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

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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-
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)(12pter>12p13.2 (11,896,468)::13q12.2(28,034,123)>13pter;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 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. Structural rearrangements involving ETV6 are common in leukemias with more than 30 characterized partner genes. 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.

Rearrangements of ETV6 with other tyrosine kinase encoding genes such as ABL1 and FGFR1 are among described drivers of clonal eosinophilia. With the growing list of recurrent rearrangements associated with eosinophilia, rearrangements involving PDGFRα/β 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. 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 cytoduction 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 neoplasms), should raise

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

<table>
<thead>
<tr>
<th>Literature</th>
<th>Age/ gender</th>
<th>Diagnosis</th>
<th>Eosinophilia %WBC</th>
<th>Genetic testing</th>
<th>Breakpoints</th>
<th>Response to TKI</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current case</td>
<td>22/M</td>
<td>T-cell lymphoblastic lymphoma (T-LBL)</td>
<td>30.9%</td>
<td>Karyotyping, MPseq</td>
<td>In-frame fusion between ETV6 exon 6 and FLT3 exon 14</td>
<td>Negative PET-CT and bone marrow biopsy following midostaurin adjuvant therapy</td>
<td>Remission following haploidentical stem cell transplant</td>
</tr>
<tr>
<td>Vu, et al. 20062</td>
<td>68/F</td>
<td>Myeloproliferative neoplasm (MPN)</td>
<td>54%</td>
<td>Karyotype, FISH, RT-PCR, RACE-PCR, sequencing</td>
<td>In-frame fusion between ETV6 exon 5 and FLT3 exon 14</td>
<td>Poor response to high-dose imatinib</td>
<td>Progression to blast phase. Death 1 month after induction chemotherapy.</td>
</tr>
<tr>
<td>Walz, et al. 201112</td>
<td>60/M</td>
<td>Bone marrow biopsy: MPN in accelerated phase</td>
<td>24%</td>
<td>Karyotype, FISH, RT-PCR, RACE-PCR, sequencing</td>
<td>In-frame fusion between ETV6 exon 4 and FLT3 exon 14</td>
<td>Sunitinib: complete cytogenetic response followed by relapse after 6 months</td>
<td>Rapid increase in myeloid blasts after 6 months. Pancytopenia following sunitinib reinitiation and acquisition of FLT3799L mutation. Death due to pancytopenia and infection.</td>
</tr>
<tr>
<td>Falchi, et al. 201414</td>
<td>40/F</td>
<td>Eosinophilia-associated MPN and myelofibrosis (MF-2) unclassified</td>
<td>12%</td>
<td>Karyotype, FISH, RACE-PCR, RT-PCR, nested PCR</td>
<td>In-frame fusion between ETV6 exon 5 and FLT3 exon 15</td>
<td>Sorafenib: complete hematologic response and partial cytogenetic response</td>
<td>Reported complete morphologic and cytogenetic remission following haplo-identical allogeneic stem cell transplant</td>
</tr>
<tr>
<td>Chonabayashi, et al. 201416</td>
<td>33/M</td>
<td>T-LBL and eosinophilia-associated MPN</td>
<td>18%</td>
<td>Karyotype, FISH, RT-PCR</td>
<td>In-frame fusion between ETV6 exon 6 and FLT3 exon 14</td>
<td>NA</td>
<td>Reported complete molecular remission for up to 6 years following match allogeneic stem cell transplant</td>
</tr>
<tr>
<td>Hosseini, et al. 201415</td>
<td>20/F</td>
<td>Extramedullary T-cell and mixed phenotype acute leukemia with eosinophilia-associated MPN</td>
<td>20%</td>
<td>Karyotype, FISH, RT-PCR</td>
<td>In-frame fusion between ETV6 exon 5 and FLT3 exon 14</td>
<td>NA</td>
<td>Allogeneic stem cell transplant complicated by severe graft-versus-host disease and death</td>
</tr>
<tr>
<td>Zhang, et al. 201816</td>
<td>49/F</td>
<td>Chronic myelomonocytic leukemia</td>
<td>Present</td>
<td>Karyotype, FISH, RT-PCR, sequencing</td>
<td>In-frame fusion between ETV6 exon 6 and FLT3 exon 14</td>
<td>NA</td>
<td>Reported remission &gt;43 months following allogeneic stem cell transplant</td>
</tr>
</tbody>
</table>
susception of an ETV6/FLT3 rearrangement. T-cell phenotypes associated with this rearrangement seem to commonly resemble T-cell lymphoblastic lymphoma, as reported in this case and other previous reports. 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.

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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