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# Characterization of leukemias with *ETV6-ABL1* fusion

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## ABSTRACT

To characterize the incidence, clinical features and genetics of *ETV6-ABL1* leukemias, representing targetable kinase-activating lesions, we analyzed 44 new and published cases of *ETV6-ABL1*-positive hematologic malignancies [22 cases of acute lymphoblastic leukemia (13 children, 9 adults) and 22 myeloid malignancies (18 myeloproliferative neoplasms, 4 acute myeloid leukemias)]. The presence of the *ETV6-ABL1* fusion was ascertained by cytogenetics, fluorescence *in-situ* hybridization, reverse transcriptase-polymerase chain reaction and RNA sequencing. Genomic and gene expression profiling was performed by single nucleotide polymorphism and expression arrays. Systematic screening of more than 4,500 cases revealed that in acute lymphoblastic leukemia *ETV6-ABL1* is rare in childhood (0.17% cases) and slightly more common in adults (0.38%). There is no systematic screening of myeloproliferative neoplasms; however, the number of *ETV6-ABL1*-positive cases and the relative incidence of acute lymphoblastic leukemia and myeloproliferative neoplasms suggest that in adulthood *ETV6-ABL1* is more common in *BCR-ABL1*-negative chronic myeloid leukemia-like myeloproliferations than in acute lymphoblastic leukemia. The genomic profile of *ETV6-ABL1* acute lymphoblastic leukemia resembled that of *BCR-ABL1* and *BCR-ABL1*-like cases with 80% of patients having concurrent *CDKN2A/B* and *IKZF1* deletions. In the gene expression profiling all the *ETV6-ABL1*-positive samples clustered in close vicinity to *BCR-ABL1* cases. All but one of the cases of *ETV6-ABL1* acute lymphoblastic leukemia were classified as *BCR-ABL1*-like by a standardized assay. Over 60% of patients died, irrespectively of the disease or age subgroup examined. In conclusion, *ETV6-ABL1* fusion occurs in both lymphoid and myeloid leukemias; the genomic profile and clinical behavior resemble *BCR-ABL1*-positive malignancies, including the unfavorable prognosis, particularly of acute leukemias. The poor outcome suggests that treatment with tyrosine kinase inhibitors should be considered for patients with this fusion.

## Introduction

*ETV6-ABL1* (*TEL-ABL*) fusion is a rare but recurrent genetic aberration found in hematologic malignancies. Given the orientation of *ETV6* (12p13) and *ABL1* (9q34) an in-frame fusion cannot be produced by a simple balanced translocation. In fact, the fusion results from a complex rearrangement involving a translocation and inversion or an insertion of *ETV6* into chromosomal band 9q34 or *ABL1* into 12p13. Alternative splicing generates two fusion transcripts - type A and B without and with *ETV6* exon 5, respectively. Both result in constitutive chimeric tyrosine kinase activity analogous to *BCR-ABL1* fusion.[1] Likewise the effect of *ETV6-ABL1* on cellular proliferation, cell survival and transforming capacity mirrors that seen in cases with *BCR-ABL1*.[2,3] However, unlike *BCR-ABL1*, *ETV6-ABL1* does not cause acute leukemia in mice; instead it induces a chronic myeloproliferation similar to *BCR-ABL1*-induced chronic myeloid leukemia.[4] In humans, the oncogenic potential of *ETV6-ABL1* is similar to that of *BCR-ABL1* and both fusions can be found in a spectrum of malignancies including myeloproliferative neoplasms (MPN), acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

There is renewed interest in *ETV6-ABL1* since the discovery of a "*BCR-ABL1*-like" (or "Ph-like") gene expression profile (GEP) among B-cell precursor (BCP) ALL cases lacking an established chromosomal abnormality (so-called B-other ALL). The *BCR-ABL1*-like GEP resembles the *BCR-ABL1* GEP because both are driven by the activation of kinase signaling.[5-8] *In vitro* studies suggest that many of these kinase activating aberrations, including *ETV6-ABL1* fusion, are sensitive to specific tyrosine kinase inhibitors (TKI).[7,9,10] In addition, there are case reports of patients responding to TKI treatment indicating that these aberrations represent a promising and relevant therapeutic target especially given the reported unfavorable prognosis of *BCR-ABL1*-like ALL.[5-7,11-14] However, the biology of this heterogeneous group of abnormalities is not fully understood and there is evidence that the prognosis of patients depends on the type of kinase activating lesion and the presence of cooperating aberrations such as *IKZF1* deletions and *JAK2* mutations.[7] Further studies, based on patients with specific gene fusions, are therefore warranted.

Here we present new data from 20 *ETV6-ABL1* patients and thoroughly scrutinized existing data from other patients with this fusion to evaluate different detection methodologies, estimate frequency, describe clinical features and outcome, and characterize the profile of copy number aberrations and gene expression of this distinct molecular subtype.

## Methods

### Patients

The cohort consisted of 44 patients with an *ETV6-ABL1* fusion and comprised newly identified cases (n=9), published cases with additional new data (n=11)[7,8,15-20] and cases with re-examined published data (n=24).[1,18,21-39] Standard diagnostics, including molecular genetics, karyotyping and fluorescence *in-situ* hybridization (FISH) were performed according to the standard practice of the local diagnostic laboratories. Basic clinical/outcome data were collected from treating centers. Detailed diagnostic procedures have been published for existing cases. For the newly described patients, diagnostic and treatment procedures and protocols were approved by local Institutional Review Boards. Informed consent was obtained in accordance with the Declaration of Helsinki.

Primary samples from the first and second MPN in lymphoid blast crisis of case 34-a-MPN used in this study were provided by the Institute Paoli Calmettes Tumor Bank (Marseille, France). The primary childhood leukemia sample of case 09-ch-ALL was provided by the Leukaemia and Lymphoma Research Childhood Leukaemia Cell Bank working with teams in the Bristol Genetics Laboratory (Southmead Hospital, Bristol), Molecular Biology Laboratory (Royal Hospital for Sick Children, Glasgow), Molecular Haematology Laboratory (Royal London Hospital, London), and Molecular Genetics Service and Sheffield Children's Hospital, Sheffield) in the UK.

### Detection of transcript variants

The expression of alternative splice variants (type A/B) was detected by end-point reverse transcriptase polymerase chain reaction (RT-PCR)[20] and quantitative RT-PCR (qRT-PCR) analyses, utilizing forward primers annealing to *ETV6* exon 5 (type B-specific: 5'- GCCCATCAACCTCTCTCATCG -3') or *ETV6* exon 4/*ABL1* exon 2 junction (type A-specific: 5'- CAGAACCATGAAGAAGAAGCCC -3'). For the schematic representation of transcript variants and position of primers see *Online Supplementary Figure S1*.

### Single nucleotide polymorphism array

Copy number alterations (CNA) were determined by single nucleotide polymorphism array (HumanOmni Express BeadChip, Illumina, San Diego, USA). DNA from bone marrow or peripheral blood cells was extracted using standard methods. DNA was labeled and hybridized according to the recommended Infinium HD assay Ultra protocol from Illumina; scanning was performed using an Illumina iScan System. GenomeStudio Software v2011.1 (Illumina) was used for genotype calling and quality control. DNA CNA and regions of uniparental disomy (UPD) were analyzed using the CNV Partition 2.4.4 algorithm plug-in within the GenomeStudio followed by visual inspection in the Illumina Chromosome Browser (display option of the GenomeStudio) and manual correction. Identified CNA/UPD were mapped against human genome assembly GRCh37/hg19. Deletions corresponding to somatic rearrangements of the immunoglobulin and T-cell receptor gene loci and CNA/UPD seen in remission samples (or those that overlapped with copy number polymorphisms listed in the Database of Genomic Variants) were excluded from further analysis.

To identify recurrently affected regions, the genome was segmented into the minimal segments using genomic coordinates for the start and end of all detected CNA/UPD from all analyzed samples. Segments affected by the same type of aberration (loss or gain or UPD) in more than two cases were called recurrently affected regions (*Online Supplementary Figure S2*). For this analysis continuous CNA/UPD in a particular sample could be segmented into several neighboring regions when they overlapped in the different sets of samples.

### Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification assays (P335-A3 ALL-IKZF1, MRC-Holland, The Netherlands) were performed according to the manufacturer's instruction. Fragments were analyzed on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Carlsbad, CA, USA) and data analyzed using Peak Scanner v1.0 and Coffalyser v9.4 software. Relative copy number was calculated after intra-sample normalization against control fragments and inter-sample normalization against control samples (healthy blood donor DNA). Probes with a ratio of 1±0.3 were assigned a normal copy number of 2, probes with a ratio of less than 0.7 and less than 0.3 were considered heterozygously and homozygously deleted, respectively, while probes with a ratio of more than 1.3 were considered amplified.

### Whole genome gene expression profiling and the BCR-ABL1-like signature

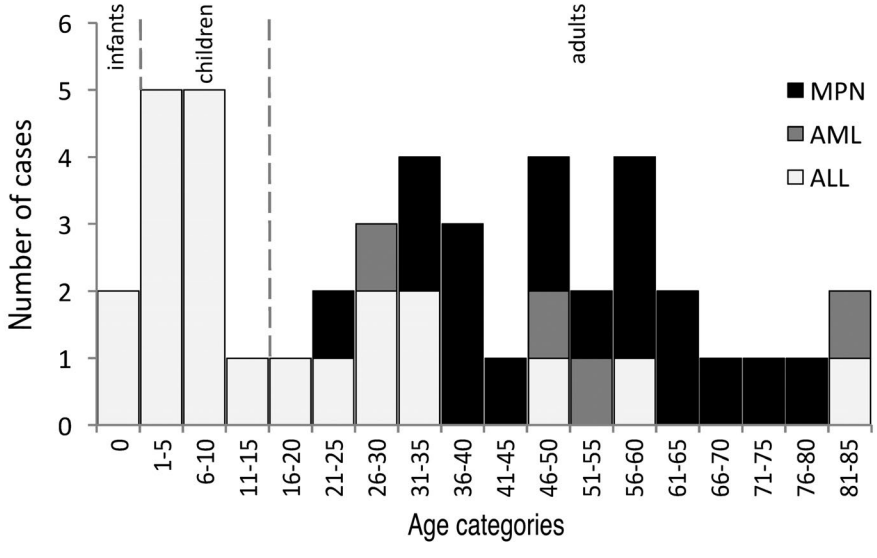
RNA was isolated from pretreatment diagnostic ALL samples and analyzed on Affymetrix Human Genome GeneChip® U133_Plus_2.0 arrays. CEL files from the previously published 1181 cases[7] (including 2 *ETV6-ABL1*-positive patients reported in this study: 06-ch-ALL and 14-a-ALL) were normalized together with another ten *ETV6-ABL1*-positive samples described here. Besides the 12 *ETV6-ABL1*-positive samples the cohort for gene expression profiling consisted of 664 children with National Cancer Institute–classified high-risk BCP-ALL, 348 adolescents (16 to 20 years old), and 167 young adults (21 to 39 years old). The cohort included 84 *BCR-ABL1*-positive ALL and 209 *BCR-ABL1*-like ALL. Expression data were generated using the default RMA conditions of the Expression Console 1.4.1 software (Affymetrix). The clustering was performed using MATLAB R2015a (MathWorks) with Euclidean distance and weighted linkage using 257 probe sets predicting *BCR-ABL1*-like status according to predictive analysis of microarrays.[7] Maximum ranges for coloring high (red) and low (green) expression were set to 10-fold the median. Information for *BCR-ABL1*-like and *BCR-ABL1* status was obtained from previously published data. The *BCR-ABL1*-like cluster was deduced from the expression patterns of its signature genes.[7]

The *BCR-ABL1*-like signature was determined using customized Taqman low density arrays at the South Australian Health & Medical Research Institute.[19]

## Results

### Disease subtype, frequency, demographics and clinical features

Of the 44 *ETV6-ABL1*-positive patients considered in this study, half were diagnosed with ALL [n=22; including one manifesting as B-lymphoblastic lymphoma (13-ch-LBL)], while the other half had chronic myeloid leukemia-




| Diagnosis | Age group | Male | Female | Total |
|---|---|---|---|---|
| ALL | *infant* | *1* | *1* | *2* |
| | *childhood* | *6* | *5* | *11* |
| | *adult* | *5* | *4* | *9* |
| | | **12** | **10** | **22** |
| **AML** | | **4** | **0** | **4** |
| **MPN** | | **12** | **5** | **18*** |
| Total | | 28 | 15 | 44 |

**Figure 1. Malignancy subtypes, age and gender distribution of the *ETV6-ABL1*-positive cases.** In childhood, all cases were diagnosed as ALL (albeit one originally manifested as B-lymphoblastic lymphoma). Nine childhood ALL were of BCP and two of T-cell origin. Notably, there were two infants in this group; however, most of the childhood ALL were diagnosed in the preschool/early school age (5-10 years). ALL is dominant until the fourth decade and then it is replaced by MPN, which account for more than 70% of cases in the age categories over 35 years. Overall, the male to female ratio is 1.9 : 1 (28:15). While gender distribution is almost even in ALL (M:F 12:10), patients with MPN were predominantly male (M:F 12:5) and all four AML cases were diagnosed in males. *The gender of one case of MPN was not reported.

like MPN (n=18) or AML (n=4) (Figure 1; Table 1). A high diagnostic white blood cell count (>50x10$^9$/L) was observed in 10/12 children and 3/7 adults with ALL. Interestingly, MPN cases presented with lower white blood cell counts (median 36x10$^9$/L with only 1/16 patients having a count >100x10$^9$/L) than those reported for patients with chronic myeloid leukemia.[40] Eosinophilia, suggested as a hallmark of *ETV6-ABL1* leukemia,[28] was present in all MPN and AML cases but only 4/13 ALL cases had an elevated absolute eosinophil count (>0.5x10$^9$/L).

Routine screening of newly diagnosed childhood ALL patients for *ETV6-ABL1* was started in 2003 and 2007 in the Czech Republic and Italy, respectively. By the end of 2014, 730 and 2795 cases had been screened by PCR. This prospective screening coupled with two smaller studies[41,42] allows us to estimate the frequency of *ETV6-ABL1* in childhood ALL at 0.17% (6/3610) which corresponds to ~1-2% of *BCR-ABL1*-like cases. Meta-analysis of the data from four studies of adult ALL cases showed a frequency of 0.38% (4/1060).[18,20,39,42] One screening study in AML found a single case (1/1197) which coupled with the fact that only four *ETV6-ABL1*-positive AML cases have been published so far suggests that *ETV6-ABL1* is very rare in AML.[18] Estimating the incidence of *ETV6-ABL1* in MPN is difficult because no systematic screening data are available.

### *Identification and location of the* ETV6-ABL1 *fusion*

Among the 22 cases of *ETV6-ABL1*-positive ALL, the fusion was originally identified by PCR screening (n=10), cytogenetics/FISH (n=6), whole transcriptome sequencing (RNAseq, n=3) or another approach (n=3). Among MPN/AML cases the majority of the fusions were originally detected by cytogenetics/FISH (16/21) and only a minority by PCR (n=2) or other techniques (n=3) (Table 1). In 14 cases, we re-analyzed samples to determine *ETV6-ABL1* splice variants. We detected both types (A and B, with type B expressed at significantly higher levels) in all but one case (patient 18-a-ALL in whom only type B was detected, possibly due to poor quality RNA) (*data not shown*).

A total of 28 cases were tested using commercial *ETV6* and/or *ABL1* FISH probes. Abnormal signal patterns indicative of *ETV6* or *ABL1* rearrangement were observed in only 19/28 (67%) cases tested with *BCR-ABL1* probes and 10/19 (53%) with *ETV6* probes. A further eight *ETV6* or *ABL1* aberrant signals were detected using specific BAC/PAC/YAC/cosmid FISH probes (7 *ETV6*, 1 *ABL1*) (Table 1 and *Online Supplementary Table S1*). In 30 patients the cytogenetic/FISH result enabled localization of *ETV6-ABL1* to a particular chromosome (*Online Supplementary Figure S3*). In most cases (18/30) the fusion was present on chromosome 12 (10/10 BCP-ALL, 7/16 MPN, 1/3 AML) whereas it was located on chromosome 9 in nine cases (7/16 MPN, 1/1 T-ALL, 1/3 AML). In three cases with more complex rearrangements involving a third chromosome the fusion signal was found on chromosome 8 (AML), 16 (MPN) and 17 (MPN).

### *Copy number aberration profile of* ETV6-ABL1*-positive leukemia*

We assessed the CNA profile of 22 samples from 18 *ETV6-ABL1* patients by high-density single nucleotide polymorphism array further refined by multiplex ligation-dependent probe amplification analysis in seven cases. The cohort comprised 15 patients with ALL and three with MPN (Figure 2; Table 1). Three MPN samples were taken at lymphoid blast crisis (LBC) and two in chronic phase (CP). Among ALL and MPN-LBC samples, we found an average of 12 regions of CNA or UPD per case (range, 2 to 29 alterations). Deletions were more common than gains (206:19). No CNA or UPD abnormalities were detected in two MPN-CP samples (*Online Supplementary Tables S2* and *S3*). Genome segmentation based on the overlap of all CNA identified 74 genomic regions which were affected in at least two patients. The majority of these regions are neighboring loci on chromosomes 7 and 9 (*Online Supplementary Figure S1*). The most prevalent aberrations detected by single nucleotide polymorphism array in ALL/MPN-LBC were deletions of *CDKN2A/CDKN2B* (16/17; 94%, although in one MPN case the deletion was apparent only at the second LBC) and deletions of *IKZF1* (15/17; 88%), followed by *PAX5* (8/17; 47%) and *BTG1* (7/17; 41%) deletions (although again in the same MPN case the *PAX5* deletion was detected only at the second LBC). In eight patients an aberration of the *ABL1* gene was detected (gain of entire *ABL1* in 4 cases, partial *ABL1* gain in 3 and partial deletion in 1 case) and three patients had partial deletions of *ETV6* exons 6-8 (associated with *ABL1* gain in all 3 cases). Taking into account the affected exons, these partial deletions/gains seem to result from the primary unbalanced genomic rearrangement creating the fusion gene rather than being secondary aberrations. Other recurrent aberrations occurring in two to five patients included deletions of *SLX4IP*, *ATP10A*, *BTLA*, *CD200* and *RB1* genes. Overall, the frequency of the aberrations is similar to that in *BCR-ABL1*-positive and *BCR-ABL1*-like (Ph-like) ALL. Notably, while the two MPN-CP samples had no detectable CNA, the MPN cases analyzed in LBC had the same CNA pattern as ALL cases (Figure 2).

### *Gene expression profile and* BCR/ABL1*-like status*

Expression profiling was performed on 12 samples from ten patients: diagnostic samples from seven ALL cases along with relapse/LBC samples from two cases of BCP-ALL and one MPN case. We compared the GEP of these *ETV6-ABL1* samples with those of a previously published cohort of 1179 BCP-ALL cases tested on the same platform at the University of New Mexico.[7] Overall, with the 257 probe sets predicting *BCR-ABL1*-like status according to predictive analysis of microarrays,[7] the GEP of all the *ETV6-ABL1* cases was consistent with the signature produced from *BCR-ABL1* cases; furthermore, in an unsupervised analysis, all *ETV6-ABL1* samples clustered within the *BCR-ABL1*-like cluster and in close vicinity to *BCR-ABL1* cases (unlike other *ABL1* and *ABL2* fusion genes) (Figure 3).

Moreover, we analyzed *ETV6-ABL1* cases by custom Taqman low-density array[19] to determine whether they would be classified as *BCR-ABL1*-like also when using a method which, unlike GEP, is applicable in routine diagnostics. Eleven of 12 cases (9 BCP-ALL, 1 T-ALL and 1 MPN-LBC) tested positive for the *BCR-ABL1*-like signature indicating that these cases do indeed share considerable similarity with other *BCR-ABL1*-like cases. The single negative sample came from a child with BCP-ALL (11-ch-ALL) (Table 1).

**Table 1.** Methods and results of the performed diagnostic/research genetic tests.

| Case ID | Cancer subtype | *ETV6/ABL1* identification | Type of transcript | Simplified cytogenetics | *ABL1* FISH | *ETV6* FISH | Fusion location (Chr) | Ph-like ALL signature * | SNP array * ¥ |
|---|---|---|---|---|---|---|---|---|---|
| 01-ch-ALL | BCP-ALL | PCR | A, B * | 46,XX | n.r. | n.r. | n.r. | pos(TLDA) | C,P |
| 02-ch-ALL | BCP-ALL | PCR | A, B * | 46,XY,inv(1),t(1;9),del(1), t(9;12),t(1;9),t(1;9;12) | normal | normal | 12 | pos(TLDA) | C,I |
| 03-ch-ALL | BCP-ALL | other | A | n.r. | n.r. | n.r. | n.r. | n.a. | n.a. |
| 04-ch-ALL | T-ALL | cytogenetics | A, B | 47,XXYc,del(6), ins(9;12),inv(9) | normal | abnormal % | 9 | n.a. | n.a. |
| 05-ch-ALL | BCP-ALL | PCR | A, B * | 46,XY | abnormal | normal | 12 | pos(TLDA) † | C,I,B |
| 06-ch-ALL | BCP-ALL | RNAseq | B | 46,XY,ins(12;9) | n.r. | n.r. | 12 | n.a. | C,I,P |
| 07-ch-ALL | BCP-ALL | RNAseq | n.r. | 46,XX,t(9;12) | n.r. | n.r. | n.r. | pos (LDA) & | n.a. |
| 08-ch-ALL | BCP-ALL | other | A, B * | no karyotype | n.r. | n.r. | n.r. | pos(TLDA) | C,I,B |
| 09-ch-ALL | BCP-ALL | cytogenetics | A, B * | 46,XX.ish ins(12;9) | abnormal | normal | 12 | pos(TLDA) | C,I,B |
| 10-ch-ALL | BCP-ALL | SNP array/ PCR ° | A, B * | no karyotype | normal | n.r. | n.r. | pos(TLDA) | C,I,P,B |
| 11-ch-ALL | BCP-ALL | cytogenetics | A, B * | 46,XY,?ins(12;9) | abnormal | normal | 12 | neg(TLDA) | I |
| 12-ch-ALL | T-ALL | PCR | A, B * | 46,XY | n.r. | n.r. | n.r. | pos(TLDA) | C |
| 13-ch-LBL | B-LBL | PCR | A, B * | 46,XY | n.r. | n.r. | n.r. | pos(TLDA) † | C,I,B |
| 14-a-ALL | BCP-ALL | RNAseq | n.r. | 46,XY | n.r. | n.r. | n.r. | n.a. | C,I,P,B |
| 15-a-ALL | BCP-ALL | PCR | A, B | 46,XX,del(9), t(9;10) | abnormal | normal | 12 | n.a. | C,I |
| 16-a-ALL | BCP-ALL | PCR | A, B | 47,XX,+5. ish t(9;12),inv(12) | abnormal | abnormal | 12 | n.a. | n.a. |
| 17-a-ALL | BCP-ALL | cytogenetics | A, B * | 45,XY,t(1;9), t(9;13),-13,t(16;22) | abnormal % | abnormal % | 12 | pos(TLDA)§ | C,I,P |
| 18-a-ALL | BCP-ALL | cytogenetics | B * | 46,XY,t(2;9), t(2;8),ins(12;9) | abnormal | normal | 12 | n.a. | n.a. |
| 19-a-ALL | BCP-ALL | PCR | A, B * | 46,XX,t(8;9;12) | abnormal | normal | 12 | n.a. | C,I |
| 20-a-ALL | BCP-ALL | PCR | A | 46,XX,t(9;12) | n.r. | n.r. | n.r. | n.a. | n.a. |
| 21-a-ALL | BCP-ALL | PCR | A | 46,XY,t(9;12) | n.r. | n.r. | n.r. | n.a. | n.a. |
| 22-a-ALL | BCP-ALL | cytogenetics | B | complex rearrangements; t(9;12) | n.r. | n.r. | n.r. | pos(TLDA) | C,I,P |
| 23-a-AML | AML-M2 | cytogenetics | A | 46,XY,t(8;12) | abnormal | abnormal % | 8 | n.a. | n.a. |
| 24-a-AML | AML-M1 | cytogenetics | B | 46,XY,t(9;12) | normal | abnormal % | 9 | n.a. | n.a. |
| 25-a-AML | AML | PCR | B | 46,XY | abnormal | normal | 12 | n.a. | n.a. |
| 26-a-AML | AML-M6 | other | B | 45,t(9;12;14), complex rearrangements | n.r. | n.r. | n.r. | n.a. | n.a. |
| 27-a-MPN | MPN | SNP | A, B | 46,XX | normal | abnormal | 9 | n.a. | n.a. |
| 28-a-MPN | MPN | cytogenetics | B | 46,XY,t(12;14) | n.r. | n.r. | 9 | n.a. | n.a. |
| 29-a-MPN | MPN | cytogenetics | n.r. | 46,XY,t(9;12) | abnormal | abnormal | n.r. | n.a. | n.a. |
| 30-a-MPN | MPN | cytogenetics | n.r. | 46,XX,t(9;12) | normal | abnormal (m) | 9 | n.a. | n.a. |
| 31-a-MPN | MPN | cytogenetics | A, B | 46,XX,t(9;12) | abnormal (i) | abnormal | 9 | n.a. | n.a. |
| 32-a-MPN | MPN | n.r. | B | n.r. | n.r. | n.r. | 12 | n.a. | n.a. |
| 33-a-MPN | MPN | other | A, B | 46,XY | normal | n.r. | n.r. | n.a. | n.a. |
| 34-a-MPN | MPN | cytogenetics | A, B * | 44,X,-Y,-7,?add(9), del(9),add(12) | abnormal | normal | 12 | pos(TLDA)§ | C[2],I[1],P[2],B[1] |
| 35-a-MPN | MPN | cytogenetics | A, B | 46,XY,del(6),t(9;12) | normal | abnormal % | 9 | n.a. | n.a. |
| 36-a-MPN | MPN | cytogenetics | n.r. | 46,XX,ins(9;12) | abnormal | n.r. | 9 | n.a. | n.a. |
| 37-a-MPN | MPN | PCR | A, B | 51,XX,t(5;9),add(17),del(5), (+8,+11,+12,+18,+19) | abnormal | abnormal § | 12 | n.a. | n.a. |
| 38-a-MPN | MPN | cytogenetics | A, B * | 46,XY,t(5;12;16) | abnormal | abnormal % | 16 | n.a. | no |
| 39-a-MPN | MPN | cytogenetics | n.r. | 46,XY,ins(12;9) | abnormal | abnormal (i) | 12 | n.a. | n.a. |
| 40-a-MPN | MPN-B-LBC | cytogenetics | n.r. | 45,XY.isht(9;9),t(9;12) | n.r. | n.r. | 12 | n.a. | C,I,P |
| 41-a-MPN | MPN-EM-T-LBC | cytogenetics | A, B | 47,XY,t(9;12),del(12), t(7;14),+19 | normal | abnormal (m) | 9 | n.a. | n.a. |

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|---|---|---|---|---|---|---|---|---|---|
| 42-a-MPN | MPN-MBC | cytogenetics | B | 45,XY,-7,t(9;12) | abnormal | abnormal % | 12 | n.a. | n.a. |
| 43-a-MPN | MPN-MBC | cytogenetics | A, B | 49,XY,+11,t(9;12), +der(12)t(9;12),+19,t(1;22) | abnormal | abnormal § | 12 | n.a. | n.a. |
| 44-a-MPN | MPN-B-LBC | cytogenetics | B | 46,XY,t(12;17) | abnormal | abnormal (m) | 17 | n.a. | n.a. |

*BCP-ALL: B-cell precursor acute lymphoblastic leukemia; T-ALL: T-lineage acute lymphoblastic leukemia; AML: acute myeloid leukemia; MPN: myeloproliferative neoplasm; EM: extramedullary; B-LBL: B-lymphoblastic lymphoma; B-LBC: B-lymphoid blast crisis; T-LBC: T-lymphoid blast crisis; MBC: myeloid blast crisis; a: adult; ch: childhood; Chr: chromosome; TLDA: Taqman low-density array; SNP: single nucleotide polymorphism; n.r.: not reported; n.a.: not available. *Analyses performed within the present study. [$]Presence of deletions involving* CDKN2A/CDKN2B *(C),* IKZF1 *(I),* PAX5 *(P) and* BTG1 *(B). [1]deletion gained at 1st recurrence; [2]deletion gained at 2nd recurrence; °CNA with the breakpoint in* ABL1 *intron 1 identified by SNP array,* ETV6/ABL1 *confirmed by PCR. [§]Analyses performed at disease recurrence (1st recurrence in 13-ch-ALL and 1st and 2nd recurrences in 05-ch-ALL and 34-a-MPN). [†]Analyses performed at initial disease and recurrence (1st recurrence in 13-ch-ALL and 05-ch-ALL and 1st and 2nd recurrences in 34-a-MPN). [&]Analysis performed on the cell line established from the initial disease. [#]Analyzed by low density array (LDA) at the University of New Mexico, USA. (i) Abnormal on interphases, normal on metaphases. (m) Abnormal on metaphases, normal on interphases. % Abnormal with specific BAC/YAC/PAC/cosmid probes used. [§]Trisomy 12.*

| | | CDKN2A/ CDKN2B* | IKZF1 | PAX5 | BTG1 | ABL1 | SLX4IP | ATP10A | CD200/ BTLA | ETV6 | RB1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ch-ALL | 01-ch-ALL | | | | | # | | | | | |
| | 02-ch-ALL $ | | 1-8 | | | | | | | | |
| | 05-ch-ALL $ | | 4-7 | | | | | | | | |
| | 05-ch-ALL-RII $ | | 4-7 | | | | | | | | |
| | 06-ch-ALL | | 1-4 | | | | | | | | |
| | 08-ch-ALL $ | | 2-8 | | | | | | | | |
| | 09-ch-ALL $ | | 2-8 | | | | | | | | |
| | 10-ch-ALL $ | | 2-7 | | | 2-12 | | | | | |
| | 11-ch-ALL | | | | | | | | | 6-8 | |
| | 12-ch-ALL | | | | | 2-12 | | | | 6-8 | |
| | 13-ch-LBL | | 2-8/4-7 | | | | | | | | |
| | 13-ch-LBL-RII | | 2-8/4-7 | | | | | | | | |
| a-ALL | 14-a-ALL | | 2-3/4-7 | | | | | | | | |
| | 15-a-ALL | | 2-8/4-7 | | | | | | | | |
| | 17-a-ALL $ | | 1-3 | | | 2-12 | | | | | |
| | 19-a-ALL $ | | 4-7 | | | | | | | | |
| | 22-a-ALL | | 1-8 | | | | | | | | |
| LBC | 34-a-MPN-RI | | 4-7 | | | | | | | | |
| | 34-a-MPN-RII | | 4-7 | | | | | | | | |
| | 40-a-MPN | | 1-8 | | | | | | | 6-8 | |
| CP | 34-a-MPN | | | | | | | | | | |
| | 38-a-MPN | | | | | | | | | | |
| **total affected ALL/LBC cases** | | **16** | **15** | **8** | **7** | **7** | **5** | **4** | **4** | **3** | **2** |
| **(%)** | | **94** | **88** | **47** | **41** | **41** | **29** | **24** | **24** | **18*** | **12** |
| *total affected ch-ALL (%)* | | *90* | *80* | *30* | *50* | *50* | *40* | *20* | *10* | *20** | *10* |
| *total affected a-ALL (%)* | | *100* | *100* | *60* | *20* | *20* | *20* | *20* | *40* | *0* | *20* |

**Figure 2. Overview and frequency of selected recurrent copy number aberrations in 22 samples (17 ALL, 3 MPN-LBC, 2 MPN-CP) from 18 *ETV6-ABL1*-positive cases.** The most prevalent aberrations detected by the SNP array in ALL/MPN-LBC were deletions of *CDKN2A/CDKN2B* and deletions of *IKZF1* followed by *PAX5* (8/17; 47%) and *BTG1* (7/17; 41%) deletions. *CDKN2A* and *CDKN2B* as well as *BTLA* and *CD200* genes were co-deleted in all cases, thus the pairs are merged into one column. Deletion of *ATP10A* was recently shown to be a recurrent aberration in BCP-ALL, associated with decreased 10-year event-free survival[56]. Gray indicates loss, black indicates a gain, deleted exons within *IKZF1*, *ABL1* and *ETV6* genes are indicated by numerals (for *IKZF1* white numerals depict losses resulting in a dominant negative *IKZF1* isoform or complete loss of *IKZF1* expression, black numerals depict haploinsufficiency with the potential exception of combined 2-3/4-7 loss which can affect either a single allele or both gene alleles and thus can result in either haploinsufficiency or complete loss). For frequencies of the selected recurrent copy number aberrations in published ALL studies see *Online Supplementary Figure S4*. a: adult; ch: childhood; LBC: lymphoid blast crisis of MPN; CP: chronic phase of MPN. [$]In these cases some of the findings from the SNP array were further refined by multiplex ligation-dependent probe amplification. [#]*ABL1* deletions were not found recurrently in the present study, thus, only *ABL1* gains were considered for CNA frequency. * The relatively high frequency of *ETV6* deletions in *ETV6-ABL1*-positive cases compared to that in *BCR-ABL1*-positive ALL probably does not reflect frequency of genuine secondary *ETV6* loss but rather results from *ETV6* loss during primary genomic rearrangement.

### *Clinical characteristics, treatment and outcome*

Response to treatment is the most important predictor of outcome in ALL.[43-45] Treatment response is determined differently per protocol but is usually measured after 1 or 2 weeks of treatment with corticosteroids ("prednisone response") and again at the end of induction therapy. In this series, both children with a poor prednisone response died whereas three of four patients with a good prednisone response remain alive after >3 years (Table 2). Residual disease was assessed at the end of induction in

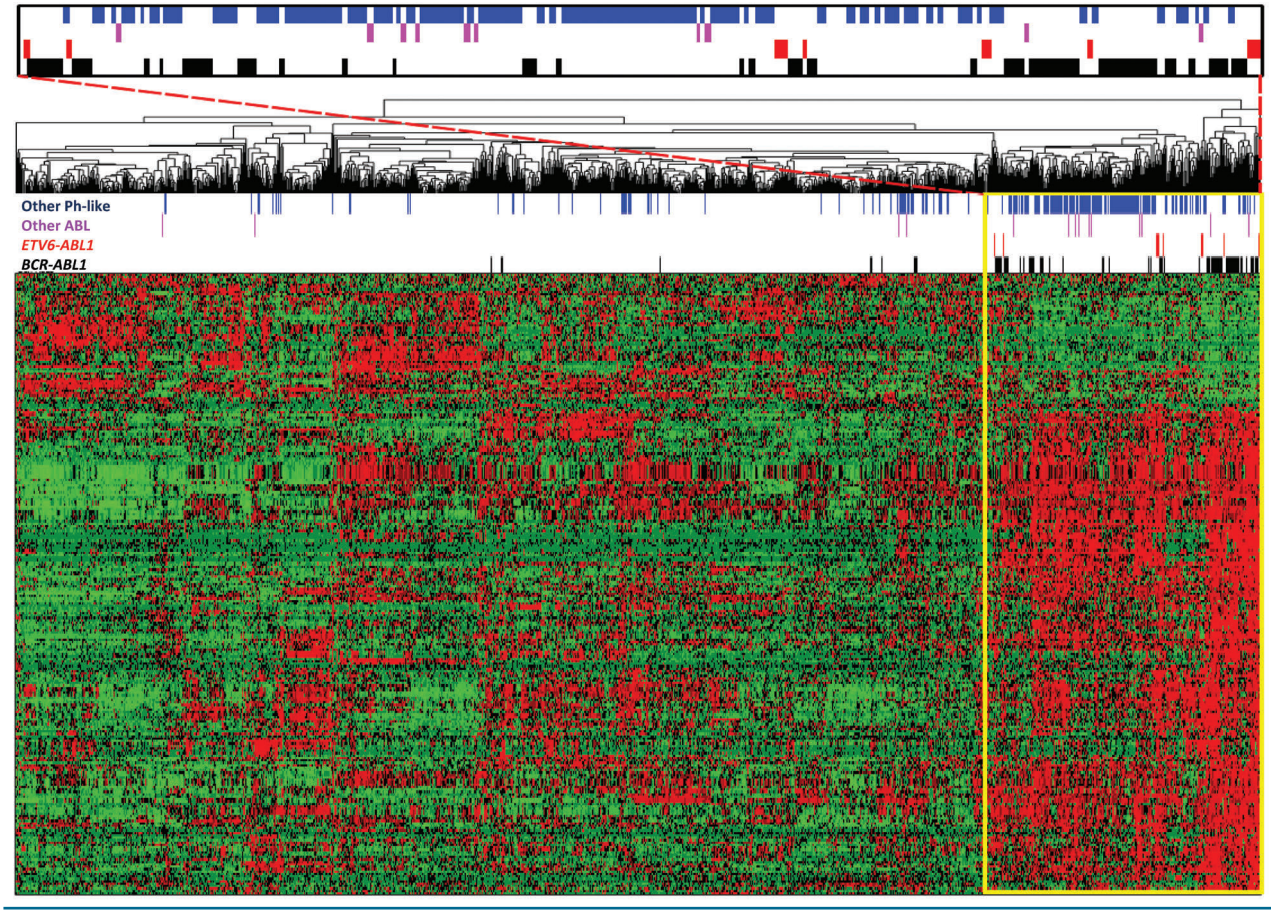


**Figure 3. Unsupervised hierarchical clustering of *ETV6-ABL1* cases with a large cohort of BCP-ALL.** Twelve *ETV6-ABL1* cases clustered with a cohort of 1179 BCP-ALL cases using data from RMA normalized U133_Plus_2.0 arrays. The 1191 samples are shown in columns, while the 257 *BCR-ABL1*-like predicting probe sets (described previously[7]) are in rows. The yellow box highlights the *BCR-ABL1*-like cluster. *BCR-ABL1*, *ETV6-ABL1* and other *ABL1* and *ABL2* fusion (*NUP214-ABL1*, *SNX2-ABL1*, *ZMIZ1-ABL1*, *RSCD1-ABL1*, *RSCD1-ABL2*, *PAG1-ABL2*, *ZC3HAV1-ABL2*) status is shown with black, red and magenta bars, respectively, across the top of the heatmap. All other Ph-like cases are shown with blue bars using the status defined in their original report.[7] The order of samples in the Ph-like cluster is enlarged on the top (with the same color coding). The order of the *ETV6-ABL1* samples as shown in the figure, from left to right is 34-a-MPN (1st LBC)/08-ch-ALL/10-ch-ALL/09-ch-ALL/06-ch-ALL/14-a-ALL/11-ch-ALL/12-ch-ALL/13-ch-LBL (1st relapse)/05-ch-ALL (2nd relapse)/05-ch-ALL (1st relapse)/34-a-MPN (2nd LBC).

ten children; four cases had no or low levels of minimal residual disease ($<10^{-4}$) whereas six cases had higher levels of residual disease ($>10^{-4}$). Of the four patients with low-risk minimal residual disease, three are alive >4 years after diagnosis whereas one patient died after a bone marrow relapse. One infant case with a borderline level of minimal residual disease ($3x10^{-4}$) is alive after a central nervous system relapse. Out of the five patients with minimal residual disease levels $\geq 10^{-3}$ at the end of induction therapy, three relapsed and died while the other two are in first remission, but only 19 and 28 months after diagnosis (Table 2). Of the nine adults with ALL, seven died, one relapsed with no further outcome data and one was in short-term remission after stem cell transplantation. The survival rate of the ALL patients is 46% in children (6/13 alive) and 13% in adults (1/8 alive). Three of the four AML patients failed to achieve a complete remission and hence died within a few months. The overall survival of *ETV6-ABL1* MPN cases is 50% (9/18 alive) but the risk of death is more than double for patients diagnosed in blast crisis. Importantly, of the 23 patients with *ETV6-ABL1*-positive malignancies in whom the cause of death could be evaluated, 19 (>80%) died of disease progression or relapse.

Although TKI were not used uniformly to treat this cohort of patients, 17 patients were given a TKI at some point during their treatment. The two children with ALL who received imatinib during their frontline treatment remain in first remission 46 and 57 months after initial diagnosis even though one experienced an isolated central nervous system relapse after 20 months. Two children were treated with TKI after relapse but both died shortly afterwards. In adult ALL, one patient (aged 81) was treated with TKI within frontline treatment and died after relapse (Table 2).

A total of five MPN patients diagnosed in CP received imatinib during frontline therapy and all are still alive. However, two cases did switch to nilotinib because of disease progression. In comparison only three out of eight MPN patients diagnosed in CP who did not receive a TKI are still alive. Two patients received a TKI at disease progression only but both subsequently died. Five of the MPN patients were diagnosed in blast crisis (Table 2). Four of these patients received a TKI during frontline treatment and the fifth patient only after relapse. Four of these MPN patients with blast crisis died, three within 1 year. One patient who received a TKI during frontline therapy remains alive after 12 months but has cytogenetically detectable residual leukemia.

**Table 2.** Demographics, clinical characteristics and outcome of *ETV6/ABL1*-positive cases.

| | | | | | | First manifestation | | | | | | | First recurrence/progression | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case ID | Cancer subtype° | Age at Dx (years) | Sex | WBC (x10⁹/L) | Eosinophilia | Treatment | TKI | SCT | Prednisone response | MRD EOI | Complete remission | Recurrence/ progression | Type (time) | Treatment | TKI | SCT | Additional recurrence(s)/ progression(s) | Outcome | Reference(s) |
| 01-ch-ALL | BCP-ALL | 0 | F | 158 | no | Interfant 06 + TKI | IM | MUD | PGR | $3x10^{-4}$ | yes | yes | CNS (20M) | AIEOP ALL REC2003 (S2) | n.r. | MUD# | no | alive (CR>46M) | p.s |
| 02-ch-ALL | BCP-ALL | 0 | M | 565 | yes* | Interfant 06 (LR) | no | no | PGR | $1x10^{-3}$ | yes | yes | CNS (8M) | ALL BFM REZ2002 (S2) + TKI | IM, DASA | UCB | yes | died (15M) | 20 |
| 03-ch-ALL | BCP-ALL | 1 | F | 204 | n.r. | n.r. | no | n.r. | n.r./n.a. | n.r./n.a. | no | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | died (3M) | 33 |
| 04-ch-ALL | T-ALL | 4 | M | 67 | yes | CHT (MR) | no | no | n.r./n.a. | n.r./n.a. | yes | yes | n.r. (15M) | n.r. | n.r. | no | no | died (22M) | 37 |
| 05-ch-ALL | BCP-ALL | 5 | M | 184 | yes | AIEOP-BFM ALL 2000 (HR) | no | no | PPR | $9x10^{-5}$ | yes | yes | BM (25M) | ALL BFM REZ2002 (S3) | no | no | yes$ | died (32M) | 20 |
| 06-ch-ALL | BCP-ALL | 5 | M | 83 | n.r. | AALL0232 | no | no | n.r./n.a. | $1x10^{-3}$ | yes | yes | CNS (12M) | n.r. | no | no | no | died (18M) | 7,8 |
| 07-ch-ALL | BCP-ALL | 5 | F | 322 | n.r. | AALL1131 (B, VHR) | no | no | n.r./n.a. | $6x10^{-2}$ | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>28M) | 7 |
| 08-ch-ALL | BCP-ALL | 7 | F | 5 | no | AIEOP-BFM ALL 2000 (SR) | no | no | PGR | neg | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>72M) | p.s. |
| 09-ch-ALL | BCP-ALL | 8 | F | 282 | no | UKALL 2003 (sch B->C)+TKI | IM | no | n.r./n.a. | $<5x10^{-5}$ | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>57M) | 16 |
| 10-ch-ALL | BCP-ALL | 8 | F | 284 | yes* | AIEOP-BFM ALL 2000 (SR) | no | no | PGR | neg | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>60M) | p.s. |
| 11-ch-ALL | BCP-ALL | 9 | M | 9 | no | UKALL 2011 (sch A->C) | no | no | n.r./n.a. | $1x10^{-3}$ | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>19M) | p.s. |
| 12-ch-ALL | T-ALL | 11 | M | 167 | n.r. | AIEOP-BFM ALL 2009 (HR) | no | yes | PPR | $2x10^{-2}$ | yes | yes | BM+MED (9M) | paliative | no | no | no | died (11M) | p.s. |
| 13-ch-LBL | B-LBL | 9 | M | 8 | no | AIEOP LNH 97 | no | no | n.r./n.a. | n.r./n.a. | yes | yes | EM (43M) | AIEOP ALL REC2003 (A) | no | aSCT | yes& | died (73M) | p.s. |
| 14-a-ALL | BCP-ALL | 19 | M | 2 | no | hyper-CVAD + rituximab | no | no | n.r./n.a. | n.r./n.a. | yes | yes | CNS (13M) | DepoCyt | no | no | yes | died (20M) | 7 |
| 15-a-ALL | BCP-ALL | 25 | F | 12 | no | modif CVAD+ CONS/i.th. CHT | no | no | PGR | n.r./n.a. | yes | yes | BM (14M) | n.r. | n.r. | no | no | died (15M) | 18 |
| 16-a-ALL | BCP-ALL | 30 | F | 227 | n.r. | IND + CONS CHT | no | PBSCT | n.r./n.a. | n.r./n.a. | yes | yes | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | 35 |
| 17-a-ALL | BCP-ALL | 30 | M | 129 | no | V/P/D/L+MTX i.th.+Ara-C | no | aSCT | n.r./n.a. | n.r./n.a. | yes | yes | BM+CNS (4M) | V/P/D | no | no | no | died (11M) | 15 |
| 18-a-ALL | BCP-ALL | 32 | M | 36 | no | UKALL12 | no | no | n.r./n.a. | n.r./n.a. | yes | yes | BM+CNS (6M) | FLAG | no | no | no | died (13M) | p.s. |
| 19-a-ALL | BCP-ALL | 33 | F | 22 | no | n.r. | n.r. | n.r. | n.r./n.a. | n.r./n.a. | yes | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. | died (18M) | 20 |
| 20-a-ALL | BCP-ALL | 49 | F | n.r. | n.r. | IND (V/P/D/L) | no | yes | n.r./n.a. | $1x10^{-1}$ | n.r. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | alive (>24M) | 39 |
| 21-a-ALL | BCP-ALL | 58 | M | n.r. | n.r. | IND (V/P/D/L) | no | no | n.r./n.a. | $1x10^{-1}$ | no | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | died (3M) | 39 |
| 22-a-ALL | BCP-ALL | 81 | M | 183 | n.r. | IND (D/V/M)/ PRED+TKI | DASA | no | n.r./n.a. | n.r./n.a. | yes | yes | BM+CNS (9M) | HD-DEXA/ VCR + TKI | PONA | no | yes | died (11M) | 7,19 |
| 23-a-AML | AML-M2 | 29 | M | 40 | yes | GIMEMA LAM99 | no | HRD | n.r./n.a. | n.r./n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (>20M) | 28 |
| 24-a-AML | AML-M1 | 48 | M | 8 | yes | Ara-C/IDA/ETO | no | no | n.r./n.a. | n.r./n.a. | no | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | died (6M) | 28 |

*continued on the next page*

*continued from the previous page*

| | | | | | | First manifestation | | | | | | | First recurrence/progression | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case ID | Cancer subtype° | Age at Dx (years) | Sex | WBC (x10⁹/L) | Eosinophilia | Treatment | TKI | SCT | Prednisone response | MRD EOI | Complete remission | Recurrence/ progression | Type (time) | Treatment | TKI | SCT | Additional recurrence(s) progression(s | Outcome | Reference(s) |
| 25-a-AML | AML | 52 | M | 83 | yes | IND (MTX/ETO/Ara-C) | no | no | n.r./n.a. | n.r./n.a. | no | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | died (4M) | 18 |
| 26-a-AML | AML-M6 | 81 | M | n.r. | n.r. | n.r. | no | n.r. | n.r./n.a. | n.r./n.a. | no | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | died (3M) | 1 |
| 27-a-MPN | MPN | 24 | F | 99 | yes | TKI | IM | no | n.r./n.a. | n.r/n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>7M) | 25 |
| 28-a-MPN | MPN | 32 | M | 29 | yes | n.r. | no | yes | n.r./n.a. | n.r/n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>36M) | 21 |
| 29-a-MPN | MPN | 36 | M | 55 | yes | HU + TKI | IM | no | n.r./n.a. | n.r/n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>60M) | 34 |
| 30-a-MPN | MPN | 44 | F | 37 | yes | HU/IFNa | no | no | n.r./n.a. | n.r/n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>6M) | 27 |
| 31-a-MPN | MPN | 46 | F | n.r. | yes | TKI | IM | no | n.r./n.a. | n.r/n.a. | yes | yes | MPN (6M) | TKI | NILO | no | no | alive (CR>22M) | 24 |
| 32-a-MPN | MPN | 49 | n.r. | n.r. | yes | n.r. | no | n.r. | n.r./n.a. | n.r/n.a. | no | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | died (3M) | 23 |
| 33-a-MPN | MPN | 53 | M | 22 | yes | HU/IFNa | no | no | n.r./n.a. | n.r/n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (CR>69M) | 29 |
| 34-a-MPN | MPN | 57 | M | 25 | yes | IFNa | no | no | n.r./n.a. | n.r/n.a. | yes | yes | B-LBC (19Y) | EWALL + TKI | IM | no | yes& | died (240M) | 17 |
| 35-a-MPN | MPN | 59 | M | 27 | yes | HU | no | no | n.r./n.a. | n.r/n.a. | no | yes | MPN (12M) | GC/TG | no | no | no | died (13M) | 37 |
| 36-a-MPN | MPN | 61 | F | 92 | yes | TKI | IM | no | n.r./n.a. | n.r/n.a. | yes | yes | MPN (18M) | TKI | DASA, NILO | no | no | alive (CR>36M) | 31 |
| 37-a-MPN | MPN | 65 | F | 25 | yes | LD-Ara-C | no | no | n.r./n.a. | n.r/n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | died (12M) | 30 |
| 38-a-MPN | MPN | 68 | M | 52 | n.r. | TKI | NILO | no | n.r./n.a. | n.r/n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | n.a. | alive (>41M) | p.s. |
| 39-a-MPN | MPN | 79 | M | 35 | yes | n.r. | no | no | n.r./n.a. | n.r/n.a. | n.r. | yes | MBC (12M) | TKI | IM | no | no | died (13M) | 26 |
| 40-a-MPN | MPN-B-LBC | 59 | M | 25 | yes | BFM-93 | no | no | n.r./n.a. | n.r/n.a. | yes | yes | MPN (37M) | TKI | IM | no | yes | died (64M) | p.s. |
| 41-a-MPN | MPN-EM-T-LBC | 31 | M | 51 | yes | hyper-CVAD+TKI | DASA | yes | n.r./n.a. | n.r/n.a. | n.r. | no | n.a. | n.a. | n.a. | n.a. | no | died (11M) | 38 |
| 42-a-MPN | MPN-MBC | 36 | M | 238 | yes | LD-Ara-C/ETO/ MTX+TKI | IM | no | n.r./n.a. | n.r/n.a. | yes | yes | MBC (9M) | n.r. | n.r. | no | no | died (8M) | 22 |
| 43-a-MPN | MPN-MBC | 38 | M | 78 | n.r. | HU + TKI | IM | no | n.r./n.a. | n.r/n.a. | yes | yes | MBC | FLAG | no | no | no | died (6M) | 32 |
| 44-a-MPN | MPN-B-LBC | 72 | M | 6 | n.r. | TKI | IM | no | n.r./n.a. | n.r/n.a. | yes | no | n.a. | n.a. | n.a. | n.a. | no | alive (CR>12M) | 36 |

*BCP-ALL: B-cell precursor acute lymphoblastic leukemia; T-ALL: T-lineage acute lymphoblastic leukemia; AML: acute myeloid leukemia; MPN: myeloproliferative neoplasm; EM: extramedullary; B-LBL: B-lymphoblastic lymphoma; B-LBC: B-lymphoid blast crisis; T-LBC: T-lymphoid blast crisis; MBC: myeloid blast crisis; a: adult; ch: childhood; Dx: diagnosis; n.r.: not reported; n.a.: not applicable; m: male; f: female; WBC: white blood cell count; TKI: tyrosine-kinase inhibitor; LR: low risk; SR: standard risk; MR: medium risk; HR: high risk; VHR: very high risk; sch: schedule; CHT: chemotherapy, IND: induction; CONS: consolidation; i.th.: intrathecal; VCR: vincristine; PRED: prednisone; HD-DEXA: high-dose dexamethasone; MTX: methotrexate; Ara-C: cytosine arabinoside; IDA: idarubicin; ETO: etoposide; HU: hydroxyurea; IFNa: interferone alfa; LD-Ara-C: low dose Ara-C; CG: glucocorticoids; TG: thioguanine; D/V/M: high-dose dexamethasone + pulses of vincristine + intrathecal methotrexate; V/P/D/L: vincristine + prednisone + daunorubicin + L-asparaginase; FLAG: fludarabine + high-dose cytarabine + granulocyte colony-stimulating factor; hyper-CVAD: cyclophosphamide + vincristine + doxorubicin + dexamethasone + methotrexate + cytarabine; modif CVAD: cytarabine + vincristine + doxorubicin + daunorubicin + dexamethasone; IM: imatinib mesylate; DASA, dasatinib; NILO: nilotinib; PONA: ponatinib; SCT: stem cell transplantation; MUD: matched unrelated donor; PBSCT: allogeneic peripheral blood SCT; HRD: haplo-identical related donor; UCB: umbilical cord blood; aSCT: autologous SCT; PPR: prednisone poor response; PGR: prednisone good response; MRD-EOI: minimal residual disease at the end of induction; CNS: central nervous system; BM: bone marrow; MED: mediastinum; M: month; p.s.: present study. ° The 1st disease manifestation*

**Only relative eosinophilia due to high WBC (1% eosinophils in differential cell count). #From the same donor as in the 1st SCT. $TKI used in the treatment of the 2nd relapse. &After the 1st recurrence and 2nd complete remission cases 13-ch-LBL and 34-a-MPN experienced 2nd and 3rd recurrences of BCP-ALL character.*

## Discussion

The *ETV6-ABL1* fusion is rare but can occur in a range of hematologic malignancies; principally BCP-ALL (sporadically manifesting as lymphoma) and MPN but also T-ALL and AML. In this respect, *ETV6-ABL1*-positive malignancies are reminiscent of *BCR-ABL1*-positive neoplasms.[46] An additional similarity lies in the non-random age distribution with acute leukemias dominating in children and young adults and MPN in older adults.[46] However, the incidence of *ETV6-ABL1* leukemia is considerably lower than that of *BCR-ABL1* leukemia, accounting for <1% of cases of ALL at any age. An accurate estimate of the frequency of *ETV6-ABL1* in MPN remains elusive as there has not been any systematic screening. However, all cases of *BCR-ABL1*-negative chronic myeloid leukemia-like MPN should be screened for this fusion as it might account for a significant proportion of this relatively rare condition.

Given that at least three chromosomal breaks are required to generate an in-frame fusion, the genomic rearrangement is not uniform across cases and typically involves cryptic insertions. Our study suggests that insertions of *ABL1* into the *ETV6* locus are favored in BCP-ALL (10/10 patients) whereas in other cases the location of the fusion was evenly distributed - we can only speculate about the biological basis and relevance of this finding or whether it is simply a coincidence. The complex and heterogeneous nature of the rearrangement, coupled with its rarity, makes routine detection challenging. Routine karyotyping is usually inconclusive and FISH with either *BCR-ABL1* or *ETV6-RUNX1* probes (including specific "break-apart" probes) can miss the fusion – the cryptic insertions are often too small to generate a visible signal split. Indeed, insertions of part of the *ABL1* gene into the *ETV6* locus on 12p were frequently missed with the *ETV6* probes (9/13 cases), and, *vice versa*, insertions of the 5' part of the *ETV6* gene into the *ABL1* locus on 9q can be missed with the *ABL1* specific probes (6/8 cases) (*Online Supplementary Figure S3*). Thus, only a combination of *ETV6* and *ABL1* probes reliably identifies the fusion by FISH.

There are two types of *ETV6-ABL1* transcript, caused by alternative splicing, but their expression was not consistently analyzed in previous studies. We detected both transcripts in all but one of the samples we analyzed, with the type B variant, including *ETV6* exon 5, expressed at higher levels. These observations do not agree with those of previous studies which reported four cases expressing only the type A transcript. However, in three cases the type B fusion could have been missed due to the primers used for RT-PCR.[33,39] In the other case, the absence of *ETV6* exon 5 in the expressed transcript was shown to result from direct disruption of this exon by a fusion-generating DNA break.[28] A chimeric kinase without *ETV6* exon 5 lacks the GRB2 binding site and has attenuated oncogenic potential analogous to *BCR-ABL1* deficient for GRB2 interaction.[28,47,48] The predominance of the type B transcript does, therefore, have a biological basis. The recently reported complex network of kinase lesions in ALL makes RNA sequencing a useful additional diagnostic tool and in three cases in this series the *ETV6-ABL1* type B fusion was identified this way. Some clinical study groups have opted to pre-select BCP-ALL for *BCR-ABL1*-like cases prior to screening for specific fusions.[7,8] However, we present an *ETV6-ABL1* BCP-ALL case classified as *BCR-ABL1*-like-negative by Taqman low density array analysis (despite clustering with other *ETV6-ABL1* and *BCR-ABL1* cases on GEP) and a T-ALL case with *ETV6-ABL1* classified as *BCR-ABL1*-like positive. Thus although methods to evaluate the *BCR-ABL1*-like signature were developed on expression data from BCP-ALL, they might also have the potential to identify T-ALL cases with kinase-activating lesions. This potential does, however, need to be evaluated further.

Genomic and gene expression profiling demonstrates the similarity of *ETV6-ABL1* and *BCR-ABL1* cases. Besides the involvement of *ABL1*, these cases have a similar profile of secondary aberrations including frequent deletions of *IKZF1*, *CDKN2A/B*, *PAX5*, *BTG1* and *RB1* which distinguish them from other ALL.[49-51] We have previously shown the prenatal origin of *ETV6-ABL1* in a 5-year old child with ALL[20] demonstrating the need for cooperating mutations to induce an overt disease. The two most frequent secondary aberrations in *ETV6-ABL1* ALL (*CDKN2A/B* and *IKZF1* loss) have both been shown to cooperate with *BCR-ABL1* during leukemogenesis in mice and likely represent cooperating lesions also in *ETV6-ABL1* leukemia.[52,53] These and other CNA recurrently found in our cohort (*ATP10*, *BTLA/CD200*) have been associated with an unfavorable prognosis in ALL overall and specifically in *BCR-ABL1* and *BCR-ABL1*-like ALL.[6,13,54-57] Interestingly, we found no secondary aberrations in MPN-CP samples but progression to LBC was accompanied by the gain of a profile similar to that of *ETV6-ABL1* ALL.

Most studies show that the *BCR-ABL1*-like subgroup has an inferior prognosis.[5-7] The final outcome was shown to depend significantly on the particular primary lesion (with childhood ALL bearing *ABL*-class aberrations having 5-year event-free survival and overall survival rates of 50% and 68%, respectively) and also on the presence of secondary aberrations (e.g. *IKZF1* deletions).[7] A recent study showed that, on current minimal residual disease-based protocols, the prognosis of *BCR-ABL1*-like patients treated with standard chemotherapy and stem cell transplantation is not inferior to that of patients with other ALL; however, only one out of 40 analyzed *BCR-ABL1*-like patients in this study bore an *ABL* gene fusion (*NUP214-ABL1*) and thus the results are not directly applicable to *ETV6-ABL1* cases.[58] Our study confirms the poor outcome associated with *ETV6-ABL1* in ALL and AML; however, we did observe long-term survivors in childhood ALL among patients with a good initial response. The number of patients in this study was small but there is corroborating evidence in the literature from patients with other kinase fusions[58] to support the notion that a TKI can be useful for treating slow-responding or high-risk ALL. Again among a small number of cases of MPN there was a trend towards improved outcome for CP patients treated upfront with a TKI (5/5 patients alive while 5/8 MPN-CP patients without TKI treatment died). In line with increased genomic complexity during LBC, patients who presented at this advanced stage did not survive despite TKI therapy. These observations are consistent with recent data in adult *BCR-ABL1* ALL which showed that the effect of a TKI is optimal when it is administered early during frontline therapy.[59]

In conclusion, the *ETV6-ABL1* fusion is a rare and complex rearrangement occurring in a variety of hematologic malignancies but especially ALL and MPN. The diagnosis is often incidental and only targeted RT-PCR screening

(e.g. in a multiplex setting with primers for *ETV6-RUNX1* and *BCR-ABL1* fusions, also detecting *ETV6-ABL1* cases), a combination of both *ETV6* and *ABL1* FISH probes or RNAseq will reliably detect all cases. *ETV6-ABL1* leukemias resemble *BCR-ABL1* leukemias in many aspects including expression profile and secondary genetic aberrations. The outcome of *ETV6-ABL1* ALL is often poor but in childhood ALL with an excellent early treatment response, continuous remissions seem to be achievable with current therapy without TKI. There is increasing evidence that among poor responding childhood ALL cases, as well as in adult acute leukemias and in MPN, the poor outcome can be abrogated by the use of a TKI which should be administered early, before progression of the disease, and thus depends on timely diagnostics. Future screening algorithms should include appropriate measures to detect *ETV6-ABL1* so that the most appropriate treatment strategy can be determined.

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