ETV6-ABL1-positive "chronic myeloid leukemia": clinical and molecular response to tyrosine kinase inhibition

While great progress has been achieved in the clinical management and molecular understanding of Ph+ chronic myeloid leukemia (CML), little is known about the optimal approach to monitor and treat patients with ETV6-ABL1$^+$ myeloproliferative neoplasms. Nine BCR-ABL1-negative CML patients with a variant *ABL1* gene (9q34) rearrangement, involving fusion to ETV6 aka TEL (12p13) have been reported thus far.[1-9] The ETV6-ABL1 fusion gene has also been identified in 3 patients with chronic myeloproliferative neoplasms other than "chronic myeloid leukemia" (cMPN) as well as 7 patients with BCR-ABL1 negative acute lymphoblastic leukemia and 4 patients with acute myeloid leukemia.[10, 11] Of the 9 cases of ETV6-ABL1$^+$ chronic myeloid leukemia, only 2 were treated with a TKI in chronic phase[4, 5] and only one of these reached a complete remission with a modest follow up of seven months (no molecular monitoring was performed).[4] Among the other published ETV6-ABL1$^+$ reports, one patient was treated with a second generation TKI for a relapsed cMPN.[12] We provide the first molecular documentation of sustained remission of ETV6-ABL1$^+$ chronic phase "chronic myeloid leukemia" (CML requires a BCR-ABL1 fusion according to the most recent 2008 WHO classification) to TKI. Molecular monitoring for the ETV6-ABL1 transcript is important given the fact that conventional karyotyping frequently fails to detect a cryptic translocation, i.e. the t(9;12). We provide evidence that treatment of ETV6-ABL1+ "chronic myeloid leukemia" with imatinib results in downregulation of C-MYC, BCL-XL, ID1 and NUP98, mediators of BCR-ABL1 transforming activity. Moreover, we found no associated mutations in *UTX*, *ASXL1*, *EZH2*, *TET2* and *IDH1/2* suggesting that ETV6-ABL1$^+$ "chronic myeloid leukemia" may be as tyrosine kinase focused as BCR-ABL1 driven disease.

The patient is a 36-year male who was found to have splenomegaly and a total white blood cell (WBC) count of 55×10^9/L (57% neutrophils, 6% lymphocytes, 1% monocytes, 3% eosinophils, 2% basophils, 7% metamyelocytes, 24% myelocytes). Lactate dehydrogenase was elevated at 653 IU/L. Bone marrow biopsy revealed myeloid hyperplasia suggestive of a myeloproliferative disorder. qRT-PCR for BCR-ABL1 translocation was negative. No BCR-ABL1 fusion signal was observed in interphase FISH analysis using BCR (22q11.2) and ASS-ABL1 (9q34) probes. Instead, 80% of interphase nuclei showed a variant signal pattern consisting of two signals for BCR and three signals for ASS-ABL1 consistent with rearrangement of ABL1 at 9q34 but not BCR at 22q11. Cytogenetic G-banding analysis and FISH showed t(9;12)(q34;p13) in an otherwise normal karyotype (Figure 1A and B). RT-PCR detected the ETV6-ABL1 translocation and the patient was diagnosed with ETV6-ABL1$^+$ CML-like disorder. Given the persistence of night sweats and fevers, and the persistent disease despite hydroxyurea at 1,000mg daily (WBC decreased to 7×10^9 cells/L after one month of hydroxyurea) imatinib mesylate, 400mg daily, was initiated. The patient tolerated imatinib and achieved a complete hematological remission after three months of treatment (WBC 5.6×10^9 cells/L). FISH testing after three months of imatinib revealed no evidence of rearrangement at the BCR or ETV6 loci (Figure 1B).

To follow the patient's response to therapy more sensitively, qRT-PCR was performed using primers for ETV6 and ABL1 (*Online Supplementary Appendix*). Quantification of the ETV6-ABL1 transcript level in peripheral blood cells one month prior to initiation of imatinib revealed 2,160×10^3 ETV6-ABL1 copies/μg RNA. After one month of treatment, ETV6-ABL1 transcript level dropped to 495×10^3 copies/μg RNA. The ETV6-ABL1 transcript became undetectable by seven months of treatment, indicating a complete and rapid molecular response. The patient's molecular response closely mirrored normalization of the WBC count (Figure 2A). We also evaluated the expression of BCR-ABL1 target genes *C-MYC*, *BCL-XL*, *ID1* and *NUP98*, pre- and post-imatinib treatment (Figure 2B). The expression of these genes closely mirrored ETV6-ABL1 expression: imatinib downregulated their expression. The patient has continued to

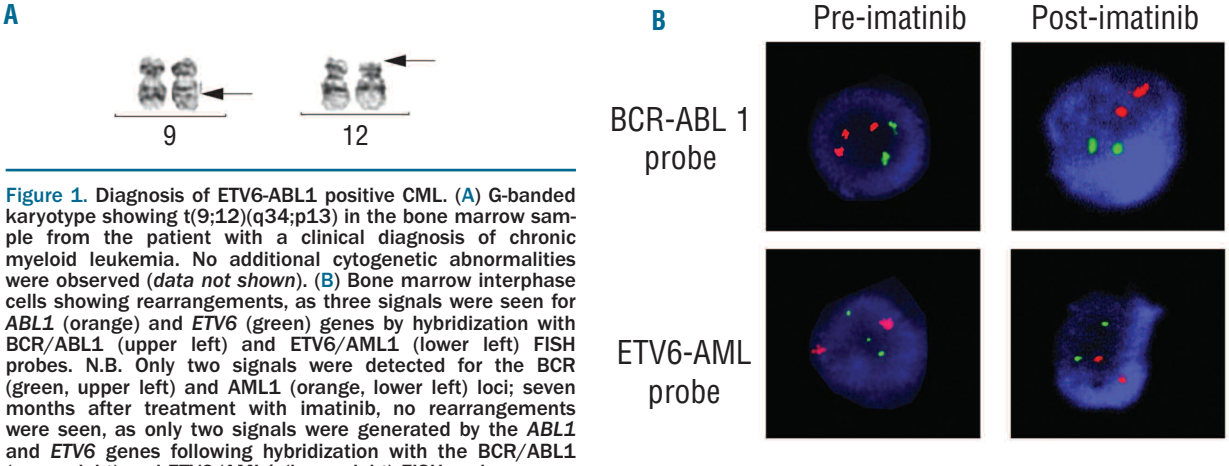

Figure 1. Diagnosis of ETV6-ABL1 positive CML. (A) G-banded karyotype showing t(9;12)(q34;p13) in the bone marrow sample from the patient with a clinical diagnosis of chronic myeloid leukemia. No additional cytogenetic abnormalities were observed (*data not shown*). (B) Bone marrow interphase cells showing rearrangements, as three signals were seen for *ABL1* (orange) and *ETV6* (green) genes by hybridization with BCR/ABL1 (upper left) and ETV6/AML1 (lower left) FISH probes. N.B. Only two signals were detected for the BCR (green, upper left) and AML1 (orange, lower left) loci; seven months after treatment with imatinib, no rearrangements were seen, as only two signals were generated by the *ABL1* and *ETV6* genes following hybridization with the BCR/ABL1 (upper right) and ETV6/AML1 (lower right) FISH probes.

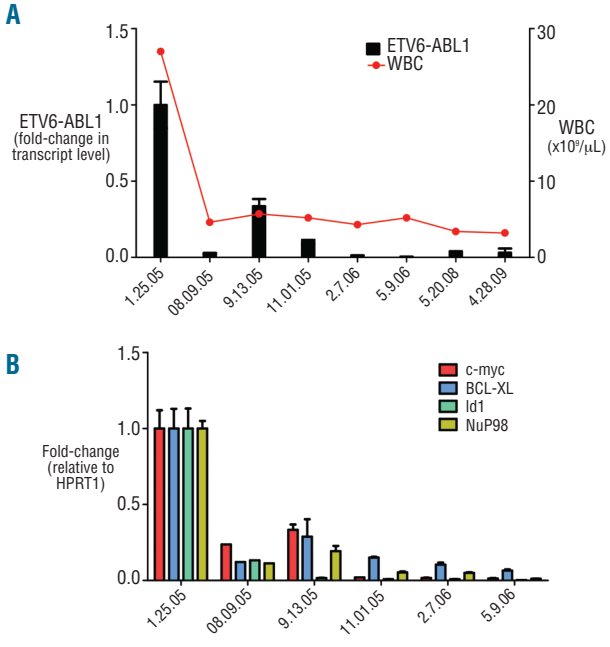

Figure 2. Response to Imatinib in an ETV6-ABL1 positive chronic myeloid leukemia. (A) White blood cell count and peripheral blood qRT-PCR analysis of ETV6-ABL1 transcript levels pre-imatinib and throughout imatinib treatment. (B) Concomitant with decreases in ETV6-ABL1 transcript levels, decreases in C-MYC, ID1, BCL-XL, and NUP-98 transcripts were also seen. The patient remains in hematological remission with no identifiable ETV6-ABL1 transcripts in the peripheral blood after approximately five years of imatinib.

do well on imatinib 400mg/day with no evidence of ETV6-ABL1 transcript by qRT-PCR for the past five years.

Somatic mutations in *UTX*, *ASXL1*, and *TET2* have been reported in chronic myeloid leukemia and mutations in *EZH2* and *IDH1/2* in myeloid malignancies other than CML. We found no somatic alterations in these genes in the DNA extracted from whole blood prior to imatinib treatment, nor when the patient was in a molecular remission.

Our studies indicate that ETV6-ABL1$^+$ "chronic myeloid leukemia" can be sensitive to imatinib and there is significant overlap of molecular targets of ETV6-ABL1 with those of BCR-ABL1, suggesting that the ETV6-ABL1 fusion protein may trigger similar oncogenic cascades as BCR-ABL1. Finally, we were able to exclude mutations in any of the recently identified "myeloid" genes including *UTX*, *ASXL1*, *EZH2*, *TET2* and *IDH1/2* suggesting that the pathogenesis of ETV6-ABL1$^+$ "chronic myeloid leukemia" may be as tyrosine kinase focused as BCR-ABL1 driven disease.

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Cytogenetically complex *SEC31A-ALK* fusions are recurrent in ALK-positive large B-cell lymphomas

Fusion tyrosine kinases involving anaplastic lymphoma kinase (ALK) are central to the pathogenesis of numerous malignancies, in which they represent impor-