Evidence for a BCR/ABL1-positive T-cell acute lymphoblastic leukemia arising in an early lymphoid progenitor cell

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Abbreviation	**Full Term**
ALL	acute lymphoblastic leukemia
MRD	minimal residual disease
FISH	fluorescence in situ hybridization
CML	chronic myelogenous leukemia
RT	reverse transcription
PCR	polymerase chain reaction
TCR	T-cell receptor
FACS	fluorescent-activated cell sorter
HSCT	hematopoietic stem cell transplant
BP	blast phase
PB	peripheral blood
BM	bone marrow

Abstract

BCR-ABL1-positive leukemias have historically been classified as either CML or Ph+ acute lymphoblastic leukemia. Recent analyses suggest there may be a wider range of subtypes. We report a patient with BCR-ABL1 fusion positive T-ALL with a previously undescribed cell distribution of the fusion gene. The examination of sorted cells by fluorescence in situ hybridization (FISH) showed the BCR-ABL1 fusion in the malignant T-cells and a subpopulation of the non-malignant B-cells, but not non-malignant T-cells, myeloid or CD34+ progenitor cells providing evidence that the fusion may have occurred in an early lymphoid progenitor.

Introduction

The BCR-ABL1 fusion gene is the hallmark of chronic myelogenous leukemia (CML) and is also present in a subtype of ALL. Hovorkova et. al[1] provided evidence that patients presenting with BCR-ABL1 fusion-positive ALL might represent a spectrum of leukemia subtypes. They demonstrated that minimal residual disease (MRD) measured by the BCR-ABL1 fusion gene polymerase chain reaction (PCR) and the immunoglobulin gene /T-cell receptor (TCR) gene PCR was concordant in the majority of cases and that the fusion gene was confined to the lymphoid blasts. However, in about 20% of the cases the MRD was discordant and the fusion gene was present not only in the malignant blasts but also in non-malignant cells suggesting that the fusion might have occurred in a multipotent progenitor cell. The BCR-ABL1 fusion gene is almost always found in malignant B-cells and has been reported only rarely in malignant T-cells.[2-4] We describe a patient with BCR-ABL1 transcripts in the malignant T-cells and in a subpopulation of non-malignant B cells. Progenitor cells, myeloid cells, non-malignant T-cells and a population of normal B-cells were BCR-ABL1 negative. The TCR beta and gamma genes

were not rearranged in the malignant T-cells. These combined results suggest that the BCR-ABL1 fusion may have occurred in an early lymphoid progenitor. Determining the cell type of origin is important, as each cell type may have distinct biological properties and may require different treatment.[5-7] Including FISH analysis for BCR/ABL1 fusions in fluorescent-activated cell sorter (FACS)-sorted cells and genomic analysis during induction may lead to a better understanding of the frequency and outcome of the different BCR-ABL1-positive leukemia subtypes.

Case description and methods

A 13-year-old male presented with a white blood cell count of 426 x 10^9/L, hemoglobin 11.5 g/dl, and platelets 115 10^9/L. The review of the peripheral blood (PB) smear showed 84% blasts, 1% myelocytes, 1% metamyelocytes, 1% bands, 3% neutrophils, 12% lymphocytes, and 1% monocytes. His creatinine was 0.6 and uric acid 7.7. A computed tomography scan was remarkable for splenomegaly and enlarged abdominal lymph nodes; there was no mediastinal mass. Blasts comprised 77% of the marrow cellularity and were positive for CD34 (minimal), CD7, CD5, CD4, CD8 (partial), CD1a (partial), CD38, cytoplasmic CD3, and TdT but negative for B-cell and myeloid markers. The cerebrospinal fluid was negative. FISH was positive for the BCR-ABL1 fusion gene. The ABL kinase domain mutation analysis of his BCR-ABL1 fusion protein (p190) was negative. Cytogenetic studies revealed no other abnormalities. He was started on four-drug induction and the tyrosine kinase inhibitor, dasatinib. His initial clinical course was complicated by tumor lysis syndrome and acute kidney injury requiring continuous venovenous hemodiafiltration.

On induction day 8, 3.5% T-lymphoblasts were detected in the PB by flow cytometry but the quantitative BCR-ABL1 reverse transcription (RT) PCR was positive at 42.51% (ratio of fusion gene transcript /ABL1 reference gene transcript multiplied by 100) in the nucleated cells. Due to this discrepancy we performed FACS followed by BCR-ABL1 FISH analysis to determine which cell populations were positive for the BCR-ABL1 fusion. His PB (day 15) and bone marrow (BM) cells (day 29) were sorted into CD5+CD3- malignant T-cells, CD3+ non-malignant T-cells, CD33+ non-malignant neutrophils, CD19+ non-malignant B-cells, and CD34+ non-malignant progenitor cells at Hematologics Inc. FISH was performed on the sorted cells with the BCR-ABL1 + ASS1 (9q34) tricolor dual fusion translocation probe, A total of 200-300 sorted interphase nuclei were examined for each cell type. Multiplex PCR with specific primers for the TCR beta and gamma chain was performed on sorted and unsorted PB (day 15). His BM at diagnosis was further characterized with targeted sequencing studies with the FoundationOne Heme panel. After completing induction therapy, he was treated with four cycles of Hyper-CVAD with ponatinib.[8] His MRD testing by RT-PCR were consistently higher in the PB compared to his BM prior to his HSCT, likely due to the lower number of lymphocytes in the BM compared to PB. [9-12] (Table 1) After recovery from his fourth cycle of chemotherapy, he received a matched-related allogeneic hematopoietic stem cell transplant (HSCT). His post-transplant BM MRD by flow cytometry has remained negative (Table 1).

Results

The FISH analysis of the CD5+CD3- malignant T-cell fraction revealed an abnormal dual fusion signal pattern (1R1G2F2A) indicative of a t(9;22) BCR-ABL1 gene rearrangement in 86% of the PB cells and 80% of the BM cells examined (Fig. 1B). In addition, the CD19+ non-malignant B-

cell fraction revealed an abnormal dual fusion signal pattern (1R1G2F2A) in 22% of the PB cells and 14% of the BM cells, suggestive of a BCR-ABL1 gene rearrangement in a sub-clone of the non-malignant B-cell compartment (Fig. 1D). Analysis of the non-malignant CD33+ neutrophil and CD3+ T-cell fractions were negative for the BCR-ABL1 gene rearrangement (Figs 1A and 1C). TCR beta and gamma chain multiplex PCR did not reveal presence of clonal amplification in the malignant T-cell fraction and the non-malignant B-cell fraction. The targeted sequencing studies revealed a mutation of FBXW7 pR465C (mutation allelic frequency (MAF) 9%) and CDKN2A 16INK4a pW110* and CDKN2A 14ARF pG125R (MAF 78%). IKFZ1 was not mutated or deleted. The number of mutations was intermediate at 7 mutations/Mb.

Discussion

The BCR-ABL1 fusion in acute leukemia with T-cell differentiation usually leads to the differential diagnosis of either CML with T-cell blast phase (BP) or de novo BCR-ABL1-positive T-ALL. The incidence of each is rare. Only 1.3% of CML-BP have a T-lymphoid BP and less than 5% of BCR-ABL1 fusion-positive ALL are T- ALL.[2,3] Distinguishing CML-BP from de novo ALL can be very challenging, especially if there is no prior history of CML, although the lack of TCR beta and gamma rearrangement would favor a leukemia arising in an early progenitor cell.[3] Hovorkova[1] et. al provide data suggesting even greater complexity with a spectrum of BCR-ABL1 fusion-positive B-cell leukemias. In 20% of the cases the BCR-ABL1 fusion was present to variable degrees in the non-malignant B and T-lymphoid cells and granulocytes. Patients with CML had BCR-ABL1 positive monocytes and granulocytes but not T cells. In their small study, the 15 ALL patients with multilineage involvement had a better outcome with HSCT than with chemotherapy alone.

BCR-ABL1-positive ALL and lymphoid blastic transformation of CML very rarely involve the T-cell lineage. The reason for the myeloid and B-cell predominance is not known. Genome-wide analysis have shown that IKFZ1 deletions occur in CML during progression to lymphoid BP in B-lymphoid blasts (84%) but rarely in T-cell blasts.[13] CDKN2A/B deletions occur in both B and T-cell BCR-ABL1-positive lymphoid blasts during progression of CML to lymphoid BP.[14] Both genomic alteration, the CDKN2A/B deletions and the lack of an IKFZ1 deletion, and the cell of origin may be related to our patient's T-cell phenotype.

Much more work is needed to understand the underlying genetic lesions and the clinical implications of multilineage involvement in BCR-ABL1-positive disease, especially as it relates to T-ALL. FISH analysis of cells sorted into lineage specific populations and genomic analysis are feasible and can be included into routine diagnostic procedures and will undoubtedly lead to a better characterization of these BCR-ABL1-positive leukemic subtypes. Although the optimal therapy for subtypes with multilineage involvement has yet to be defined, we believe the current standard practice is HCST analogous to CML that presents in BP. [15]

Authorship

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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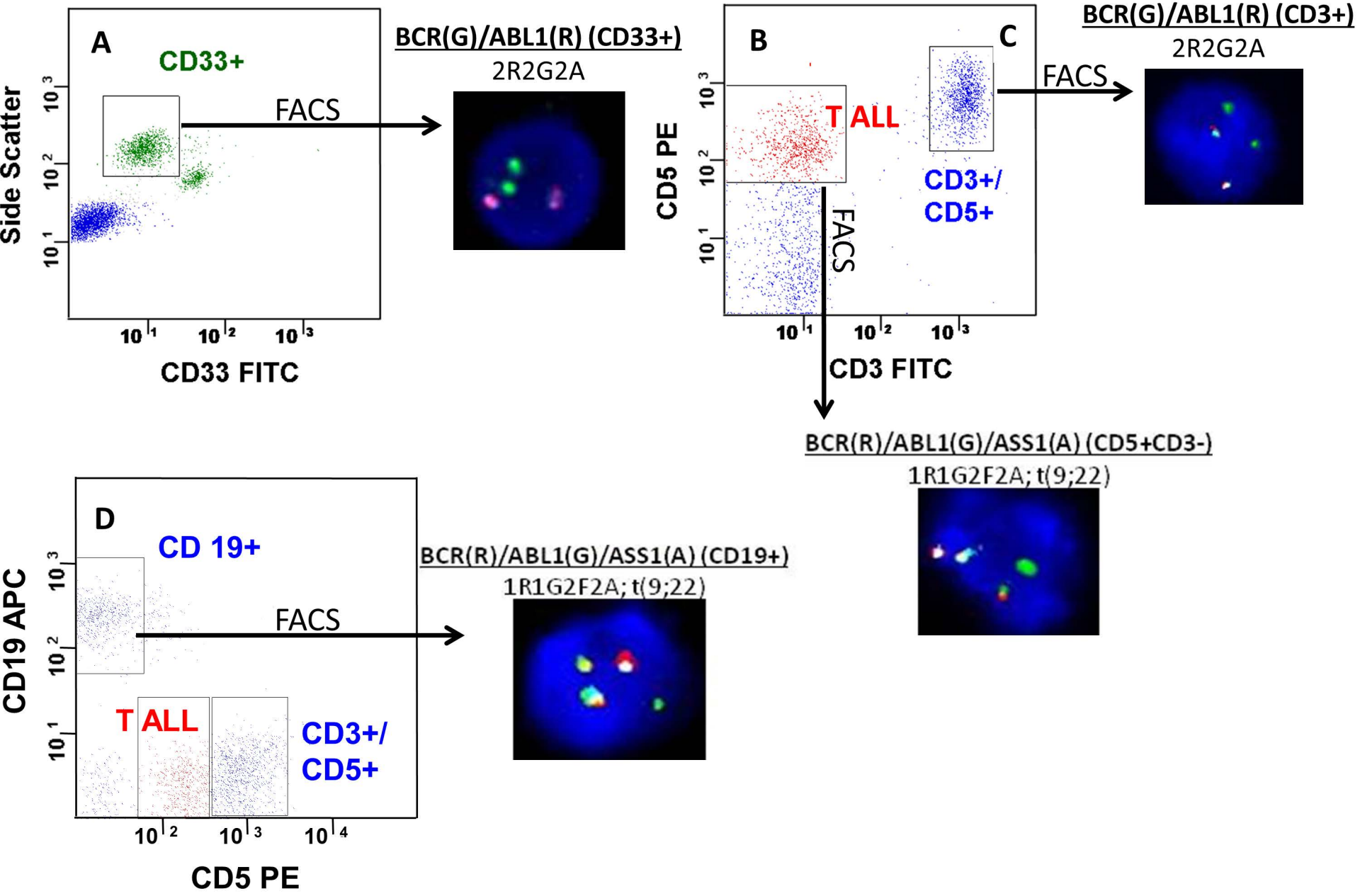
TABLE 1 RT-PCR and MRD results during treatment

Timepoint	BCR-ABL % residual disease RT-PCR		MRD % by flow cytometry	
	PB	BM	PB	BM
Induction day 8	42.5		3.5	
Induction day 29	19.5	6.8		4.3
Hyper-CVAD Cycle 2	0.2	0.02		0.1
Hyper-CVAD Cycle 4	0.3	0.02		0.05
4 months post HSCT	0.02	0.04	<0.02	<0.02

BM, bone marrow; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; PB, peripheral blood; Hyper-CVAD, hyperfractionated therapy of cyclophosphamide, vincristine, doxorubicin and dexamethasone.


A
CD33+
FACS
Side Scatter
CD33 FITC
BCR(G)/ABL1(R) (CD33+)
2R2G2A
B
C
T ALL
CD3+/
CD5+
FACS
CD5 PE
CD3 FITC
BCR(G)/ABL1(R) (CD3+)
2R2G2A
BCR(R)/ABL1(G)/ASS1(A) (CD5+CD3-)
1R1G2F2A; t(9;22)
D
CD 19+
FACS
CD19 APC
CD5 PE
T ALL
CD3+/
CD5+
BCR(R)/ABL1(G)/ASS1(A) (CD19+)
1R1G2F2A; t(9;22)