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Two episodes of Ph+ acute leukemia with divergent Ig/TCR rearrangements in two patients with persistent *BCR::ABL1* positivity: a 17-year follow-up

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Author Contributions

CMZ and PMH cared for the patients and took overall responsibility for the study. ESo performed molecular MRD analyses for both patients. IMS, ML and RP provided critical input for the manuscript. ESa and ARS wrote the initial draft and the revised version of the manuscript with CMZ and critical input from all authors. ESa prepared figures and tables with input from all authors.

Conflict of Interest Statement

CMZ reports consultancy for Incyte, Kura Oncology, Syndax, BMS, Novartis, and Gilead; institutional trial support from Kura Oncology, Gilead, Jazz, Daiichi Sankyo, Takeda, AbbVie, and Pfizer; advisory or board roles with Novartis, Incyte, and Sanofi; and leadership roles (paid or unpaid) within the ITCC Hem Malignancies Committee, the Dutch MREC Society (Chair), and MREC Utrecht (Chair). ARS serves on a data monitoring committee for a Miltenyi Biotec trial, has consulted for Eusa Pharma and Sanofi, and participated in educational activities for Roche, SERB, and Eusa Pharma. The remaining authors declare no competing interests.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon request.

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To the Editor:

Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph⁺ ALL) remains a biologically and clinically heterogeneous disease. We wish to contribute to the ongoing discussion on diagnostic and monitoring challenges, in line with previously published findings(1), by reporting two paediatric patients with Ph⁺ ALL that highlight persistent *BCR::ABL1* positivity despite sustained minimal residual disease (MRD) negativity by *Ig/TCR*-based assays but both experienced a second disease episode after a long interval harbouring different *Ig/TCR* targets.

The Philadelphia chromosome translocation (t(9;22)) results in the molecular juxtaposition of two genes, *BCR* (*breakpoint cluster region*) and the proto-oncogene *ABL1*, to form an aberrant *BCR::ABL1* fusion gene on the derivative chromosome 22. This fusion is the hallmark genetic alteration of chronic myeloid leukemia (CML) but it is also detected in approximately 2-5% of paediatric and 25-30% of adult cases of acute lymphoblastic leukemia (ALL), and classified as Philadelphia chromosome-positive ALL (Ph⁺ ALL)(2). Whereas the breaks in *ABL1* occur in the same region, two different breakpoint cluster regions affect the *BCR* gene. More than 90% of children with Ph⁺ ALL show a break in the 'minor' breakpoint cluster region between the *BCR* exons 1 and 2 resulting in a fusion protein of 190 kDa (p190)(3). In contrast, the rearrangement of *ABL1* with the 'major' breakpoint cluster region within *BCR* (p210) is the usual finding in CML(4) and an occasional finding in Ph⁺ ALL(3).

Ph⁺ ALL is almost exclusively of B lineage, with less than 2% of cases of T lineage leukemia according to the paediatric Children's Oncology Group (COG) and the European group (EsPhALL)(5-7). In contrast to adults, CML in paediatric patients is rare (1-2.2 cases per million per year, with 7-10% being diagnosed with blast phase)(8). Children with CML in blast phase also present with a distinct predominance of the lymphoid phenotype (70-80% vs. 20-30% in adults) and a varying landscape of additional chromosomal aberrations(9). Those leukemic features complicate the distinction between paediatric *de novo* CML in lymphoblastic blast phase (CML-BP) and Ph⁺ ALL. In recent years it became clear that also Ph⁺ ALL cases are more heterogeneous than previously thought and that distinct subtypes, correlate with multilineage versus lymphoid-only *BCR::ABL1* involvement and differ in outcome(10). This is also reflected in the recent consensus classification of myeloid neoplasms and acute leukemias with the term Ph⁺ ALL with multilineage involvement(11). In 20-30% of children (and more than 30% of adults(10, 12)) diagnosed with Ph⁺ ALL the *BCR::ABL1* fusion is present in a wider clone, involving myeloid cells, non-ALL B-cells and T-cells and termed CML-like ALL(13). The overall prognosis of CML-like ALL is similar to Ph⁺ ALL, but whereas relapses are more common in typical Ph⁺ ALL, early deaths from treatment toxicity are more common in CML-like ALL, highlighting the need for early distinction and tailored therapy(13). Additionally, diagnosing underlying CML may be relevant given the fact that hematopoietic stem cell transplantation (HSCT) remains the only curative option for CML-BP(11).

Ph⁺ ALL is commonly monitored using two main PCR-based targets: clonal immunoglobulin and T-cell receptor (*Ig/TCR*) gene rearrangements, and the quantification of *BCR::ABL1* levels, either at DNA or RNA level(14). This may complicate the decision on HSCT indication, as also outlined in a recent article by Short and colleagues if *Ig/TCR* MRD becomes negative while the *BCR::ABL1* fusion transcript remains detectable(15, 16). Of note, Short and colleagues here refer to

molecular MRD using next-generation sequencing based technologies and not conventional *Ig/TCR PCR* MRD measurements.

As these discrepancies occur frequently, the COG and EsPhALL consortia only consider MRD *Ig/TCR* gene rearrangements for risk stratification as long as informative results for *Ig/TCR* MRD are available(5).

Here, we report two paediatric patients with Ph+ ALL who, despite achieving repeated negative MRD by *Ig/TCR*-based assays, showed persistent *BCR::ABL1* positivity, with a 2nd episode of Ph+ ALL, harboring different *Ig/TCR* rearrangements compared to the first episode after long time-intervals. Both cases demonstrate the importance of long-term observation and raise additional questions about optimal management for these patients. This study has been approved by the local ethics committee and informed consent was obtained from both patients.

The first patient is a boy of 20 years, who was initially diagnosed at age two with a p190 *BCR::ABL1* -positive pre-B ALL, and was treated on the EsPhALL-2004 protocol, and randomized to the arm without imatinib. The *Ig/TCR*-based MRD declined rapidly to <0.01% by the end of induction (EOI), whereas RNA-based qPCR *BCR::ABL1* transcripts persisted at 1–4% throughout therapy, yet the child completed treatment in continuous complete remission and no further MRD follow-up was done. Eleven years later, at the age of 13, he developed a new episode of p190 *BCR::ABL1*-positive leukemia that demonstrated the same *BCR::ABL1* fusion at DNA level but a different *Ig/TCR* rearrangement, favoring the interpretation of a second malignancy rather than a classic late relapse. He was retreated according to the EsPhALL2010 protocol with initially imatinib, later dasatinib (**Figure 1**) which yielded negativity by *Ig/TCR* MRD at EOI, but DNA-based qPCR *BCR::ABL1* MRD remained persistently detectable at levels of 2–11%. HSCT at relapse was considered but because the patient did not have a suitable donor and given the rapid response it was decided not to consolidate with HSCT. Notably, discontinuation of therapy after maintenance did not provoke any expansion of the *BCR::ABL1*-positive cells. Tyrosine kinase inhibitor (TKI) monotherapy for over 4 years, including dasatinib, ponatinib, and asciminib, failed to eradicate the residual *BCR::ABL1* positivity (**Figure 1**), using a DNA-based qPCR that was developed in the meantime. He is currently without any therapy for over 1 year. More detailed analyses of marrow subsets revealed that *BCR::ABL1* positivity involved a small population of T- and B-lymphocytes, with no evidence of myeloid involvement. This patient remains in complete remission more than four years after the second course of therapy, with negative *Ig/TCR PCR* (**Table 1&Figure 1**).

At the age of 10, the second patient - a now 19-year-old man - was diagnosed with p190 *BCR::ABL1*-positive, hyperdiploid pre-B ALL. *Ig/TCR* MRD negativity was obtained at the end of consolidation (EOC) with the EsPhALL-2010 protocol that included imatinib. Six years after completing treatment he developed a new episode of p190 *BCR::ABL1*-positive leukemia, harboring the identical *BCR::ABL1* fusion at DNA level but demonstrated, similar to the first patient, different *Ig/TCR* rearrangements, also favouring the interpretation of a second malignancy. He received only mild chemotherapy followed by chimeric antigen receptor T cells (CART), also because of a cardiomyopathy after first line chemotherapy, which resulted in swift clearance of *Ig/TCR* MRD on day 28 after CART. Although he lost CART-cells soon after infusion, he has remained in continuous complete remission for the next two and a half years. MRD for *BCR::ABL1* using DNA-based qPCR remained at approximately 3%. Despite sequential therapies with imatinib, dasatinib, and asciminib, the *BCR::ABL1* MRD levels using a DNA-based qPCR have remained stable in the range of 0.2–1%, also

without treatment now for over 1 year. Immunophenotypic sorting of the remission bone marrow showed *BCR::ABL1* positivity in about 2% of T-cells and 2% of normal B-cells, while myeloid cells were negative. *Ig/TCR* MRD PCR in the subpopulations has not been performed (**Table 1&Figure 1**).

The overall complication burden was comparable to that of other patients undergoing ALL induction. Patient 2 developed cardiomyopathy between the two episodes, which guided the decision to proceed with CAR-T therapy. During the first episode, he experienced methotrexate-induced encephalopathy without seizures, tachycardia secondary to TKI treatment, vincristine-associated neuropathy, and pneumococcal pneumonia. His cardiac function showed a shortening fraction of 30% in 2019, improved to low-normal in 2020, and declined again to 22% at relapse in 2022; treatment with an angiotensin converting enzyme (ACE) inhibitor resulted in full recovery, and his cardiac function is currently normal. Patient 1 relapsed in January 2019 and subsequently developed septic arthritis in January 2020. He also suffered from gastrointestinal bleeding in July 2018, followed by a veno-occlusive disease (VOD)-like complication that was successfully managed with defibrotide, and later experienced several cytomegalovirus (CMV) reactivations during chemotherapy. In summary, patient 2 experienced more complications during the first episode, whereas patient 1 was more affected during the second episode. In both patients, TKIs were discontinued and exchanged due to lack of activity in eliminating the *BCR::ABL1*-positive cells, further supporting the notion that, likely because of their pre-leukemic nature, these cells do not depend on *BCR::ABL1* signaling. Blinatumomab was considered but not pursued, as the IG-PCR was negative and therefore no clear indication was present. When asciminib became available, it was initiated as a purely clinical decision, given that *BCR::ABL1* levels had not decreased and the clones were not dependent. These experiences highlight the phenomenon referred to as “CML-like” biology in Ph+ ALL or multilineage involvement, wherein persistent *BCR::ABL1* positivity extends beyond the original blast population and involves multiple hematopoietic compartments, with a ‘dormant’ persisting lymphoid *BCR::ABL1* clone(13). In the 2 patients reported by us, *BCR::ABL1* positivity seemed to be restricted to the lymphoid progenitor compartment and no discordances between *BCR::ABL1* transcript levels were noted between bone marrow and peripheral blood. As emphasized in the studies by Zuna and colleagues(13), this scenario reflects a stem-cell–driven disease in which the *BCR::ABL1* clone extends beyond the ALL blast population, and these cells may serve as a reservoir for future disease episodes. Zuna and colleagues have also demonstrated the presence of the *BCR::ABL1* clone in T cells, myeloid cells, and normal B cells, reinforcing the concept that some Ph+ ALL cases may originate in a multipotent progenitor(17). In both our patients, *Ig/TCR*-based MRD served as a more definitive predictor of clinical remission status. Unfortunately, additional molecular/genomic testing to compare the additional mutational profile, apart from *BCR::ABL1* positivity between the first and second episodes was not feasible as relevant material was not preserved. Mutational profiling of *BCR::ABL1* was performed once in each patient: for patient 1, it was negative before initiation of asciminib, and for patient 2, it was negative at the diagnosis of the second leukemia. These children achieved negative *Ig/TCR* MRD, remained in morphological remission for several years, and despite persisting *BCR::ABL1* positivity showed no immediate disease progression even when TKI therapy was withheld. Of note, although *BCR::ABL1* levels remained variably positive, both patients were clinically asymptomatic following their follow-up after the first episode of leukemia. During the first year, they were monitored monthly, and subsequently every three months. As they remained without clinical

symptoms, diagnostic bone marrow aspirations were discontinued after the first year. At the time of relapse, however, both patients presented with a combination of clinical symptoms, peripheral blood count abnormalities and increasing *BCR::ABL1* positivity. As both patients experienced a second *BCR::ABL1*-positive ALL a significant number of years later, it is evident that even in an apparently indolent state, *BCR::ABL1* positive cells leukemias are in a pre-leukemic state which favours clonal evolution and leukemic transformation when additional hits occur. The possibility that further episodes might occur introduces the question whether HSCT is indicated to eradicate the *BCR::ABL1*-positive reservoir. At present, we have advised the families that a future Ph+ leukemia episode may be an indication for HSCT to achieve definitive eradication of the *BCR::ABL1* reservoir.

As immunotherapeutic strategies such as CARTs are increasingly integrated into clinical practice, it will become important to distinguish patients with (CD19+)-B cell-restricted *BCR::ABL1* expression from those with a broader, CML-like involvement - particularly to understand whether *BCR::ABL1* can be fully eradicated and if such treatment can be considered definitive, which was not achieved in patient 2.

In conclusion, these observations reinforce the concept that *BCR::ABL1*-based MRD alone does not consistently predict relapse risk in Ph+ ALL. Although some patients may harbor seemingly inactive *BCR::ABL1*-positive cells for many years without clinical progression but, the possibility of later leukemic transformation appears genuine. Identifying clear biomarkers that distinguish quiescent from malignant-prone residual clones would greatly assist in personalizing both the intensity and duration of therapy, including the role of allogeneic HSCT. While immunotherapies and TKIs offer clinical benefit, they do not eradicate the underlying pre-leukemic clone. Therefore, prospective follow-up of this subgroup is essential to determine whether outcome differences truly exist and to guide evidence-based, individualized treatment strategies.

References:

1. Zuna J, Hovorkova L, Krotka J, et al. Posttreatment positivity of BCR::ABL1 in acute lymphoblastic leukemia: Should we keep track? *Am J Hematol.* 2023;98(10):E269-E271.
2. Foa R, Chiaretti S. Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia. *N Engl J Med.* 2022;386(25):2399-2411.
3. Suryanarayan K, Hunger SP, Kohler S, et al. Consistent involvement of the bcr gene by 9;22 breakpoints in pediatric acute leukemias. *Blood.* 1991;77(2):324-330.
4. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. *Blood.* 2000;96(10):3343-3356.
5. Biondi A, Gandemer V, De Lorenzo P, et al. Imatinib treatment of paediatric Philadelphia chromosome-positive acute lymphoblastic leukaemia (EsPhALL2010): a prospective, intergroup, open-label, single-arm clinical trial. *Lancet Haematol.* 2018;5(12):e641-e652.
6. Hunger SP, Tran TH, Saha V, et al. Dasatinib with intensive chemotherapy in de novo paediatric Philadelphia chromosome-positive acute lymphoblastic leukaemia (CA180-372/COG AALL1122): a single-arm, multicentre, phase 2 trial. *Lancet Haematol.* 2023;10(7):e510-e520.
7. Slayton WB, Schultz KR, Kairalla JA, et al. Dasatinib Plus Intensive Chemotherapy in Children, Adolescents, and Young Adults With Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia: Results of Children's Oncology Group Trial AALL0622. *J Clin Oncol.* 2018;36(22):2306-2314.
8. Meyran D, Petit A, Guilhot J, et al. Lymphoblastic predominance of blastic phase in children with chronic myeloid leukaemia treated with imatinib: A report from the I-CML-Ped Study. *Eur J Cancer.* 2020;137:224-234.
9. Sembill S, Ampatzidou M, Chaudhury S, et al. Management of children and adolescents with chronic myeloid leukemia in blast phase: International pediatric CML expert panel recommendations. *Leukemia.* 2023;37(3):505-517.
10. Bastian L, Beder T, Barz MJ, et al. Developmental trajectories and cooperating genomic events define molecular subtypes of BCR::ABL1-positive ALL. *Blood.* 2024;143(14):1391-1398.
11. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood.* 2022;140(11):1200-1228.
12. Kim JC, Chan-Seng-Yue M, Ge S, et al. Transcriptomic classes of BCR-ABL1 lymphoblastic leukemia. *Nat Genet.* 2023;55(7):1186-1197.
13. Zuna J, Hovorkova L, Krotka J, et al. Minimal residual disease in BCR::ABL1-positive acute lymphoblastic leukemia: different significance in typical ALL and in CML-like disease. *Leukemia.* 2022;36(12):2793-2801.
14. Pfeifer H, Cazzaniga G, van der Velden VHJ, et al. Standardisation and consensus guidelines for minimal residual disease assessment in Philadelphia-positive acute lymphoblastic leukemia (Ph + ALL) by real-time quantitative reverse transcriptase PCR of e1a2 BCR-ABL1. *Leukemia.* 2019;33(8):1910-1922.
15. Short NJ, Jabbour E, Macaron W, et al. Ultrasensitive NGS MRD assessment in Ph+ ALL: Prognostic impact and correlation with RT-PCR for BCR::ABL1. *Am J Hematol.* 2023;98(8):1196-1203.
16. Cazzaniga G, Lanciotti M, Rossi V, et al. Prospective molecular monitoring of BCR/ABL transcript in children with Ph+ acute lymphoblastic leukaemia unravels differences in treatment response. *Br J Haematol.* 2002;119(2):445-453.
17. Hovorkova L, Zaliova M, Venn NC, et al. Monitoring of childhood ALL using BCR-ABL1 genomic breakpoints identifies a subgroup with CML-like biology. *Blood.* 2017;129(20):2771-2781.

Table 1

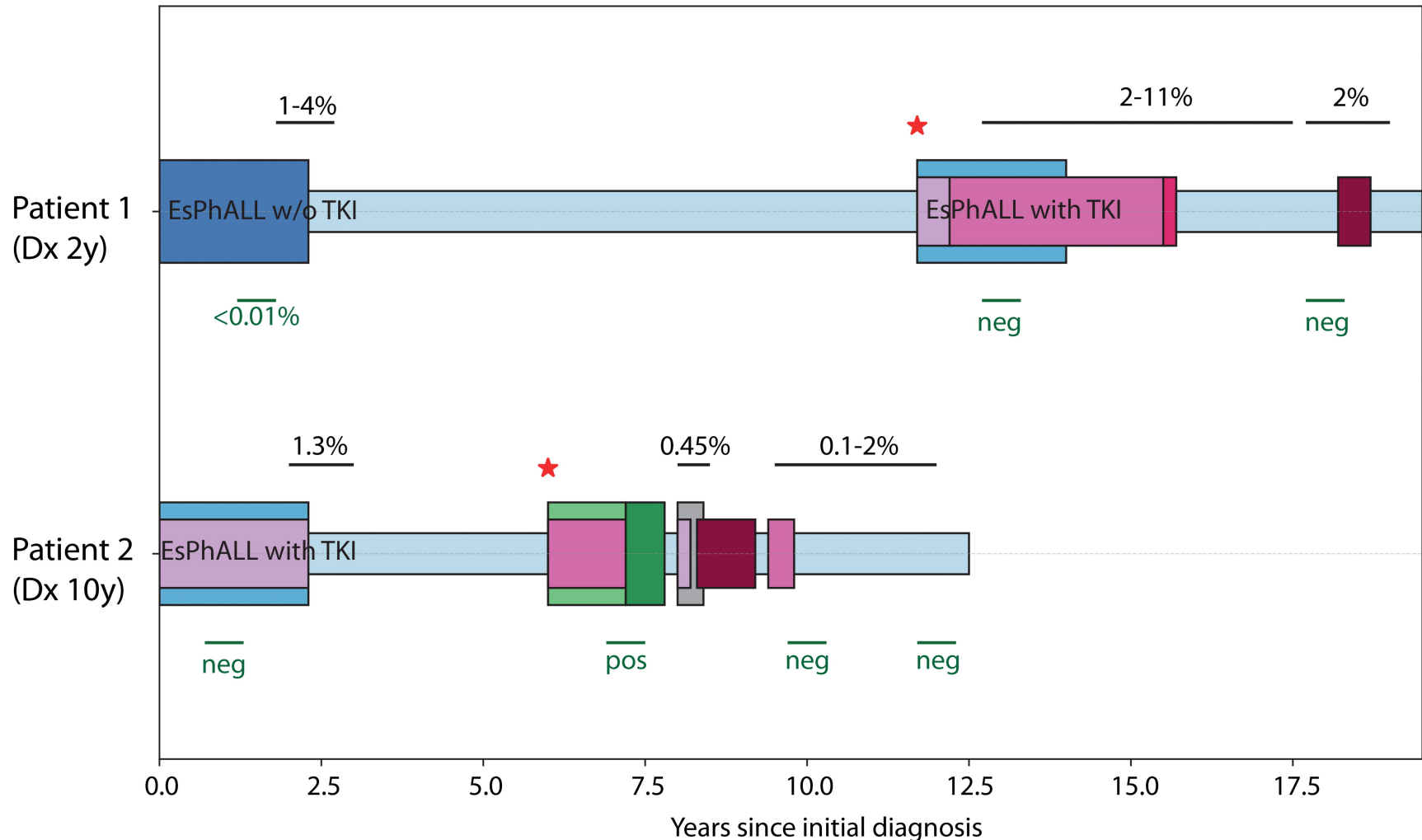
Patient	Episode	Diagnosis	Flow cytometry	PCR MRD	<i>BCR::ABL</i> , other
1	1	Pre-B-ALL CNS 2	CD19 ⁺ , CD34 ^{dim} , CD45 ⁺ , CD10 ⁺ , CD20 ⁺ , TdT ⁺ , HLA-DR ⁺ , cylgM ⁺ , sIgM ⁻	Targets: VH3.30-DH2.2-JH4b and DH2.15-JH4b negative EOI	t(9;22) (q34;q11) (<i>BCR::ABL 1</i>) p190
	2	Common-ALL CNS 2	CD19 ⁺ , CD34 ⁺ , CD45 ⁻ , CD10 ⁺ , CD20 ⁻ , TdT ⁺ , CD22 ^{weak} , cylgM ⁺ , CD66c ⁺ , CD123 ⁺ , NG2 ⁻ , CD38 ⁻	Targets: Vg11-Ig2.3 and DH2.2-JH6c negative EOI	t(9;22) (q34;q11) (<i>BCR::ABL 1</i>) p190
2	1	Pre-B-ALL CNS 1	CD19 ⁺ , CD34 ⁺ , CD45 ⁻ , CD10 ⁺ , CD20 ⁺ , CD22 ⁺ , TdT ⁺ , cylgM ⁺ , CD123 ^{weak+} , CD66c ⁺ , NG2 ⁻ , CD38 ⁺ , CD81 ⁺	Target: IgH VH3.7-JH6 negative EOC	t(9;22) (q34;q11) (<i>BCR::ABL 1</i>) p190 hyperdiploid
	2	Pre-B-ALL CNS 1	CD19 ⁺ , CD34 ⁺ , CD45 ^{dim} , CD10 ⁺ , CD20 ⁻ , CD66c ^{variable+} , CD123 ^{variable+}	Targets: IgH (Vh3-15-Dh3-9-Jh5b) and TRCD (Vd2-Ja29) negative d28 after CAR-T infusion	t(9;22) (q34;q11) (<i>BCR::ABL 1</i>) p190

Table 1: Clinical presentation and leukemia-specific findings. Abbreviations: **EOC**, end of consolidation; **EOI**, end of induction; **Pre-B-ALL**, precursor B-cell acute lymphoblastic leukemia; **CNS**; **CAR-T**, chimeric antigen receptor T-cell therapy; **PCR MRD**, polymerase chain reaction minimal residual disease; **TdT**, terminal deoxynucleotidyl transferase; **CD**, cluster of differentiation; **cylgM**, cytoplasmic immunoglobulin M; **sIgM**, surface immunoglobulin M; **IgH**, immunoglobulin heavy chain; **VH, DH, JH**, variable, diversity, and joining segments of immunoglobulin genes; **TRCD**, T-cell receptor delta; **bold**, flow cytometry markers differing between first and second episode

Figure 1

Figure 1. Swimmer plot of treatment history and molecular responses in the two patients. Abbreviations: ALL, acute lymphoblastic leukemia; *BCR::ABL1*, Breakpoint Cluster Region-Abelson Tyrosine Kinase 1 fusion gene; **EsPhALL**, European intergroup study protocol for Philadelphia chromosome-positive ALL; **TKI**, tyrosine kinase inhibitor; **Ig/TCR MRD**, immunoglobulin/T-cell receptor minimal residual disease; **MRD**, minimal residual disease; **CAR-T**, chimeric antigen receptor T-cell therapy; **Off Tx**, off treatment; **Dx**, diagnosis; **Neg**, negative; **Pos**, positive; **Imatinib**, first-generation tyrosine kinase inhibitor; **Dasatinib**, second-generation tyrosine kinase inhibitor; **Ponatinib**, third-generation tyrosine kinase inhibitor effective against T315I mutations; **Asciminib**, allosteric *BCR::ABL1* inhibitor, STAMP inhibitor targeting the myristoyl binding pocket.

Timeline of two patients with *BCR::ABL1*-positive ALL



EsPhALL without TKI
EsPhALL with TKI
Imatinib

Dasatinib
Ponatinib
Asciminib

Remission Induction:
Dexamethasone
CAR-T cells
Loss of CAR-T

Off Tx
BCR::ABL1 MRD
IG/TCR MRD
★ second Ph⁺ ALL