost of NGS-based sequencing methods capable of detecting structural rearrangements, there is potential for providing timely, cost-effective testing results that could be actionable for treating clinicians. This case is a perfect example to demonstrate superior performance of MPseq when identifying cryptic structural abnormalities across the entire genome and guiding treatment.

In summary, *ETV6/FLT3* rearrangement falls in the spectrum of oncogenic tyrosine kinase fusions causing eosinophilia and are potentially targetable by *FLT3* inhibitors. However, it remains unclear how this rearrangement leads to neoplastic lymphoid and/or myeloid phenotypes. The adoption of novel NGS-based methods such as MPseq will hopefully facilitate the characterization of more cases and increase our understanding of the underlying pathogeneses and presentations associated with *ETV6/FLT3* rearrangements. □

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Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereports *and will be published next month in* CAP TODAY.

1. Which of the following statements is true about *FLT3*?

a. *FLT3* mutations are identified in about five percent of patients with newly diagnosed myeloid leukemias.
b. *FLT3*-ITD indicates the internal tandem duplications in the tyrosine kinase domain.
c. *FLT3* activators have been approved for clinical use, leading to therapeutic paradigms for AML with *FLT3* mutations.
d. It is recommended to screen for *FLT3* mutations by the National Comprehensive Cancer Network guidelines for new acute leukemia cases.

2. Which of the following tests could potentially refine the breakpoints of a translocation detected in chromosomal analysis?

a. Chromosome microarray.
b. NGS-based mate-pair sequencing.
c. MLPA.
d. STR analysis.

3. Which of the following tyrosine kinase fusion genes is *not* recognized by the 2016 WHO classification of hematopoietic tumors as part of the diagnostic criteria for myeloid/lymphoid neoplasms with eosinophilia?

a. *ETV6-RUNX1.*
b. *ETV6-PDGFRB.*
c. *PCM1-JAK2.*
d. *ZMYM2-FGFR1.*