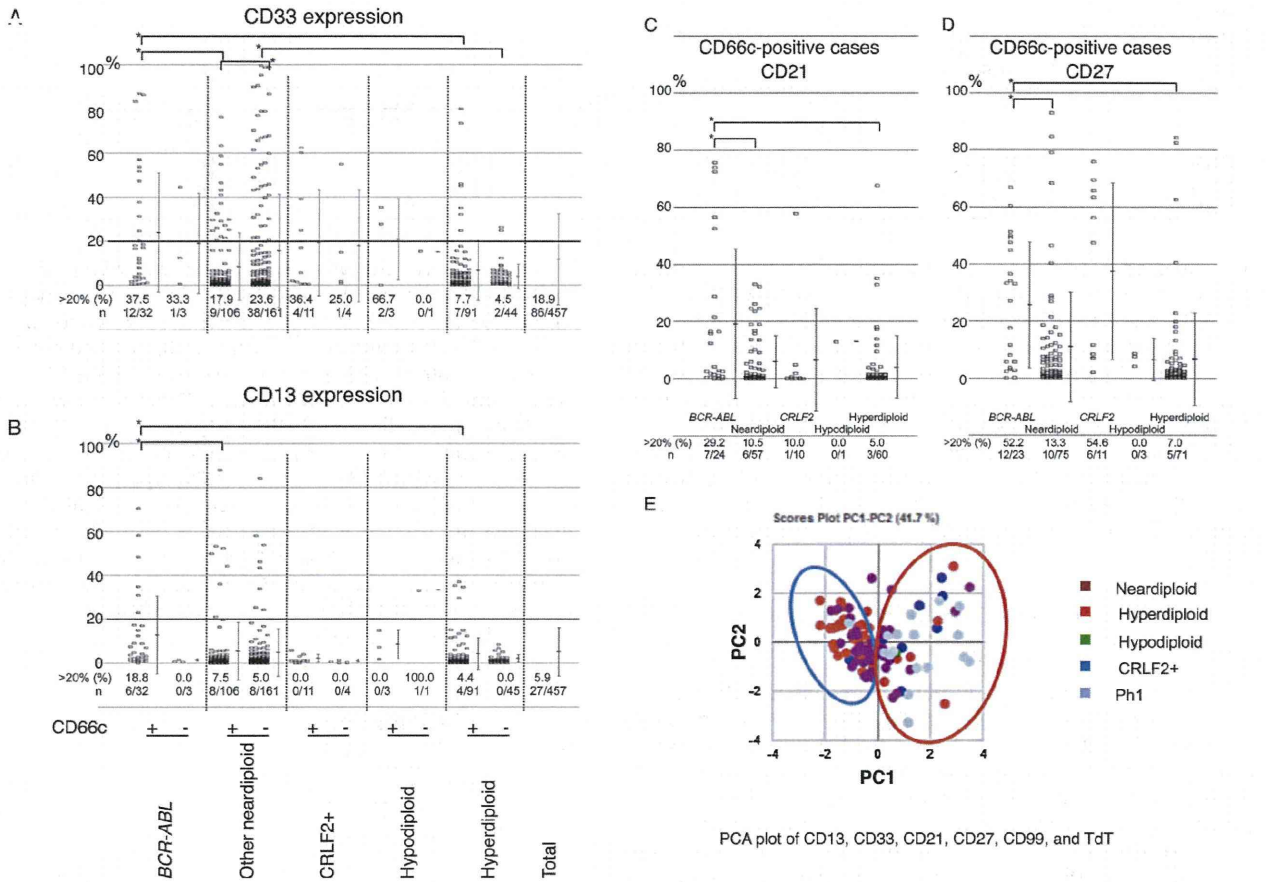

Fig. 2. Correlation between CD66c positivity and the expression of myeloid antigens, CD21 and CD27 in different genotype categories of acute lymphoblastic leukemia (ALL). The positivity (percentage) of CD33 (A) and CD13 (B) of B-cell precursor ALL was plotted on a scattergram categorized by the CD66c expression and genotype, as in Fig. 1. The positivity (percentage) of CD21 (C) and CD27 (D) of CD66c-positive B-cell precursor ALL was plotted on a scattergram categorized by the genotype as indicated in the figure. The percentage of positive cases (more than 20% expression in blasts) in each group is listed below. $^{*}p < 0.01$, using Student's *t*-test. (E) Principal components analysis (PCA) was performed on CD66c-positive B-cell precursor ALL cases. PCA plot of 6 antigen expression, including CD13, CD33, CD21, CD27, CD99, and TdT, is presented with two PCA axes (PC1 vs PC2).

In an attempt to explore immunophenotypic characteristics distinguishing *BCR-ABL*-positive ALLs and other CD66c-positive ALLs, we observed relatively high expression of CD21 and CD27 in *BCR-ABL*-positive ALLs. The molecule CD21 is a mature B-cell antigen and its expression in BCP-ALL is very limited [19]. The CD27 molecule is a member of the TNF receptor family and known as a marker of mature memory B cells, while some malignant and nonmalignant B precursors also express this antigen [20]. Among CD66c-positive cases, CD21 expression was revealed to be relatively high in *BCR-ABL*-positive cases (Fig. 2C), and CD27 expression was high in both *BCR-ABL*-positive and CRLF2-overexpressing cases (Fig. 2D).

To further assess the biological relevance of the expression of above antigens in CD66c-positive ALL, we performed multivariate analyses by employing PCA. As shown in Fig. 2E, PCA plot using the expression data of 6 antigens, including CD13, CD33, CD21, CD27, CD99, and TdT, could roughly separate BCR-ABL-positive and CRLF2-positive cases from remaining chimeric gene-negative cases expressing CD66c.

3.4. Correlation between risk factors and CD66c expression

We next examined the correlation between CD66c expression and risk classification in chimeric gene-negative cases. In our protocol, the patients were stratified into three risk groups, namely, standard risk (SR), intermediate risk (IR), and high risk (HR), based on presenting features (age and the leukocyte count before starting the treatment) and, then, reclassified into them three categories 7 days later according to the sensitivity to oral prednisolone monotherapy, using the cut-off counts of 1000 blasts/μL [21]. As shown in Table 3, hyperdiploidy/CD66c-positive cases were more frequently classified into SR at diagnosis, while no difference was observed at reclassification on Day 8, indicating that hyperdiploid/CD66c-positive cases tend to have favorable initial presenting features but exhibit poor response for steroid. On the other hand, neardiploid/CD66c-negative cases tended to be more frequently classified into IR and HR at the initial classification, and the tendency appeared to be more significant at reclassification on Day 8 (Table 3), indicating that neardiploid/CD66c-negative have unfavorable presenting features as well as poor steroid sensitivity. After 5-year observation, however, no significant difference in the subsequent prognosis between CD66c-positive and -negative groups was observed (data not shown).

4. Discussion

Upon precisely analyzing CD66c expression in a large cohort of childhood BCP-ALL, we further extended the previous findings, and clearly identified the characteristics of CD66c expression as follows: First, among BCP-ALL possessing well-known chimeric genes, CD66c expression is highly selective in *BCR-ABL*-positive

Table 3
Risk classification and CD66c expression.

Hyper/	HR		IR		SR			Total	HR+IR/SR	
	(Case no.)								(Ratio)	
CD66c+	1		12		27			40	0.48	Initial
	HR-SCT	HR	HR	IR	HR	IR	SR			
	0	1	4	8	7	0	20		1.00	Day-8
Hyper/	HR		IR		SR					
CD66c-	0		8		8			16	1.00	Initial
	HR-SCT	HR	HR	IR	HR	IR	SR			
	0	0	1	7	0	0	8		1.00	Day-8
Diploid/	HR		IR		SR					
CD66c+	7		24		30			61	1.03	Initial
	HR-SCT	HR	HR	IR	HR	IR	SR			
	4	3	2	22	0	0	30		1.03	Day-8
Diploid/	HR		IR		SR					
CD66c-	14		48		31			93	2.00	Initial
	HR-SCT	HR	HR	IR	HR	IR	SR			
	8	6	4	44	3	0	28		2.32	Day-8

Hyper, hyperdiploid; Diploid, neardiploid; HR, high risk; IR, intermediate risk; SR, standard risk; SCT, stem-cell transplantation; Initial, risk classification based on presenting features; Day-8, re-risk classification after 7-day oral prednisolone monotherapy.

cases, while CD66c is never expressed in cases possessing not only *ETV6-RUNX1* and *MLL-AF4*, but also *MLL-AF9*, *MLL-ENL*, and *E2A-PBX1*. Second, among BCP-ALL cases without well-known chimeric genes, CD66c expression also exhibits some selectivity that correlates with genetic abnormalities and CRLF2-positive and probably hypodiploid states, and, as in hyperdiploidy cases tend to express CD66c at a high frequency. The results were schematically summarized in Fig. 3A. Above data indicate that CD66c expression has a close correlation with definite set of genetic abnormalities, although it is not limited to a specific one.

The overexpression of *CRLF2* arises from a translocation juxtaposing *CRLF2* to the *IGH* enhancer or an interstitial deletion (*CRLF2-P2RY8*) and has been reported to be found in 4.7% to 17.5% of BCP-ALL cases as assessed by real-time PCR [22–29]. In this study, however, we found only 15 CRLF2-positive cases (2.2%) in our cohort by flow cytometry. Although the precise reason for the inconsistency in the frequency of CRLF2 overexpression between previous reports by real-time PCR and our data of flow cytometry is remaining unclear, it is possibly due to the difference of detection methods including diagnostic criteria for positive case.

Most recently, a subtype of BCP-ALL including CRLF2-overexpressing cases has been called "Ph-like ALL" and identified to be sharing a transcriptional signature that significantly overlaps with a *BCR-ABL*-positive ALL and accompanied by high rates of relapse and poor overall survival [30]. Besides CRLF2-overexpressing cases, our preliminary results indicate that other Ph-like ALL cases also tend to frequently express CD66c (data not shown).

As well as CRLF2 overexpression [22–29], both *BCR-ABL*-positive [13,31] and hypodiploid patients are well known to show a poor prognosis [13–15]. In contrast, hyperdiploid BCP-ALL patients are generally accompanied by a relatively favorable therapeutic outcome [12,13]. Therefore, the expression of CD66c itself is not directly linked to the prognosis, whereas the genetic abnormalities accompanying CD66c expression are important to make a prognosis for BCP-ALL patients. Concerning the chimeric gene-negative cases, our data further indicated that the combination of CD66c expression and chromosome number abnormalities is closely related to risk classification and steroid sensitivity. Thus the genetic findings must be paid attention when CD66c expression is detected.

Since *CRLF2*-overexpressing BCP-ALLs and *BCR-ABL*-positive cases share overlaped transcriptional signature as we described above [24], the transcription of CD66c might be regulated by a common downstream factor in both pathways. Similarly, CD66c expression in hyper- and hypodiploid cases might also share the same pathway, whereas the precise mechanism that induces the aberrant expression of CD66c in BCP-ALL is unclear. In the

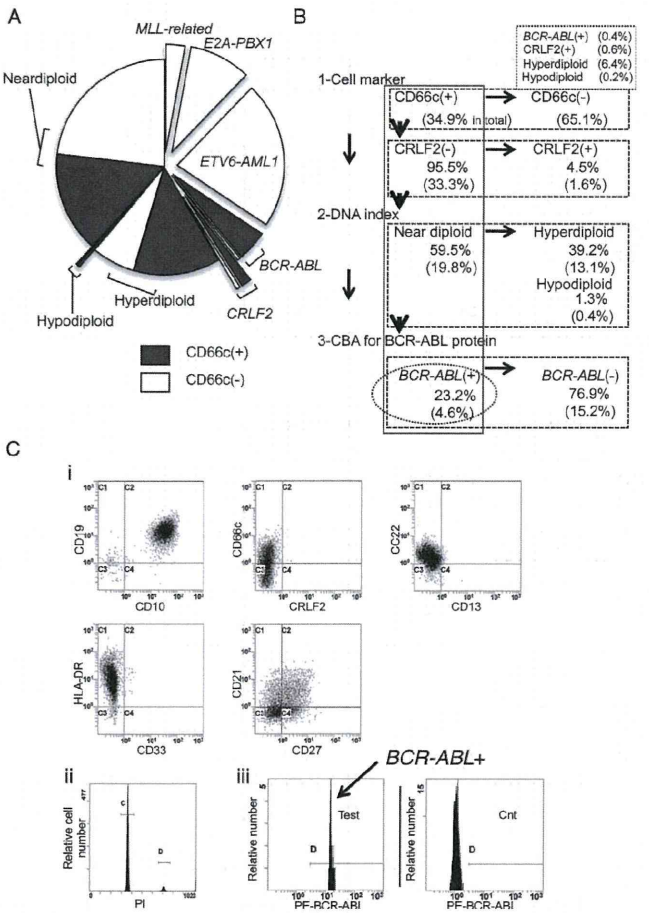

Fig. 3. Summary of CD66c expression and diagnostic flow to detect BCR-ABL-positive acute lymphoblastic leukemia (ALL) by flow cytometry. (A) Summary of CD66c expression and correlation with genetic abnormalities. (B) An initial diagnostic flow of the subclassification of BCP-ALL using flow cytometry is indicated. In the case of neardiploid B-cell precursor ALL that is CD66c-positive and CRLF2-negative, it is recommended to perform the Cytometric Bead Array (CBA) for BCR-ABL protein. Cases expressing any of the myeloid antigens, CD21, or CD27 and exhibiting extreme elevation of peripheral blood white blood cell counts are highly probable of being BCR-ABL-positive. The frequency (%) of each subclass in our study is indicated as a percentage. The number in parentheses indicates the frequency in the total cases. (C) Case diagnosed as BCR-ABL-positive ALL by flow cytometry. The leukemic cells exhibit CD19+, CD10+, HLA-DR+, CD22+, CD66c+, CRLF2-, myeloid-antigen-, CD21+, and CD27+. The DNA-index analysis revealed neardiploidy, and CBA for BCR-ABL protein was positive.

case of hyperdiploid ALLs, a significant correlation between the chromosomal location of upregulated genes and the presence of trisomies/tetrasomies was observed, and, thus, the reflection of a gene-dosage effect has been suggested [32]. On the other hand, hyperdiploid ALL is characterized by a nonrandom gain of chromosomes commonly including chromosomes X, 4, 6, 10, 14, 17, 18, and 21, but CD66c is located on chromosome 19 [33]. Considering the findings, the expression of CD66c in hyperdiploid cases should not be mediated by the gene-dosage effect.

It was reported that the expression of CD13, CD33, CD15, and CD65 tended to be mutually exclusive with CD66c [6]. Since both *ETV6-RUNX1*-positive ALL frequently expressing CD33 and CD13 [17] and ALL with *MLL*-related chimeric genes commonly expressing CD15 and CD65 [18] are highly concentrated in neardiploid/CD66c-negative cases, it is quite reasonable that the expressions of CD66c and other myeloid antigens tend to be mutually exclusive. Therefore, we excluded BCP-ALL cases possessing well-known chimeric genes lacking CD66c expression and then analyzed the correlation between CD66c expression and that of other myeloid antigens. However, upon excluding *ETV6-RUNX1*-positive cases from the analysis, neardiploid/CD66c-negative cases still exhibited a significantly higher expression of CD33. On the other hand, both *BCR-ABL*-positive and CRLF2-expressing cases exhibited frequent expression of CD33 besides CD66c. Further investigation of the underlying mechanisms that induce the aberrant expression of CD66c and other myeloid antigens should be conducted in the future.

Based on our analysis, we propose an initial diagnostic flow of the prognosis-based subclassification of BCP-ALL using flow cytometry. As presented in Fig. 3B and C, in addition to a regular diagnostic panel, the immunocytological detection of CD66c and CRLF2 in combination with PI staining should be a useful tool for the initial diagnosis of BCP-ALL. By assessing cases with PI staining, more than one-third of the patients should be diagnosed as hyperdiploid, and hypodiploid cases might be rarely detected. After the exclusion of hyper- and hypodiploid BCP-ALL cases, less than 10% of the cases will be CRLF2-positive. In the remaining cases, approximately one quarter of the cases should be *BCR-ABL*-positive ALL. As we presented in Fig. 2E by PCA, the coexpression of myeloid antigens, CD21 or CD27 with CD66c, as well as extreme elevation of peripheral blood white blood cell counts, suggests the presence of a *BCR-ABL* chimeric gene at a high probability, while the findings are not definitive. However, by utilizing the recently developed CBA for BCR-ABL protein, we can make a final diagnosis of *BCR-Abl*-positive ALL at the initial presentation of the patient without waiting for the results of RT-PCR or chromosomal analysis. Since the effectiveness of tyrosine kinase inhibitors as first-line treatment has been reported [31,34], the prompt diagnosis of *BCR-ABL*-positive ALL is important. In our pilot study on 20 patients suspected of *BCR-ABL*-positive ALL, including 5 cases subsequently confirmed as true *BCR-ABL*-positive, the results showed a complete concordance between prior CBA for BCR-ABL fusion proteins and following real-time PCR for *BCR-ABL* chimeric genes (a typical result was presented in Fig. 3C).

In conclusion, CD66c expression is not always specific for *BCR-ABL*-positive ALL, whereas it is frequently associated with some genetic abnormalities, which are important for the prognosis. Although further analysis is needed to elucidate the underlying genetic characteristics as well as clinico-pathological features of CD66c-positive neardiploid BCP-ALL cases, our observations should shed light on the significance of CD66c expression in BCP-ALL.

Conflict of interest statement

The authors have no conflict of interest to declare with regards to this work.

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Contributions. NK designed the research study, performed research, analyzed data and wrote the paper. OT, KI, MM, DH, KK, HO, MK, HS, AM, TK, NT and KK performed the research. AK, JF, YH AO analyzed data. TK, AM and KS analyzed data and wrote the paper.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2013.10.008.

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CASE REPORT

Poor responses to tyrosine kinase inhibitors in a child with precursor B-cell acute lymphoblastic leukemia with *SNX2-ABL1* chimeric transcript

Aki Masuzawa[1], Chikako Kiyotani[1], Tomoo Osumi[1], Yoko Shioda[1], Kazutoshi Iijima[2,3], Osamu Tomita[2], Kazuhiko Nakabayashi[4], Keisuke Oboki[5], Kazuki Yasuda[6], Hiromi Sakamoto[7], Hitoshi Ichikawa[7], Kenichiro Hata[4], Teruhiko Yoshida[7], Kenji Matsumoto[5], Nobutaka Kiyokawa[2], Tetsuya Mori[1]

[1]Division of Pediatric Oncology, National Center for Child Health and Development, Setagaya-ku; [2]Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, Setagaya-ku; [3]Department of Industrial Chemistry, Faculty of Engineering, Tokyo University of Science, Shinjuku-ku; [4]Department of Maternal-Fetal Biology, National Research Institute for Child Health and Development, Setagaya-ku; [5]Department of Allergy and Immunology, National Research Institute for Child Health and Development, Setagaya-ku; [6]Department of Metabolic Disorder, Diabetes Research Center, National Center for Global Health and Medicine, Shinjuku-ku; [7]Division of Genetics, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan

Abstract

In addition to *BCR*, various rare fusion partners for the *ABL1* gene have been reported in leukemia. We have identified the fusion gene *SNX2-ABL1* in a pediatric case of acute lymphoblastic leukemia (ALL), which has only once previously been reported in an adult patient. Cytogenetic analysis detected this fusion gene arising from a t(5;9)(q22;q34) translocation. ALL cells carrying a *SNX2-ABL1* fusion exhibited a *BCR-ABL1*+ ALL-like gene expression profile. The patient poorly responded to dasatinib but partially responded to imatinib. Treatment using tyrosine kinase inhibitors requires further investigation to optimize the genotype-based treatment stratification for patients with *SNX2-ABL1* fusion.

Key words *SNX2-ABL1*; precursor B-cell acute lymphoblastic leukemia; tyrosine kinase inhibitors; imatinib; dasatinib

Correspondence Nobutaka Kiyokawa, Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, 2-10-1, Okura, Setagaya-ku, Tokyo 157-8535, Japan. Tel / Fax: +81 3 3417 2496; e-mail: kiyokawa-n@ncchd.go.jp

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Constitutively activated tyrosine kinase ABL1 plays an important role in several leukemias (1). The most frequent *ABL1* fusion gene is *BCR-ABL1*, which results from t (9;22)(q34;q11) in patients with chronic myeloid leukemia (CML) and Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL). Targeting BCR-ABL1 fusion proteins with tyrosine kinase inhibitors (TKIs) has been a major breakthrough in the management of patients with CML and Ph+ALL. Imatinib, a first-generation specific inhibitor of ABL1, binds ABL1 kinase in its inactive conformation and specifically inhibits activated kinases. Dasatinib, a second-generation inhibitor of ABL1, binds ABL1 both in its active and inactive conformation, resulting in a higher potency than imatinib and is active against most imatinib-resistant ABL1 kinase domain mutants (2). A number of *ABL1*-related fusion genes that have various fusion partners other than *BCR*, including *NUP214* (3–5), *RANBP2* (5), *RCSD1* (6), *ZMIZ1* (7), and *ETV6* (8), have been described. They are uncommon but important therapeutically because they also have sensitivities to TKIs (6, 9). Ernst T *et al.* reported the first case of the *SNX2-ABL1* fusion gene from t(5;9)(q23;q34) in an adult patient with B-cell precursor (BCP-)ALL who transiently responded to imatinib after early relapse (10). Here, we describe the second reported case of *SNX2-ABL1* fusion gene-positive BCP-ALL. The patient exhibited a *BCR-ABL1*+ ALL-like gene expression profile whereas poorly responded to dasatinib but partially responded to imatinib.

Materials and methods

Whole transcriptome sequencing

Total RNA was extracted from bone marrow-derived leukemic cells of the patient using the miRNeasy Mini Kit (Qiagen, Valencia, CA, USA). After qualification using Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA), 1 μg of total RNA was prepared for sequencing with the TruSeq RNA sample preparation kit v2, Set B (Illumina, Inc., San Diego, CA, USA) according to the manufacture's protocol. The resulting cDNA libraries were quantified and qualified using KAPA Library Quantification Kit (KAPA Biosystems, Inc., Woburn, MA, USA) and Agilent High Sensitivity DNA Kit (Agilent), respectively. The cDNA libraries with average length of 380 bp were loaded on to the cBot (Illumina) for clustering on a flow cell, then sequenced using a HiSeq1000 (Illumina). A paired-end (2 × 101) run was performed using the SBS Kit (Illumina). Real-time analysis and basecalling was performed using the HiSeq Control Software Version 1.5 (Illumina). The sequence data have been deposited in DNA Data Bank of Japan Sequence Read Archive (DDBJ, http://trace.ddbj.nig.ac.jp/dta/index_e.html) under the accession number DRA001161. The chimeric transcripts were investigated in a way similar as described previously by employing deFuse, an algorithm for gene fusion discovery (11, 12).

Polymerase chain reaction (PCR) and sanger sequencing

One microgram of total RNA was transcribed to cDNA using the ReverTra Ace qPCR RT Master Mix (TOYOBO Co., LTD., Osaka-shi, Osaka, Japan). For detection of the *SNX2-ABL1* fusion transcript, PCR was carried out using the primers described previously (10). The PCR products were analyzed by electrophoresis on a 2.0% agarose gel. As an internal control for the intactness of the RNA, cDNA of the GAPDH gene was also amplified. PCR products were cloned into pGEM-T Easy vector system (Promega, Madison, WI, USA). Sequencing reactions of the cloned PCR products were carried out using Big Dye Terminator Cycle Sequencing for an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Gene expression profiling

We analyzed the gene expression profile of the patient by employing SurPrint G3 Human GE 8x60K v2 Microarray (Agilent) according to the manufacture's protocol. To assess similarity of the gene expression profile between our case and the signature of *BCR-ABL1*+ ALL, gene set enrichment analysis (GSEA) (5) was performed. Ranked gene list of the top up- and down-regulated genes in the signatures of *BCR-ABL1*+ ALL in comparison with chimera-negative BCP-ALL were created with public repositories of databases of *BCR-ABL1*+ ALL (16 cases) and BCP-ALL without recurrent chromosomal changes (19 cases) obtained from a gene expression data set from the St. Jude Children's Research Hospital web site (http://www.stjuderesearch.org/ALL1) (13). We then determined the fraction of the top 300 differentially expressed probe sets in our case and performed GSEA.

Case report

A 7-yr-old boy was diagnosed with BCP-ALL based on an initial peripheral white blood cell (WBC) count of 115.4 × 10^9/L with 93% of lymphoblast and immunophenotyping (CD19+, CD10+, cCD79a+, TdT+, CD34+, HLA-DR+). The cytogenetic analysis revealed t (5;9) (q22;q34), described in more detail below. Treatment was initiated according to the standard chemotherapy for childhood ALL based on the ALL-BFM95 regimen. The patient achieved a morphological remission after the induction course. Despite continuing the postinduction chemotherapy, the patient experienced a bone marrow relapse 15 months after the initial diagnosis. After the relapse, he could not achieve a second remission, because response to the chemotherapy became limited. The patient received an umbilical cord blood transplantation with refractory disease. He achieved a second morphological remission after the transplantation. However, at 6 months from transplantation, the patient developed bone marrow relapse again. Since that time he has never achieved a remission despite salvage chemotherapies.

At the time of diagnosis, a clonal karyotype, 46XY, t (5;9) (q22;q34) was detected. Since the involvement of the 9q34 breakpoint had suggested the presence of an *ABL1*-related fusion gene, we performed whole-transcriptome sequencing on the bone marrow specimens at the initial diagnosis and first relapse and identified in-frame fusion transcripts of *SNX2* and *ABL1*. The presence of fusion transcripts of *SNX2-ABL1* was further confirmed by RT-PCR (Fig. 1A) and a Sanger sequencing (Fig. 1B). The sequencing of PCR products showed that *ABL1* exon 4 was fused in frame to *SNX2* exon 3. Upon a search using Basic Local Alignment Search Tool (BLAST), we found that the *SNX2-ABL1* sequence of our patient was completely identical with that reported by Ernst T *et al.* (6) in an adult case with ALL. We analyzed the gene expression profile of the patient and GSEA revealed that our patient had a gene expression profile that was enriched in that of *BCR-ABL1*+ ALL (Fig. 1C).

During the course, the patient received TKIs without other anti-leukemia drugs (Table 1). After the first relapse, imatinib was introduced at a dose of 240 mg/m^2/d once daily. Although the patient had never achieved a remission, the disease stabilized for 2 months after starting imatinib. The first course of imatinib was discontinued because of the

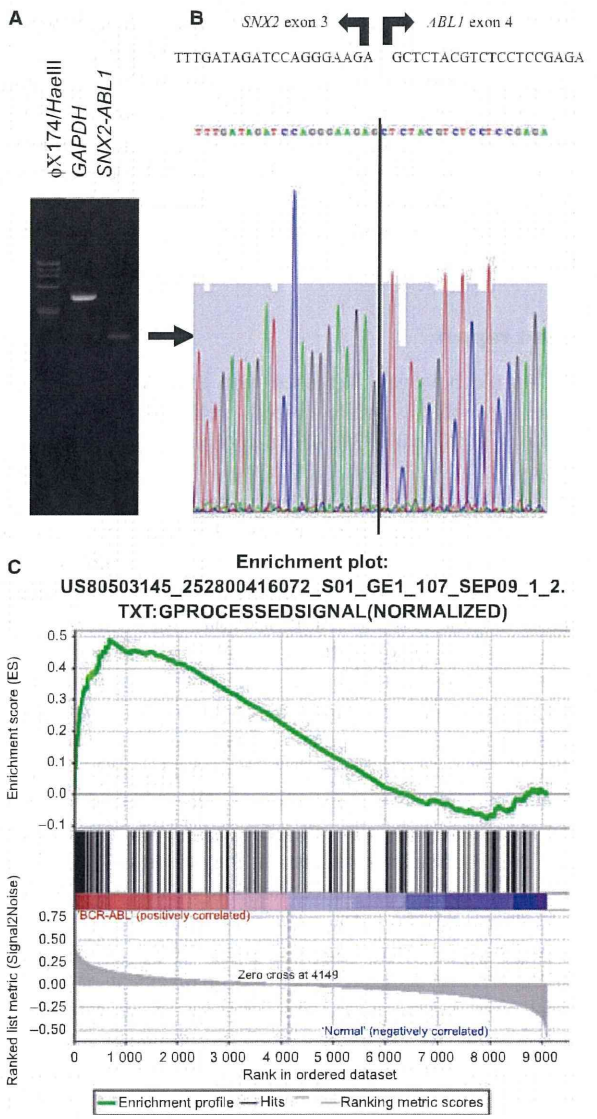

Figure 1 *SNX2-ABL1* fusion gene resulting from t(5;9)(q22;q34). (A) PCR product (B) amplified *SNX2-ABL1* mRNA fusion transcript. (C) Gene set enrichment analysis for *BCR-ABL1*-like signature.

umbilical cord blood transplantation (Table 1, column 1, 2). The patient received TKIs again after the second relapse. Because response to imatinib worsened (Table 1, column 3, 4), dasatinib was introduced at a dose of 80 mg/m^2/d once daily. However, the number of blast cells in peripheral blood rapidly increased (Table 1, column 5). Dasatinib was attempted again at the same dose after reduction of blast cells using vincristine and prednisolone. Again, the number of blast cells in peripheral blood rapidly increased (Table 1, column 6). At 3 months after discontinuing dasatinib, starting imatinib at a dose of 600 mg/m^2/d once daily rapidly decreased the number of blast cells in peripheral blood (Table 1, column 6).

Discussion

We have reported herein an *SNX2-ABL1* fusion resulting from the rearrangement of chromosome 9q34 and 5q22 in a pediatric BCP-ALL case. As we demonstrated, our patient with *SNX2-ABL1* exhibited a gene expression profile of *BCR-ABL1*+ ALL-like signature. In the literature, *SNX2-ABL1* has been reported only once in an adult patient with BCP-ALL (10), and thus, this is the second reported case to the best of our knowledge. SNXs are a family of oligomeric proteins that contain a variety of domains for protein-protein and protein-lipid interactions and are found distributed between membranes and the cytosol (14). While the precise function of SNX protein is not known, it is understood that SNXs are involved in various aspects of protein trafficking. SNX2 exhibits interactions with platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin classes of receptor tyrosine kinases (15). Therefore, it is expected that fusion with SNX2 induces the constitutive activation of ABL1 kinase, but the details are still unclear.

Most *ABL1* fusions reported to date, including *BCR-ABL1*, result in the partner gene fusing to *ABL1* exons 2 (3, 16, 17). However, as in the report by Ernst (10), *SNX2-ABL1* fusion in this patient revealed that *SNX2* exon 3 was fused in frame to *ABL1* exon 4. Such a fusion may lack the amino acid W127-K183 to form an intact Src homology 2 (SH2) domain (18, 19). Since SH2 domain is postulated to be important for the regulation of ABL1 kinase acivity, *SNX2-ABL1* lacking SH2 domain may activate pathways different from those activated by BCR-ABL1. From a clinical point of view, it is therefore possible that patients with *SNX2-ABL1* show a clinical course different from those with *BCR-ABL1* or *BCR-ABL1*-like alterations. Indeed, the patient showed a transient and partial response to imatinib at 600 mg/m^2/d with a reduction in blast cells and the WBC count, while the effect of dasatinib seemed to be rather limited. The patient described by Ernst (106) also experienced early relapse after the initial chemotherapy, transiently responded to treatment with imatinib, and died despite salvage chemotherapies. While prognostic relevance of *SNX2-ABL1* is unknown, the clinical course of this case is very similar to that of the patient described by Ernst. We further confirmed the poorer sensitivity of *SNX2-ABL1* kinase toward TKIs in compared to BCR-ABL1 kinase by introducing *SNX2-ABL1* into murine Ba/F3 cells (unpublished data).

Although more studies are needed to assess the outcomes associated with treatment using tyrosine kinase inhibitors in patients with *SNX2-ABL1* fusion, therapeutic molecules designed to disrupt the SH2 domain of *ABL1* would be ineffective for patients with BCP-ALL possessing an *SNX2-ABL1* aberration. Therefore, possibilities of genotype-based treatment stratification should be explored, especially for chromosomal aberrations like *SNX2-ABL1*. Further investigation should be directed toward the identification of novel

Table 1 Responses to TKI treatment in a child with *SNX2-ABL1* positive precursor B-cell acute lymphoblastic leukemia

	TKI Dose, Duration	Previous anti-leukemia treatment other than TKI (within 2 wk before starting TKI)	Day after starting TKI WBC count/mm^3 (Blast%)			
			At the time of starting TKI	~7 d	~14 d	14 d~
1	Imatinib	Day −16~ to −12	Day 0	Day +6	Day +12	Day +24
	200 mg/d 1×, 24 d	DEX, 6MP, VDS, MTX, Dauno, IFO, IT	830 (39%)	1700 (0.5%)	3420 (0%)	1270 (1%)
2	Imatinib	Day −2~ to −1	Day 0	Day +5	Day +14	Day +34
	200 mg/d 1×, 34 d	VP16	860 (0%)	1390 (0%)	660 (0%)	1320 (0%)
3	Imatinib	–	Day0	Day +7	Day +13	Day +32
	200 mg/d 1×, 32 d		11 180 (26%)	10 990 (22%)	10 120 (24.5%)	21 960 (86%)
4	Imatinib	–	Day 0	NE	Day +10	NE
	300 mg/d 1×, 14 d		18 170 (60%)		21 960 (77%)	
5	Dasatinib	–	Day 0	Day +7	NE	NE
	70 mg/d 1×, 7 d		21 960 (86%)	55 190 (94%)		
6	Dasatinib	Day −12~ to −1	Day-1	Day +5	Day +13	NE
	70 mg/d 1×, 13 d	PSL, VCR	4040 (42%)	9440 (64%)	66 360 (93%)	
7	Imatinib	Day −25~ to −12	Day 0	Day +6	Day +14	Day +24
	500 mg/d 1×, 24+ days	DEX, CA	66 250 (93%)	16 410 (95%)	3430 (53%)	1390 (42%)

TKI, tyrosine kinase inhibitor, DEX, dexamethasone, 6-MP, mercaptopurine, VDS, vindesine, MTX, methotrexate, Dauno, daunorubicin, IFO, ifosfamide, IT, intrathecal chemotherapy, VP16, etoposide, PSL, prednisolone, VCR, vincristine, CA, cytarabine, NE, not evaluated.

genetic abnormalities, which would aid in developing risk-stratified treatment regimens for patients with BCP-ALL.

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Conflict of interest

The authors declare no conflict of interest.

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Sensitivity of SNX2-ABL1 toward tyrosine kinase inhibitors distinct from that of BCR-ABL1

Osamu Tomita[a,b], Kazutoshi Iijima[a,c], Takeshi Ishibashi[a,b], Tomoo Osumi[d], Kenichiro Kobayashi[a], Hajime Okita[a], Masahiro Saito[e], Tetsuya Mori[d], Toshiaki Shimizu[e], Nobutaka Kiyokawa[a,*]

[a] Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, Setagaya-ku, Tokyo, Japan
[b] Department of Pediatrics and Adolescent Medicine, Juntendo University Graduate School of Medicine, Bunkyo-ku, Tokyo, Japan
[c] Department of Industrial Chemistry, Faculty of Engineering, Tokyo University of Science, Shinjuku-ku, Tokyo, Japan
[d] Division of Pediatric Oncology, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan
[e] Department of Pediatrics, Juntendo University Faculty of Medicine, Bunkyo-ku, Tokyo, Japan

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ABSTRACT

We introduced *SNX2-ABL1*, a novel *ABL1*-related chimeric transcript lacks SH3 and SH2 domains, into murine Ba/F3 cells and compared their function with that of *BCR-ABL1*. After the expression of SNX2-ABL1 proteins, Ba/F3 cells acquired an ability to proliferate in an IL-3-independent manner. Upon treatment with both imatinib and dasatinib, BCR-ABL1-expressing Ba/F3 cells underwent rapid apoptosis, whereas SNX2-ABL1-expressing Ba/F3 cells showed poorer sensitivity toward these TKIs and could proliferate in the presence of a low dose of dasatinib. Therefore, other TKIs with a more selective effect against this chimeric kinase should be used for the treatment of patients with *SNX2-ABL1*+ ALL.

1. Introduction

Among B-cell precursor (BCP)-acute lymphoblastic leukemia (ALL), approximately one-third of cases express well-characterized chimeric transcripts [1,2]. Since the abnormal function of subsequent chimeric proteins is associated with leukemogenesis, the presence of chimeric transcripts is closely correlated with the biological characteristics of ALL cells as well as clinical outcomes of patients. For example, *BCR-ABL1* is a well-characterized one identified in approximately 5% of childhood ALL originating from the specific chromosomal translocation t(9;22)(q34;q11), designated as Philadelphia (Ph) translocation [3–5]. ABL1 is a tyrosine kinase known to be constitutively activated upon fusion with BCR protein, which causes the abnormal proliferation and immortalization of leukocytes [6], and *BCR-ABL1*-positive (Ph+) ALL is accompanied by an extremely unfavorable outcome compared to other BCP-ALL [7,8]. However, the therapeutic application of recently developed tyrosine-kinase inhibitors (TKIs) has significantly improved the early outcome of Ph+ ALL patients [9,10].

Imatinib (IC50: 0.6 μM) is the first TKI approved for the treatment toward Ph+ leukemia that competes with ATP for its binding site in ABL1 tyrosine kinase domain in inactive conformations and thus inhibits the tyrosine kinase activity of BCR-ABL1 [4,11]. Currently, the combination of chemotherapy with imatinib is the frontline regimen for both adult and pediatric patients with newly diagnosed Ph+ ALL [12,13], whereas the evidence of imatinib-resistant mutations or clones in Ph+ ALL, even at diagnosis, is increasing and the long-term advantage from using imatinib is still controversial [14]. Dasatinib (IC50: <1 nM) is a second-generation ATP-competitive TKI originally identified as a potent inhibitor of Src family kinases and binds to ABL1 with less stringent conformational requirements, exhibiting 300 times higher potency than imatinib although with less selectivity. Dasatinib can bind to ABL1 both in its active and inactive conformations and inhibit most imatinib-resistant BCR-ABL1 mutations [15–18]. A recent study indicated the feasibility, safety, and efficacy of dasatinib for adult Ph+ ALL and, thus, has advocated the use of the inhibitor alone as a first-line treatment for patients with Ph+ ALL [18].

In addition to dasatinib, another second- and third-generation TKIs have been developed to override the imatinib-resistance mechanisms [13,19,20]. For example, nilotinib (IC50: <30 nM) and bafetinib (IC50: 5.8 nM) have been developed based on the chemical

* Corresponding author. Tel.: +81 3 3417 2496; fax: +81 3 3417 2496.
E-mail address: kiyokawa-n@ncchd.go.jp (N. Kiyokawa).

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