

# Efficacy of Tyrosine Kinase Inhibitor Therapy in a Chemotherapy-refractory B-cell Precursor Acute Lymphoblastic Leukemia With *ZC3HAV1-ABL2* Fusion

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Acute lymphoblastic leukemia (ALL) is the most common form of pediatric malignancy, encompassing multiple entities characterized by both distinct clinical and biological features as well as treatment response.<sup>1</sup> Underlying genetic alterations is a key determinant to explain such heterogeneity and inform disease classification, especially among B-cell precursor (BCP)-ALL.<sup>2</sup> Thus, high hyperdiploidy and the *ETV6-RUNX1* fusion are associated with a favorable outcome. By contrast, *KMT2A* rearrangements and the *BCR-ABL1* fusion confer adverse prognosis. Until recently, a large proportion of BCP-ALL patients lacking such aberrations (named B-other ALL) was allocated to the intermediate prognostic subgroup.<sup>3</sup> Great advances have been made to unravel the genetics of this

subgroups leading to the discovery of an entity of high-risk ALL with *BCR-ABL1*-like properties.<sup>4</sup> Patients with *BCR-ABL1*-like ALL have a gene expression profile similar to that of *BCR-ABL1*-positive ALL but lack the *BCR-ABL1* fusion gene. *BCR-ABL1*-like ALL has a poor outcome and frequently harbors deletions in *IKZF1* or other B-lymphoid key genes.<sup>4</sup> Detection of *BCR-ABL1*-like ALL is clinically important due to the presence of targetable kinase-activating alterations, which represent the hallmark of this entity.<sup>5</sup> Here, we report the case of a young adult with a refractory BCP-ALL displaying a rare *ZC3HAV1-ABL2* fusion. *ABL2* is a member of the Abelson family of nonreceptor tyrosine kinase proteins closely related to *ABL1*. To date, the efficacy of tyrosine kinase inhibitors (TKI) on this particular genetic alteration remains unknown.<sup>5</sup>

The patient was an 18-year-old male, with no previous medical history, referred to our Hematology Department for hyperleukocytosis incidentally discovered during a blood donation. The full blood count showed: white blood cell count  $171 \times 10^9/L$  (including 90% of blast cells), hemoglobin concentration 9.3 g/dL, and platelet count  $107 \times 10^9/L$ . Clinical examination demonstrated asthenia, pallor, and splenomegaly without adenopathy. Bone marrow aspirate was of high cellularity with 90% of peroxidase negative-blast cells. Additionally, a lumbar puncture showed central nervous system involvement. Immunophenotyping confirmed the diagnosis of BCP-ALL with the expression of CD34, HLA-DR, and B lymphoid markers CD19, CD10, CD20, and CD22 and the negativity of myeloid and T-lymphoid markers. Conventional cytogenetics showed a normal karyotype 46,XY in all metaphases. Fluorescent in situ hybridization (FISH) and molecular studies were negative for *ETV6-RUNX1*, *BCR-ABL1*, *TCF3-PBX1*, and *KMT2A* fusions. *IKZF1* deletion encompassing exons 4 to 8, as well as *PAX5* and *EBF1* deletions were detected by multiplex ligation probe amplification (MLPA P335 kit, MRC Holland, Amsterdam, the Netherlands).<sup>6</sup>

First-line treatment was initiated according to the CAALL-F01 clinical trial (A French Protocol for the Treatment of Acute Lymphoblastic Leukemia in Children and Adolescents; ClinicalTrials.gov Identifier NCT02716233). Evaluation after 7 days of prednisolone revealed the persistence of  $6.9 \times 10^9/L$  blast cells in the peripheral blood. The patient was then treated in the high-risk group. Induction therapy included vincristine 1.5 mg/m<sup>2</sup> IV (D8,

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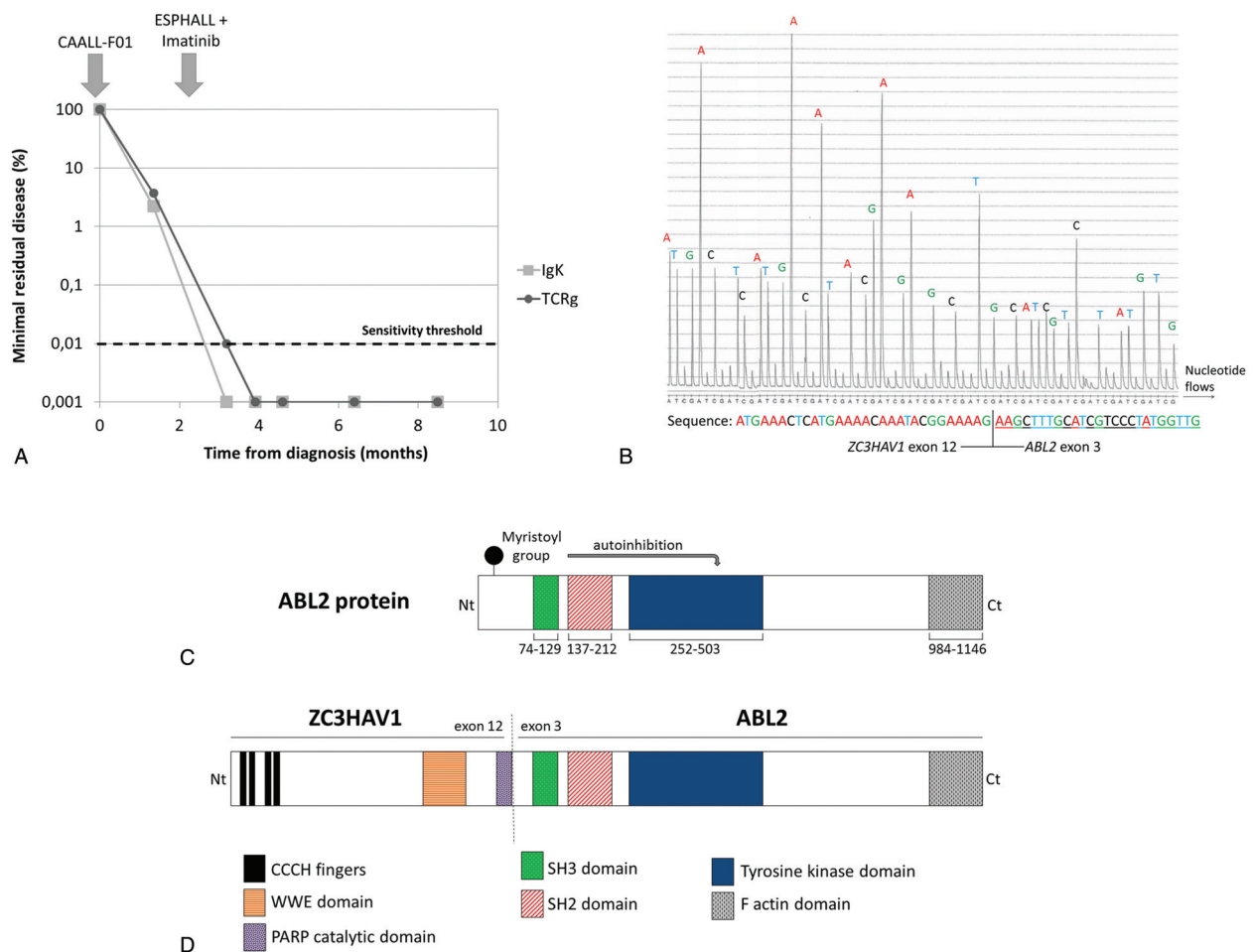
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**Figure 1. Clinical and molecular data.** (A) Minimal residual disease monitoring in bone marrow using rearrangements of immunoglobulin light chain kappa and T-cell receptor gamma as leukemia-specific markers. (B) *ZC3HAV1-ABL2* fusion sequence determined by reverse transcriptase-multiplex ligation probe amplification. The sequencing is performed by cyclic flow of nucleotides (A, T, C, G). Each nucleotide incorporation gives a strong signal (peak indicated with A, T, C or G), which is proportional to the number of nucleotides incorporated. The sequence deduced is shown below with indication of the *ZC3HAV1* part and *ABL2* parts. (C) Domain organization of *ABL2*. (D) Domain organization of the putative *ZC3HAV1-ABL2* fusion protein.

D15, D22, and D29), daunorubicin 30 mg/m<sup>2</sup> IV (D8, D15, D22, and D29), prednisolone 60 mg/m<sup>2</sup> per day PO (D1–D7, and D8–D28 then tapered over 1 week), peg-asparaginase 2500 IU/m<sup>2</sup> IV (D12 and D26), intrathecal methotrexate (D1), and triple intrathecal (methotrexate, cytarabine, and corticoid; D13 and D24). Bone marrow aspirate at D35 revealed induction failure with positivity of minimal residual disease (MRD) above 10<sup>-2</sup> for Ig/TCR markers using standard procedures<sup>7</sup> (approximately 3% residual leukemic blasts; Fig. 1A) and for leukemia-associated immunophenotype in flow cytometry (not shown). Considering the patient to have a B-other ALL, together with age and poor treatment response, we performed reverse transcriptase multiplex ligation probe amplification (RT-MLPA) to extend the panel of transcript fusion detection<sup>8</sup> as well as single nucleotide polymorphism (SNP)-array karyotyping. Remarkably, the RT-MLPA detected a fusion between the exon 12 of *ZC3HAV1* and the exon 3 of *ABL2* exon 3 (Fig. 1B). Additionally, SNP-array (Cytoscan High Density, Affymetrix, Thermo Fisher Scientific, Waltham, Massachusetts, United States) confirmed deletions in *IKZF1* (7p12.2), *EBF1* (5q33.3), and *PAX5* (9p13.2) and revealed small deletions in *BTLA* (3q12.2), *PBX3* (9q33.3), *VPREB1* (22q11.22), as well as larger copy-number aberrations at 15q and Xp regions (Table 1).

The *ZC3HAV1-ABL2* fusion has been rarely described in BCP-ALL.<sup>9,10</sup> The *ZC3HAV1* gene (*Zinc Finger CCCH-Type Containing, Antiviral 1*), located at 7q34, encodes an RNA-binding protein, which acts as a tumor suppressor and regulates various processes during cell development and homeostasis.<sup>11</sup> The *ABL2* gene (*Abelson tyrosine-protein kinase 2*), located at 1q25.2, encodes a nonreceptor tyrosine kinase protein that belongs to the Abelson family. *ABL2* shares a high degree of sequence conservation and a similar domain organization with *ABL1*. *ABL2* functions may overlap with those of *ABL1* and include cytoskeleton organization, cell proliferation, adhesion, and migration.<sup>12</sup> Activity of *ABL2* is regulated by an autoinhibitory mechanism. Notably, *ABL2* is maintained in an inactive conformation by an N-terminal myristoyl group binding to a hydrophobic pocket in the kinase domain<sup>10</sup> (Fig. 1C). Thus, the fusion of *ZC3HAV1* to the N-terminal part of *ABL2* is supposed to disrupt this autoinhibitory mechanism and enhance the tyrosine kinase activity to induce leukemia (Fig. 1D). Additionally, crystallization experiments of the *ZC3HAV1* protein have revealed that its N-terminal part, containing the 4 tandem CCCH-type zinc-finger motifs, was involved in protein dimerization,<sup>13</sup> a feature shared with many *ABL1* fusion partners.

Table 1

## Copy-Number Abnormalities Found by Single Nucleotide Polymorphism Array.

Type	Chr.	Start	End	Size, kbp	Cytoband Start	Cytoband End	CN State	Genes
Loss	3	112135341	112220396	85	q13.2	q13.2	1.0	<i>BTLA</i>
Loss	4	71547865	71774144	226	q13.3	q13.3	1.0	<i>UTP3, RUFY3, GRSF1, MOB1B</i>
Loss	5	158280198	158524504	244	q33.3	q33.3	1.0	<i>EBF1</i>
Loss	7	50307124	50482012	174	p12.2	p12.2	1.0	<i>IKZF1</i>
Loss	9	37033145	37258316	225	p13.2	p13.2	1.0	<i>PAX5, EBLN3, ZCCHC7</i>
Loss	9	128499613	128607420	107	q33.3	q33.3	1.0	<i>LOC51145, PBX3</i>
Loss	15	74559477	102374741	27,815	q24.1	q26.3	1.0	304 genes
Loss	20	33393133	33677239	284	q11.22	q11.22	1.0	<i>NCOA6, HMGB3P1, GGT7, ACSS2, GSS, MYH7B, MIR499A, MIR499B, TRPC4AP</i>
Loss	22	22571858	22603418	31	q11.22	q11.22	1.0	<i>VPREB1</i>
Gain	X	168566	26583721	26,415	p22.33	p21.3	2.0	168 genes

For each aberration, chromosome location, size, copy number state, and genes involved are indicated. Chromosomal locations were obtained from the Human (GRCh37/hg19—February 2009) assembly of the UCSC Genome Browser (<http://genome.ucsc.edu/>).

Considering those findings, our patient was subsequently treated according to the ESPHALL trial (ClinicalTrials.gov Identifier NCT00287105).<sup>14</sup> He received imatinib 500 mg/d (D1–D21) in combination with dexamethasone, vincristine, high-dose methotrexate, high-dose cytarabine, high-dose asparaginase, and cyclophosphamide. He finally achieved complete remission after the first consolidation course with negativity of MRD based on Ig/TCR monitoring and immunophenotyping. Due to this complete response, the indication of hematopoietic stem cell transplantation was not retained. Subsequent MRD evaluations confirmed MRD negativity (Fig. 1A).

*BCR-ABL1*-like ALL is a common subtype of ALL, especially in adolescents and young adults (AYA) in whom it could account for more than 25% of BCP-ALL cases.<sup>15</sup> Identifying such ALL is essential since patients could benefit of treatment combinations with TKIs. Particularly, significant results have been reported with TKIs in patients with chemotherapy-refractory ALL harboring *ABL1*<sup>16</sup> or *PDGFRB*<sup>17</sup> fusions while experience in patients with *ABL2* fusions is limited.<sup>10</sup> This report suggests that *ABL2* fusions could respond to TKIs in vivo. Further studies and international collaboration are required to better define management and prognosis of patients bearing these rare rearrangements. In clinical practice, the detection of *BCR-ABL1*-like fusions, which are diverse and often cryptic, can be assessed by FISH analyses (using split FISH probes targeting the tyrosine kinase genes) or molecular methods such as RT-MLPA or RNA sequencing.<sup>18</sup> Because of major treatment implications, such experiments should be consistently performed in patients with B-other ALL and/or suggestive findings (AYA, poor treatment response, evocative pattern of microdeletions by MLPA or SNP-array).

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