ORIGINAl ARTiCLE

Characterization of pediatric Philadelphia-negative B-cell precursor acute lymphoblastic leukemia with kinase fusions in Japan

T Imamura1, N Kiyokawa2, M Kato3, C Imai5, Y Okamoto3, M Yano1, K Okhi6, Y Yamashita7, Y Kodama3, A Saito8, M Mori9, S Ishimaru10, T Deguchi11, Y Hashii12, Y Shimomura13, T Hori13, K Kato14, H Goto15, C Ogawa16, K Koh9, T Taki17, A Manabe18, A Sato19, A Kikuta20, S Adachi21, K Horibe22, A Ohara23, A Watanabe24, Y Kawano5, E Ishii25 and H Shimada26

Recent studies revealed that a substantial proportion of patients with high-risk B-cell precursor acute lymphoblastic leukemia (BCP-ALL) harbor fusions involving tyrosine kinase and cytokine receptors, such as ABL1, PDGFRB, JAK2 and CRLF2, which are targeted by tyrosine kinase inhibitors (TKIs). In the present study, transcriptome analysis or multiplex reverse transcriptase–PCR analysis of 373 BCP-ALL patients without recurrent genetic abnormalities identified 29 patients with kinase fusions. Clinically, male predominance (male/female: 22/7), older age at onset (mean age at onset: 8.8 years) and a high white blood cell count at diagnosis (mean: 94 200/μl) reflected the predominance of National Cancer Institute high-risk (NCI-HR) patients (NCI-standard risk/HR: 8/21). Genetic analysis identified three patients with ABL1 rearrangements, eight with PDGFRB rearrangements, two with JAK2 rearrangements, three with IGH-EPOR and one with NCOR1-LYN. Of the 14 patients with CRLF2 rearrangements, two harbored IGH-EPOR and PDGFRB rearrangements. IKZF1 deletion was present in 16 of the 22 patients. The 5-year event-free and overall survival rates were 48.6 ± 9.7% and 73.5 ± 8.6%, respectively. The outcome was not satisfactory without sophisticated minimal residual disease-based stratification. Furthermore, the efficacy of TKIs combined with conventional chemotherapy without allogeneic hematopoietic stem cell transplantation in this cohort should be determined.

Blood Cancer Journal (2016) 6, e419; doi:10.1038/bcj.2016.28; published online 13 May 2016

INTRODUCTION

Modern therapeutic strategy of pediatric acute lymphoblastic leukemia (ALL), such as minimal residual disease-based risk-adopted treatment, progress of chemotherapy, supportive care and allogeneic hematopoietic stem cell transplantation (allo-HSCT) greatly improved the prognosis of pediatric ALL.1 However, approximately 15–20% patients experience relapse and show dismal prognosis.1 Additionally, late effect is also the considerable problem for the heavily treated patients, especially for the patients who received allo-HSCT. Thus it is quite important to treat high-risk ALL patients such as Ph+ ALL patients with less toxic chemotherapeutic regimen.2

Recent comprehensive genomic analyses revealed the genetic landscape of high-risk pediatric B-cell precursor ALL (BCP-ALL).3–7 In particular, a number of chimeric fusions, including those involving tyrosine kinase and cytokine receptors, were identified in a subgroup of BCP-ALL designated as Ph-/BCR-ABL-like ALL.6,7 Because some of these genetic alterations may be treated by molecular targeted therapies, these patients may benefit from specific kinase inhibitor treatment.8,9 However, a comprehensive analysis of the clinical characteristics of patients with these kinase fusions is necessary, despite recent reports providing clinical information on these patients.7,10 The present study aimed to clarify the clinical characteristics of Philadelphia-negative BCP-ALL patients with kinase fusions in Japan by performing a genetic analysis of pediatric BCP-ALL to identify patients with kinase fusions. Clinical information was collected retrospectively and data were analyzed to identify prognostic factors in these patients.

1Department of Pediatrics, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto, Japan; 2Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, Tokyo, Japan; 3Division of Pediatrics, The University of Tokyo, Tokyo, Japan; 4Division of Pediatrics, Department of Homeostatic Regulation and Development, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; 5Department of Pediatrics, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; 6Department of Hematology/Oncology, Gunma Children’s Medical Center, Shibakawa, Japan; 7National Hospital Organization Nagoya Medical Center, Clinical Research Center, Nagoya, Japan; 8Department of Hematology and Oncology, Hyogo Prefectural Children’s Hospital, Kobe, Japan; 9Department of Hematology/Oncology, Aichi Children’s Medical Center, Nagakute, Japan; 10Department of Hematology/Oncology, Saitama Children’s Medical Center, Saitama, Japan; 11Department of Hematology/Oncology, Tokyo Metropolitan Children’s Medical Center, Tokyo, Japan; 12Department of Pediatrics, Mie University, Tsu, Japan; 13Department of Pediatrics, Osaka University, Osaka, Japan; 14Department of Pediatrics, Aichi Medical University School of Medicine, Nagakute, Japan; 15Division of Pediatric Hematology/Oncology, Ibaraki Children’s Hospital, Mito, Japan; 16Division of Hemato-Oncology and Regenerative Medicine, Kanagawa Children’s Medical Center, Yokohama, Japan; 17Department of Pediatric Oncology, National Cancer Center Hospital, Tokyo, Japan; 18Department of Molecular Diagnostics and Therapeutics, Kyoto Prefectural University of Medicine, Kyoto, Japan; 19Department of Pediatrics, St Luke’s International Hospital, Tokyo, Japan; 20Department of Hematology and Oncology, Miyagi Children’s Hospital, Sendai, Japan; 21Department of Pediatrics, Fukushima Medical School, Fukushima, Japan; 22Department of Human Health Sciences, Graduate School of Medicine, Kyorin University, Tokyo, Japan; 23Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan; 24Department of Pediatrics, Toho University, Tokyo, Japan; 25Department of Pediatrics, Nakadori General Hospital, Akita, Japan; 26Department of Pediatrics, Ehime University Graduate School of Medicine, Toon, Japan and 27Department of Pediatrics, School of Medicine, Keio University School of Medicine, Tokyo, Japan. Correspondence: Dr T Imamura, Department of Pediatrics, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kaji-cho, Hirokoji, Kaminoy-ku, Kyoto 602-8566, Japan.

E-mail: imamura@koto.kpu-m.ac.jp
Received 28 March 2016; accepted 6 April 2016
MATERIALS AND METHODS
Patient cohort and samples
Diagnostic samples of bone marrow or peripheral blood were obtained from patients with pediatric B-CP-ALL enrolled in and treated under clinical trial protocols of the Japan Association of Childhood Leukemia Study Group (JACLS)-ALL02 study (n = 1252),11,12 Tokyo Children’s Cancer Study Group (TCCSG)-LO4-16 (n = 150), L06-16 (n = 194), L07-16 (n = 274) and L09-16 (n = 607) studies,13 Childhood Cancer and Leukemia Study Group (CCLSG)-ALL2004 study (n = 325)14 and Kyushu-Yamaguchi Childhood Cancer Study Group (KYCCSG)-ALL02 study (n = 156).15 The diagnosis of B-CP-ALL was based on morphological findings of bone marrow aspirates and immunophenotypic analyses of leukemic cells by flow cytometry. Conventional cytogenetic analyses and molecular studies were performed as part of the routine work-up in each protocol.11–15 Patients with recurrent fusion transcripts, including ETV6-RUNX1, E2A-PBX1, MLL-related fusion and BCR-ABL, and those with high hyperdiploidy were excluded that defined as B-other cases in this study. Down syndrome-related B-CP-ALL was also excluded from this study. Informed consent was obtained from the guardians of patients according to the Declaration of Helsinki, and genetic study protocols were approved by the institutional review boards of the participating institutes.

mRNA sequencing (mRNA-seq) and multiplex reverse transcriptase–PCR (mRT-PCR)
mRNA-seq or mRT-PCR analyses were performed in 373 B-CP-ALL patients with sufficient RNA samples. mRNA-seq was performed in 92 patients in the TCCSG cohort and 17 patients in the JACLS cohort (total 109 patients) according to previously described methods.16,17 Briefly, the cDNA libraries were loaded on to the cBot (Illumina, Inc., San Diego, CA, USA) for clustering on a flow cell and then sequenced using a HiSeq1000 (Illumina). A paired-end 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 chimeric transcripts were investigated by employing defuse and TopHat-Fusion, algorithms for gene fusion.17 All kinase fusions determined by mRNA-seq were validated by RT-PCR and Sanger sequencing, mRT-PCR was used in 264 patients (68 in the TCCSG cohort, 95 in the JACLS cohort, 83 in the CCLSG cohort and 18 in the KYCCSG cohort) to determine the presence of 15 kinase fusions, including ZMYM1-ABL1, SNX2-ABL1, FOXP1-ABL1, SFOB-ABL1, EML1-ABL1, NUP214-ABL1, RCD1-ABL1, ETV6-ABL1, RBANP2-ABL1, STRN3-JAK2, BCR-JAK2, PAXS-JAK2, EBF1-PDGFRB, ATFI7-PDGFRB and P2RY8-CRLF2. The primers used in this study are listed in Supplementary Table S1.6 The consort diagram of genetic analysis of 373 patients is described in Figure 1. The comparison of clinical characteristics of analyzed and non-analyzed patients in each cohort, such as TCCSG, JACLS, CCLSG and KYCCSG, are summarized in Supplementary Table S2. Although clinical characteristics were not significantly different between the analyzed and non-analyzed groups in TCCSG, CCLSG and KYCCSG cohorts, National Cancer Institute high-risk (NCI-HR) patients were more in the analyzed group in the JACLS cohort.

Determination of IKZF1 deletion and JAK2 mutation
To identify the copy number abnormality of IKZF1, PAXS, EBF1, ETV6, CDK4,2 and CDKN2B in patients with kinase fusions, the SALSA Multiplex Ligation-dependent Probe Amplification (MLPA) Kit P335-A4 (MRC Holland, Amsterdam, The Netherlands) was used as described previously.18 Screening of JAK2 exons 16, 20 and 21 (gene accession number NM_004972) mutations was performed in patients with CRLF2 rearrangement, as described previously.19

Gene set enrichment analysis
Gene expression profiles of the patients’ samples analyzed by mRNA-seq were obtained as previously described.16 To assess similarity of gene expression profile between the kinase fusion-positive cases and the signature of BCR-ABL1-positive cases, gene set enrichment analysis was performed as previously described.16

Statistical analysis
Event-free survival (EFS) and overall survival (OS) rates were estimated using the Kaplan–Meier method, and differences were compared using the log-rank test. A P-value < 0.05 (two-sided) was considered significant. EFS and OS were defined as the times from diagnosis to event (any death, relapse, secondary malignancy or failure of therapy) and from diagnosis to death from any cause or to the last follow-up, respectively. Patients without an event of interest were censored at the date of last contact. Hazard ratios for probability of relapse between subgroups were calculated

| Table 1. Kinase fusions identified in this study |
|-----------------|-----------------|-----------------|-----------------|
| Kinase gene     | Fusion partners | Patients (n)    | S’ Genes        |
| ABL1            |                 | 3               | SNX1, ZIM21, ETV6 |
| PDGFRB          |                 | 2               | EBF1, ATFI7      |
| CRLF2           |                 | 3               | P2RY8, IgH, CSF2RA |
| JAK2            |                 | 2               | PAXS, OFD1      |
| EPOR            |                 | 1               | IgH            |
| LYN             |                 | 1               | NCO1R          |
| **Total**       |                 | **10**          |                 |

*There were two patients who harbored two kinase fusions. One had ATFI7-PDGFRB and P2RY8-CRLF2, and another patient had IgH-EPOR and CSF2RA-CRLF2.

Figure 1. A consort diagram of genetic analysis of 373 patients. Samples were obtained from patients treated in the TCCSG (n = 160), JACLS (n = 112), CCLSG (n = 83) and KYCCSG (n = 18) cohorts. Ninety-two of the 160 in the TCCSG cohort and 12 of the 112 in the JACLS cohort were analyzed by mRNA-seq. The remaining 264 patients were analyzed by mRT-PCR. The kinase-activating fusions were identified in 16 of the 109 by mRNA-seq and in 13 of the 264 by mRT-PCR.

| Table 2. IKZF1 and JAK2 status of kinase fusion-positive patients in this study |
|-----------------|-----------------|-----------------|-----------------|
| Kinase fusion   | IKZF1 WT       | Deletion        | JAK2 WT        | Mutation        |
| P2RY8-CRLF2     | 6               | 3               | 1              | 1†              |
| IgH-CRLF2       | 0               | 1               | 0              | 1              |
| CSF2RA-CRLF2+IgH-EPOR | 0 | 1             | 0              | 0               |
| EBF1-PDGFRB     | 0               | 5               | 1              | —              |
| ATFI7-PDGFRB+P2RY8-CRLF2 | 0 | 1             | 0              | 1               |
| JAK2            | 0               | 1              | 0              | 1              |
| OFD1-JAK2       | 0               | 1              | 0              | 1              |
| IgH-EPOR        | 0               | 1              | —              | —              |
| SNX2-ABL1       | 0               | 0              | 1              | —              |
| ZMYM1-ABL1      | 0               | 0              | 1              | —              |

Abbreviations: ND, not determined; WT, wild type. = 631 81 a 1

Blood Cancer Journal
RESULTS
Identification of kinase fusions in BCP-ALL
Twenty-nine patients with kinase fusions were identified (Table 1, Supplementary Table S3), of whom 16 were identified by mRNA-seq and 13 by mRT-PCR. All kinase fusions identified by mRNA-seq were confirmed to be in-frame with intact tyrosine kinase domain by RT-PCR and Sanger sequencing. The involved exons in each kinase fusion except CRLF2-related ones and IgH-EPOR are listed in Supplementary Table S4. It was likely that mRNA-seq was more sensitive to detect the kinase fusion (16 of the 109, 14.7% by mRNA-seq vs 13 of the 264, 4.9% by mRT-PCR), simply because only mRNA-seq can detect a novel kinase fusion. However, comparing the detection frequency of the 15 kinase fusions that were included in the mRT-PCR system, we identified 9 of the 109 (8.3%) by mRNA-seq and 13 of the 264 (4.9%) by mRT-PCR assay. Therefore, the sensitivity of two detection methods is not significantly different (9/109 vs 13/264, \(P = 0.32\)).

Regarding chimeric fusions, three patients had ABL1 rearrangements (SNX2, ZMIZ1 and ETV6), eight had PDGFRB rearrangements (EBF1 in six patients and ATF7IP\(^18\) in two patients), two had JAK2 rearrangements (PAX5 and OFD1\(^20\)), three had IgH-EPOR and one had NCOR1-LYN\(^20\). Of the 14 patients with CRLF2 rearrangements (P2RY8 in 11 patients, IgH in 2 patients and CSF2RA\(^21\) in 1 patient), 2 harbored IgH-EPOR and ATF7IP-PDGFRB (Table 1). MLPA analysis detected IKZF1 deletions in 16 of the 22 (72.7%) patients (Table 2, Supplementary Table S3). Mutational analysis of JAK2 was performed.

Figure 2. Gene set enrichment analysis plot of the patients with NCOR1-LYN, OFD1-JAK2 and CSF2A-CRLF2. The enrichment score (ES) is shown at the bottom of each graph. The positive ES means significant enrichment of the BCR-ABL1 gene expression signature.
in 12 of the 14 patients with CRLF2 rearrangement and identified 2 of the 12 (16.7%) patients with JAK2 R683-activating mutations (Table 2). The results of MLPA analysis in 29 kinase fusion-positive patients are summarized in Supplementary Table S3. Gene set enrichment analysis revealed that gene expression profile of the patients harboring kinase fusion except for P2RY8-CRLF2 was similar to that of BCR-ABL1-positive patients (Figure 2).

Clinical characteristics and outcomes of BCP-ALL patients with kinase fusions

Analysis of the clinical characteristics of the 29 patients showed male predominance (male/female: 22/7), older age at onset (median age at diagnosis: 8.8 years) and high white blood cell (WBC) count at diagnosis (median WBC count at diagnosis: 94 200/μl), which reflected the predominance of NCI-HR patients (NCI-standard risk (SR)/HR: 8/21) (Table 3). Thirteen of the 29 (44.8%) patients showed poor response to initial prednisolone (PSL) therapy. Ten patients (34.5%) who received allo-HSCT were in the first complete remission (CR), and six of them (60%) maintained first CR, and six of them (60%) maintained first CR. Four patients (13.8%) did not respond to the initial treatment, and two of them relapsed. The remaining two patients died of treatment-related complications, including transplant-related mortality. Twenty-nine patients were alive in CR by allo-HSCT. The remaining two patients died of first or second relapse. Six patients died of death and 54.6 ± 15.0% in negative B-others patients in the JACLS cohort. The difference was not statistically significant (log-rank P = 0.089). Because all of the 17 patients in the JACLS cohort have IKZF1 deletion, the prognosis of these patients was poor irrespective of the presence of kinase-activating fusions. According to the NCI risk classification, the 5-year EFS rate was 57.1 ± 18.7% in the SR group and 44.4 ± 11.2% in the HR group. The 5-year OS rate was 85.7 ± 13.2% in the SR group and 65.8 ± 10.5% in the HR group (Figures 3b and c). Although univariate analysis was performed to determine the factors related to inferior EFS or OS in 29 patients, none of the covariates such as age at diagnosis, WBC count at diagnosis, NCI risk, initial PSL response, IKZF1 deletion or allo-HSCT in first CR were statistically significant (Table 4). According to the type of chimeric fusions, among patients with ABL-class rearrangements, such as ABL1, PDGFRB and LYN, which could be targets of imatinib or dasatinib, the 5-year EFS and OS rates were 63.6 ± 14.5% and 90.0 ± 9.5%, respectively (Supplementary Figures S2A and B). Among patients with JAK-class rearrangements, such as JAK2, CRLF2 and EPOP, which could be targets of JAK2 inhibitors, the 5-year EFS and OS rates were 37.5 ± 12.1% and 62.5 ± 12.1%, respectively (Supplementary Figures S2A and B). There were no significant differences in EFS and OS between the two genetic subgroups (log-rank P = 0.42 and 0.18, respectively). No significantly different factors, such as age at onset, initial WBC count, NCI risk, IKZF1 deletion, initial PSL response, and the number of patients receiving allo-HSCT in first CR, were observed between the two genetic subgroups (Table 5).

DISCUSSION

Roberts et al.7 identified kinase fusions in 172 of the 264 patients with Ph-like ALL using mRNA-seq. Although 106 (61.6%) of the 172 patients harbored CRLF2-related fusions (45 P2RY8-CRLF2 and 61 IgH-CRLF2), the chimeric fusions identified in the remaining 66 patients varied. In detail, 36 (20.9%) patients harbored ABL-class rearrangements, including ABL1, ABL2, CSF1R and PDGFRB; 28 (16.3%) patients harbored EPOP or JAK2 rearrangement; and the remaining 2 (1.2%) patients harbored rare kinase fusions, such as ETV6/NTRK3 and ZFAND3 DGKH. IKZF1 deletion was present in 45 (68.2%) of the 66 patients with kinase fusions, excluding CRLF2-related fusions. In addition, 68 (55%) of the 126 patients with CRLF2 rearrangement harbored JAK2-activating mutation.

In the present study, we analyzed 373 patients with BCP-ALL without hyperdiploid karyotype and recurrent fusions, such as ETV6-RUNX1, E2A-PBX1, MLL-related fusions and BCR-ABL, because we did not have enough data for gene expression profiling, which is mandatory for the diagnosis of Ph-like ALL. We identified 29 kinase fusion-positive patients: 14 patients with CRLF2 rearrangements, 12 patients with ABL-class rearrangements, and 5 patients with EPOP or JAK2 rearrangement. Our retrospective

Table 3. Clinical characteristics of 29 patients with kinase fusions

| Characteristic          | Value |
|-------------------------|-------|
| Age (years)             | 8.8   |
| Range                   | 1.9–16|
| WBC count               |       |
| Median (×10³/μl)        | 94.2  |
| Range                   | 0.6–420|
| Sex                     |       |
| Male                    | 22    |
| Female                  | 7     |
| NCI                      |       |
| SR                      | 8     |
| HR                      | 21    |
| PSL response            |       |
| PGR                     | 16    |
| PPR                     | 13    |
| Induction failure       |       |
| Yes                     | 4     |
| No                      | 25    |
| Allo-HSCT in first CR   |       |
| Yes                     | 10    |
| No                      | 19    |
| Outcome                 |       |
| Alive                   | 21    |
| Dead                    | 8     |

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR, complete remission; HR, high risk; NCI, National Cancer Institute; PGR, prednisolone good responder; PPR, prednisolone poor responder; PSL, prednisolone; SR, standard risk; WBC, white blood cell.

In the survival analysis, the 5-year EFS and OS rates were 48.6 ± 9.7% and 73.5 ± 8.6%, respectively, with a median follow-up period of 6.7 years (Figure 3a). To determine prognostic significance of kinase-activating fusions, we compared 5-year EFS between kinase fusion-positive (n = 16) and -negative (n = 93) in mRNA-seq cohort. The 5-year EFS rate was 41.0 ± 13.0% in the kinase-activating fusion-positive group and 67.0 ± 7.7% in the fusion-negative group, respectively. The prognosis of kinase-activating fusion-positive patients was significantly inferior to that of kinase-negative patients (log-rank P = 0.035). When mRNA-seq cohort was split into two groups based on the clinical trial, such as TCSGG (n = 92) and JACLS cohorts (n = 17), the 5-year EFS rate was 38.1 ± 19.9% in activating kinase-positive patients and 68.9 ± 9.3% in activating kinase-negative patients in the TCSGG cohort. The difference was statistically significant (log-rank P = 0.039). On the other hand, the 5-year EFS was 50.0 ± 20.4% in activating kinase fusion-positive B-others patients and 54.6 ± 15.0% in negative B-others patients in the JACLS cohort. The difference was not statistically significant (log-rank P = 0.89). Because all of the 17 patients in the JACLS cohort have IKZF1 deletion, the prognosis of these patients was poor irrespective of the presence of kinase-activating fusions. According to the NCI risk classification, the 5-year EFS rate was 57.1 ± 18.7% in the SR group and 44.4 ± 11.2% in the HR group. The 5-year OS rate was 85.7 ± 13.2% in the SR group and 65.8 ± 10.5% in the HR group (Figures 3b and c). Although univariate analysis was performed to determine the factors related to inferior EFS or OS in 29 patients, none of the covariates such as age at diagnosis, WBC count at diagnosis, NCI risk, initial PSL response, IKZF1 deletion or allo-HSCT in first CR were statistically significant (Table 4). According to the type of chimeric fusions, among patients with ABL-class rearrangements, such as ABL1, PDGFRB and LYN, which could be targets of imatinib or dasatinib, the 5-year EFS and OS rates were 63.6 ± 14.5% and 90.0 ± 9.5%, respectively (Supplementary Figures S2A and B). Among patients with JAK-class rearrangements, such as JAK2, CRLF2 and EPOP, which could be targets of JAK2 inhibitors, the 5-year EFS and OS rates were 37.5 ± 12.1% and 62.5 ± 12.1%, respectively (Supplementary Figures S2A and B). There were no significant differences in EFS and OS between the two genetic subgroups (log-rank P = 0.42 and 0.18, respectively). No significantly different factors, such as age at onset, initial WBC count, NCI risk, IKZF1 deletion, initial PSL response, and the number of patients receiving allo-HSCT in first CR, were observed between the two genetic subgroups (Table 5).
analysis confirmed three types of kinase rearrangements, including CRLF2, ABL1-class and JAK2/EPOR, which were dominant, although we may have missed several patients with kinase fusions owing to lack of data in 264 patients who were not analyzed by mRNA-seq. A high prevalence (16 of the 22, 72.7%) of IKZF1 deletion in these patients was also confirmed in our study, suggesting that kinase fusion-positive BCP-ALL patients are biologically similar to Ph+ ALL patients. However, JAK2 mutation was present in only 2 (16.7%) of the 12 patients with CRLF2 rearrangements, which was a lower rate than that (55%) reported in previous studies. The reason for this discrepancy is unclear. Because other genetic alterations related to the JAK-STAT pathway, such as activating mutations in the IL7R, FLT3, JAK1 and JAK3 genes and the deletion of SH2B3, were not investigated in our study, these genetic alterations may have replaced JAK2-activating mutations in our cohort.

We confirmed that the clinical characteristics of patients with kinase fusions are those commonly associated with Ph+ and Ph-
Table 5. Comparison of the characteristics between two genetic subgroups

| PDGFRB ABL1 LYN JAK2 EPOR CRLF2 | P |
|----------------------|---|
| Age (years) at diagnosis | |
| < 10 | 6 | 10 | 0.92 |
| > 10 | 6 | 7 |
| WBC (μl) at diagnosis | |
| < 50000 | 3 | 8 | 0.27 |
| > 50000 | 9 | 9 |
| NCI risk | |
| SR | 2 | 6 | 0.41 |
| HR | 10 | 11 |
| IKZF1 status | |
| WT | 1 | 7 | 0.18 |
| del | 7 | 8 |
| PSL response | |
| PGR | 5 | 11 | 0.5 |
| PPR | 7 | 7 |
| Relapse or IF | |
| + | 5 | 10 | 0.59 |
| - | 7 | 7 |
| Survival | |
| Alive | 10 | 11 | 0.41 |
| Dead | 2 | 6 |
| Allo-HSCT in first CR | |
| + | 5 | 5 | 0.69 |
| - | 7 | 12 |

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR, complete remission; HR, high risk; IF, induction failure; NCI, National Cancer Institute; PGR, prednisolone good responder; PPR, prednisolone poor responder; PSL, prednisolone; SR, standard risk; WBC, white blood cell; WT, wild type.

like ALL, such as older age at diagnosis, male predominance and high WBC count at diagnosis.⁶,⁷,²³,²⁴ In terms of treatment response, we showed that the prevalence of PSL poor responders was high in this subgroup, compared with the findings of the BFM2000 study (44.8% vs 6.3%, P < 0.01).²⁵ Compared with the kinase-activating fusion-negative patients in this study, the rate of induction failure was also high in this subgroup (12.9% vs 2.1%, P = 0.004). However, the PSL poor responders and induction failure rates in our study did not differ significantly from those reported in the Ph+ ALL cohort (44.8% vs 21.4%, P = 0.07 and 13.8% vs 14%, P = 0.86, respectively).²⁶ These findings suggested clinical similarities between Ph+ ALL and BCP-ALL with kinase fusions other than BCR-ABL.

The prognostic impact of Ph-like signature is controversial.⁷,¹⁰,²³,²⁶ In addition, prognostic significance of kinase-activating fusions should be determined. In the present study, the 5-year EFS rates of kinase fusion-positive patients was significantly inferior to those of kinase fusion-negative patients in the mRNA-seq cohort, which included non-biased B-others (log-rank P = 0.035). The limitation of this study was that mRNA-seq was performed only in 109 of the 373 (29.2%) patients. Complete genetic study of larger cohort is needed to clarify the prognostic significance of kinase-activating fusions in pediatric BCP-ALL.

Although information about risk factors associated with Ph-like ALL is limited, Roberts et al.⁷,¹⁰ suggested that age at onset and minimal residual disease status are related to poor prognosis. In the present study, the small sample size prevented identification of risk factors related to inferior EFS and OS in our cohort, such as age at diagnosis, NCI risk and the presence of IKZF1 deletion. Furthermore, we focused on analyzing differences in the biological or clinical characteristics between genetic subgroups, such as those with ABL-class rearrangements and JAK-class rearrangements. Although EFS and OS tend to be low among patients with JAK-class rearrangements, there were no statistically significant differences between groups (data not shown). Furthermore, we could not find any difference in clinical and biological features between the two genetic subgroups (Table 5). However, our study was limited by its small sample size, its retrospective nature, the heterogeneity of treatment protocols and no data of minimal residual disease status. Thus further studies using larger cohorts are required to determine the prognostic factors in this unique subgroup. International collaborating studies are warranted in the near future.

Finally, the rapid identification of tyrosine kinase inhibitor-eligible patients is mandatory. The establishment of systematic screening systems is currently in progress in the pediatric study groups. For example, the Children’s Oncology Group used a low-density gene expression array to identify Ph-like ALL.²² We are currently developing fluorescence in situ hybridization protocols for the detection of ABL1, PDGFRB and JAK2 rearrangements, which are targetable and relatively frequent genetic abnormalities. In the future, efficient screening systems for the identification of rare but treatable kinase fusions should be established to enable the design of tailored therapy protocols for patients with high-risk BCP-ALL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants for Clinical Cancer Research and Research on Measures for Intractable Diseases from the Japanese Ministry of Health, Labor and Welfare, by grants-in-aid for scientific research from the Japanese Ministry of Education, Culture, Sports, Science and Technology and by the Practical Research for Innovative Cancer Control from Japan Agency for Medical Research and development, AMED (15ek010666h0002).

REFERENCES

1. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukemia. Lancet 2013; 381: 1943–1955.
2. Schultz KR, Carroll A, Heerema NA, Bowman WP, Aledo A, Slayton WB et al. Long-term follow-up of imatinib in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: Children’s Oncology Group Study AALL0031. Leukemia 2014; 28: 1467–1471.
3. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med 2009; 360: 470–480.
4. Holmfeldt L, Wei L, Diaz-Flores E, Walsh M, Zhang J, Ding L et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet 2013; 45: 242–252.
5. Chen IM, Harvey RC, Mullighan CG, Gastier-Foster J, Wharton W, Kang H et al. Outcome modeling with CRLF2, IKZF1, JAK, and minimal residual disease in pediatric acute lymphoblastic leukemia: a Children’s Oncology Group Study. Blood 2012; 119: 3512–3522.
6. Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell 2012; 22: 153–166.
7. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang D, Pei K et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med 2014; 371: 1005–1015.
8. Weston BW, Hayden MA, Roberts KG, Bowyer S, Hsu J, Fedorov G et al. Tyrosine kinase inhibitor therapy induces remission in a patient with refractory EBF1-PDGFRB-positive acute lymphoblastic leukemia. J Clin Oncol 2013; 31: e413–e416.
9. Lengline E, Beldjord K, Dombret H, Soulier J, Boissel N, Clappier E. Successful tyrosine kinase inhibitor therapy in a refractory B-cell precursor acute lymphoblastic leukemia with EBF1-PDGFRB fusion. Haematologica 2013; 98: e146–e148.
10. Roberts KG, Pei D, Campagna D, Payne-Turner D, Li Y, Chen C et al. Outcomes of children with BCR-ABL-like acute lymphoblastic leukemia treated with risk-
Supplementary Information accompanies this paper on Blood Cancer Journal website (http://www.nature.com/bcj)