Novel *FIP1L1::KIT* fusion in a myeloid neoplasm with eosinophilia, T-lymphoblastic transformation, and dasatinib response

Myeloid/lymphoid neoplasms with eosinophilia (MLN-eo) with tyrosine kinase fusions are a family of hematolymphoid diseases with common shared features of blood eosinophilia and activating fusions involving tyrosine kinase genes.[1,2] *FIP1L1::PDGFRA* fusion is the most common genetic lesion in this class and is caused by a cytogenetically cryptic interstitial deletion at 4q12 that includes the *CHIC2* locus and that leads to fusion of the 5′ *FIP1L1* and 3′ *PDGFRA* genes, with retention of the *PDGFRA* tyrosine kinase domain.[3-6] Constitutive tyrosine kinase activation induces hematopoietic proliferation through stimulation of downstream targets involving the STAT5 pathway. MLN-eo with *FIP1L1::PDGFRA* fusion most commonly manifests as a myeloid neoplasm morphologically resembling chronic eosinophilic leukemia, but blastic manifestations, including T-lymphoblastic leukemia/lymphoma sometimes occur.[6,7] *KIT*, located 3′ to *PDGFRA* at 4q12, is a member of the same class III receptor tyrosine kinase family as *PDGFRA*, but despite the physical proximity and similar protein structure, *FIP1L1::KIT* fusions have not been described. We report a novel *FIP1L1::KIT* fusion in a myeloid neoplasm with eosinophilia that underwent blastic transformation with a T-cell phenotype.

A 79-year-old female was seen in consultation for leukocytosis. Laboratory work-up showed a white blood cell count (WBC) of 18.2×10^9/L, absolute neutrophil count of 12×10^9/L, absolute eosinophil count of 2.5×10^9/L, absolute monocyte and absolute lymphocyte count of 1.6×10^9/L each. She had normal hemoglobin and platelet counts. Bone marrow biopsy showed a hypercellular marrow (90%), left-shifted granulocytic hyperplasia, increased eosinophils, and otherwise morphologically unremarkable hematopoiesis. *BCR::ABL1* RT-PCR, *JAK2* V617F mutation testing, and karyotypic analyses were normal. Given the monocytosis, a presumptive diagnosis of chronic myelomonocytic leukemia (CMML) was rendered. Since the patient was asymptomatic, she was followed with observation. Three months later, routine testing revealed a WBC of 166×10^9/L, hemoglobin of 113 g/L and platelet count of 31×10^9/L. The peripheral blood showed leukocytosis with a myeloid left shift, eosinophilia, monocytosis and basophilia (Figure 1A). The bone marrow was hypercellular with myelomonocytic expansion, dysplasia in the myeloid series and no increase in blasts, consistent with the prior diagnosis of CMML (Figure 1B, C). Mast cells were mildly increased but were phenotypically normal, without aggregates and with only very rare spindled forms. Given the eosinophilia, fluorescence *in situ* hybridization (FISH) studies to assess for eosinophilia-associated rearrangements were initiated. Meanwhile, the patient received hydroxyurea and decitabine initially. This was complicated by cytopenia, after which her counts recovered, and her absolute eosinophil count rose. The FISH studies were negative for *PDGFRA, PDGFRB, FGFR1,* and *JAK2* rearrangements; however, the *PDGFRA* FISH assay demonstrated loss of both the *CHIC2* and *PDGFRA* signals, with retention of the signal 5′ of *FIP1L1* (Figure 1D). The karyotype was normal, but whole-genome microarray analysis revealed a ~1.274 Mb loss in the 4q12 region with breakpoints within the *FIP1L1* and *KIT* genes, suggesting a *FIP1L1::KIT* fusion (Figure 1E). These findings were confirmed by RNA-sequencing studies that demonstrated an in-frame, intraexonic fusion joining exon 16 of FIP1L1 and exon 11 of *KIT*, with retention of the *KIT* tyrosine kinase domain (NM_030917.4:r.–198_1372::NM_000222.3:r.1679_*2158) (Figure 1F). The fusion breakpoint was confirmed in the specimen's genomic DNA by Sanger sequencing (Figure 1G). High-throughput DNA-sequencing studies identified a *STAG2* nonsense mutation. Based on these findings, the treatment was changed to dasatinib 20 mg daily. The patient's blood counts normalized within 1 month, consistent with a good response to treatment. After over 1 year of treatment, she suffered from intermittent gastrointestinal symptoms and infections resulting in prolonged interruptions of dasatinib. Consequently, the patient reported progressive night sweats and cervical lymphadenopathy. Her WBC increased to 20×10^9/L with 17% eosinophils. A repeat bone marrow biopsy showed a hypercellular marrow with myeloid predominant hematopoiesis and mild to moderate myelofibrosis. She could not tolerate higher doses of dasatinib and elected not to switch to an alternative agent. Four months later, the patient presented to the emergency department with shortness of breath. Her WBC was 3.8×10^9/L (WBC trend in Figure 2A). Imaging showed bilateral moderate pleural effusions, diffuse lymphadenopathy, and moderate splenomegaly. She underwent an axillary lymph node biopsy that showed sheets of blasts expressing the T-cell-associated markers CD3 (cytoplasmic), CD2, CD7, and CD5 (<75% of blasts), with expression of some myeloid- and immaturity-associated markers such as CD34 (partial), CD33, CD117, and TdT (partial). Staining for CD1a was negative (Figure 2B, D). FISH on the lymph node confirmed loss of 4q12. The bone marrow was hypercellular with increased

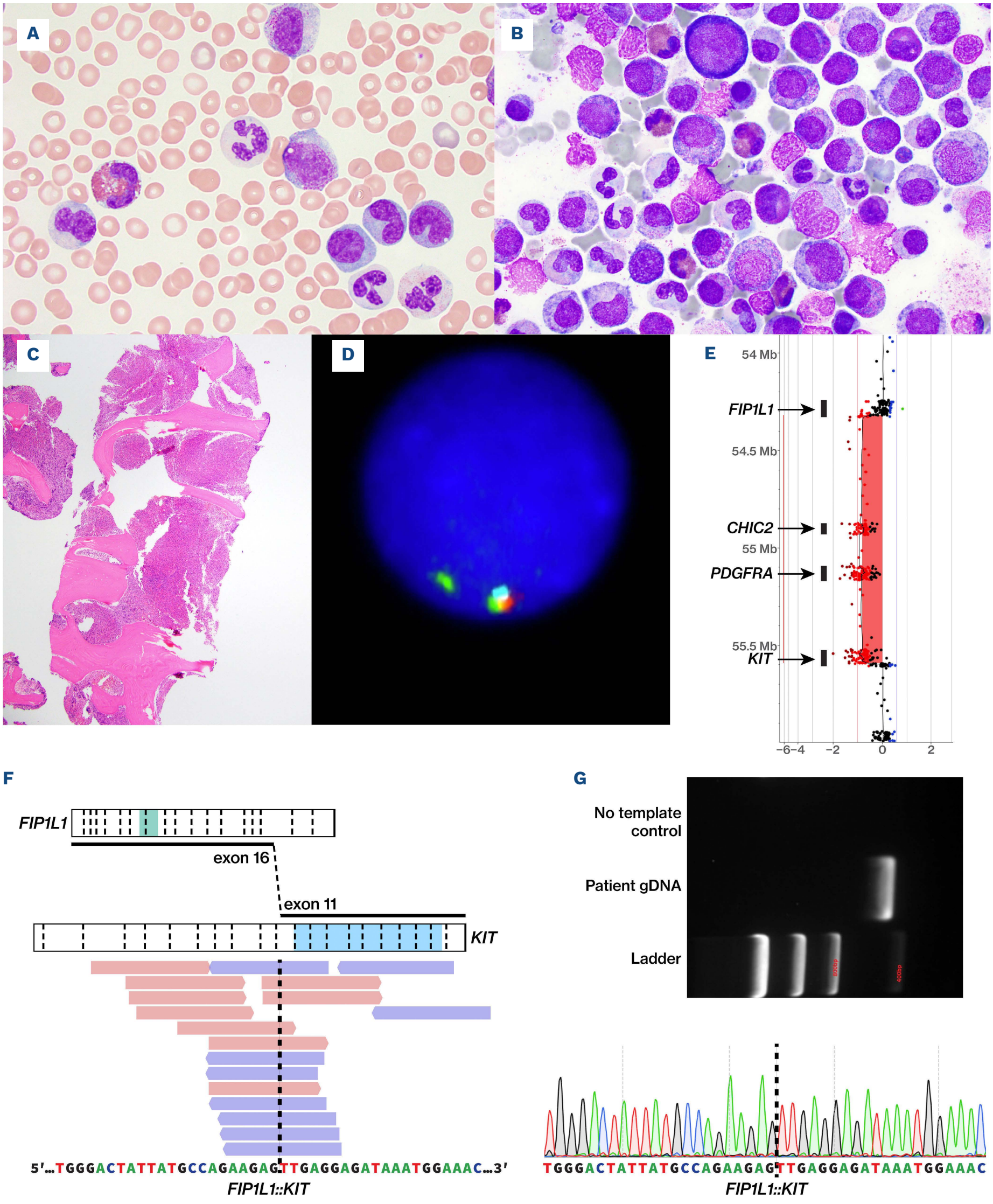

Figure 1. Pathologic and molecular characteristics of the patient's disease at diagnosis. (A) Representative image of the Wright-Giemsa-stained blood smear at diagnosis, exhibiting granulocytic left shift, granulocytic dysplasia, monocytosis, and eosinophilia (original magnification ×1,000). (B) Wright-Giemsa-stained bone marrow aspirate demonstrating left-shifted granulocytic matu-

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ration, granulocytic dysplasia, and increased monocytic and eosinophilic precursors (original magnification ×1,000). (C) Hematoxylin and eosin-stained bone marrow core biopsy demonstrating marked hypercellularity (original magnification ×40). (D) Fluorescence *in situ* hybridization pattern showing a loss of both the red (*CHIC2*) and aqua (3′ to P*DGFRA*) signals with retention of the green (5′ to *FIP1L1*) signal on one chromosome, with a normal green-red-aqua fusion pattern on the other chromosome. (E) Array comparative genomic hybridization assay demonstrating an ~1.274 Mb deletion of chromosome 4q12, with loss of exons 17-19 in *FIP1L1* and exons 1-10 of *KIT*. (F) Illustration of RNA-sequencing findings that identified an in-frame, intra-exonic fusion of exon 16 of *FIP1L1* with exon 11 of *KIT*. The green shading in *FIP1L1* represents the FIP1 motif, while the blue shading in *KIT* represents the tyrosine kinase domain. (G) The breakpoints were confirmed in the genomic DNA by amplifying a product using primers targeting intron 15 of *FIP1L1* and intron 11 of *KIT* (gel image). Sanger sequencing of this polymearse chain reaction product confirmed the fusion sequence identified by RNA sequencing (bottom panel). The dashed lines indicate the fusion site.

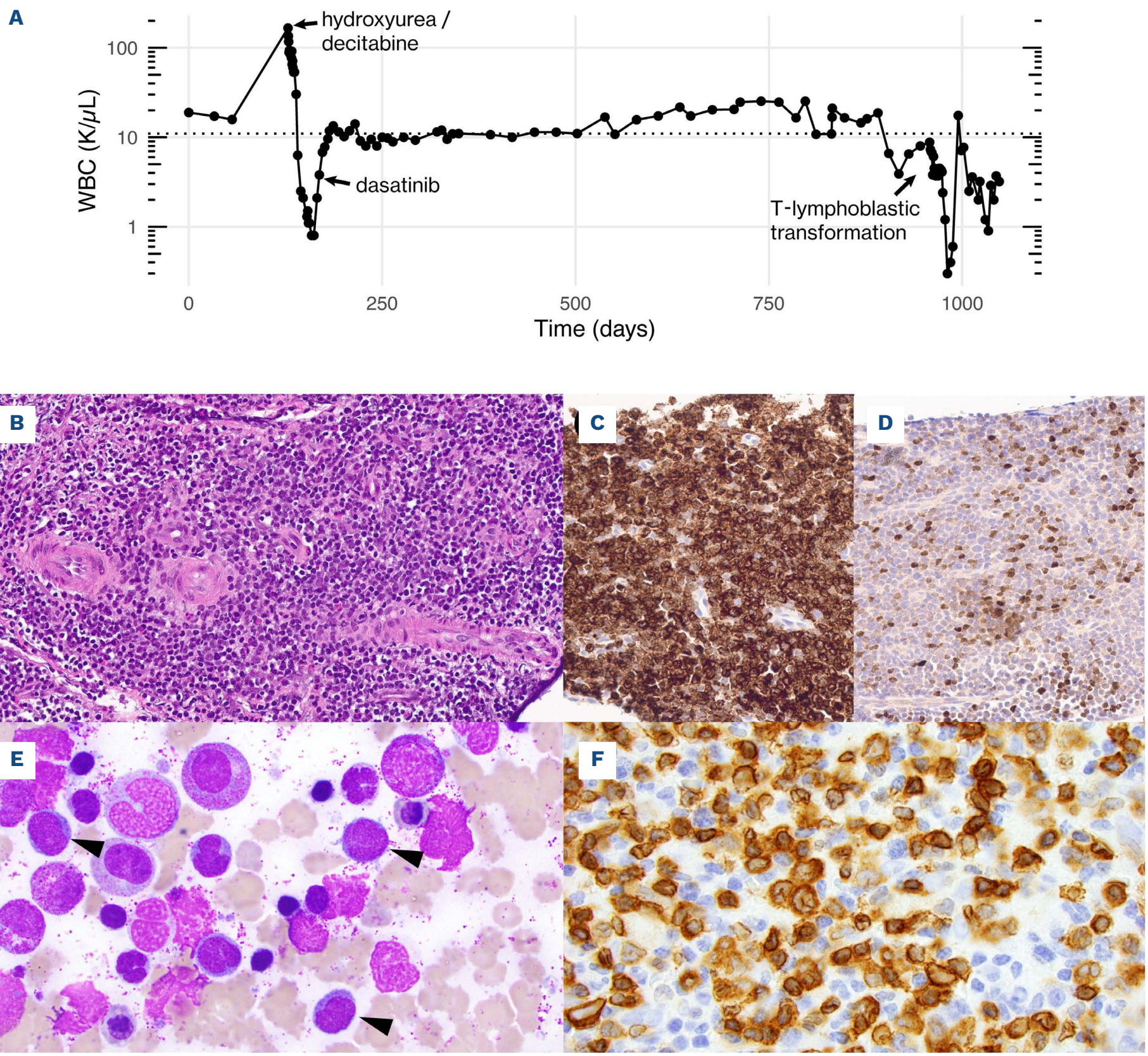

Figure 2. Response to therapy over time and subsequent relapse. (A) Plot showing the patient's white blood cell count over time (plotted on a log scale) and response to tyrosine kinase inhibition. The dotted line indicates a value of 11 K/μL. (B) Hematoxylin and eosin-stained section of the patient's left axillary lymph node, demonstrating sheets of mononuclear cells morphologically consistent with blasts (original magnification ×400). Immunohistochemical stains demonstrated that the blasts were strongly positive for CD3 (C) and partially expressed TdT (D) (original magnifications ×400). (E) Concurrent bone marrow aspirate demonstrated an increased blast population (arrowheads), along with maturing myeloid cells (original magnification ×1,000). (F) Immunohistochemical staining for CD3 in the bone marrow biopsy showed increased, morphologically atypical blastoid cells (original magnification ×1,000).

blasts with a T-lineage phenotype as seen in the lymph node, along with maturing myeloid cells (Figure 2E, F). The findings were diagnostic of a blastic transformation of the myeloid neoplasm, and the blasts exhibited a phenotype compatible with an early T-precursor leukemia. Repeat genetic testing on the bone marrow demonstrated persistent evidence of *FIP1L1::KIT* fusion, as FISH and microarray studies showed persistent loss of the *CHIC2* and *PDGFRA* loci at 4q12, along with karyotypic evolution: 46,XX,dic(7;20)(p11.2;q13.3),del(12)(p13p11.2)[6]/46XX[14]. DNA sequencing identified a persistent mutation of *STAG2*, with new mutations in *RUNX1, DNMT3A,* and *WT1*. Given the transformation to T-cell lymphoblastic leukemia, the patient received one cycle of cyclophosphamide, vincristine, dexamethasone (mini-HyperCVD), and intrathecal methotrexate, with minimal response. Lymphadenopathy progressed despite two cycles of nelarabine. Consequently, dasatinib was restarted at a dose of 100 mg daily, with significant improvement in cervical and axillary lymphadenopathy.

We report a case of a myeloid neoplasm with monocytosis and eosinophilia harboring *FIP1L1::KIT* fusion that underwent a blastic transformation with an early T-precursor immunophenotype. *KIT*, a receptor tyrosine kinase that plays a key role in hematopoiesis, is recurrently mutated in myeloid neoplasms such as systemic mastocytosis and acute myeloid leukemia, but only very rare reports have described activating fusions involving *KIT* in myeloid neoplasms.[8] To our knowledge, the *FIP1L1::KIT* fusion has not been previously described; however, the *FIP1L1::KIT* fusion structure resembles that of *FIP1L1::PDGFRA*, with the entire intracellular domain with tyrosine kinase activity present at the C-terminal end of the fusion gene.

Imatinib is a tyrosine kinase inhibitor (TKI) that inhibits signaling mediated by KIT and PDGFRA.[9,10] Imatinib results in clinical and molecular response when used in patients with MLN-eo with *FIP1L1::PDGFRA*.[3,4,11-13] Dasatinib, a second-generation TKI, inhibits the growth of MLN-eo with *FIP1L1::PDGFRA* through several mechanisms, including the dephosphorylation of *FIP1L1::PDGFRA*.[14] In *in vitro* studies, dasatinib had a 67-fold higher potency than imatinib in the inhibition of PDGFRA in animal and human cells.[15] Dasatinib resulted in a clinical response in our patient both initially and after blastic transformation. Her initial relapse occurred following multiple dasatinib interruptions, similar to the reported cases of relapse in MLN-eo with *FIP1L1::PDGFRA* after imatinib discontinuation.[13]

Both the 5th edition of the World Health Organization Classification of Hematolymphoid Tumors and the International Consensus Classification of Myeloid Neoplasms and Acute Leukemias include a category of MLN-eo with tyrosine kinase gene fusions, and in each classification specific tyrosine kinase genes involved in the fusions in this category are listed.[1,2] *KIT* fusions are not specifically mentioned in either classification system, though the World Health Oraganization classification does include the possibility of rare fusions involving unspecified kinases as belonging to the category. Our findings, including the patient's long-term response to single-agent dasatinib, support that *KIT* fusions are targetable genetic lesions in myeloid/lymphoid neoplasms and support the explicit inclusion of *KIT* fusions in this group of neoplasms.

KIT fusions are presumably rare in myeloid neoplasms. The identification of the *KIT* fusion in this patient was fortuitous, as it was identified in the microarray and *PDGFRA*-directed FISH studies since it was caused by an interstitial deletion in a genomic region of known interest. It is likely that *FIP1L1::KIT* fusions are very rare, since many laboratories assess this region in cases with eosinophilia. Our results, however, provide a rationale to further work up unusual FISH signal patterns in this region, as they may indicate a variant, clinically important fusion. Furthermore, our routine testing would likely not identify other *KIT* fusion partners, and *KIT* fusions are not included in many commercially available fusion panels such as anchored multiplexed polyerase chain reaction platforms, presumably since *KIT* is not currently recognized as a fusion partner in MLN-eo. This case emphasizes the utility and clinical relevance of broad, unbiased genomic testing in myeloid neoplasms to identify unexpected druggable targets.

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Contributions

JK, SC, BR, NA, SAY, and NGB performed pathologic review, diagnostic testing and contributed to manuscript writing. RF and RA performed clinical, diagnostic and response evaluations, and provided patient care. AA, TSW, NGB, SAY and RF drafted the manuscript. All authors reviewed and approved the final manuscript.

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Data-sharing statement

For additional information please contact the corresponding author.

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