Nationwide study of pediatric B-cell precursor acute lymphoblastic leukemia with chromosome 8q24/MYC rearrangement in Japan

Nationwide study of pediatric B-cell precursor acute lymphoblastic leukemia with chromosome 8q24/*MYC* rearrangement in Japan

Kimiyoshi Sakaguchi[1,2], Toshihiko Imamura[2,3], Sae Ishimaru[4,5], Chihaya Imai[6], Hidemi Shimonodan[7], Naoto Fujita[8], Keiko Okada[9], Takeshi Taketani[10], Rie Kanai[10], Hisamichi Tauchi[11], Motohiro Kato[12], Yasuko Kojima[13], Arata Watanabe[14], Takao Deguchi[15], Yoshiko Hashii[16], Nobutaka Kiyokawa[17], Tomohiko Taki[18], Akiko M. Saito[2], Keizo Horibe[2], Atsushi Manabe[19], Atsushi Sato[20], and Katsuyoshi Koh[21]

1 Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan.

2 Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan.

3 Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan.

4 Department of Hematology and Oncology, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan.

5 Department of Pediatric Oncology, National Cancer Center Hospital, Tokyo, Japan.

6 Department of Pediatrics, Niigata University, Niigata, Japan.

7 Department of Pediatrics, Miyazaki Prefectural Miyazaki Hospital, Miyazaki, Japan.

8 Department of Pediatrics, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Hiroshima, Japan.

9 Department of Pediatric Hematology/Oncology, Osaka City General Hospital, Osaka, Japan.

10 Department of Pediatrics, Shimane University Faculty of Medicine, Izumo, Japan.

11 Department of Pediatrics, Ehime University, Toon, Japan.

12 Division of Stem Cell Transplant and Cellular Therapy, Children's Cancer Center, National Center for Child Health and Development, Tokyo, Japan.

13 Department of Pediatrics, Toho University Omori Medical Center, Tokyo, Japan.

14 Department of Pediatrics, Nakadori General Hospital, Akita, Japan.

15 Department of Pediatrics, Mie University, Tsu, Japan.

16 Department of Pediatrics, Osaka University, Suita, Japan.

17 Department of Pediatric Hematology and Oncology Research, Research Institute, National Center for Child Health and Development, Tokyo, Japan.

18 Department of Medical Technology, Kyorin University Faculty of Health Sciences, Mitaka, Japan.

19 Department of Pediatrics, St. Luke's International Hospital, Tokyo, Japan.

20 Department of Hematology/Oncology, Miyagi Children's Hospital, Sendai, Japan.

21 Department of Hematology/Oncology, Saitama Children's Medical Center, Saitama, Japan.

Corresponding author:

Kimiyoshi Sakaguchi, M.D., Ph.D.

Department of Pediatrics, Hamamatsu University School of Medicine,

1-20-1 Handayama, Higashi-ku, Hamamatsu, Shizuoka 431-3192, Japan

Phone: +81-53-435-2312

FAX: +81-53-435-2311

Email: k-saka@hama-med.ac.jp

Word Counts:

Abstract, 246 words; **main text**, 1367 words; **number of tables**, 3; **number of figures**, 1; **number of supplemental files**, 2

Short running title: BCP-ALL with 8q24/*MYC* rearrangement in Japan

Keywords

B-cell precursor acute lymphoblastic leukemia, 8q24/*MYC* rearrangement, Burkitt lymphoma/leukemia, immunophenotype, double-hit lymphoma/leukemia

Abbreviations

8q24-r	8q24 rearrangement
ALL	acute lymphoblastic leukemia
BCP	B-cell precursor
BL	Burkitt lymphoma/leukemia
CCLSG	Japanese Childhood Cancer and Leukemia Study Group
DHL	double-hit lymphoma/leukemia
HCT	hematopoietic cell transplantation
JACLS	Japan Association Childhood Leukemia Study Group
LDH	lactate dehydrogenase
TCCSG	Tokyo Children's Cancer Study Group
TdT	terminal deoxynucleotidyl transferase

UA	uric acid

Abstract

Background

Rearrangements of chromosome 8q24/*MYC* (8q24/*MYC*-r), resulting from t(8;14)(q24;q32), t(2;8)(p11;q24), or t(8;22)(q24;q11), are mainly associated with Burkitt lymphoma/leukemia (BL) and rarely observed in patients with B-cell precursor acute lymphoblastic leukemia (BCP-ALL). The characteristics of BCP-ALL with 8q24/*MYC*-r are poorly understood.

Procedure

A retrospective nationwide study of data from patients with pediatric BCP-ALL with 8q24/*MYC*-r in Japan was conducted to clarify the clinical and biological characteristics associated with 8q24/*MYC*-r BCP-ALL.

Results

Ten patients with BCP-ALL with 8q24/*MYC*-r, including three with double-hit leukemia (DHL) (two with t(8;14)(q24;q32) and t(14;18)(q32;q21), and one with t(8;14) and t(3;22)(q27;q11)), were identified. Patients with BCP-ALL with 8q24/*MYC*-r had higher median age, and uric acid (UA) and lactate dehydrogenase (LDH) levels, than those without 8q24/*MYC*-r. All patients were initially treated with ALL-type chemotherapy; however, four, including one with DHL, were switched to BL-type chemotherapy, based

on cytogenetic findings. One patient relapsed after standard-risk ALL-type chemotherapy, and two patients with DHL did not attain complete remission with chemotherapy; all three died within 11 months. The other seven patients treated with BL-type or high-risk ALL-type chemotherapy are alive without disease.

Conclusions

The clinical and laboratory features of BL with IG-*MYC* rearrangement, displaying a BCP immunophenotype (Wagener et al. and Herbrueggen et al. termed it as preBLL), are similar to those of BCP-ALL with 8q24/*MYC*-r. Low-risk ALL-type chemotherapy may not be appropriate for them, and further studies are required to establish an adequate therapeutic strategy. Further studies of DHL to identify new treatment strategies are also needed.

Introduction

The hallmarks of Burkitt lymphoma/leukemia (BL) are 8q24/*MYC*-related chromosomal translocations, including t(8;14)(q24;q32), t(8;22)(q24;q11), and t(2;8)(p12;q24), alongside a mature B-cell immunophenotype, elevated uric acid (UA) and lactate dehydrogenase (LDH) at diagnosis, bulky disease, and FAB-L3 morphology [1]. Nevertheless, 8q24/*MYC* rearrangement (8q24/*MYC*-r) is also occasionally observed in acute lymphoblastic leukemia with a B-cell precursor immunophenotype (BCP-ALL), rather than a mature B-cell immunophenotype [2]. Due to the rarity of BCP-ALL with 8q24/*MYC*-r, its characteristics are poorly understood. In addition, leukemia/lymphoma with *BCL2* or *BCL6* and *MYC* rearrangements, known as double-hit lymphoma/leukemia (DHL) [3], is reported to exhibit a BCP-ALL immunophenotype [4]. Childhood DHL is extremely rare, and its characteristics are poorly described [4]. Herein, we report the clinical and biological characteristics of ten patients with BCP-ALL with 8q24/*MYC*-r, including three patients with DHL, in Japan.

Patients and Methods

Patients with 8q24/*MYC*-r BCP-ALL were primarily from among the 4043 patients enrolled in the Japan Association Childhood Leukemia Study Group (JACLS) ALL-02 study (n = 1252) [5]; the Tokyo Children's Cancer Study Group (TCCSG) L99-

15 (n = 770), L04-16 (n = 150), L06-16 (n = 194), L07-16 (n = 274), and L09-16 (n = 607) studies [6,7]; the Japanese Childhood Cancer and Leukemia Study Group (CCLSG) ALL2000 MRD (n = 305) and ALL2004 studies (n = 326) [8]; and the Kyushu–Yamaguchi Childhood Cancer Study Group ALL-02 study (n = 165) [9]. Disease classification as either BCP-ALL or Burkitt-ALL was determined by flow cytometric analysis, according to the Japanese Pediatric Leukemia/Lymphoma Study Group criteria [10] (Supplementary Table S1), which are based on the European Group for the Immunological Characterization of Leukemias criteria [11]. The presence of 8q24/*MYC*-r was confirmed by G-banding, *IGH-MYC* fusion, or *MYC* split signal by fluorescence in situ hybridization. Patient data analyses included the following: age, sex, and extramedullary disease; laboratory data, including white blood cell count, serum UA level, serum LDH level, and FAB classification of leukemic blasts; ALL cell cytogenetic data, including G-banding, fluorescence in situ hybridization data, and leukemic blast immunophenotype; and details of treatments and outcomes. This study was approved by the Ethics Committee of Hamamatsu University School of Medicine.

Results

Clinical characteristics of patients with BCP-ALL carrying 8q24/*MYC* rearrangements

Nine patients (0.2%) with BCP-ALL carrying 8q24/*MYC*-r were identified from among the 4043 patients enrolled in nine clinical studies in Japan (**Table 1**). An additional patient with BCP-ALL carrying t(8;14)(q24;q32), who was not enrolled in any clinical study, was identified at a participating hospital and included in this study (patient #10 in **Table 1**). FAB classification of leukemic blasts showed that eight of ten patients had L1/2 morphology, and that leukemic blasts in all patients, including two with L3 morphology, expressed CD10, and CD19, but not surface μ, κ, or λ immunoglobulins, consistent with a BCP-ALL immunophenotype (**Table 1**). The clinical characteristics of these ten patients were compared with those of other patients with BCP-ALL in the JACLS ALL-02 trial, and they had relatively higher median age, higher UA and LDH levels, and were predominantly male (**Table 2**).

Double-hit leukemia

Patients with BCP-ALL carrying 8q24/*MYC*-r included three so-called DHL patients: two with t(8;14)(q24;q32) and t(14;18)(q32;q21), and one with t(8;14) and t(3;22)(q27;q11) (patients #7, #8, and #9 in **Table 1**). Fluorescence in situ hybridization analysis confirmed the rearrangement of *MYC* and *BCL2* in the two patients with t(8;14) and t(14;18). Leukemic blasts from the majority of patients with 8q24/*MYC*-r expressed CD20 but not CD34 or terminal deoxynucleotidyl transferase (TdT), consistent with a

mature B-cell immunophenotype; however, samples from two of three DHL patients were CD20-negative and TdT-positive (patients #8 and #9 in **Table 1**). Further, central nervous system involvement was observed in two of the three patients with DHL (patients #7 and #9 in **Table 1**).

Treatments and outcomes

All patients with 8q24/*MYC*-r were initially treated with ALL-type induction therapy, and three patients had maintained complete remission (CR) until the last follow-up, following only ALL-type chemotherapy (**Table 1, Fig. 1**, Supplementary Table 2). Four patients, including one with DHL, were switched to BL-type chemotherapy because of their cytogenetic findings, and all of them maintained complete remission (**Table 1, Fig. 1**, Supplementary Table 2). One patient relapsed after standard-risk ALL chemotherapy, and two patients with DHL did not attain complete remission with chemotherapy; all three received allogeneic hematopoietic cell transplantation but died within 11 months (**Table 1, Fig. 1**, Supplementary Table 2).

Discussion

Wagener et al. and Herbrueggen et al. reported that BL with IG-*MYC* rearrangement displaying a BCP immunophenotype, which they termed preBLL, has biological similarities to BCP-ALL [12,13]. They described preBLL blasts as having

genetic abnormalities similar to BCP-ALL, such as aberrant VDJ recombination and/or activating *NRAS* and/or *KRAS* mutations. We also identified ten patients with BCP-ALL carrying 8q24/*MYC*-r in this study.

To compare the clinical and immunological features of our BCP-ALL patients carrying 8q24/*MYC*-r to those of preBLL, we conducted a literature survey, which identified 11 papers reporting 32 pediatric patients diagnosed with BCP-ALL carrying 8q24/*MYC*-r. Of these, two patients lacking flow cytometric analysis data on surface κ or λ immunoglobulins, and four patients without 8q24/*MYC*-r detection at initial diagnosis, were excluded from our analysis. Therefore, 26 patients whose karyotype data and immunophenotyping data diagnostic for BCP-ALL were complete and available were analyzed [2,4,12,14-21] (**Table 3**). The blast immunophenotypes reported in these publications were similar to those of our patients, except for positivity for TdT expression (positive TdT expression: 12/17 vs. 2/7 in our cohort), although we cannot explain this discrepancy. Ideally, we should investigate whether aberrant VDJ recombination was associated with IG translocation in our patients; however, we could not perform further genetic studies, due to a lack of sufficient sample material. By contrast, the 26 patients identified from the literature showed quite similar clinical and laboratory features to those of our patients, including relatively older median age (11.8

years vs. 8 years), and higher UA (median 12.9 vs. 7.1 mg/dl) and LDH (median 10,554 vs. 2882 IU/l) levels. Thus, we consider that the cases of BCP-ALL with 8q24/*MYC*-r identified in our study should be classified as preBLL.

Short course, high-intensity chemotherapy regimens are the standard treatments for BL. These chemotherapeutic regimens comprise alkylating agents, etoposide, antimetabolites, vincristine, steroids, and high dose methotrexate [22]. The JACLS NHL-B02p, Japanese Pediatric Leukemia/Lymphoma Study Group B-NHL03, NHL B-cell type, and NHL-BFM95 regimens are categorized as BL-type chemotherapy [23-25]. By contrast, the standard treatment for ALL is long-term chemotherapy that comprises three phases: induction, consolidation, and maintenance [26]. Treatment intensity categories are classified according to the original risk group to which treatment protocols were applied as follows: JACLS ALL-02 SR and CCLSG ALL2004 SR are categorized as standard-risk ALL-type chemotherapy [5,8]; while JACLS ALL-02 HR, JACLS ALL-02 ER, JACLS ALL-02 F, TCCSG L99-15 HR, TCCSG L99-1502 HEX, TCCSG L0416 HEX, and CCLSG ALL2004 salvage 1 are classified as high-risk ALL-type chemotherapy [5,8,27,28]. A standard chemotherapeutic regimen for BCP-ALL with 8q24/*MYC*-r has yet to be established, while the outcomes of children and adolescents with preBLL described by Herbrueggen et al. appeared to be favorable when treated with regimens used for

mature B-cell NHL, rather than ALL, despite the biological similarities to BCP-ALL [12]. In this study, all four patients treated with BL-type chemotherapy, and three of five patients treated with high-risk ALL-type chemotherapy, are alive without disease (**Table 1, Fig. 1**, Supplementary Table 2). Further, in our literature survey, 10 of 12 patients treated with BL-type chemotherapy were alive without disease; however, two of four patients initially treated with ALL-type chemotherapy died of disease (**Table 3**). Although it is possible that chemotherapy for low-risk ALL is insufficient to treat BCP-ALL with 8q24/*MYC*-r, further studies are required to establish an adequate therapeutic strategy for this relatively rare ALL subtype.

Two of the patients with DHL included in our study died of disease progression, despite highly intensive chemotherapy oriented to high-risk ALL, accompanied by allogeneic hematopoietic cell transplantation, suggesting that this disease subtype is an aggressive form of BCP-ALL. Two of three patients with DHL in the literature survey also died of disease (**Table 3**). Further studies to assess new treatment strategies, such as use of a BCL2 inhibitor [29] or anti-CD19 chimeric antigen receptor T-cell therapy [30], are warranted to identify a cure for this extremely aggressive disease.

Conflict of interest statement

There are no competing financial interests.

Acknowledgments

We gratefully acknowledge the work of past and present members of JACLS, TCCSG, CCLSG, Kyushu–Yamaguchi Childhood Cancer Study Group, Japanese Pediatric Leukemia/Lymphoma Study Group, and Japan Children's Cancer Group.

References

1. Blum KA, Lozanski G, Byrd JC. Adult Burkitt leukemia and lymphoma. *Blood* 2004;104(10):3009-3020.
2. Navid F, Mosijczuk AD, Head DR, et al. Acute lymphoblastic leukemia with the (8;14)(q24;q32) translocation and FAB L3 morphology associated with a B-precursor immunophenotype: the Pediatric Oncology Group experience. *Leukemia* 1999;13(1):135-141.
3. Kanungo A, Medeiros LJ, Abruzzo LV, Lin P. Lymphoid neoplasms associated with concurrent t(14;18) and 8q24/c-MYC translocation generally have a poor prognosis. *Mod Pathol* 2006;19(1):25-33.
4. Liu W, Hu S, Konopleva M, et al. De Novo MYC and BCL2 Double-hit B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) in Pediatric and Young

Adult Patients Associated With Poor Prognosis. *Pediatr Hematol Oncol* 2015;32(8):535-547.

5. Hasegawa D, Imamura T, Yumura-Yagi K, et al. Risk-adjusted therapy for pediatric non-T cell ALL improves outcomes for standard risk patients: results of JACLS ALL-02. *Blood Cancer J* 2020;10(2):23.

6. Kato M, Koh K, Manabe A, et al. No impact of high-dose cytarabine and asparaginase as early intensification with intermediate-risk paediatric acute lymphoblastic leukaemia: results of randomized trial TCCSG study L99-15. *Br J Haematol* 2014;164(3):376-383.

7. Kato M, Manabe A, Koh K, et al. Treatment outcomes of adolescent acute lymphoblastic leukemia treated on Tokyo Children's Cancer Study Group (TCCSG) clinical trials. *Int J Hematol* 2014;100(2):180-187.

8. Hyakuna N, Shimomura Y, Watanabe A, et al. Assessment of corticosteroid-induced osteonecrosis in children undergoing chemotherapy for acute lymphoblastic leukemia: a report from the Japanese Childhood Cancer and Leukemia Study Group. *J Pediatr Hematol Oncol* 2014;36(1):22-29.

9. Okamoto Y, Koga Y, Inagaki J, et al. Effective VCR/DEX pulse maintenance therapy in the KYCCSG ALL-02 protocol for pediatric acute lymphoblastic

leukemia. *Int J Hematol* 2016;103(2):202-209.

10. Iwamoto S, Deguchi T, Ohta H, et al. Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/Lymphoma Study Group. *Int J Hematol* 2011;94(2):185-192.

11. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). *Leukemia* 1995;9(10):1783-1786.

12. Herbrueggen H, Mueller S, Rohde J, et al. Treatment and outcome of IG-MYC(+) neoplasms with precursor B-cell phenotype in childhood and adolescence. *Leukemia* 2019.

13. Wagener R, Lopez C, Kleinheinz K, et al. IG-MYC (+) neoplasms with precursor B-cell phenotype are molecularly distinct from Burkitt lymphomas. *Blood* 2018;132(21):2280-2285.

14. Rawlinson NJ, Baker P, Kahwash SB. Burkitt's leukemia with an atypical immunophenotype: report of a case and review of literature. *Lab Hematol* 2011;17(4):27-31.

15. Kaplinsky C, Rechavi G. Acute lymphoblastic leukemia of Burkitt type (L3 ALL) with t(8;14) lacking surface and cytoplasmic immunoglobulins. *Med*

Pediatr Oncol 1998;31(1):36-38.

16. Hassan R, Felisbino F, Stefanoff CG, et al. Burkitt lymphoma/leukaemia transformed from a precursor B cell: clinical and molecular aspects. *Eur J Haematol* 2008;80(3):265-270.

17. Sato Y, Kurosawa H, Fukushima K, Okuya M, Arisaka O. Burkitt-Type Acute Lymphoblastic Leukemia With Precursor B-Cell Immunophenotype and Partial Tetrasomy of 1q: A Case Report. *Medicine (Baltimore)* 2016;95(10):e2904.

18. Gupta AA, Grant R, Shago M, Abdelhaleem M. Occurrence of t(8;22)(q24.1;q11.2) involving the MYC locus in a case of pediatric acute lymphoblastic leukemia with a precursor B cell immunophenotype. *J Pediatr Hematol Oncol* 2004;26(8):532-534.

19. Demina I, Zerkalenkova E, Illarionova O, et al. Heterogeneity of childhood acute leukemia with mature B-cell immunophenotype. *J Cancer Res Clin Oncol* 2019;145(11):2803-2811.

20. Pegoraro L, Palumbo A, Erikson J, et al. A 14;18 and an 8;14 chromosome translocation in a cell line derived from an acute B-cell leukemia. *Proc Natl Acad Sci U S A* 1984;81(22):7166-7170.

21. Uemura S, Hasegawa D, Yokoi T, et al. Refractory double-hit

lymphoma/leukemia in childhood mimicking B-precursor acute lymphoblastic leukemia at initial presentation. *Rinsho Ketsueki* 2017;58(2):143-149.

22. Giulino-Roth L, Goldman S. Recent molecular and therapeutic advances in B-cell non-Hodgkin lymphoma in children. *Br J Haematol* 2016;173(4):531-544.

23. Fujita N, Mori T, Mitsui T, et al. The role of hematopoietic stem cell transplantation with relapsed or primary refractory childhood B-cell non-Hodgkin lymphoma and mature B-cell leukemia: a retrospective analysis of enrolled cases in Japan. *Pediatr Blood Cancer* 2008;51(2):188-192.

24. Tsurusawa M, Mori T, Kikuchi A, et al. Improved treatment results of children with B-cell non-Hodgkin lymphoma: a report from the Japanese Pediatric Leukemia/Lymphoma Study Group B-NHL03 study. *Pediatr Blood Cancer* 2014;61(7):1215-1221.

25. Woessmann W, Seidemann K, Mann G, et al. The impact of the methotrexate administration schedule and dose in the treatment of children and adolescents with B-cell neoplasms: a report of the BFM Group Study NHL-BFM95. *Blood* 2005;105(3):948-958.

26. Hunger SP, Mullighan CG. Acute Lymphoblastic Leukemia in Children. *N Engl J Med* 2015;373(16):1541-1552.

27. Manabe A, Ohara A, Hasegawa D, et al. Significance of the complete clearance of peripheral blasts after 7 days of prednisolone treatment in children with acute lymphoblastic leukemia: the Tokyo Children's Cancer Study Group Study L99-15. *Haematologica* 2008;93(8):1155-1160.

28. Takahashi H, Kajiwara R, Kato M, et al. Treatment outcome of children with acute lymphoblastic leukemia: the Tokyo Children's Cancer Study Group (TCCSG) Study L04-16. *Int J Hematol* 2018;108(1):98-108.

29. Li L, Pongtornpipat P, Tiutan T, et al. Synergistic induction of apoptosis in high-risk DLBCL by BCL2 inhibition with ABT-199 combined with pharmacologic loss of MCL1. *Leukemia* 2015;29(8):1702-1712.

30. Nandagopal L, Mehta A. Treatment approaches of hard-to-treat non-Hodgkin lymphomas. *Expert Rev Hematol* 2017;10(3):259-273.

Figure Legends

Fig. 1 Schematic representation of the clinical course of ten patients with BCP-ALL with 8q24/*MYC* rearrangement.

Blue arrow, standard-risk ALL-type chemotherapy; yellow arrow, high-risk ALL-type chemotherapy; red arrow, BL-type chemotherapy; gray arrow, palliative therapy; black arrow, observation; RT, rituximab; H, hematopoietic cell transplantation; R, relapse; F, induction failure; C, complete remission; †, death; BCP, B-cell precursor; ALL, acute lymphoblastic leukemia; BL, Burkitt lymphoma/leukemia.

Figure 1

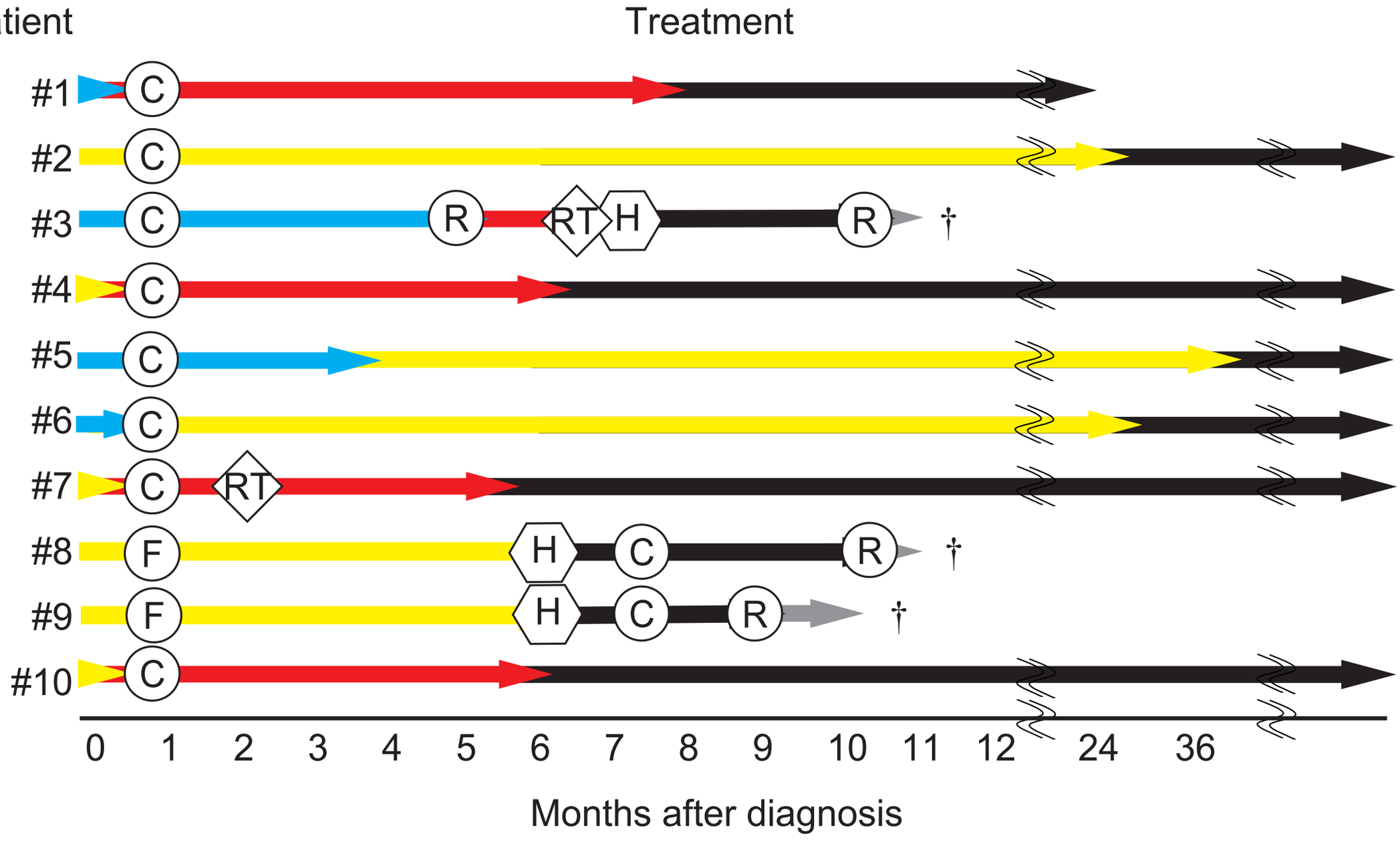
Patient
Treatment
#1
C
#2
C
#3
C
R
RT
H
R
†
#4
C
#5
C
#6
C
#7
C
RT
#8
F
H
C
R
†
#9
F
H
C
R
†
#10
C
0
1
2
3
4
5
6
7
8
9
10
11
12
24
36
Months after diagnosis

TABLE 1 Clinical and biological characteristics of ten patients with BCP-ALL with 8q24/*MYC* rearrangement

Patient	Age (y)	Sex	Extramedullary disease	Morphology	Laboratory data			Cytogenetic data	Immunophenotypic data								Treatment (Treatment after relapse)	Outcome
					WBC (/µL)	UA (mg/dl)	LDH (IU/L)		CD 10	CD 19	CD 20	CD 34	TdT	µ	κ	λ		
1	1.4	M	No	**L3**	1400	**7.1**	**3498**	46,XY,**t(8;14)(q24;q32)**, der(14)t(1;14)(q12;p13), der(15)t(1;15)(q12;p13) [19/20]	+	+	+	-	N/A	-[s]	-	-	SR ALL type → **BL type**	ANED20 m+
2	16.1	M	No	L1	6730	3.7	**837**	46,XY,**t(8;14)(q24;q32)**, add(9)(p13),add(13)(q32) [19/20]	+	+	+	-	-	-[cs]	-	-	HR ALL type	ANED121 m+
3	4.9	M	No	L2	6900	N/A	**2157**	46,XY,**t(8;14)(q22;q32)** [15/20] ***IgH-MYC* FISH 21%**§	+	+	+	-	-	-[cs]	-	-	SR ALL type (**BL type** + HCT)	Relapse 5m DOD11 m
4	7.8	M	No	**L3**	3430	**12.2**	**12 660**	46,XY,ins(1;?)(q21;?),**t(8;14)(q24;q32)**,add(13)(q	+	+	+	-	N/A	-[c]	-	-	HR ALL	ANED136 m+

								34) [1/20] ***IgH-MYC* FISH 16%**									type → **BL type**	
5	4.2	M	No	L2	**29 100**	4.7	**3240**	46,XY,ins(1;?)(q21;?),der(4),t(1;4)(q21;q31),**t(8;14)(q24;q32)** [2/19]	+	+	N/A	-	-	-[cs]	-	-	SR ALL type → HR ALL type	ANED114 m+
6	9.6	M	No	L1	2400	5.8	196	46,XY,add(5)(p11),**t(8;14)(q24;q32)**,t(11;16)(q23;p13) [6/20]	+	+	N/A	+	N/A	N/A	-	-	SR ALL type → HR ALL type	ANED122 m+
7	8.2	M	**CNS**	L1	2680	**18.7**	**1966**	46,XY,t(3;22)(q27;q11),**t(8;14)(q24;q32)**,dup(12)(q13q24),del(13)(q?) [4/10] ***IgH-MYC* FISH 97%**	+	+	+	-	-	-[c] +[s]*	+ *	+ *	SR ALL type → **BL type**	ANED104 m+
8	14.0	M	No	L2	**23 400**	**7.2**	**5586**	46,XY,**t(8;14)(q24;q32)**,t(14;18)(q32;q21) [18/19] ***IgH-MYC* FISH 92%**	+	+	-	-	+	-[cs]	-	-	HR ALL type + HCT	Relapse 10m DOD11 m

								***IgH-BCL2* FISH 90%**											
9	11.3	M	**CNS**	N/A	14 470	6.9	**2523**	46,XY,**t(8;14)(q24;q32)**, t(14;18)(q32;q21) [6/8] ***MYC* split FISH 90.4%** ***IgH-BCL2* FISH 90.4%**	+	+	-	-	+	-[cs]	-	-	HR ALL type + HCT	Relapse 8m DOD10 m
10	5.0	F	**Kidney**	L1	6400	**9.8**	**8525**	47,XX,+i(1)(q10),**t(8;14)(q24;q32)** [20/20] ***IgH-MYC* FISH 54%**	+	+	-	-	-	+[c] -[s]	-	-	SR ALL type → **BL type**	ANED66 m+

[c]Cytoplasmic

[s]Surface

§This data was obtained when the ALL was relapsed.

*False positive: these results were considered false positives because κ and λ were positive simultaneously.

BCP, B-cell precursor; ALL, acute lymphoblastic leukemia; y, years; N/A, not assessed; M, male; F, female; CNS, central nervous system; WBC, white blood cell; UA, uric acid; LDH, lactate dehydrogenase; FISH, fluorescence in situ hybridization; TdT, terminal deoxynucleotidyl transferase; BL, Burkitt lymphoma/leukemia; SR, standard-risk; HR, high-risk; ANED, alive with no evidence of disease; DOD, dead of disease; m, months after diagnosis.

Supplemental TABLE S1 Proposed immunophenotypic criteria for de novo cases of acute lymphoblastic leukemia [11]

T-lineage ALL	1. $CD3^+$ 2. Express CD2, CD5, CD7, or CD8
B-lineage ALL	
Early pre-B ALL	Express at least two B-lineage markers (CD19, CD20, CD22, or CD79a)
Pre-B ALL*	1. Express at least two B-lineage markers (CD19, CD20, CD22, or CD79a) 2. Negative for surface membrane immunoglobulin κ or λ light chains 3. Express cytoplasmic and/or surface immunoglobulin μ heavy chains
B-ALL	1. Express at least two B-lineage markers (CD19, CD20, CD22, or CD79a) 2. Express surface membrane immunoglobulin κ or λ light Chains
ALL with aberrant myeloid-associated antigen expression	
My Ag^+ T-lineage ALL	1. $CD3^+$ and express CD2, CD5, CD7, or CD8 2. $CD79a^-$ 3. MPO^- and express myeloid-associated markers (CD13, CD15, CD33, or CD65)
My Ag^+ B-lineage ALL	1. Express at least two B-lineage markers (CD19, CD20, CD22, or CD79a) 2. $CD3^-$ 3. MPO^- and express myeloid-associated markers (CD13, CD15, CD33, or CD65)

BCP-ALL or mature B-ALL were classified by FCM according to the JPLSG criteria.

BCP-ALL includes early pre-B ALL and pre-B ALL. Mature B-ALL includes B-ALL.

*Pre-B ALL cases include transitional pre-B cases.

My, myeloid; Ag^+, antigen positive.

TABLE 2 Clinical characteristics of BCP-ALL with 8q24/*MYC* and BCP-ALL from JACLS ALL-02

Phenotype		BCP-ALL with 8q24/*MYC*	BCP-ALL from JACLS ALL-02
n		10	1091
Age (y)	< 10	7 (70%)	896 (82.1%)
	≥ 10	3 (30%)	195 (17.9%)
Median Age (y)		8.0 (1.4–16.1)	4 (1–18)
Sex	Male	9 (90%)	578 (53%)
	Female	1 (10%)	513 (47%)
WBC (/μL)	< 20 000	8 (80%)	799 (73.2%)
	≥ 20 000	2 (20%)	292 (26.8%)
Median WBC (/μL)		6565 (1400–29 100)	7100 (370–816 000)
UA (mg/dl)	< 7	4 (40%)	878 (84.6%)
	≥ 7	6 (60%)	160 (15.4%)
Median UA (mg/dl)		7.1 (3.7–18.1)	4.7 (0.7–53)
LDH (IU/L)	< 500	1 (10%)	550 (52.1%)
	≥ 500	9 (90%)	505 (47.9%)
Median LDH (IU/L)		2881.5 (196–12 660)	476 (7.35–28 900)

BCP, B-cell precursor; ALL, acute lymphoblastic leukemia; JACLS, Japan Association Childhood Leukemia Study Group; WBC, white blood cell; UA, uric acid; LDH, lactate dehydrogenase.

Supplemental TABLE S2 Treatment administered and outcomes of ten patients with BCP-ALL with 8q24/*MYC* rearrangement

Patient	Treatment	Recurrence	HCT	Outcome	
1	*JACLS ALL-02 SR*[a] → **JACLS NHL-B02p Group 4**[c]	No	No	ANED	20 m+
2	JACLS ALL-02 HR[b]	No	No	ANED	121 m+
3	*JACLS ALL-02 SR*[a] → <relapse> → **JPLSG B-NHL03 Group 4**[c], rituximab, and so on → <non CR> → CBT → <relapse> → <death>	Yes (5 m)	Yes (8 m)	DOD	11 m
4	TCCSG L99-1502 HEX[b] → **NHL B-cell type group IV**[c]	No	No	ANED	136 m+
5	*CCLSG ALL2004 SR*[a] → CCLSG ALL2004 salvage 1[b]	No	No	ANED	114 m+
6	*JACLS ALL-02 SR*[a] → JACLS ALL-02 HR[b]	No	No	ANED	122 m+
7	JACLS ALL-02 HR[b] → **JPLSG B-NHL03 Group 4**[c]	No	No	ANED	104 m+
8	JACLS ALL-02 ER[b] → <non CR> → JACLS ALL-02 F[b] → <non CR> → PBSCT → <CR> → <relapse> → palliative care → <death>	Yes (10 m)	Yes (6 m)	DOD	11 m
9	TCCSG L0416 HEX[b] → <non CR> → TCCSG L0416 (VCR+DEX+L-asp) + RT (30 Gy/15 fr) → <non CR> → BMT → <CR> → <relapse> → palliative care → <death>	Yes (8 m)	Yes (6 m)	DOD	10 m
10	TCCSG L99-15 HR[b] → **NHL-BFM95 R4**[c]	No	No	ANED	66 m+

[a]*JACLS ALL-02 SR* and *CCLSG ALL2004 SR* are standard-risk ALL-type chemotherapy.

[b]JACLS ALL-02 HR, JACLS ALL-02 ER, JACLS ALL-02 F, TCCSG L99-1502 HEX, TCCSG L0416 HEX, and CCLSG ALL2004 salvage 1 are high-risk ALL chemotherapy.

[c]**JACLS NHL-B02p Group 4**, **JPLSG B-NHL03 Group 4**, **NHL B-cell type group IV,** and **NHL-BFM95 R4** are BL-type chemotherapy.

BCP, B-cell precursor; ALL, acute lymphoblastic leukemia; JACLS, Japan Association Childhood Leukemia Study Group; TCCSG, Tokyo Children's Cancer Study Group; CCLSG, Japanese Childhood Cancer and Leukemia Study Group; NHL, non-Hodgkin lymphoma; SR, standard risk; HR, high risk; HEX, extremely high risk; ER, extremely high risk; F, induction failure; BFM, Berlin-Frankfurt-Münster; CBT, cord blood transplantation; PBSCT, peripheral blood stem cell transplantation; BMT, bone marrow transplantation; VCR, vincristine; DEX, dexamethasone; L-asp, L-asparaginase; RT, irradiation; fr, fraction; m, months after diagnosis; ANED, alive with no evidence of disease; DOD, dead of disease.

TABLE 3 Review of the literature on BCP-ALL with 8q24/*MYC* rearrangement

Patient	Age (y)	Sex	Morphology	Extramedullary disease	Laboratory data			Cytogenetic data	Immunophenotypic data							Treatment	Outcome	Ref
					WBC (/µL)	UA (mg/dl)	LDH (IU/L)		CD 10	CD 19	CD 20	CD 34	TdT	κ	λ			
1	4	F	**Atypical L3**	No	**33 000**	**14**	**30 109**	46,XX,**t(8;14)(q24;q32)** [11] / 47,idem,+i(1)(q10) [7] / 46,idem,der(22)t(1;22)(q11;p11) [2]	+	+	-	-	N/A	-	-	**BL type**	**ANED 64 m+**	2
2	14	M	**L3**	**Kidney**	8300	**17**	**10 629**	47,XY,+i(1)(q10),**t(8;14)(q24;q32)** [6] / 46,XY [10]	+	+	-	-	+	-	-	**BL type**	DOD 6 m	2
3	13	F	**L3**	No	**20 000**	**>19**	**45 000**	46,XX,**t(8;14)(q24;q32)** [11] / 46,idem,inv(2)(p11q12) [33] / 46,XX [11]	+	+	-/+	+	+	-	-	**BL type**	**ANED 36 m+**	2
4	14	F	**L3**	No	**50 300**	**11.7**	**21 432**	46,XX,dup(1)(q21q44),**t(8;14)(q24;q32)** [4] / 46,X,der(X)t(X;1)(p2	+	+	-/+	-	-/+	-	-	**BL type**	**ANED 29 m+**	2

								2;q23),**t(8;14)(q24;q32)** [6] / 47,XX,+i(1)(q10),**t(8;14)(q24;q32)** [10]											
5	6	M	**L3**	**right maxillary sinus**	12 700	**9.9**	**5090**	46,XY,**t(8;14)(q24;q32)**, t(14;17)(q32;q21) [cp4] / 46,idem,dup(1)(q32q21) [2] / 46,XY [4]	+	+	+	-	+	-	-	**BL type**	**ANED 13 m+**	2
6	12	F	**L3**	No	**68 600**	**26.7**	**7012**	46,XX, **t(8;14)(q24;q32)** [7] / 46,XX [2]	+	+	+	N/A	-	-	-	**BL type**	**ANED 36 m+**	12
7	6	M	**L3**	**CNS**	N/A	N/A	N/A	**t(8;14)(q24;q32)** int del(2)(q14;q31)	+	+	+	+	N/A	-	-	N/A	N/A	13
8	4	M	**L3**	**Retro-gastric mass**	**32 400**	**9.5**	**14 990**	**t(8;14)(q24;q32)**	+	+	-	-	+	-	-	ALL type[b]	**ANED 24 m+**	14
9	10	F	**L3**	No	4400	N/A	**10 554**	46,XY,**t(8;14)(q24;q32)**,t(2;4)(p13;q27),der(1)(pter!q32.1::q32.1!q21.1::q11!qter)	+	+	+	N/A	-	-	-	**BL type**	**ANED 48 m+**	15

10	8	F	L2/**L3**	No	4100	N/A	**17 393**	**t(8;22)(q24.1;q11.2)**	+	+	-	N/A	+	-	-	**BL type**	N/A	16
11	1	M	**L3**	**Skull bones**	N/A	N/A	N/A	**t(8;14)(q24:q32)**	+	+	-	-	N/A	-*	-*	N/A	N/A	17
12	14	M	**L3**	**Mediastinum**	N/A	N/A	N/A	**t(8;14)(q24:q32)**	+	+	+	-	N/A	-*	-*	N/A	N/A	17
13	10	M	N/A	**Pancreas**	N/A	N/A	N/A	**t(8;14)(q24:q32)**	+	+	+	-	N/A	-*	-*	N/A	N/A	17
14	13	M	**L3**	**Intestine, liver, kidney**	N/A	N/A	N/A	**t(8;14)(q24:q32)**	+	+	+	-	N/A	-*	-*	N/A	N/A	17
15	16	M	**L3**	**Ileum**	N/A	N/A	N/A	**t(8;22)(q24:q11)**	+	+	+	-	N/A	-*	-*	N/A	N/A	17
16	15	F	**L3**	N/A	N/A	N/A	N/A	**t(8;14)(q24:q32)**	+	+	+	-	N/A	-*	-*	N/A	N/A	17
17	2.5	M	L1/**L3**	**Epidural, spinal, bone, retrobulbar**	16 700	N/A	**13 027**	46,XY,dup(1)(q21q31),**t(8;14)(q24;q32)**,der(15)t(1;15)(q12;p13)[19] / 46,XY [1]	+	+	-	-	-	-	-	**BL type** + maintenance	Relapse	18
18	13.4	M	N/A	No	**25 300**	N/A	**9979**	47,XY,**t(2;8)(p12;q24)**,+21 [11]	+	+	-	-	+	-	-	ALL type → **BL**	Relapse	18

																	type + mainte nance		
19	5.5	M	**L3**	**Ascites , pleural effusio n**	**55 100**	N/A	**5544**	46,XY,dup(1)(q11q43),**t(8;14)(q24;q32)** [5] / 46,XY [10]	+	+	+	-	+	-	-	**BL type**	Relapse	18
20	15.4	F	N/A	**CNS, Bones, epidur al, salivar y gland**	3450	N/A	**6608**	47,XX,+idic(1)(p11), **t(8;14)(q24;q32)** [15]	+	+	+	-	-	-	-	**BL type** + mainte nance	**ANED 16 m+**	18
21	14.3	M	**L3**	**CNS**	**21 490**	N/A	**2392**	46,XY,**t(8;22)(q24;q 11)**,add(19)(p13.3) [21]	+	+	-	-	+	-	-	**BL type** + mainte nance	**ANED 35 m+**	18
22	11.6	F	**L3**	No	9730	N/A	**11 997**	46,XX, **t(8;22)(q24;q11)** [20]	+	+	N/ A	-	+	-	-	**BL type**	**ANED 96 m+**	18
23	15	M	L2	N/A	**24 600**	N/A	N/A	46,XY,**t(8;14)(q24;q 32)**,	+	N/ A	N/ A	N/ A	+	-	-	ALL type	DOD 4 m	19

								t(14;18)(q21;q32)[a]											
24	10	F	L2	**CNS**	11 600	N/A	**3268**	48, XX,+i(1)(q10),+7, **t(8;22)(q24;q11.2)**,t(14;18)(q32;q21) [14] / 55,idem,+X,+2,+6,-7,+der(8)t(8;22),+10,+11,+14,+16 [3] / 64,XXX,i(1)(q10),-3,-7,**t(8;22)(q32;q21)**,der(8)t(8;22),-9,-12,t(14;18)(q32;q21)×2,?add(19)(p13),-21 [3]	+	+	- / +	-	+	-	-	ALL type → RTX + **BL type** +HCT	DOD 7 m	20
25	15	F	N/A	**CNS**	11 400	N/A	**1243**	46,XX,**t(8;9)(q24.1;p13)**,der(14) t(14;18)(q32;q21),del(18)(q21) [2] / 46,idem,dup(1)(q11q44) [14] / 47,idem,dup(1)(q11q44), +der(9)t(8;9) [3]	+	+	+	+	+	-	-	ALL type[c]	**ANED 53 m+**	4

26	8	M	L2	**Intraocular**	N/A	N/A	N/A	**t(8;14)(q24:q32)**, t(14;18)(q32;q21)	+	+	+	-	N/A	-*	-*	N/A	N/A	17

*Because these leukemia cells were B-II or B-III immunophenotype.

[a]Recombination of the *BCL2* locus with the *Cμ* locus was detected.

[b]Modified ALL-BFM 95 protocol.

[c]COG AALL0232.

BCP, B-cell precursor; ALL, acute lymphoblastic leukemia; y, years; Ref, reference number; F, female; M, male; N/A, not assessed; ANED, alive with no evidence of disease; DOD, dead of disease; m, months after diagnosis; RTX, rituximab; BFM, Berlin-Frankfurt-Münster; COG, Children's Oncology Group.