Myeloid/lymphoid neoplasms with eosinophilia/basophilia and *ETV6-ABL1* fusion: cell-of-origin and response to tyrosine kinase inhibition

ETV6-ABL1 rearrangements have been reported in a spectrum of hematologic malignancies, including B- or T-acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and myeloproliferative neoplasms (MPN).[1-11] Mostly reported as single cases, *ETV6-ABL1* rearranged MPN shows clinical features mimicking chronic myeloid leukemia (CML) and empirically responds to tyrosine kinase inhibitors (TKI). Therefore, these cases are commonly diagnosed as Philadelphia chromosome negative CML (Ph^- CML), CML with atypical ABL1 fusions, or even atypical CML. Transformation to AML, B-ALL and T-ALL has also been observed in a subset of cases.[3-8] In addition, eosinophilia is a hallmark in nearly all cases, proportionally much greater than seen in CML. On the other hand, a few *de novo* AML and ALL cases with this fusion also present with eosinophilia,[9,10] raising the possibility of a progression from an underlying chronic phase of myeloid/lymphoid neoplasm, similar to those seen in *PDGFRA, PDGFRB, FGFR1* and *PCM1-JAK2* rearrangements. Here we report six patients with myeloid/lymphoid proliferation and *ETV6-ABL1* fusions and review the literature. Our findings support the classification of such cases as myeloid/lymphoid neoplasms with eosinophilia/basophilia and *ETV6-ABL1* fusion, similar to the category of myeloid/lymphoid neoplasms with eosinophilia and rearrangements of *PDGFRA, PDGFRB,* or *FGFR1*, or with *PCM1-JAK2* listed in the World Health Organization classification.[12]

A search for cases at Memorial Sloan Kettering Cancer Center from January 2014 to December 2019 was performed and identified five patients with *ETV6-ABL1* fusions and we also include a case from University Hospital Cleveland Medical Center (Cleveland, OH, USA). The clinicopathologic features including laboratory findings, pathologic evaluation, and cytogenetic and molecular results are summarized in Table 1. Two female and four male patients were included, with a median age of 49.5 years (range 23-88 years). All six patients presented with myeloid proliferation and eosinophilia: four patients were diagnosed as CML, atypical CML or CML with atypical *ABL1* fusion, one as essential thrombocythemia (ET) based on morphologic findings and peripheral blood counts, and one as myeloid/lymphoid neoplasm with eosinophilia and *ETV6-ABL1* fusion. Five patients (with the exception of Patient 4) were treated with either first- or second-generation TKI (imatinib, dasatinib and nilotinib) and showed complete cytogenetic response a few months (range 2-6 months) after initiation of treatment. Patient 1 had cytogenetic and morphologic relapse after imatinib treatment for 10 years (*ABL1* mutational analysis failed) but again achieved cytogenetic remission 2 months after switching to dasatinib treatment. This patient continued to have cytogenetic remission 5 years after dasatinib. Patient 4 had a cryptic rearrangement not detected by routine karyotyping and was initially managed as ET. The patient failed multiple lines of treatment (hydroxyurea, Heat Shock Protein 90 inhibitor, ruxolitinib, anagrelide, and α-interferon), and progressed four years later to AML with marked basophilia. RNA-based sequencing studies revealed *ETV6-ABL1* fusion, confirmed by fluorescence *in situ* hybridization (FISH) analysis. Combined imatinib and cytarabine treatment was initiated; however, the patient died shortly after due to comorbid complications. Patient 5 responded to nilotinib treatment for 2 years then progressed to B-ALL (*ABL1* mutational analysis failed) and obtained complete remission after HyperCVAD. Patient 6 presented as myeloid sarcoma and T-lymphoblastic lymphoma (TLL) in two separate foci of the same neck node with no increased blasts in the marrow. She was treated with dasatinib for 8 weeks. Her lymphadenopathy and eosinophilia both resolved.

All patients had peripheral eosinophilia, ranging from 1.7-44.5x10^9/L. Patient 1 had 11% eosinophils in peripheral blood (PB) at relapse. Three (Patients 2-4) had eosinophilia in the marrow at the time of diagnosis. Peripheral basophilia was documented in Patients 3 and 5 at presentation, in Patient 1 at relapse (2% basophils in PB) and in Patient 4 at transformation (10% basophils in PB). Leukocytosis was widely variable, ranging from 9-374 x10^9/L. Patient 3 had anemia and thrombocytopenia. Patient 4 had marked thrombocytosis but no splenomegaly. Patient 5 had anemia. Patients 2-6 had diagnostic marrow biopsy for review that showed 90-100% cellularity and markedly increased M:E ratio. Blasts were not increased in any of the six patients at diagnosis. Megakaryocyte morphology was highly variable: both small and large forms in Patient 2, predominantly large forms in Patient 4, increased hypolobated forms in Patients 5 and 6, unremarkable in the other two cases (Figure 1A). There was no overt dysplasia in myeloid or erythroid lineages observed in any of the cases (*data not shown*).

FISH analysis using *ETV6* and/or *ABL1* break-apart probes detected the presence of the *ETV6* rearrangement in metaphase cells in four patients (Patients 1, 2, 4 and 5): *ABL1* rearrangement in Patient 2, an extra normal fusion signal in Patients 1 and 6 (*ABL1* gain), and normal signal pattern in both Patients 4 and 5. Patient 6 had no *ETV6* FISH testing but only *ABL1*. FISH was not performed on the diagnostic sample from Patient 3. Next generation RNA sequencing (RNAseq NGS) with a customized 199-gene panel (Archer FusionPlex) identified the *ETV6-ABL1* transcripts involving the same breakpoints with *ETV6* exon 5 and ABL1 exon2 in all six cases (Figure 1D and *Online Supplementary Table S1*). Next generation DNA sequencing was performed using FoundationOne Heme (Foundation Medicine 406 gene panel, Patients 1, 4 and 6) and MSK-IMPACT Heme (400 gene panel, Patients 2, 3 and 5). Patients 1 and 2 were positive for *ARID2* truncating mutations. In addition, Patient 1 had *TP53* point mutation while Patient 2 had *CDKN1B* truncating mutations. Patient 5 was positive for *SETD2* mutation. Patients 3, 4 and 6 were negative for additional mutations (*Online Supplementary Table S2*). While *ARID2* defect has been associated with megakaryocytic dysplasia,[13] in our study, one patient harboring an *ARID2* mutation had no megakaryocytic atypia (Patient 1) whereas the other showed variable megakaryocyte morphology (Patient 2), suggesting that the functional significance of *ARID2* mutations in such cases needs further investigation. Although *CDKN1B* expression level was reported to be a potential biomarker for prognostication in acute myeloid leukemia,[14] the biological role of this mutation in this entity remains unclear.

To investigate the downstream signaling pathway activation of *ETV6-ABL1* fusion, phosphorylation levels of STAT3, STAT5 and ERK were evaluated by immunochemistry using antibodies specific for phosphorylated proteins on the bone marrow biopsy from Patient 4. Although phospho-STAT3 was not increased, phospho-STAT5 showed a markedly increased signal, suggestive of a spe-

cific STAT5 activation as a downstream target (Figure 1B). ERK1/2 phosphorylation was also increased (Figure 1C).

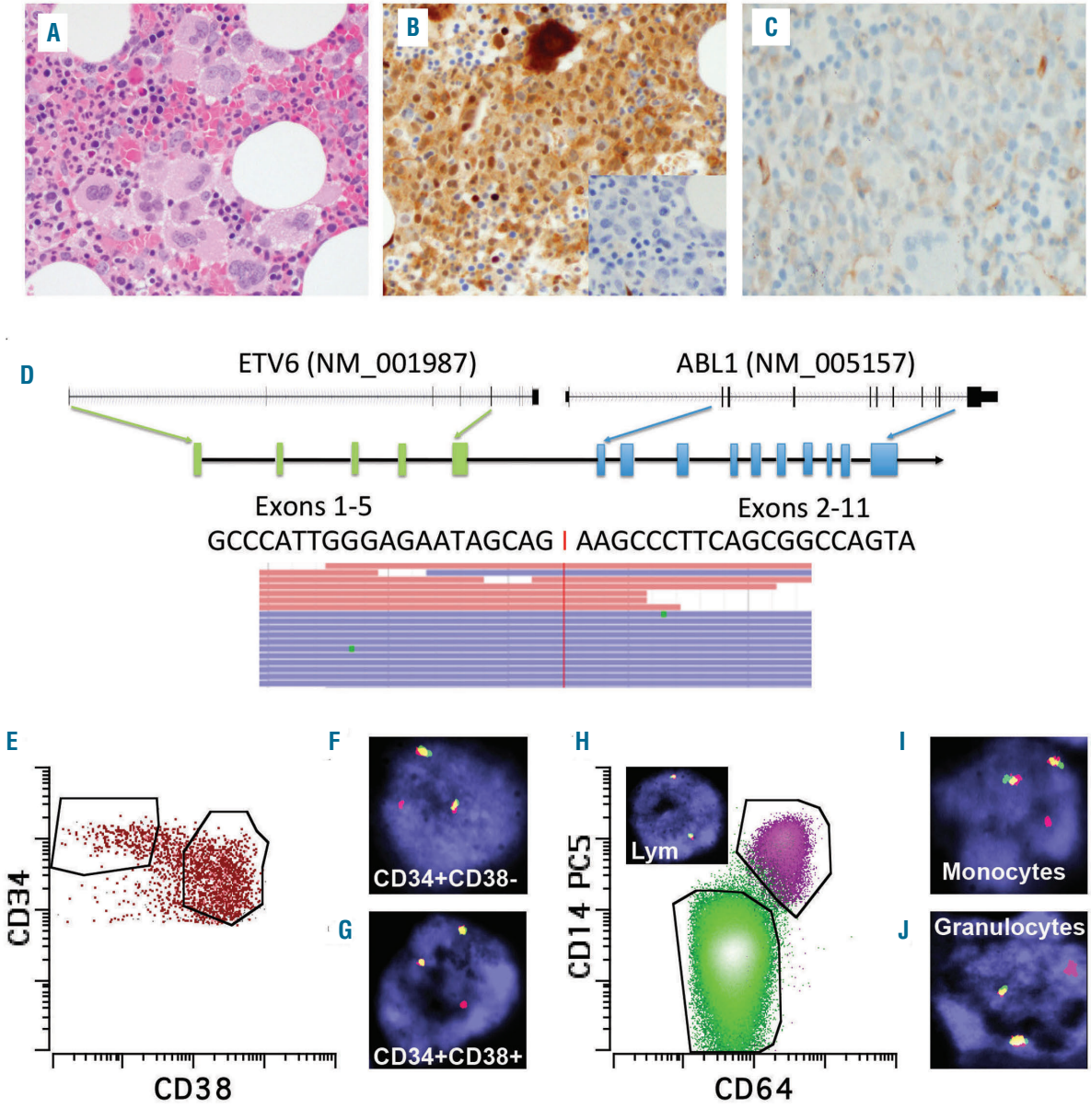
In order to study the cell-of-origin of the *ETV6-ABL1* fusions, various cell populations from two patients (Patients 1 and 4) were sorted based on immunophenotype by flow cytometry, including $CD34^+CD38^-$ (hematopoietic stem cells and early progenitors, HSPC), $CD34^+CD38^+$ (late committed progenitors), monocytes, granulocytes and lymphocytes. FISH studies were performed on the sorted cells. *ETV6* rearrangements were observed in $CD34^+CD38^-$, $CD34^+CD38^+$, monocytes, and granulocytes but not in mature lymphocytes (Figure 1F-K), supporting the view that *ETV6-ABL1* fusions originate from HSPC.

Table 1. Clinicopathologic features of myeloid neoplasms with ETV6-ABL1 fusions.

Patient ID	1	2	3	4	5	6
Age/Sex	34 yrs/M	45 yrs/M	23 yrs/M	55 yrs /F	54 yrs/M	88 yrs/F
Splenomegaly	Yes	Yes	Yes	No	Yes	No
WBC ($x10^9$/L)	27	17.2	374	9	217	14.5
Hb (g/dL)	13.9	11.6	6	12.5	7.8	11.7
PLT ($x10^9$/L)	236	203	76	818	191	417
Abs Eo($x10^9$/L) at presentation	1.7	1.7	44.5	9.2	2.0	2.6
Abs Baso($x10^9$/L) at presentation	0.5	0.2	3.7	0.2	2.1	0.6
BM cellularity	Normal*	100%	100%	100%	>90%	75%
Megakaryocytes	Unremarkable*	Ranging from small to large, clustering	Unremarkable	Increased, large, clustering	Increased, hypolobated	Increased, hy-polobated
M:E ratio	7.3*	10:1	>10:1	10:1	10:1	3.4:1
Eo (%) on aspirate	3*	32	12	10	6	7
Blast (%)	Not increased*	Not increased	Not increased	Not increased	2	4
Initial diagnosis	CML with atypical fusion	Atypical CML	CML with atypical fusion	Essential thrombocythemia (ET)	CML	Myeloid/ lymphoid neoplasm with eosinophilia and gene rearrangement
Karyotype	46,XY,t(9;12)(q34;p13)[9]/46,XY[11]	46,XY,t(9;12)(q34.1;p13)[16]/46,idem,del(7)(q22q36)[1]/46,XY[11]	46,XY	46,XX	45,XY,-7[17]/46,idem,+11 [4]	53,XX,+X,+8,+10,+11,+14,+18,+19[7]/46,XX[13]
ETV6 FISH	Positive	Positive	NA	Positive	Positive	NA
Mutations	*ARID2*	*ARID2*	Absent	Absent	*SETD2*	Absent
	TP53	*CDKN1B*				
Fusions	*ETV6-ABL1*	*ETV6-ABL1*	*ETV6-ABL1*	*ETV6-ABL1*	*ETV6-ABL1*	*ETV6-ABL1*
Treatment	Imatinib, Dasatinib	Dasatinib	Dasatinib	Hydroxyurea, Heat shock protein Inhibitor, ruxolitinib, Anagrelide, interferon Imatinib, cytarabine	Nilotinib	Dasatinib
Response to TKI	Diagnosed in 2005 achieved cytogenetic remission on imatinib; relapsed in 2015, achieved cytogenetic/ molecular remission on dasatinib. Alive	Diagnosed in 02/2018, achieved cytogenetic/ molecular remission on dasatinib; Alive	Diagnosed in 10/2018, achieved cytogenetic/ molecular remission on dasatinib; Alive	Treated as ET in 2011-2016; progressed to AML in 2016 and treated with induction and imatinib died in a month from complications	Leukocytosis in 2016, diagnosed and treated with nilotinib until 2018, progressed to BALL, achieved remission with HyperCVAD, alive	Marked improvement in lymphadenopathy; resolution of eosinophilia, alive

*At relapse in 2015. WBC: white blood cell count; PLT: platelets; yrs: years; mons: months; M: male; F: female; NA: not available; CML: chronic myeloid leukemia; TKI: tyrosine kinase inhibitor; MPAL: mixed phenotype acute leukemia.

All six *ETV6-ABL1* rearranged patients showed myeloid proliferation and eosinophilia, a common characteristic of *ETV6* rearranged myeloid neoplasms, such as *ETV6-PDGFRA, ETV6-PDGFRB*, and *ETV6-JAK2*.[15] Review of the literature identified 21 additional cases of MPN and four cases of AML with *ETV6-ABL1* fusions (median age 48 years, 18 male, 7 female) (*Online Supplementary Table S3*). PB absolute eosinophilia was reported in 13 of 14 patients who had available data for evaluation. Interestingly, 8 of 14 patients also had peripheral basophilia (defined by >1% basophils). Six out of 21 MPN cases with *ETV6-ABL1* rearrangements progressed



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Patient	CD34+CD38-	CD34+CD38+	Monocytes	Granulocytes	Lymphocytes
1	n/a	33.3% [6/18]	34% [34/100]	25% [8/32]	0% [0/100]
4	75% [3/4]	87% [87/100]	85% [85/100]	86% [86/100]	0% [0/100]

Figure 1. Myeloid neoplasms with *ETV6-ABL1* fusions. (A) Hematoxylin & Eosin staining of a bone marrow biopsy specimen (Patient 4) showed hypercellularity, increased M:E ratio, highly variable megakaryocytes morphology, predominantly large in size. (B) Immunohistochemistry showed markedly increased phospho-STAT5 signals but not phospho-STAT3 (inset). (C) Immunohistochemistry showed mildly increased phospho-ERK1/2 signals. (D) Schematic illustration of *ETV6-ABL1* fusions, bidirectional RNA sequencing reads, and transcript sequence of the in-frame fusion product detected by Archer FusionPlex with exons 1-5 of *ETV6* fused to exons 2-11 of ABL1. (F-K) Flow cytometry sorted cell populations, including $CD34^{+}CD38^{-}$ (enriched for hematopoietic stem cells, HSC), $CD34^{+}CD38^{+}$ (hematopoietic progenitors/blasts), monocytes, granulocytes and lymphocytes (Lym) (F and I). Fluorescence *in situ* hybridization (FISH) analysis with an *ETV6* break-part probe set (Abbott Molecular) shows a split *ETV6* signal pattern. The 5′ and 3′ ETV6 were labeled with green and red, respectively. *ETV6* rearrangement was observed in $CD34^{+}CD38^{-}$ (G), $CD34^{+}CD38^{+}$ (H), monocytes (J), and granulocytes (K) but not in mature lymphocytes (inset in I). (L) A summary of *ETV6* FISH results on flow sorted cell populations from two patients. n/a: not available.

to acute leukemia (3 AML, 2 B-ALL, and 1 T-ALL) and all died shortly after transformation despite the addition of TKI to the standard chemotherapy.[3-8] In contrast, four other MPN patients (with no increased blasts) who received TKI achieved long-term survival (longest survival 9 years),[1,2,11] similar to the experience at our institution. Therefore, it appears to be of paramount importance to identify this fusion and incorporate TKI treatment at an early stage of disease. High index of suspicion is critical to initiate proper testing in patients presenting with signs of MPN and eosinophilia. With disease progression, TKI response may be limited. Considering the clinicopathologic similarities (myeloid proliferation, eosinophilia, basophilia, and transformation to AML, B-ALL and T-ALL) between these cases and sensitivity to TKI treatment, we propose classifying them as one group: myeloid/lymphoid neoplasms with eosinophilia/basophilia and *ETV6-ABL1* fusion. Our findings do not favor a diagnosis of Ph^- CML or atypical CML based on the pathologic features, eosinophilia and potential transformation to T-ALL.

Due to the cryptic nature of t(9;12), the incidence of *ETV6-ABL1* fusion might have been underestimated in the literature. The presence of additional *ABL1* signal by FISH studies in the absence of *BCR-ABL1* fusion is a clue to search for other *ABL1* fusions in a subset of cases. FISH analysis using *ETV6* and *ABL1* break-apart probes and RNA-based NGS assay are able to detect the fusion with high sensitivity. RNAseq NGS assay has recently been developed for fusion detection in hematologic malignancies, which can overcome the technical barriers in the identification of *ETV6-ABL1* fusion by traditional methods. Our laboratory's panel has extensive *ETV6* and *ABL1* coverage, facilitating the detection of both *ETV6-ABL1* and any other novel *ETV6* or *ABL1* fusions. With the wide application of RNAseq assay, we expect that more fusions will be detected in hematologic malignancies. RNAseq assay can also elucidate transcript type, critical in determining "A *versus* B".[16] Type A involves *ETV6* exon 4, whereas type B involves exon 5, joining to *ABL1* exon 2; type B is the prevalent transcript form (17 of 18 cases in *Online Supplementary Table S3*), detected in five patients with *ETV6-ABL1* fusions in our institution. As a member of Ets family of transcription factors, when ETV6 fuses to a receptor tyrosine kinase, the fusion protein displays an elevated tyrosine kinase activity. Type B has higher kinase activity since *ETV6* exon 5 includes a direct binding site for the SH2 domain of the GRB2, which enhances the PI3-kinase and MAP Kinase pathways.[17] Consistently, ERK phosphorylation was increased in *ETV6-ABL1* positive marrow cells. In addition, STAT5 phosphorylation was also upregulated similar to observations in *BCR-ABL1* CML, although this needs to be confirmed in more patients.

In summary, our approach combining flow cytometry sorting technology and downstream FISH studies clearly demonstrated *ETV6-ABL1* fusions in sorted HSC, myeloid progenitors/blasts, granulocytes and monocytes but not lymphocytes, suggesting an HSC origin. The mechanism of this fusion driving myeloid/lymphoid differentiation remains to be investigated. Patients with these fusions typically present initially as myeloid/lymphoid proliferation with eosinophilia/basophilia and show hypersensitivity to TKI treatment. Early identification by FISH (particularly by *ETV6* probes) and/or RNA sequencing and potential for such therapy is advantageous based on the current and published experience with this rare neoplasm. We propose to classify this group of disease as myeloid/lymphoid neoplasms with eosinophilia/basophilia and *ETV6-ABL1* fusion to facilitate and unify the clinico-pathologic and molecular diagnosis and subsequent clinical management. Development of a quantitative polymerase chain reaction assay similar to *BCR-ABL1* fusion is needed for future disease and therapy monitoring.

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