

B-lymphoblastic lymphoma with *TCF3-PBX1* fusion gene

Lymphoblastic lymphoma (LBL) is a rare subtype of non-Hodgkin lymphoma (NHL) seen primarily in children or young adults. The frequency of T-cell lymphoblastic lymphomas (T-LBL) are predominant and frequency of B-cell LBL (B-LBL) are only 10~20% of LBL. Therefore, B-LBL account for 1 ~11% of NHL.¹⁻³

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is a cancer that originates from Pro-B to pre-B cells and proliferates in the bone marrow. The tumors originating from mature B cells proliferate at the extramedullary and are categorized as lymphoma. However, the origin of B-LBL is also the pro-B to pre-B cell stage. From a cell biology point of view, the differentiation stage of B-LBL and BCP-ALL are quite similar. The genetic and cell biological difference between B-LBL and BCP-ALL has not been fully elucidated. Therefore, the reason why B-LBL proliferate in the extramedullary and not in the bone marrow has not yet been determined.

BCP-ALL is one of the best-characterized neoplasms by cytogenetic and molecular genetic analysis. Molecular characterization of BCP-ALL results in important prognostic associations. However, due to the rarity of B-LBL, and limitation to the accessibility of tumor cells, cytogenetic and molecular genetic analysis are limited in B-LBL. The translocation t(1;19)(q23;p13) causing *TCF3-PBX1* fusion gene is a well-known chromosome abnormality in childhood pre-B ALL, being present in 3~5% of all ALL. Historically, *TCF3-PBX1* positive leukemia had been associated with a poor prognosis but has lost its prognostic significance in the context of modern ALL chemother-

apy, especially that which contains high-dose methotrexate.^{4,5} Whilst little is known about the molecular cytogenetic signature of B-LBL, a few reports describe the hyperdiploidy as a universal abnormal karyotype in B-LBL.^{6,7} *TCF3-PBX1* positive B-LBL has not been reported, and the clinical features and prognosis of LBL with this fusion is unclear.

Although *TCF3-PBX1* positive B-LBL is not unexpected to occur in B-LBL, the translocation appears to not have been previously described in literature and therefore is a novel finding worth reporting. Survey for *TCF3-PBX1* positive B-LBL detected by the evidence of the t(1;19)(q23;p13.3) translocation, RT-PCR positivity, or presence of fusion mRNA detected by RNA sequencing was performed at fourteen local core hospitals with pediatric hematology-oncology patients in their care. Seven cases of *TCF3-PBX1* positive B-LBL were identified in five institutes. Table 1 summarizes the clinical and radiologic features for 7 patients of B-LBL with *TCF3-PBX1*. The detailed clinical course, radiological findings and pathological findings of each patient are described in the *Online Supplementary Material*. The mean age at diagnosis was 12.5 years (range, 8-16 years). Three cases involved bone lesion. Although, microscopically, the number of blast cells in bone marrow was in the normal range in 6 cases, bone marrow minimal disseminated disease (MDD) was positive in 3 out of 3 evaluated cases. None of the patients had cytopenia or appearance of blast cells in the peripheral blood at the time of diagnosis. Laboratory findings showed only moderate to highly increased levels of lactate dehydrogenase (LDH) levels. Increased soluble IL2 receptor levels were mild to moderate. According to the St. Jude classification for NHL in children, 2 had stage III disease and 5 had stage IV. The karyotyping analysis

Table 1. The cases and their clinical information.

Case	Age (Years)	sex	Location of tumor	Bone marrow lymphoblast cell (%), MDD	Stage	Regimen	Outcome
1	15	F	Femur, ovary, thorax	0 N.E	III	*ALB-NHL-14	Relapsed just after termination of maintenance therapy. Retrieval therapy in progress
2	11	F	Femur, tibia	0.8 RT-PCR (+)	IV	ALB-NHL-03	NED in CR1, CR for 2.5 years
3	8	M	Mediastinum, atrium, thorax, CSF	0 N.E	IV	NHL-BFM95	Relapse just after termination of maintenance therapy. HCT under non-remission status, DOD
4	9	F	Extracranial tissue, jaw, ilium, lumbar vertebrae, rib, kidney	1.2 RT-PCR (+)	IV	AIEOP-BFM 2000	NED in CR1, CR for 5.1 years
5	15	F	Pancreas, thoracic vertebrae	1.0 FCM (+)	IV	NHL-BFM95	NED in CR1, CR for 1.9 years
6	16	M	Nasal cavity	0 N.E	III	NHL-BFM95	NED in CR1, On treating
7	14	M	Palate, jaw	16 N.E	IV	TLK-88	Relapsed during maintenance therapy. HCT under non-remission status, DOD

F: female; M: male; MDD: minimal disseminated disease; NED: No evidence of disease; N.E: not examined; RT-PCR: reverse transcription polymerase chain reaction; (+): positive; CR: complete remission; DOD: dead of disease; CSF: cerebrospinal fluid; FCM: flow cytometry. *Ongoing clinical trial, UMIN000018780, https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000021371

Table 2. Cytogenetic findings of the cases.

Case	Karyotype analysis using G-banding	TCF3-PBX1 FISH
1	46,XX,add(5)(q11.2),der(19)t(1;19)(q23;p13.3)	N.E.
2	47,XX,+i(1)(q10),t(1;19)(q23;p13.3)	N.E.
3	46,XY,add(1)(q21),add(9)(q22) (sample at relapsed)	positive
4	N.E.	positive
5	No mitotic phase available	positive
6	50,XY,t(1;19)(q23;p13.3),+4,+5,+8,der(15)t(1;15)(q12;p11.2),-19,+20,+20	N.E.
7	46,XY,+1,der(1;15)(q10;q10),t(1;19)(q23;p13),46,idem,-9,+r1	N.E.

N.E: not examined.

was evaluable in 5 cases (Table 2). Although case 3 did not demonstrate t(1;19), case 1, 2, 6 and 7 were positive for t(1;19). There were no common additional abnormal cytogenetic changes in these 5 cases. *TCF3-PBX1* fluorescence *in situ* hybridization (FISH) was performed in case 3, 4 and 5 and revealed the presence of the *TCF3-PBX1* fusion gene. There were no specific common features regarding the cell surface marker expression between these cases (see *Online Supplementary Material*). All cases were treated with NHL-BFM95-based chemotherapeutic regimen, and 3 out of 7 cases relapsed during or immediately after completion of maintenance therapy. There were no common additional abnormal cytogenetic changes in these 3 relapsed cases. Two patients died due to tumor progression after receiving hematopoietic cell transplantation. It is interesting to compare the clinical similarities and differences between B-LBL with *TCF3-PBX1* and preB-ALL with *TCF3-PBX1*. Compared with patients with other B-ALL, those with *TCF3-PBX1* were relatively older (median 6.9 vs. 4.6 years).⁸ The age distribution of the children in our series suggests that this condition affects older children compared with *TCF3-PBX1* positive PreB-ALL. It is also interesting to note that most of the children had bone or invasive bone involvement, and most had low level bone marrow disease detected at initial staging.

Recent advances in molecular analysis for leukemia gives us valuable information which is directly applicable to therapeutic intervention. However, due to the rarity of the disease, the molecular pathogenesis of B-LBL has not yet been elucidated. Cytogenetic analysis of 16 evaluable B-LBL cases revealed 6 normal karyotype, 5 hyperdiploidy and 5 other structural abnormalities such as t(12;17), t(4;11), t(9;12) or del 2p.⁶ Another cytogenetic analysis of 9 cases revealed 3 normal karyotype, 1 hyperdiploidy, and 4 other nonspecific structural abnormalities.⁷ Single-nucleotide polymorphism (SNP) microarray analysis revealed that 26% of cases carried *CDKN2A/B* deletions, 48% of cases were hyperdiploid, 13% of cases carried *IKZF1* deletion, and 17% of cases carried *PAX5* deletions, which was similar to the reported frequency in BCP-ALL. The gene expression profile analysis of T-ALL and T-LBL revealed that the T-ALL cluster was completely segregated with T-LBL, and immunohistochemical analysis based on gene expression profiles confirmed overexpression of *MLL* (*KMT2A*) in T-LBL tumor cells compared to T-ALL and CD47 in T-ALL tumors cells when compared to T-LBL.⁹ However, limited studies have been performed in B-LBL. Immunoglobulin light chain lambda (IGL) locus deletions consistent with normal light chain rearrangement were observed in 22% of B-LBL

cases, compared with only 1% in BCP-ALL samples. None of the B-LBL cases showed abnormal, isolated *VPREB1* deletion adjacent to IGL locus, which was identified in 25% of BCP-ALL.¹⁰ Other reports described that 71% of B-LBL was characterized by high-hyperdiploidy.¹¹ However, comprehensive genomic or transcriptome analysis has not been performed for B-LBL. These observations suggest that hyperdiploidy is one of the major genomic signatures of B-LBL and BCP-ALL. Due to the similarity of cell biology phenotype and limited genomic analysis between B-LBL and BCP-ALL, it can easily be speculated that the fusion gene observed in BCP-ALL may also be observed in B-LBL. However, few reports have described the fusion gene in B-LBL. There are several case reports with *KMT2A* (*MLL*) rearranged B-LBL.¹²⁻¹⁷ *BCR-ABL1* positive LBL has also been reported.¹⁷ *TCF3-PBX1* positive B-LBL has not been reported. Until now, the reason why a limited case developed as an LBL and not as an ALL, whilst having the same genetic alteration, has not been determined. Genetic base comparison between LBL and ALL will give us further insight.

In patients with B-LBL, event free survival (EFS) was almost 80~90%.^{11,18} In the case of T-LBL, 6q loss is significantly related to poor outcome.¹⁹ However, what affects the outcome in B-LBL is still unknown. In our study, 3 out of 7 patients relapsed after standard childhood LBL chemotherapy. Due to the limited number of cases, it is hard to predict a poor outcome of *TCF3-PBX1* positive LBL; more cases therefore need to be studied. Historically, the prognosis of *TCF3-PBX1* positive ALL was improved by intensification of chemotherapy, which is one of a number of options to improve survival of *TCF3-PBX1* positive LBL until prognostic factors are revealed. To this end, routine cytogenetic analysis and comprehensive chimera message RNA analysis using biopsy specimen is required in LBL. Gene signature oriented therapy based on these results may contribute to improving the outcome of B-LBL, as has previously been successfully demonstrated in BCP-ALL.

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