Successful tyrosine kinase inhibitor therapy in a refractory B-cell precursor acute lymphoblastic leukemia with *EBF1-PDGFRB* fusion

The advent of tyrosine kinase inhibitors (TKI) has profoundly changed the current therapeutic approach in some hematologic malignancies, including *BCR-ABL1*-positive acute lymphoblastic leukemia (ALL). Recently, next-generation genomic methods have uncovered various alterations in ALL that lead to deregulation of kinases and cytokine receptors, suggesting the potential interest of TKI treatment in some patients with high-risk ALL.[1] However, in order to translate these insights gained from large-scale genomic profiling studies into clinical practice, the challenge is now to identify at diagnosis those mutations that would make patients eligible for testing such targeted therapy.

We report a case of refractory B-cell precursor ALL (BCP-ALL) where the identification of a genomic alteration activating kinase signaling, the *EBF1-PDGFRB* fusion, allowed the patient to benefit from early introduction of imatinib treatment, with subsequent cytological remission and profound MRD response. This report highlights the promising use of TKI in high-risk BCP-ALL with kinase alterations while emphasizing the importance of screening for such alterations at diagnosis,

A 16-year old male presented with general weakness, stage II dyspnea, dry cough and 7% weight loss over one month. He reported no past medical or surgical history. Complete blood count and morphological observation revealed a hyperleukocytosis at $167x10^9$/L with 86% blast cells. The patient was referred to the Adolescents and Young Adults Hematology Unit of St. Louis Hospital, Paris, where physical examination revealed soft painless axillar and inguinal lymph nodes as well as liver and spleen enlargement. Neurological and testis examination was normal. A bone marrow aspirate (BMA) revealed an infiltration of 84% blasts of lymphoid morphology. Flow cytometry analysis showed a population of CD45 low positive cells expressing HLA-DR, CD19, CD10, CD20, CD22, CD79a, TdT but not cytoplasmic μ chains, leading to the diagnosis of EGIL-BII BCP-ALL. These cells also displayed aberrant expression of CD33 and CD36 but not CD13. Cerebrospinal fluid examination showed no blast cells. DNA index was 1.0 and karyotype was normal. Fluorescent in situ hybridation (FISH) and molecular analyses for *BCR-ABL1, MLL, ETV6-RUNX1* and *TCF3-PBX1* translocations were negative. The patient was treated according to the high-risk Group B of the FRALLE 2000 national pediatric ALL trial.[2] Following seven days of steroid treatment and one methotrexate intrathecal infusion, 25,600 blasts per microliter (59% of leukocytes) were detected in peripheral blood, demonstrating a poor response to pre-phase. He then received a BFM-like 5-drug induction course with prednisone, vincristine, native L-asparaginase, daunorubicin and cyclophosphamide. At Day 21 peripheral blast clearance was still not complete with 550 blasts per microliter (13% of leukocytes), so BMA was not performed and induction was pursued according to the most intensive treatment Group B2 of FRALLE 2000. At Day 43 post-induction, BMA was indicative of complete remission but Ig/TCR-based minimal residual disease (MRD) measured at $2x10^{-1}$ indicated persistent leukemia.

At this time, results from array-CGH analysis revealed several cryptic genomic abnormalities including an intragenic IKZF1 Δ(2-7) deletion and a 5q33 microdeletion with breakpoints located just in the *EBF1* and *PDGFRB* genes (Figure 1A). EBF1 is a transcription factor essential for B-cell lineage differentiation,[3] and *PDGFRB* is a membrane receptor which includes an intracellular tyrosine kinase domain normally activated in response to ligand binding and receptor dimerization. PDGFRB is known to be implicated in a chromosomal rearrangement, *ETV6-PDGFRB*, observed in a subtype of myeloproliferative neoplasms.[4] The chimeric oncoprotein ETV6-PDGFRB is constitutively phosphorylated, triggering downstream signaling,[5] but importantly it can be successfully targeted by TKI, such as imatinib.[6] In addition, an *EBF1-PDGFRB* fusion transcript has been recently reported in a specific subtype of BCP-ALL and was associated with in vitro sensitivity to TKI treatment.[1] RT-PCR and sequencing confirmed that the microdeletion we observed by array-CGH did result in the expression of an *EBF1-PDGFRB* fusion transcript (Figure 1B and C). Therefore, considering the very poor response after high-risk induction regimen in this young patient, and the potential responsiveness to TKI, it was decided to immediately introduce an imatinib treatment in combination with conventional chemotherapy. The patient was given imatinib continuously at a dose of 400 mg/d from Day 53, along with successive consolidation blocks including dexamethasone, vincristine, etoposide, cytarabine, high-dose methotrexate and inthathecal chemotherapy. Following 20 days of imatinib and the first consolidation block, MRD assessed on TCRD and IKZF1 Δ(2-7)[7] genomic markers was quantified at $4x10^{-3}$ (Figure 2). During the following two months of imatinib treatment and consolidation courses, MRD continued to decline to a non-quantifiable level close until 10^{-5}. Subsequently, imatinib therapy was stopped while the patient underwent a conditioning regimen before allogeneic bone marrow transplantation (BMT) with an HLA-identical sibling donor. It was planed to reintroduce imatinib in case of MRD positivation. MRD evaluations performed so far, at Day 30 and Day 105 post-BMT, showed no detectable leukemic blasts with a sensitivity of 10^{-5}.

BCP-ALL is a heterogeneous disease that comprises distinct entities characterized by recurring genetic alterations. However, a number of BCP-ALLs remain uncharacterized by conventional cytogenetic and molecular analyses, especially in patients aged 10 years or over. Recently, gene-expression profiling studies in pediatric patients identified a distinct group of BCP-ALL characterized by a gene-expression signature similar to that of *BCR-ABL1*-positive ALLs, associated with frequent *IKZF1* deletion and a dismal prognosis.[8,9] In a subsequent work based on RNA-sequencing, the St. Jude group identified in such cases a large variety of genomic alterations, including *EBF1-PDGFRB*, leading to deregulation of kinase and cytokine receptor signaling. Although these findings suggest that targeted therapy may be of great help to treat those high-risk ALLs, so far there is no simple laboratory method to recognize the BCR-ABL1-like gene-expression profile or to identify the large range of kinase-deregulating genomic lesions. In the case reported here, we were fortunate that the *EBF1-PDGFRB* fusion could be detected by prospective array-CGH analysis. Recently, Weston *et al.*[10] reported a similar case with *EBF1-PDGFRB* fusion in a refractory leukemia, who also benefited from TKI treatment. Collectively, these data suggest that *EBF1-PDGRFB* fusion distinguishes a rare subtype of BCP-ALL associated with poor response to chemotherapy. Considering the major

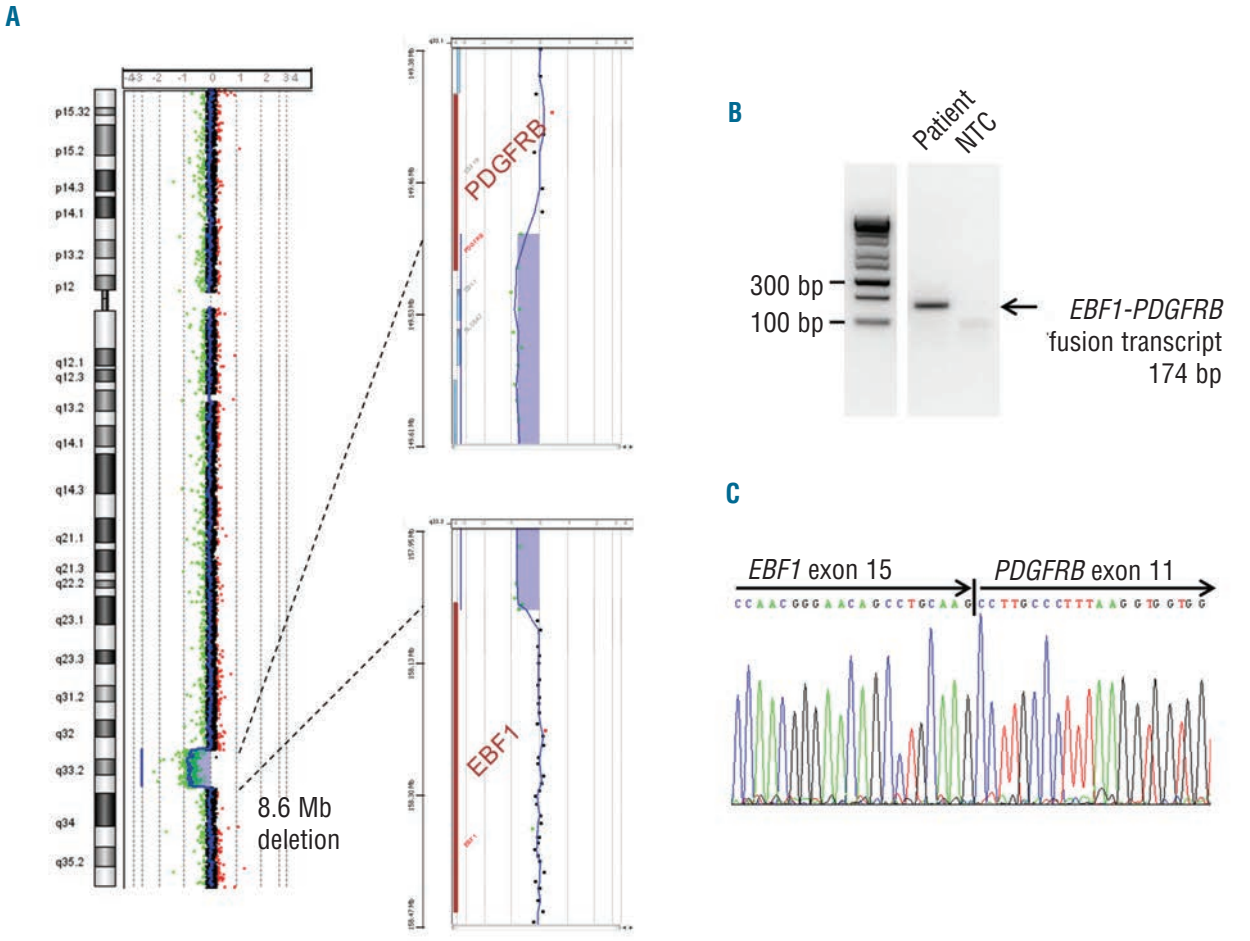

Figure 1. (A) Array-CGH plots showing 5q33 microdeletion that fuses *EBF1* and *PDGFRB* genes. SurePrint G3 180K array, analysis using Agilent Genomic Workbench software with the ADM-2 algorithm (*www.agilent.com*). (B) RT-PCR of the *EBF1-PDGFRB* fusion transcript using the following primers: EBF1-forward 5'-AAGAGTGCTTTCGCACCAGT-3; PDGFRB-reverse 5'- GGGCAGAGCATTGCTGTAGA-3'. (C) Electropherogram of the transcript fusion sequence after direct Sanger sequencing of the RT-PCR product.

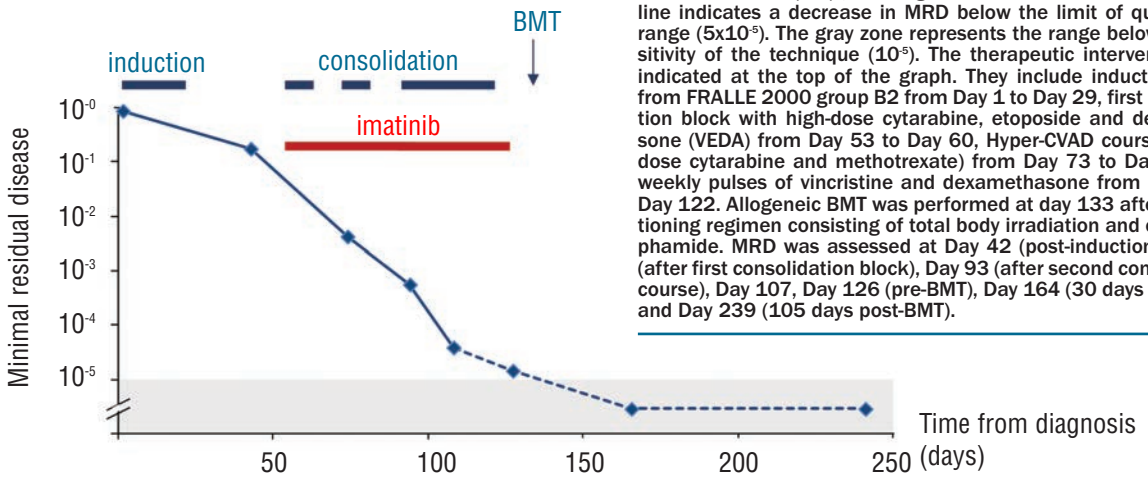

Figure 2. Minimal residual disease (MRD) in bone marrow as a function of time, evaluated by real-time genomic quantification of molecular markers according to EuroMRD guidelines. The molecular markers used were a VD2DD3 rearrangement and intragenic *IKZF1* deletion Δ(2-7)[7], which gave consistent results. The dotted line indicates a decrease in MRD below the limit of quantitative range ($5x10^{-5}$). The gray zone represents the range below the sensitivity of the technique (10^{-5}). The therapeutic interventions are indicated at the top of the graph. They include induction phase from FRALLE 2000 group B2 from Day 1 to Day 29, first consolidation block with high-dose cytarabine, etoposide and dexamethasone (VEDA) from Day 53 to Day 60, Hyper-CVAD course B (high-dose cytarabine and methotrexate) from Day 73 to Day 76, four weekly pulses of vincristine and dexamethasone from Day 93 to Day 122. Allogeneic BMT was performed at day 133 after a conditioning regimen consisting of total body irradiation and cyclophosphamide. MRD was assessed at Day 42 (post-induction), Day 73 (after first consolidation block), Day 93 (after second consolidation course), Day 107, Day 126 (pre-BMT), Day 164 (30 days post-BMT) and Day 239 (105 days post-BMT).

impact of TKI treatment in these observations, a systematic screening of this lesion is warranted, at least in poor responder patients. It may also be necessary to evaluate the use of the different available TKIs and their optimal dose in this leukemia subtype. Finally, since the majority of BCP-ALL in adolescents and adults is not fully characterized at the genomic level, and given that they more frequently respond poorly to chemotherapy, a systematic approach to identify other signaling alterations at diagnosis should be implemented in order to provide tailored therapy to these patients.

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References

1. Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell. 2012;22(2):153-66.
2. Strullu M, Corradini N, Audrain M, Orsonneau JL, Bouige D, Thomare P, et al. Silent hypersensitivity to Escherichia coli asparaginase in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2010;51(8):1464-72.
3. Lin H, Grosschedl R. Failure of B-cell differentiation in mice lacking the transcription factor EBF. Nature. 1995;376(6537):263-7.
4. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell. 1994;77(2):307-16.
5. Carroll M, Tomasson MH, Barker GF, Golub TR, Gilliland DG. The TEL/platelet-derived growth factor beta receptor (PDGF beta R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF beta R kinase-dependent signaling pathways. Proc Natl Acad Sci USA. 1996;93(25):14845-50.
6. Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med. 2002;347(7):481-7.
7. Caye A, Beldjord K, Mass Malo K, Drunat S, Soulier J, Gandemer V, et al. Breakpoint-specific multiplex PCR allows the detection of IKZF1 intragenic deletions and minimal residual disease monitoring in B-cell precursor acute lymphoblastic leukemia. Haematologica. 2013;98(4):597-601.
8. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009;360(5):470-80.
9. Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009;10(2):125-34.
10. Weston BW, Hayden MA, Roberts KG, Bowyer S, Hsu J, Fedoriw G, et al. Tyrosine Kinase Inhibitor Therapy Induces Remission in a Patient With Refractory EBF1-PDGFRB-Positive Acute Lymphoblastic Leukemia. J Clin Oncol. 2013;31(25):e413-6.