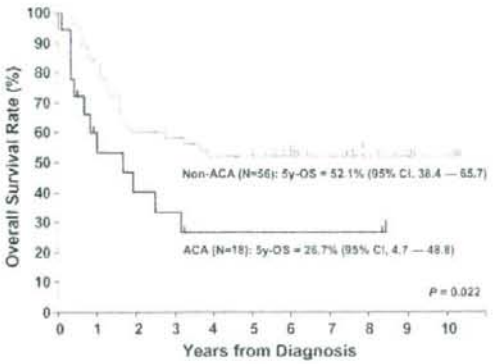

Fig. 2. Overall survival estimates for 74 infants with ALL and *MLL* gene rearrangements in the MLL96 and MLL98 studies; a comparison between patients with additional chromosomal abnormalities and patients with sole 11q23 abnormality excluding normal karyotype with *MLL* gene rearrangements. Median follow-up period: 78 months (range, 8–124 months).

Table 3
Multivariate analysis of prognostic factors in *MLL* rearranged ALL infants

	Parameter estimates	Risk ratio (95% CI)	*P*-value
Age, less than 6 months	0.724	2.063 (1.026–4.146)	0.041
Additional chromosomal abnormalities	0.418	1.519 (0.771–2.993)	0.226
t(4;11)(q21;q23)	0.345	1.413 (0.744–2.683)	0.290
WBC ≥ 300,000/uL	0.387	1.473 (0.771–2.812)	0.239
CNS leukemia	1.166	3.209 (1.497–6.881)	0.002
Registered in the MLL98 study	0.387	1.472 (0.756–2.865)	0.254

CI, confidence intervals; WBC, white blood cell; CNS, central nervous system.

(95% CI, 38.4–65.7%) ($P = 0.022$) (Fig. 2). In a multivariate analysis, only age at diagnosis (younger than 6 months) and positive central nervous system leukemia were significant prognostic factors for poor outcome in this study (Table 3).

4. Discussion

This study demonstrated that complex chromosomal abnormalities were associated with poor outcome in infant ALL with *MLL* gene rearrangements. The previous study described by Moorman et al. showed different findings, in that no prognostic effect of additional chromosomal abnormalities was observed in infants and children with ALL and 11q23 abnormalities [17]. However, it is difficult to simply compare between the study by Moorman et al. and ours as follows. First, Moorman et al. collected data from several cooperative study groups, which comprise different treatment cohorts. Secondly, accurate analyses of karyotypes and *MLL* gene rearrangements were not performed in all patients. Thirdly, the EFS rate in this previous study was too low to evaluate the effect of the additional chromosomal abnormalities in infants with *MLL*-rearranged ALL.

Moorman et al. stated that the frequency of additional chromosomal abnormalities depends on the different 11q23 translocations: high frequency of +X in t(4;11) and t(11;19), involvements in chromosomes 6, 9, and 12 in del(11)(q23) and other 11q23 [17]. In our study, several novel translocations were observed: t(2;4)(q31;q32), t(9;11)(p22;q13), t(2;9)(p10;q10), and t(6;11)(p10;q10). Other frequent chromosomal changes were +X and involvements of chromosomes 4, 7, and 11. In our study, a three-way 11q23 translocation was observed in four patients: t(4;11;15), t(4;11;5), t(4;11;9), and t(4; 11; 21). Different three-way translocations have been also detected in several other reports [15,17,19]. Complex structural chromosomal changes were observed in four patients, including insertion of a 4q21 fragment to the 11q23 locus or insertion of 10p12 to the 11q23 locus in our study. Kowarz et al. described ten patients with three-way translocation or complex structural chromosomal changes in MLL-AF4$^+$/AF4-MLL$^-$ ALL [16]. These findings indicate that complex chromosomal changes in leukemic cells disrupt several genes owing to the "cut and paste" recombination mechanism [16].

Recently, the functions of the partner genes fused to *MLL* gene located in 11q23 locus have been clarified: *AF4* at 4q21, *AF9* at 9p22, *ENL* at 19p133, *ELL* at 19p13.1, *AFX* at Xq13, and *AF6q21* at 6q21 are all transcription factors; *CBP* at 16p13 is a transcriptional coactivator; *AF1q* at 1q21 is a growth factor; and *AF17* at 17q21 is a dimerization protein [7]. In addition, several known genetic changes, such as *p53*, *p16*, and *RAS* mutations, are present in some cases in addition to *MLL* gene rearrangement, which might indicate the essential role of additional genetic changes in combination with *MLL* gene translocation in leukemogenesis [20]. Disruption of the Ikaros gene is also detected as an additional alteration in infant ALL [21]. Table 4 summarizes the genes at the breakpoint region of complex chromosomal abnormalities observed in our study, which have been reported only in hematologic malignancies, such as leukemia or lymphoma [22–29]. The function of each gene varies: *PMS1* at 2q31 and *FANCG* at 9p13 are a mismatch or DNA repair gene [23,27]; *Pax5* also located at 9p13, a differentiation factor of B-cells; and *HOXD13* also located at 2q13, a homeobox gene [24,28]. *PML* at 15q22, usually observed as *PMR-RARα* in acute promyelocytic leukemia with t(15;17), and *E2A* at 19p13, usually observed as *E2A-PBX1* in pre-B ALL with t(1;19), are both transcription factors [34,37]. Other genes such as *CHIC2* at 4q11 is associated with exocytosis, *SYK* and *NR4A3* at 9q22 are a tyrosine kinase and membrane receptor, respectively [29], and *CCND1* (*BCL1*) at 11q13 is associated with cell cycle [31]. Thus, if these genes are functionally disrupted after chromosomal changes, this could promote leukemogenesis.

In our study, the overall survival was significantly worse in the ACA group than that in the non-ACA group, but ACA was

Table 4
Breakpoint of chromosomes and possible located genes

Breakpoint	Located genes	Function	Associated translocation	Associated disease	Reference
1q32			t(1;13)(q32;q14)	Diffuse large B-cell lymphoma	[22]
2q31	*PMS1*	Mismatch repair gene	t(2;12)(q31;p13)	Non-Hodgkin lymphoma, MDS	[23]
	HOXD13	Homeobox gene	t(2;11)(q31;p15)	Therapy-related AML	[24]
4q11	*CHIC2*	Exocytosis	t(4;12)(411;p13)	AML	[25]
7p11			dic(7;9)(p11-13;p11)	Pre-B ALL	[26]
9p13	*FANCG*	DNA repair	t(2;9)(p11;p13)	Pre-B ALL	[27]
	Pax5	B-cell differentiation	t(7;9)(q11;p13)	B-ALL	[28]
9q22	*SYK*	Tyrosine kinase	t(5;9)(q33;q22)	Peripheral T-cell lymphoma	[29]
	NR4A3	Membrane receptor	t(9;12)(q22;p12)	MDS	[29]
11p11			t(11;14)(p11;q32)	Splenic marginal-zone B-cell lymphoma	[30]
11q13	*CCND1 (BCL1)*	Cell cycle control	t(11;14)(q13;q32)	Mantle cell lymphoma, others	[31]
	MYBOV (Cyclin D)			Multiple myeloma	[32]
15q22	*PML*	Transcription factor	t(5;15)(q33;q22)	CML	[33]
			t(15;17)(q22;q21)	APL	[34]
16p11			t(3;16)(q27;p11)	Diffuse large B-cell lymphoma	[35]
			t(16;21)(p11;q22)	AML	[36]
19p13	*E2A*	Transcription factor	t(1;19)(q23;p13)	Pre-B ALL	[37]
			t(2;19)(p11;p13)	AML	[38]
	LYL1	Transcription factor	t(7;19)(q34;p13)	T-ALL	[39]
			t(17;19)(q22;p13)	ALL	[37]

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia.

not a significant factor in the multivariate analysis. However, given that young age and central nervous system leukemia are significant prognostic factors by multivariate analysis, it is likely that the poor survival outcome seen in the ACA group is associated with the combination of young age, positive central nervous system leukemia and ACA. Since another report showed no effect of additional chromosomal changes in *MLL* positive infant ALL [17], an analysis of the data from a greater number of patients treated with identical treatment protocols is underway to address this issue. In our study, the genes affected by the chromosomal changes varied among the patients, and the function of each gene was different. However, it can be postulated that some genetic alterations induced by additional chromosomal changes might be associated with leukemogenesis and disease progression in *MLL* positive infant ALL.

5. Conflict of interest

All the authors do not have any commercial or other associations that might pose a conflict of interest.

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Contributions. H. Tauchi, D. Tomizawa and E. Ishii contributed to the analysis and interpretation of data, writing the article. M. Eguchi, M. Eguchi-Ishimae, N. Kinukawa and Y. Hayashi contributed to the analysis and interpretation of data. M. Hirayama and N. Miyamura contributed to the data collection and analysis. K. Koh and K. Horibe contributed to the study conception, revising and approving the final version of the article.

References

[1] Tomizawa D, Koh K, Sato T, Kinukawa N, Morimoto A, Isoyama K, et al. Outcome of risk-based therapy for infant acute lymphoblastic leukemia with or without an *MLL* gene rearrangement, with emphasis on late effects: a final report of two consecutive studies, MLL96 and MLL98, of the Japan Infant Leukemia Study Group. Leukemia 2007;21:2258–63.

[2] Hilden JM, Dinndorf P, Meerbaum SO, Sather H, Villaluna D, Heerema NA, et al. Analysis of prognostic factors of acute lymphoblastic leukemia in infants: report on CCG 1953 from the Children's Oncology Group. Blood 2006;108:441–51.

[3] Pieters R, Schrappe M, De Lorenzo P, Hann I, De Rossi G, Felice M, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet 2007;370:240–50.

[4] Isoyama K, Eguchi M, Hibi S, Kinukawa N, Ohkawa H, Kawasaki H, et al. Risk-directed treatment of infant acute lymphoblastic leukemia based on early assessment of MLL gene status: results of the Japan Infant Leukemia Study (MLL96). Br J Haematol 2002;118:999–1010.

[5] Kosaka Y, Koh K, Kinukawa N, Wakazono Y, Isoyama K, Oda T, et al. Infant acute lymphoblastic leukemia with *MLL* gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation. Blood 2004;104:3527–34.

[6] Greaves MF. Infant leukemia biology, aetiology, and treatment. Leukemia 1996;10:372–7.

[7] Felix CA, Lange BJ. Leukemia in infants. Oncologist 1999;4:225–40.

[8] Eguchi M, Eguchi-Ishimae M, Greaves M. Molecular pathogenesis of MLL-associated leukemias. Int J Hematol 2005;82:9–20.
[9] Megonial MD, Rappaport EF, Jones DH, Kim CS, Nowell PC, Lange BJ, et al. Panhandle PCR strategy to amplify MLL genomic breakpoints in treatment-related leukemias. Proc Natl Acad Sci USA 1997;94:11583–8.
[10] Pui C-H, Crist WM. Biology and treatment of acute lymphoblastic leukemia. J Pediatr 1994;124:491–503.
[11] Heerema NA, Arthur DC, Sather H, Albo V, Feusner J, Lange BJ, et al. Cytogenetic features of infants less than 12 months of age at diagnosis of acute lymphoblastic leukemia: impact of the 11q23 breakpoint on outcome: a report of the Children's Cancer Group. Blood 1994;83:2274–84.
[12] Hilden JM, Frestedt JL, Moore RO, Heerema NA, Arthur DJ, Reaman GH, et al. Molecular analysis of infant acute lymphoblastic leukemia: MLL gene rearrangement and reverse transcriptase-polymerase chain reaction for t(4;11)(q21;q23). Blood 1995;86:3876–82.
[13] Reaman GH, Sposto R, Sensel MG, Lange BJ, Feusner JH, Heerema NA, et al. Treatment outcome and prognostic factors for infants with acute lymphoblastic leukemia treated on two consecutive trials of the Children's Cancer Group. J Clin Oncol 1999;17:445–55.
[14] Corral J, Lavenir I, Impey H, Warren AJ, Forster A, Larson TA, et al. An Mll-AF9 fusion gene made by homogenous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. Cell 1996;85:853–61.
[15] Pui CH, Behm FG, Downing JR, Hancock ML, Shurtleff SA, Ribeiro RC, et al. 11q23/MLL rearrangement confers a poor outcome in infants with acute lymphoblastic leukemia. J Clin Oncol 1994;12:909–15.
[16] Kowarz E, Burmeister T, Lo Nigro L, Jansen MW, Delabesse E, Klingebiel T, et al. Complex MLL rearrangements in t(4;11) leukemia patients with absent AF4–MLL fusion allele. Leukemia 2007;21:1232–8.
[17] Moorman AV, Raimondi SC, Pui CH, Baruchel A, Biondi A, Carroll AJ, et al. No prognostic effect of additional chromosomal abnormalities in children with acute lymphoblastic leukemia and 11q23 abnormalities. Leukemia 2005;19:557–63.
[18] Shaffer LG, Tommerup N, eds. ISCN 2005: An international system for human cytogenetic nomenclature, 2005. Basel, Switzerland: S. Karger; 2005.
[19] Cimino G, Lanza C, Elia L, Lo Coco F, Gaidano G, Biondi A, et al. Multigenetic lesions in infant acute leukaemias: correlations with ALL-1 gene status. Br J Haematol 1997;96:308–13.
[20] Johansson B, Moorman AV, Secker-Walker LM. Derivative chromosomes of 11q23-translocations in hematologic malignancies. European 11q23 Workshop participants. Leukemia 1998;12:828–33.
[21] Sun L, Heerema N, Crotty L, Wu X, Navara C, Vassilev A, et al. Expression of dominant negative and mutant isoforms of the antileukemic transcription factor Ikaros in infant acute lymphoblastic leukaemia. Proc Natl Acad Sci USA 1999;96:680–5.
[22] Nanjangud G, Rao PH, Hegde A, Teruya-Feldstein J, Donnelly G, Qin J, et al. Spectral karyotyping identifies new rearrangements, translocations, and clinical associations in diffuse large B-cell lymphoma. Blood 2002;99:2554–61.
[23] Sato Y, Bohlander SK, Kobayashi H, Reshmi S, Suto Y, Davis EM, et al. Heterogeneity in the breakpoints in balanced rearrangements involving band 12p13 in hematologic malignancies identified by fluorescence in situ hybridization: TEL (ETV6) is involved in only one half. Blood 2007;90:4886–93.
[24] Raza-Egilmez SZ, Jani-Sait SN, Grossi M, Higgins MJ, Shows TB, Aplan PD. NUP98-HOXD13 gene fusion in therapy-related acute myelogenous leukemia. Cancer Res 1998;58:4269–73.
[25] Cools J, Bilhou-Nabera C, Wlodarska I, Cabrol C, Talmant P, Bermard P, et al. Fusion of a novel gene, BTL, to ETV6 in acute myeloid leukemias with a t(4;12) (911–912; p 13) Blood 1999; 94:1820–4.
[26] Heerema NA, Nachman JB, Sather HN, La MK, Hutchinson R, Lange BJ, et al. Deletion of 7p or monosomy 7 in pediatric acute lymphoblastic leukemia is an adverse prognostic factor: a report from the Children's Cancer Group. Leukemia 2004;18:939–47.
[27] Lu XY, Harris CP, Cooley L, Margolin J, Steuber M, Rao PH, et al. The utility of spectral karyotyping in the cytogenetic analysis of newly diagnosed pediatric acute lymphoblastic leukemia. Leukemia 2002;16:2222–7.
[28] Bousquet M, Broccardo C, Quelen C, Meggetto F, Kuhlein E, Delsol G, et al. A novel PAX5-ELN fusion protein identified in B-cell acute lymphoblastic leukemia acts as a dominant negative on wild-type PAX5. Blood 2007;109:3417–23.
[29] Kuno Y, Abe A, Emi N, Iida M, Yokozawa T, Towatari M, et al. Constitutive kinase activation of the TEL-Syk fusion gene in myelodysplastic syndrome with t(9;12)(q22;p12). Blood 2001;97:1050–5.
[30] Cuneo A, Bardi A, Wlodarska I, Selleslag D, Roberti MG, Bigoni R, et al. A novel recurrent translocation t(11;14)(p11;q32) in splenic marginal zone B-cell lymphoma. Leukemia 2001;15:1262–7.
[31] Kobayashi H, Kitano K, Saito H, Aoki K, Narita A, Terada N, et al. Overexpression of the PRAD1 oncogene in a patient with prolymphocytic leukemia with t(11;14)(q13;q32). Cancer Genet Cytogenet 1995;84:69–72.
[32] Janssen JW, Vaandrager JW, Heuser T, Jauch A, Kluin PM, Geelen E, et al. Concurrent activation of a novel putative transforming gene, myeov, and cyclin D1 in a subset of multiple myeloma cell lines with t(11;14)(q13;q32). Blood 2000;95:2691–8.
[33] Rappold I, Iwabuchi K, Date T, Chen J. Tumor suppressor p53 binding protein 1 (53BP1) is involved in DNA damage-signaling pathways. J Cell Biol 2001;153:613–20.
[34] Pandolfi PP, Alcalay M, Fagioli M, Pandolfi PP, Mencarelli A, Lo Coco F, et al. Genomic variability and alternative splicing generate multiple PML/RAR alpha transcripts that encode aberrant PML proteins and PML/RAR alpha isoforms in acute promyelocytic leukaemia. EMBO J 1992;11:1397–407.
[35] Ueda C, Akasaka T, Kurata M, Maesako Y, Nishikori M, Ishinohasama R, et al. The gene for interleukin-21 receptor is the partner of BCL6 in t(3;16)(q27;p11), which is recurrently observed in diffuse large B-cell lymphoma. Oncogene 2002;21:368–76.
[36] Berkowicz M, Rosner E, Resnitzky P, Mamon Z, Ben-Bassat I, Ramot B. Acute nonlymphocytic leukemia with t(16;21). Cancer Genet Cytogenet 1990;47:139–40.
[37] Hunger SP. Chromosomal translocations involving the E2A gene in acute lymphoblastic leukemia: clinical features and molecular pathogenesis. Blood 1996;87:1211–24.
[38] Larson RA, Wernli M, Le Beau MM, Daly KM, Pape LH, Rowley JD, et al. Short remission durations in therapy-related leukemia despite cytogenetic complete responses to high-dose cytarabine. Blood 1988;72:1333–9.
[39] Mellentin JD, Smith SD, Cleary ML. LYL1 a novel gene altered by chromosomal translocation in T cell leukemia, codes for a protein with a helix–loop–helix DNA binding motif. Cell 1989;58:77–83.

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白血病委員会

B 前駆細胞型急性リンパ性白血病の治療

渡 辺 新

中通総合病院小児科

Treatment of Childhood Acute Lymphoblastic Leukemia with B-Cell Precursor Phenotype

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Abstract Although more than 80% of children with acute lymphoblastic leukemia of the B-cell precursor phenotype (BCP-ALL) has been cured by the 4 groups' clinical study in Japan, the remaining 20% of patients underwent some events including induction failure, relapse, secondary cancer and also suffered from late complication. Japanese Pediatric Leukemia/ Lymphoma Study Group (JPLSG) was organized in 2003 and the JPLSG protocols have been already started for three distinct and rare types of ALL, including mature B-ALL, infant ALL and Ph$^+$ALL. We are just planning to start JPLSG T-cell ALL protocol in 2009. For the purpose of finding the standard treatment of BCR-ALL in Japan, central nervous system leukemia directed treatment is important to avoid pre-symptomatic cranial radiotherapy. Then, for the best cure by the best treatment, it is importanr that risk-directed stratification also contain not only age and WBC count at onset, but also genetic subtype of BCP-ALL, early response to treatment and introduction of selected antileukemic drugs being tested in clinical trials.

要 旨 小児急性リンパ性白血病（ALL）の 8 割以上は B 前駆細胞型（BCP-ALL）であり，本邦では 4 グループによる多施設共同研究により治癒率は向上したが，約 20%の児に，寛解導入不能，再発，2 次がんが生じていることと，晩期合併症が問題となっている．2003 年に結成された日本小児白血病リンパ腫研究グループ（JPLSG）により B-ALL，乳児 ALL，Ph$^+$ALL の全国共通臨床試験が始まり，2009 年には T-ALL の統一治療研究が開始される．BCP-ALL 治療において，よりよい治療による，よりよい治癒を目指すために重要なことは，予防的頭蓋照射の全廃を見据えた中枢神経系白血病の予防法導入と，従来の年齢・白血球数による層別化に加えて，白血病細胞の染色体・遺伝子異常と治療反応性に基づいた新たな層別化と，新規開発薬剤を導入した至適治療法選択が重要となる．

Key words: acute lymphoblastic leukemia, B-cell precursor phenotype, central nervous system leukemia, minimal residual disease, clofarabine

I. はじめに

小児急性リンパ性白血病（ALL）は，わが国で年間約 600 例が発症しており，そのうち T 細胞型と成熟 B 細胞型を除いた B 前駆細胞型 ALL（B-cell precursor ALL: BCP-ALL）は年間約 500 例前後発症すると推定され，国内では CCLSG, TCCSG, KYCCSG, JACLS の 4 グループを中心に多施設共同治療研究が施行されてきており，国内外の臨床研究において BCP-ALL では年代とともに治療成績の改善傾向が認められ，90 年代の臨床研究においては 80%近い無病生存率（EFS）が達成されているが[1,2]，逆にいえば依然として 20%以上の患児において寛解導入不能・再発・2 次がん・死亡といった event が生じ，一部の症例では多剤耐性から治療抵抗性となっていることになる．この背景にはリスク因子に基づいた層別化により治療戦略を立てていく場合，メソトレキセート（MTX）（1953 年発売），6-メルカプトプリン（6MP）（1953 年発売），ビンクリスチン（VCR）（1963 年発売），シタラビン（AraC）（1969 年発売）と，BCP-ALL に汎

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用される抗白血病剤は発売から40年以上経過しているものが中心で，それらを組み合わせた治療強化には限界があり，20年以上にわたって新しい抗白血病薬が開発されなかったことが1つの要因として考えられる．2003年に結成された日本小児白血病・リンパ腫研究グループ（Japanese Pediatric Leukemia/Lymphoma Study Group: JPLSG）は，わが国の小児がんの主要な研究グループであるCCLSG/TCCSG/KYCCSG/JACLSがすべて参加する形で構成され，活発な活動を展開してきている．ALLの中でもとくに難治性で希少疾患である成熟B細胞性ALL，乳児ALLとフィラデルフィア染色体陽性（Ph⁺）ALLの3疾患に関してJPLSGの全国共通臨床試験（B-NHL03, Ph⁺ ALL04, MLL03）が開始され，診断・治療の標準化が図られてきている．また，難治性T細胞性ALL（T-ALL）にnelarabine（アラノンG®）が認可されたことを受けて，JPLSGのT-ALLの全国共通臨床試験が開始される運びとなっている．本稿ではBCP-ALLに関してもっとも新しいリスク因子に基づく層別化と治療選択の現況について概説するとともに，新規薬剤の臨床的意義についても考察する．

II. BCP-ALLにおけるリスク因子に基づく層別化の基本的考え方

本邦におけるCCLSGの多施設共同研究は1981年のALL-811研究から始まっているが，同じ年の1月からDana-Farber Cancer Institute（DFCI）グループは次のような考えに基づいてprotocol 81-01を開始している[4]．すなわち，1973～1980年における治療経験を踏まえて，患児をstandard risk（SR）とhigh risk（HR）の2群に層別化し，SR群では治療関連毒性を減らしつつ最大の治療効果を得ることを目標とした．一方，HR群では再発を防ぐための治療強化が優先され，それに伴う治療関連毒性は容認するという，きわめて明確な戦略を打ち出した．SR群は年齢2～9歳であって，1）白血球数20,000/μl以上，2）T細胞性，3）縦隔腫瘤，4）中枢神経系浸潤，の4つの特徴を1つも認めない群とし，SR群の基準を満たさない群をHR群として，治療戦略の骨子はHD-MTX/ADR/VCR/PSLの4剤による寛解導入と，それに引き続いて行う週1回のintensive L-asparaginase therapyであった．Median follow-up 35カ月とやや短いものの，SR群の4年event-free-survival（4y-EFS）：86±4%，HR群の4y-EFS：71±4%と，最新研究と比べても見劣りしない成績を報告しているが，この「治療関連毒性の容認」に関しては，心筋障害と脳腫瘍を中心とした二次がんなどの晩期合併症の発病率は追跡期間が長くなるほど増えることが判明したことから，新たな転換期を迎えてきている．最近，発表されたALL-BFM 95研究[5]（1995～2000）および本邦のCCLSG-ALL 941研究[6]（1994～2000）では，ともに晩期合併症の軽減を意図した治療軽減が行われたにもかかわらず，BFM95では6y-EFS全体（2,169名）で79.6%±0.9%，SR群：89.5±1.1%，MR群：79.7±1.2%，HR群：79.6±0.9%，という成績が得られている．また，CCLSG-ALL 941ではLR群にanthracyclineおよびアルキル化剤をまったく使わないという試みを行って，6y-EFS: 73.4±5.2%という成績を報告しており，いかに晩期障害を抑えつつ治癒率を向上させるかが大きな課題となっている．

BCP-ALLにおける初発時の年齢と白血球数の予後因子としての重要性はT-ALLに比べより大きいものがあり[7]，リスク因子の共通化に向けてCTEP/NCI（ローマ/NCI）ワークショップで提案されたリスク基準では，白血球数5万未満かつ1.00～9.99歳をSR，それ以外をHRに分類し，米国CCG，POG，DFCI，St. Jude小児研究病院のBCP-ALL症例解析から，SR群（患者比率68%）：4y-EFS 80.3%，HR群：4y-EFS 63.9%としている[8]．現在，多くの臨床研究では，初診時データとして1）*TEL-AML1*，2）hyperdiploidy（染色体数50以上），3）*BCR-ABL*，4）*MLL*融合遺伝子，5）3種のtrisomy（4, 10, 17），6）中枢神経系白血病の有無（CNS1, 2, 3），7）睾丸浸潤の有無の7点を加え，さらに治療反応性（BFMグループでは早期プレドニゾロン反応性とday33: BMおよびday78: BMのPCR法によるMRD[9]，CCG/ POG: COGではday8: BMとday15: BMの芽球比率およびday29: BMのFCM法によるMRD[10]をみることで，予後がきわめて良好な群と，きわめて不良な群を抽出し，とくに*TEL-AML1*を有する群は独立した予後良好群とした前方視的研究が行われてきている[11]．

抗原受容体遺伝子再構成を利用した微小腫瘍残存（MRD）定量法の開発は，個々のALL患児の白血病細胞の化学療法反応性に合わせた新たな層別化に結びつけられてきている．ドイツBFMグループは，T細胞受容体（TCR）γ，δ鎖，免疫グロブリン遺伝子κ鎖（Igκ），およびTAL1遺伝子再構成を指標とし，治療開始後，5, 12, 22～25, 28～33, 70, 80週，2年目，3年目に骨髄MRD定量を行い，すべての測定時点においてMRDが独立した予後因子となることを証明した[12]．

Fig. 1に小児がん白血病研究グループ（CCLSG）のALL 2004 protocolにおける，初発時データと第15週のMRDに基づいた2段階の層別化と治療プロトコールを示した．対象は乳児ALLを含まない1歳以上19歳未満

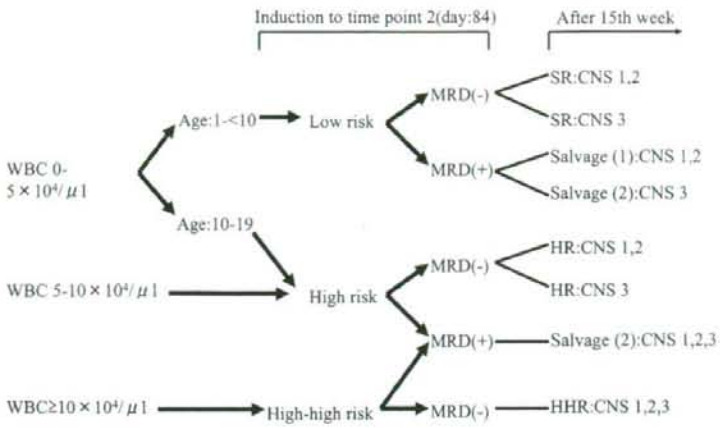

Fig. 1 Two step stratification based on age/WBC and time point 2 (day: 84) MRD (CCLSG ALL 2004 protocol)

の未治療 ALL で，プレドニゾロン単独 7 日間先行投与期間中に成熟 B 細胞性 ALL および，t(9;22) または *BCR-ABL* 融合遺伝子を有することが判明したものは含まず，初発時の年齢と白血球数で標準危険群（SR 群：白血球 0〜5 万かつ年齢 1〜10 歳），高危険群（HR 群：白血球 5〜10 万または年齢10〜19 歳），超高危険群（HHR 群：白血球 10 万以上）の 3 群に分ける．次に中枢神経系浸潤に関して CNS1（髄液赤血球数が 10/μl 未満で細胞診が陰性），CNS2（髄液赤血球数が 10/μl 未満で白血球数が 5/μl 未満かつ細胞診が陽性），CNS3（髄液赤血球数が 10/μl 未満で白血球数が 5/μl 以上かつ細胞診が陽性）の 3 群に分類し，髄液赤血球数 10/μl 以上では traumatic lumbar puncture として末梢血の赤血球・白血球の比率で補正した．CNS1 と CNS2 は第 15 週までの髄注回数が違うだけで第 15 週以降は同じレジメンとし，CNS3 には頭蓋照射を行うため別レジメンとした．MRD 量は，TCR，Igκ 遺伝子再構成を指標とし，寛解時の骨髄 DNA と初診時の DNA を，健常人の白血球 DNA で段階希釈したものを症例（クローン）特異的プライマーを用いて増幅し，両者を比較することで判定する．第 15 週以降の治療レジメン決定のための MRD 定量は初回完全寛解判定時の骨髄（ポイント 1）と，第 13 週の骨髄（ポイント 2）の 2 点で行い，これまでの International BMF Group，CCLSG の研究結果に基づき，定量結果 10^{-3} 以上を陽性：MRD（＋）とした[7]．図に示すように 2 段階の層別化後に第 15 週以降の治療レジメンが決定され，MRD 陰性群には SR, HR, HHR のレジメン治療が行われ，CNS3 および HHR 群に頭蓋照射が導入される．MRD 陽性群には，より強い強化療法を組み込んだ Salvage（1）および Salvage（2）が行われ，予定どおり研究継続中である．

III. 小児 ALL によくみられる染色体・遺伝子異常と臨床的特徴（Table 1, 2）

BCP-ALL で多く認められる染色体異常に，染色体数 50 以上の高 2 倍体，t(12;21)(p12-13;q22) により生じる *ETV6-CBFA2*（*TEL-AML1*），4 番・10 番・17 番染色体の trisomy があり，これらはいずれも 1〜10 歳に多く，代謝拮抗剤を基本にした化学療法で 5y-EFS が 85〜90%と推定される予後良好群である[3]．また，t(1;19)(q21;q23) により生じる *E2A-PBX1* は Pre-B cell 型 ALL でみられ，中枢神経系浸潤を起こしやすいことが知られていたが，最近の強化された化学療法を行うことで小児では予後良好群に含まれるものの，成人 ALL では依然として予後不良である[4]．最近報告された小児 ALL の約 2%に FISH 法で認められる 21 番染色体内の AML1 遺伝子の増幅（intrachromosomal amplification）も Pre-B 型 ALL に多く，予後不良であることが報告されている[5]．一方，t(4;11)(q21;q23) および他の 11q23 による MLL 変異は乳児・若年成人に認められた場合に予後不良となり[6]，t(9;22)(q34;q11) により生じる BCR-ABL は白血球 5 万以上または 10 歳以上の NCI-HR 群で予後不良となり，染色体・遺伝子異常と年齢との関係が認められている[7]．BCP-ALL と T-ALL を比較すると，早期治療反応不良は共通の予後不良因子であるが，NCI-HR 群は BCP-ALL でのみ予後不良となる（Table 2）．

IV. 治療レジメン

BCP-ALL に対する多くの代表的な治療レジメンは，1）寛解導入相，2）中枢神経系白血病予防相，3）中間

Table 1 Clinical and biological features of the more common genetic subtypes of childhood B lineage ALL[13]

Subtype	Frequency (%)	Molecular genetic alteration	Associated features	Estimated 5-year event-free survival (%)
Hyperdiploidy >50 choromosomes	27–29	Unknown	Predominant BCP-ALL; age between 1 and 10 years; low leukocyte count; favorable prognosis with antimetabolite-based therapy	91 (SE 3)
t(12;21)(p12-13;q22)	20–25	*ETV6-CBFA2* fusion (also termed TEL-AML1)	Predominant BCP-ALL; age between 1 and 10 years; pseudodiploidy; favorable prognosis with antimetabolite-based therapy	89 (SE 3)
t(1;19)(q23;p13)	3–4	*E2A-PBX1* fusion	Pre-B phenotype; increased leukocyte count; pseudodiploidy; black race; CNS leukemia; improved outcome with intensive therapy	86 (SE 7)
t(4;11)(q21;q23) and other 11q23 translocations	4–8	*MLL-AF4* fusion (other *MLL* rearrangements)	CD10 /CD15^{-} BCP-ALL; hyperleukocytosis; infant age group predominantly; CNS leukemia; dismal outcome in infants	32 (SE 12)
t(9;22)(q34;q11)	3–4	*BCR-ABL* fusion	Predominant BCP-ALL; increased leukocyte count; older age; dismal outcome in the subgroup with WBC $\leq 5\times10^4/\mu l$ or age $\geq$ 10 years	37 (SE 12)
t(8;14)(q24,q32.3), t(2;8)(p12;q24) or t(8;22)(q24;q11)	2	Associated *MYC* overexpression with *IGH*, *IGK*, or *IGL* rearrangement	B-cell phenotype; L3 morphology; male predominance; bulky extramedullary disease; favorable prognosis with short-term intensive chemotherapy with high-dose methotrexate plus cytarabine plus cyclophosphamide	75–85
dic(9;12)(p11-12;?p12)	1	Unknown	BCP-ALL phenotype; male predominance; excellent outcome with antimetabolite-based therapy	80–90

BCP-ALL: Precursor B cell acute lymphoblastic leukemia.

Table 2 Prognostic factors in childhood acute lymphoblastic leukemia (ALL)[7]

Leukemia subtype	Favorable prognostic factors	Unfavorable prognostic factors
BCP-ALL	Hyperdiploidy (>50 chromosomes); *TEL-AML1* fusion; Trisomies 4,10 and 17	Poor early response; *MLL* rearrengement in infants; Philadelphia chromosomes; Leukocyte count > $5\times10^4/\mu l$; Age > 10 years at diagnosis
T-ALL	*HOX11* overexpression; t(11;19) with *MLL-ENL* fusion	Poor early response; Low dose-intensity chemotherapy

維持療法相，4）再寛解導入相，5）連続維持療法相，の5相から成り立っている．NCI-SR/HRによるリスク分類に基づいて最近の治療研究では，SR群はVCR/L-asp＋steroidの3剤，HR群はこれにanthracyclineを加えた4剤で初回寛解導入を開始されることが多く，寛解導入に用いられるレジメンと同じ内容で再寛解導入相が組み入れられることが多い[18]．半減期が長いことと中枢神経系への到達率の高い点からデキサメサゾンのプレドニゾロンに対する優位性の報告は多いが投与量設定にばらつきがあり[19,20]，ランダム化比較試験による検討が進んでいる．また，L-asparaginase製剤の優劣が報告されているが，至適投与量が投与されることで各薬剤の優劣は少なくなる可能性が指摘されている[21]．寛解導入後の強化療法の目的は薬剤耐性化した白血病細胞の排除であり，MTX大量療法＋6MPなどが行われるが，MTXの指摘投与量はALLの遺伝子型/表現型と個々の患児の薬物代謝により変わってくる．すなわち，SR群ではMTX量として1～2 g/m^{2}がほとんどの患児にとって至適量となるが，HR群やT-ALLでは5 g/m^{2}といった高用量が必要となり，33.6 g/m^{2}といった超大量は不要だが，ロイコボリンによる救済のタイミングと投与量も重要となる[22]．BCP-ALLでは維持療法相を含む2～2.5年の治療期間が行われるが，おそらく2/3の児が1年で治癒しているとしても，それらの児を抽出できる手段がない現状では全員に維持療法を行う必要があり[23]，代表的なレジメンとしては6MP連日内服とMTX週1回内服が行われることが多い．

V. 中枢神経系指向性治療（Table 3）

中枢神経系再発予防は BCP-ALL の治癒率向上にとってきわめて重要であるが，予防的頭蓋照射（pre-symptomatic cranial radiotherapy: pCRT）後には 2 次がん，認知障害，内分泌障害，成長障害といった重篤な晩期障害を生じるため[24]，照射量の削減が試みられてきたが，12Gy に減量した pCRT 後にも 15 年の追跡調査で 1.7%（95%CI 0.1～3.4）で 2 次がんが認められていることなどから[25]，近年の小児 ALL に対する臨床プロトコールにおける pCRT 対象群は 2～20%に抑えられてきており，pCRT の全廃から髄注療法への変更が進んできている[26]．現行のプロトコールで照射対象とされるのは，T-ALL，BCP-ALL で白血球数 10 万以上，MRD 高値，予後不良染色体異常，および CNS3 などであるが，2 歳未満児への照射は避けるか減量されることが多く，CNS3 も含めて pCRT を全廃した臨床研究も少なからず進行してきている（Table 3）．これを担保する理論として，照射後の 2 次がんの発生率が 20 年後に 20.9%に達し，とくに 2 次性脳腫瘍の死亡率がきわめて高いことおよび，髄注療法を強化することの有効性と，中枢神経系単独再発後に初めて照射を行った群の治癒率が高いことなどから，頭蓋照射は中枢神経系再発後に備えて控えておくべきだという意見が上がってきている．pCRT で注意すべきは 18～24Gy の頭蓋照射により，通常は照射時に遮蔽されていない甲状腺に，散乱放射線として 13～132 cGy がかかってしまい，2 次がんとしての甲状腺癌が生じる可能性が指摘されていることであり，甲状腺癌は小児 ALL 治療後の 2 次がんの 10%弱を占めていることである[27]．この散乱放射線被爆量は報告されている被爆甲状腺癌の閾値：200cGy を下回るが[28]，かつて行われた頭部白癬に対する 10cGy の少量照射後にも若年小児では 2 次発癌の報告がみられることなどから，厳重な甲状腺遮蔽に加え，やはり予防的頭蓋照射の全廃を可能にする方法が求められることになる．また，最近の報告では，抗白血病薬の薬物代謝に関連する蛋白質をコードする遺伝子多型と中枢神経系再発頻度との関連性が示唆されており，MTX 耐性と関連するビタミン D 受容体遺伝子の多型や[29]，インターロイキン 15 の発現過多と発症時の中枢神経系浸潤との関連が報告されており[30]，将来のオーダーメイド治療における個別の至適中枢神経系白血病予防法選択の手がかりとなっていくことが期待できる．

Table 3 Current use of prophylactic cranial irradiation in childhood ALL in selected study group[25]

Study group	Indication (dose of cranial irradiation)	Estimated proportion of patients (%)
AIEOP	T-ALL and WBC＞$10\times10^4/\mu l$; BCP-ALL and PPR, t(4;11), t(9;22), Induction failure, MRD＞10^{-3} on day 78 (12Gy for age 1-＜2 years, 18Gy for age≥2 years); CNS3 (12Gy for age 1-＜2 years, 18Gy for age≥2 years)	15
BFM	Age＞1 year and T-ALL or high-risk BCP-ALL (12Gy), CNS3 (12Gy for age 1-＜2 years, 18Gy for age≥2 years)	10
COG	High-risk T-ALL (12Gy); BCP-ALL and slow early response, MRD＞10^{-1} at day 28 induction or t(9;22) (12Gy); CNS3 (18Gy)	20
DCOG	PPR, MRD≥10^{-1} at day 33 induction and at day 79, t(4;11), t(9;22), or induction failure (12Gy)	3
DFCI	T-ALL (12Gy); BCP-ALL and WBC＞$10\times10^4/\mu l$, rearranged *MLL* gene, hypodiploidy, or MRD≥10^{-3} at day 28 induction (12Gy); CNS3 (18Gy)	20
EORTC	None	0
FRALLE	T-ALL and WBC≥$10\times10^4/\mu l$, PPR, M2/M3 marrow at day 21 induction or MRD≥10^{-2} at day 35 to day 42; BCP-ALL and t(9;22), t(4;11), PPR, M2/M3 marrow at day 21 or MRD≥10^{-3} at day 35 to 42 (18Gy for age＞4 years; none for younger patients); CNS3 (18Gy for age＜4 years, 24Gy for age＞4 years)	13
JACLS	T-ALL and WBC≥$10\times10^4/\mu l$ (12Gy); CNS3 (12Gy)	6
NOPHO	Age＞5 years and WBC≥$10\times10^4/\mu l$ or T-ALL with mediastinal mass (18Gy); CNS3 (24Gy, optional 12Gy spinal radiation)	5
SJCRH	None	0
UKALL	Age＞2 years and CNS3 (24Gy)	2
CCLSG	WBC≥$10\times10^4/\mu l$; MRD≥10^{-3} on day 84 and WBC≥$10\times10^4/\mu l$ or age≥10 years (18Gy); CNS3 (18Gy)	13

AIEOP: Associazion Italiana di Ematologia ed Oncologia Pediatrica, BFM: Berlin-Frankfurt-Münster, COG: Children's Oncology Group, DCOG: Dutch Childhood Oncology Group, DFCI: Dana-Farber Cancer Institute consortium, EORTC: European Organisation for Research and Treatment of Cancer, FRALLE: French Acute Lymphoblastic Leukemia Study Group, JACLS: Japan Association of Childhood Leukemia Study Group, NOPHO: Nordic Society of Pediatric Hematology, SJCRH: St Jude Children's Research Hospital, UKALL: United Kingdom Medical Research Council Working Party on Childhood Leukemia, CCLSG: Children's Cancer and Leukemia Study Group of Japan, PPR: Prednisone Poor Responses, MRD: Minimum Residual Disease.

VI. *TEL-AML1*

BCP-ALLでは，t(12;21）によって生じる*TEL-AML1*融合遺伝子を有する症例は25%にのぼり，もっとも多くみられるキメラ遺伝子である．その予後について最近，前方視的な研究結果が報告され，独立した予後因子として認められてきている．POG AlinC 16治療研究では[31]，2,676例のBCP-ALL登録例において926例に適切な遺伝子解析が行われ，244例（26%）に*TEL-AML1*融合遺伝子を認めている．この研究ではlow-risk（LR）群としてNCI-SR基準に加えて4番，10番染色体のtrisomyか，予後不良のキメラ遺伝子がなく，DNA indexが1.16以上の群としたため，通常は高2倍体と共存しない*TEL-AML1*は融合遺伝子陽性群の比率はLR群で152例中7名（5%）と低く，SR群470名中173名（37%），PR群304名中64名（21%），全体で926名中244名（26%）であった．LR群とSR群にはanthracycline，epipodophylotoxin，アルキル化剤がまったく入らず，pCRTも行わない治療プロトコールが施行された．観察期間7.8年で5y-EFSはTEL-AML1群で86±2%，germline TEL群で72±2%（$p<0.0001$），NCI-SR群において*TEL-AML1*群は有意に予後がよく（5y-EFS, 88±3% vs. 78±2%: $p=0.0011$），NCI-HR群でも有意に予後良好（5y-EFS, 81±5% vs. 62±3%: $p=0.0032$）であった（Fig. 2）．また，day15: BMでM1 marrowであった群を早期治療反応良好群とすると，予想どおり*TEL-AML1*群における早期治療反応良好群は有意に予後がよく（5y-EFS, 87±2% vs. 71±12%: $p=0.043$），germline *TEL*群でも早期治療反応不良群は有意に予後良好（5y-EFS, 75±2% vs. 56±6%: $p=0.0032$）であった．さらに早期治療反応良好群においても*TEL-AML1*群はgermline *TEL*群と比べて有意に予後良好（5y-EFS, 87±2% vs. 75±2%: $p=0.0001$）であった．注目すべき点は，*TEL-AML1*群では診断から5年以上経ってから生じるlate eventが198名中4名（2%）であったのに対し，germline *TEL*群では467名中19名（4%）と多かった点である．多変量解析でも*TEL-AML1*融合遺伝子を認めないことが独立した予後不良因子（$p=0.0002$）となり，また，同様の報告は他施設からも報告されている．したがって，*TEL-AML1*融合遺伝子陽性かつ早期治療反応良好群は，現在の代謝拮抗剤を中心とした晩期障害の少ない治療が推奨される．

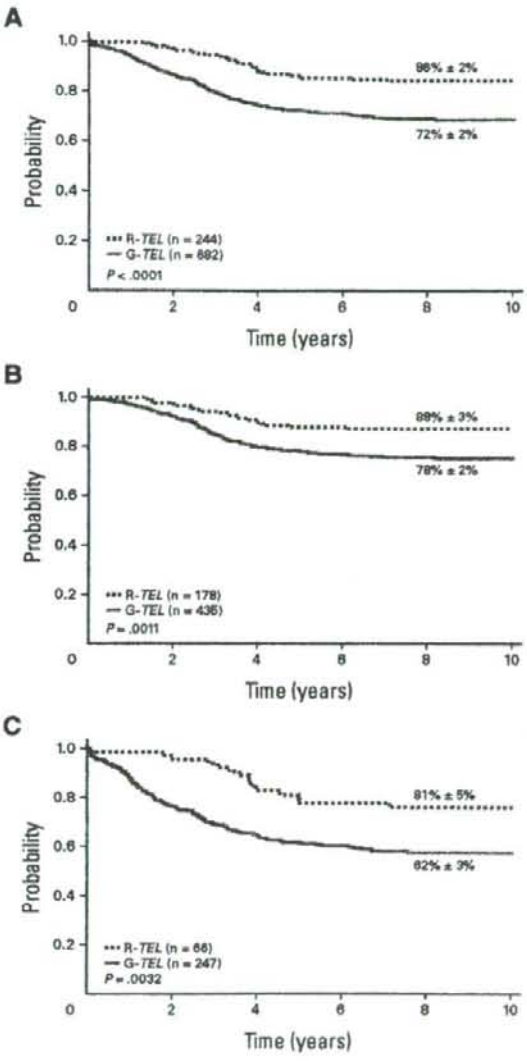

Fig. 2　Event-free survival (EFS) estimates of patients treated on ALinC 16 and their relation to *TEL* gene status (A) EFS estimates of patients with *TEL* rearrangements (R-*TEL*) compared with those of patients with germline *TEL (G-TEL)*. (B) EFS estimates of patients with National Cancer Institute (NCI) standrad-risk ALL shown in relation to *TEL* status. (C) EFS estimates of patients with NCI high-risk ALL shown in relation to *TEL* status.[31]

VII. 治療新規薬剤の導入（Table 4）

BCP-ALLの普遍的な原因が未解明である以上，普遍的なターゲット治療の開発は困難であるが，新しい機序の抗白血病剤や分子標的療法薬は近年，続々と開発されつつある（Table 4）．抗CD22抗体であるepratuzumabやubiquitin proteasome pathwayの阻害薬であるbortezomibは，現在，COGの再発ALL program（COG AALL07P1）で第II相臨床試験が進行中であるが，BCP-

Table 4 Selected antileukemic drugs being tested in clinical trials[18]

	Mechanism of action	Subtype of leukemia targeted
Clofarabine	Inhibits DNA polymerase and ribonucleotide reductase; disrupt mitochondria membrane	All
Rituximab	Anti-CD20 chimeric murine-human monoclonal antibody	CD20-positive
Epratuzumab	Anti-CD22 humanized monoclonal antibody	CD22-positive
Alemtuzumab	Anti-CD52 humanized monoclonal antibody	CD52-positive
Imatinib mesilate	ABL kinase inhibition	BCR-ABL positive
Nilotinib	ABL kinase inhibition	BCR-ABL positive
Dasatinib	BCR-ABL kinase inhibition	BCR-ABL positive
MK-0457	Aurora kinase inhibition	BCR-ABL positive
Lestaurtinib; midstaurin; tandutinib; sinitinib malate; IMC-EB10	FMS-like tyrosine kinase 3 inhibition	MLL rearraged; hyperdiploid
Tifarnib; lonafarnib	Farnesyltransferase inhibition	All
Azacytidine; decitabine; temozolomide	DNA methyltransferase inhibition	All
Romidepsin; vorinostat; valproic acid; MD-27-275; AN-9	Histone deacetylase inhibition	All
Silolimus; temsirolimus; everolimus; AP-23573	Mammalian target-of-rapamycin inhibition	All
Bortzezomib	Inhibition of ubiquitin proteasome pathway	All
Flavopiridol	Serine-threonine cyclin-dependent kinase	All
Oblimersen	Downregulation of BCL2	All
17-AAG	Heat shock protein-90 inhibitor	BCR-ABL positive; ZAP-70-positive

ALL にもっとも期待されている新薬としては clofarabine が挙げられる[32]．Clofarabine は fludarabine と cladribine の両方の特徴をもつ新しいアデノシンアナログで，ara-CTP の集積を強化しつつ，epipodophylotoxin，アルキル化剤によって生じた DNA 損傷の回復を阻害することが期待され，clofarabine: 30 mg/m^2×5 days＋etoposide: 100 mg/m^2×5 days＋cyclophosphamide: 440 mg/m^2×5 days の併用療法が初回寛解導入不能例や再発後の難治例に試みられている（CLO21800205）[33]．

VIII. おわりに

Clofarabine をはじめ，Table 4 に挙げた新規薬剤の多くは欧米では認可されたにもかかわらず，本邦では未承認薬がほとんどであり，nelarabine が難治性 T-ALL に承認された以外は，発売のめどすらたっていない薬剤が多いことは，きわめて憂慮すべき状況であるといわざるをえない．小児の BCP-ALL は近年，フォローアップ期間が 20～30 年を超えてきて，晩期合併症が大きく取り上げられてきているが[34,35]，小児では BCP-ALL を治癒させた後，60 年以上の余生があり，その QOL は今後も問われ続けていくことになる．よりよい治療とよりよい治癒はすべての患児・家族の望みであり，旧来の治療薬の組み合わせによる QOL 向上には限界があることを踏まえ，新薬の承認を含めた治療法のさらなる改善が期待される．

引 用 文 献

1) Pui CH, Relling MV, Downing JR : Acute lymphoblastic leukemia. N Engl J Med **350**: 1535–1548, 2004
2) Moorman AV, Harison CJ, Buck GA, et al: Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): Analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALL XII/Eastern Cooperating Oncology Group (ECOG) 2993 trial. Blood **109**: 3189–3197, 2007
3) Nachman JB, Heerema NA, Sather H, et al: Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood **110**: 1112–1115, 2007
4) Clavell LA, Gelber RD, Cohen HJ, et al: Four-agent induction and intensive asparaginase therapy for treatment of childhood acute lymphoblastic leukemia. N Engl J Med **315**: 657–663, 1986
5) Anja Moricke, Alfred Roiter, Martin Zimmermann, et al: Risk-ajusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: Treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood **111**: 4477–4489, 2008
6) Watanabe A, Katano N, Kikuta A, et al: *Strategy of cumulative dose reduction of drugs with late effects, using escalating dose of anti-metabolites with or without mega-dose chemotherapy plus autologous peripheral blood stem cell rescue for treatment of childhood acute lymphoblastic leukemia: Children's Cancer and Leukemia Study Group of Japan (CCLSG), CCLSG

ALL 941 Protocol Study. Blood **102**: 783, 2003
7) Schrappe M: Treatment strategy for childhood and adolescent ALL. HEMATOLOGY 2004, American Society of Hematology, Education Program Book. AMGEN oncology: p120, 2004
8) Smith M, Arthur D, Camitta B, et al: Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol **14**: 18–24, 1996
9) Davies SM, Borowitz MJ, Rosner GL, et al: Pharmacogenetics of minimal residual disease response in children with B-precursor acute lymphoblastic leukemia: A report from the Children's Oncology Group. Blood **111**: 2984–2990, 2008
10) Rubnitz JE, Wichlan D, Devidas M, et al: Prospective analysis of *TEL* gene rearrangements in childhood acute lymphoblastic leukemia: A Children's Oncology Group. J Clin Oncol **26**: 2186–2191, 2008
11) Romana SP, Mauchauffe M, Le Coniat M, et al: The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. Blood **85**: 3662–3670, 1995
12) Okamoto T, Yokota S, Katano N, et al: Minimal residual disease in early phase of chemotherapy reflect poor outcome in children with acute lymphoblastic leukemia: A retrospective study by the Children's Cancer and Leukemia Study Group in Japan. Leuk Lymphoma **43**: 1001–1006, 2002
13) Pui CH: Childhood Leukemias 16 Acute Lymphoblastic Leukemia. Cambridge University Press 439–472, 2006
14) Landau H, Lamanna N: Clinical manifestations and treatment of newly diagnosed acute lymphoblastic leukemia in adults. Curr Hematol Malig Rep **1**: 171–179, 2006
15) Moorman AV, Richards SM, Robinson HM, et al: Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). Blood **109**: 2327–2330, 2007
16) Pui CH, Gaynon PS, Boyett JM, et al: Outcome of treatment in childhood acute lymphoblastic leukemia with rearrangements of 11q23 chromosomal region. Lancet **359**: 1909–1915, 2002
17) Schultz KR, Pullen DJ, Sather HN, et al: Risk- and response-based classification of childhood B precursor acute lymphoblastic leukemia: A combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). Blood **109**: 926–935, 2007
18) Pui CH, Robinson LL, Look AT: Acute lymphoblastic leukemia. Lancet **371**: 1030–1043, 2008
19) Mitchell CD, Richards SM, Kinsey SE, et al: Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukemia: Results of the UK Medical Research Council ALL97 randomized trial. Br J Haematolo **129**: 734–745, 2005
20) Bostrom BC, Sensel MR, Sather HN, et al: Dexamethasone versus prednisone and daily oral versus weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: A report from the Children's Cancer Group. Blood **101**: 3809–3817, 2003
21) Hawkins DS, Park JR, Thomson BG, et al: Asparaginase pharmacokinetics after intensive polyethylene glycol-conjugated L-asparaginase therapy for children with relapsed acute lymphoblastic leukemia. Clin Cancer Res **10**: 5335–5341, 2004
22) Pui CH, Relling MV, Evans WE: Is mega dose of methotrexate beneficial to patients with acute lymphoblastic leukemia? Leuk Lymphoma **47**: 2431–2432, 2006
23) Jacobs SS, Stock LC, Bostrom BC, et al: Substitution of oral and intravenous thioguanine for mercaptopurine in a treatment regimen for children with standard-risk acute lymphoblastic leukemia: A collaborative Children's Oncology Group/Natuonal Cancer Institute pilot trial (CCG-1942). Pediatr Blood Cancer **49**: 250–255, 2007
24) Geenen MM, Cardous-Ubbink MC, Kremer LCM et al: Medical assessment of adverse health outcomes in long-term survivors of childhood cancer. JAMA **297**: 2705–2715, 2007
25) Pui CH, Howard S: Current management and challenges of malignant disease in CNS in paediatric leukemia. Lancet Oncol **9**: 1–12, 2008
26) Vilmer E, Suciu S, Ferster A, et al: Long-term results of three randomized trials (58831, 58832, 58881) in childhood acute lymphoblastic leukemia: A CLCG-EORTC report. Leukemia **14**: 2257–2266, 2000
27) Perel Y, Leverger G, Carrere A, et al: Second thyroid neplasms after prophylactic cranial irradiation for acute lymphoblastic leukemia. Am J Hematol **59**: 91–94, 1988
28) Tucker MA, Morris Jones PH, Boice JD Jr, et al: Therapeutic radiation at a young age is linked to secondary thyroid cancer. Cancer Res **51**: 2885–2888, 1991
29) Rocha JCC, Cheng C, Liu W, et al: Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. Blood **105**: 4752–4758, 2005
30) Cario G, Izraeli S, Teichert A, et al: High interleukin-15 expression characterized childhood acute lymphoblastic leukemia with involvement of the CNS. J Clin Oncol **25**: 4752–4758, 2005
31) van Dongen JJ, Seriu T, Panzer-Grumayer, et al: Prognostic value of minimal residual disease in acute lymphoblastic leukemia in childhood. Lancet **352**: 1731–1738, 1998
32) Conrey SJ: New agents in the treatment of childhood leukemias and myelodysplastic syndrome. Curr Oncol Rep **7**: 399–405, 2005
33) Hayes MP: Briefing package for pediatric oncology subcommittee (ODCA) meeting, 20 October 2005: Product, Clofarabine, Clolar™. P1-23: 2005
34) Mody R, Li S, Dover DC, et al: Twenty-five-year follow-up among survivor of childhood acute lymphoblastic leukemia: A report from the Childhood Cancer Survival Study. Blood **111**: 5515–5523, 2008
35) Oeffinger KC, Meriens AC, Sklar CA, et al: Chronic health conditions in adult survivors of childhood cancer. N Engl J Med **355**: 1572–1582, 2006

Outcome of Recurrent or Refractory Acute Lymphoblastic Leukemia in Infants With *MLL* Gene Rearrangements: A Report From the Japan Infant Leukemia Study Group

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Background. Despite the poor outcome of recurrent or refractory acute lymphoblastic leukemia (ALL) in infants with *MLL* gene rearrangement, few studies have focused on this specific group. We conducted a retrospective analysis of infants with recurrent or refractory ALL from two previous consecutive Japanese studies to clarify the characteristics and prognostic factors among these patients ***Procedure.*** All recurrent or refractory ALL infants with *MLL* gene rearrangement (MLL-R) who were registered in two consecutive Japanese nation-wide multicentric trials (MLL96 and MLL98; between 1995 and 2001) were eligible for the study. ***Results.*** Among 80 MLL-R ALL infants, 34 cases of recurrence and 5 induction failures occurred. The median duration of first remission was 5 months (range, 0–28 months). All patients underwent various salvage chemotherapies; remission was achieved in 40.5% (15/37). A total of 23 patients received subsequent hematopoietic stem cell transplantations (HSCT): 9 in remission, 12 without remission, and 2 with unknown status. With median follow-up period of 5.5 years, the 5-year overall survival (OS) rate after the second-line treatment was 25.6% ± 6.9%. Young age (<3 months) and central nervous system involvement at initial diagnosis were associated with poor outcome; however, failure to achieve remission after salvage therapy was the sole independent poor prognostic factor in multivariate analysis ($P = 0.01$). ***Conclusions.*** The prognosis of infants with recurrent or refractory MLL-R ALL is extremely poor despite alternative treatments including HSCT; therefore, it is necessary to develop novel treatment strategies. Pediatr Blood Cancer © 2009 Wiley-Liss, Inc.

Key words: infant acute lymphoblastic leukemia; *MLL* gene; recurrent; refractory

INTRODUCTION

The outcome of acute lymphoblastic leukemia (ALL) in infants of less than 12 months of age with *MLL* gene rearrangement remains poor, with recently published long-term event-free survival (EFS) rates of 22–54% [1–4]. More than 50–60% of newly diagnosed cases develop recurrent or refractory disease during or following treatment; however, few reports have focused on the clinical course and outcome of these specific groups. The results of a small number of studies with few cases suggest that the outcomes of recurrent or refractory ALL infants with *MLL* gene rearrangement are dismal and rarely rescued [2,4].

The Japan Infant Leukemia Study Group conducted three consecutive series of nationwide clinical studies for newly diagnosed ALL in infants (the MLL96, MLL98, and MLL03 studies) [5,6]. In the present report, we analyzed the outcome of 39 recurrent or refractory ALL infants with *MLL* gene rearrangement who were registered in either of the first two studies (MLL96 and MLL98), to clarify the characteristics and prognostic factors in this group.

METHODS

Patients

We analyzed the data regarding all consecutive recurrence and induction failures that occurred in two consecutive studies for newly diagnosed infant ALL (the MLL96 and MLL98 studies). Of the 80 ALL infants with *MLL* gene rearrangements (MLL-R) who registered in these two studies between December 1995 and December 2001, 34 relapsed and 5 were resistant to induction chemotherapy (induction failure). Patient characteristics are listed in Table I.

Informed consent, provided according to the Declaration of Helsinki, was obtained from the patients' guardians on registration of the two studies. The rearrangement of an *MLL* gene in each patient was determined by Southern blot analysis and/or split-signal fluorescence *in situ* hybridization (FISH), as described previously [5–7].

First-Line Treatment

Details of the therapeutic regimens used in the MLL96 and MLL98 studies are described elsewhere [5–7]. Briefly, MLL-R ALL infants received induction therapy and three courses of postremission intensification therapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) during the first remission, whenever 5/6 or 6/6 HLA-matched related donor, 6/6-matched unrelated donor, or 4 to 6/6-matched unrelated cord blood donor was available. The protocol-specified conditioning regimen comprised either total-body irradiation (TBI; 12 Gy in six fractions, twice daily on days −7 to −5) or busulfan (BU; 35 mg/m^2/dose

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TABLE I. Main Characteristics of 39 Patients With Recurrent/Refractory Infant ALL and *MLL* Gene Rearrangements

	Induction failure	Relapse pre-HSCT	Relapse post-HSCT	Total
Total no. of patients	5	21	13	39
Age at onset, months				
<3	2	9	1	12
3 to <6	1	8	6	15
≥6	2	4	6	12
Sex				
Male	4	9	4	17
Female	1	12	9	22
WBC count at onset, $\times 10^9$/L				
<100	2	4	4	10
100 to <300	1	9	5	15
≥300	2	8	4	14
CNS disease at onset				
Positive	2	4	2	8
Traumatic tap	0	0	1	1
Negative	3	12	9	24
Unknown	0	5	1	6
Karyotype[a]				
t(4;11)(q21;q23)	1	14	7	22
t(11;19)(q23;p13)	1	1	3	5
t(9;11)(p22;q23)	2	0	2	4
Other 11q23	0	2	1	3
No11q23 abnormalities	1	2	0	3
Unknown	0	2	0	2
First-line treatment				
MLL96	4	13	6	23
MLL98	1	8	7	16
Time to relapse				
Median, months	—	3 (0–22)	12 (4–28)	5 (0–28)
Relapse < 12 months	—	19	6	25
Relapse ≥ 12 months	—	2	7	9
Site of relapse				
Bone marrow	—	20	10	30
Combined BM/CNS	—	1	0	1
CNS	—	0	2	2
Testicular	—	0	1	1

ALL, acute lymphoblastic leukemia; BM, bone marrow; CNS, central nervous system; HSCT, hematopoietic stem cell transplantation; WBC, white blood cells. [a]All patients were confirmed as *MLL* rearranged by Southern blotting and/or split-signal fluorescence *in situ* hybridization (FISH), including three cases with no 11q23 abnormalities and two unknown cases by normal karyotypic analysis.

orally, 4 times a day on days −8 to −5) with a combination of etoposide (60 mg/kg intravenously on day −4) and cyclophosphamide (60 mg/kg intravenously on days −3 and −2).

Second-Line Treatment

Second-line treatment differed among the patients because the choice of treatment for recurrence or induction failure varied among the hospitals. Table II provides an outline of second-line treatment regimens used for these patients. Indication of subsequent HSCT was also determined according to the choice of each institution.

Evaluation of Late Effects

Late effects were also analyzed, including cardiac, pulmonary, renal, endocrine, dental, orthopedic, dermatologic, ophthalmologic, auditory, psychological, growth and development, and occurrence of secondary malignancies. Medical records regarding these issues were reviewed by each physician in the participating centers; these data were collected via a questionnaire sent to each participating center.

Statistical Considerations

Overall survival (OS) rate was estimated using the Kaplan–Meier method and standard errors (SEs) were estimated using the Greenwood formula; these were then compared using the log-rank test. Confidence intervals (CIs) were computed with a 95% confidence level. A Cox regression model was used for multivariate analysis. *P* values, when cited, are two-sided, with a value of 0.05 or less taken to indicate statistical significance.

TABLE II. Second-Line Chemotherapy Regimens Used for 39 Patients With Recurrent/Refractory Infant ALL and *MLL* Gene Rearrangements

Second-line chemotherapy regimen	No. of patients received	No. of patients achieved CR	No. of patients alive in CCR
AML-oriented	11	4	4
Ara-C civ/MIT/VP-16/TIT	7	3	3
Ara-C civ/IDA/VP-16	1	0	0
HDCA/MIT/VP-16/TIT	2	1	1
HDCA	1	0	0
ALL-oriented	11	7	2
VCR/PSL/L-asp/DRB/TIT	3	3	1
VCR/Dexa/PSL/L-asp/pirarubicin/IT-MTX	2	1	0
VCR/PSL/L-asp/MIT	1	1	1
VCR/Dexa/L-asp/IFO/VP-16/HD-MTX/TIT	1	1	0
VCR/Dexa/CPM/HD\MTX/TIT	1	0	0
VCR/L-asp/IFO	1	0	0
HD-MTX	1	1	0
VCR/PSL	1	0	0
AML/ALL-hybrid	5	3	2
Ara-C/pirarubicin/VP-16/PSL/L-asp/TIT	3	1	1
Ara-C/DXR/VP-16/VCR/Dexa/CPM/TIT	1	1	0
HDCA/DRB/VCR/PSL/L-asp/CPM/TIT	1	1	1
Others	2	0	0
BHAC-AMP	1	0	0
CXR/TIT	1	0	0
No (HSCT on disease)	1	0	0
N/A	9	2	2

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ara-C civ, continuous intravenous infusion of cytarabine; BHAC-AMP, combination of enocitabine/6-mercaptopurine/predonisone/aclarubicin; CCR, continuous complete remission; CPM, cyclophosphamide; CR, complete remission; CXR, cranial radiation; Dexa, dexamethasone; DRB, daunorubicin; DXR, doxorubicin; HDCA, high-dose cytarabine; HD-MTX, high-dose methotrexate; HSCT, hematopoietic stem cell transplantation; IDA, idarubicin; IFO, ifosfamide; IT-MTX, intrathecal methotrexate; L-asp, L-asparaginase; MIT, mitoxantrone; N/A, data not available; PSL, prednisone; TIT, triple intrathecal therapy; VCR, vincristine; VP-16, etoposide.

RESULTS

Outcome and Survival of Relapsed/Refractory Infant ALL With *MLL* Gene Rearrangements

Figure 1 summarizes the outcomes of 39 infants with recurrent or refractory MLL-R ALL. Among the 39 patients, 5 were refractory to induction chemotherapy (induction failure) and 34 relapsed, of which 21 occurred before HSCT in first remission (CR1) and 13 occurred after HSCT in CR1. The source of donor for these 13 infants was bone marrow transplantation (BMT) from related donor (R-BMT) in 3, peripheral blood stem cell transplantation from related donor (R-PBSCT) in 1, BMT from unrelated donor (U-BMT) in 2, unrelated cord blood transplantation (U-CBT) in 6, and autologous HSCT in 1. The median duration of CR1 in all 34 recurrent ALL infants was 5 months (range, 0–28 months).

Of the five infants with refractory ALL, two achieved the first complete remission by AML-type induction chemotherapy: one continued conventional AML-type chemotherapy alone and is alive in remission with a follow-up period of 4 years; the other received U-CBT in CR1 and had relapse in bone marrow (BM) 6 months later, but was rescued by a second transplantation (U-BMT) and is now in CR2 with a follow-up period of 3 years. The remaining three patients never achieved CR: one received AML-type induction chemotherapy; one received BHAC-AMP (combination of enocitabine, mercaptopurine, prednisone, and aclarubicin); and one received the "Phase A" intensification course of MLL96 (combination of cytarabine, etoposide, pirarubicin, prednisone, L-asparaginase, and triple intrathecal therapy). Of these three patients, one received R-BMT but died of interstitial pneumonia; the other two patients died of progressive disease.

Among the 34 patients with recurrence, 32 were evaluable for response: 13 (38.2%) achieved CR2 with various re-induction chemotherapies (Table II). Subsequently, 8 of these 13 patients underwent HSCT (R-PBSCT in 2, R-BMT in 1, U-BMT in 3, and U-CBT in 2); the other 5 patients relapsed and eventually died of progressive disease. Five of the 8 patients undergoing HSCT in CR2 are alive in remission with a median follow-up period of 8 years (range, 3–10 years); 2 experienced a second relapse, and 1 died of veno-occlusive disease (VOD); of the two patients with second relapse after HSCT in CR2, one is alive in CR3 (follow-up period, 3 years) and the other died of progressive disease.

Among the remaining 19 patients who did not achieve CR2, 11 patients underwent HSCT without remission: R-BMT in 2, R-PBSCT in 2, U-BMT in 4, and U-CBT in 3. Of the 11 patients, 2 patients are alive in CR2 with follow-up periods of 5 and 8 years, 3 died of HSCT-related severe infectious complications, and 6 relapsed and died of progressive disease. The remaining 8 patients without CR2 died of progressive disease, with a median period of 6.5 months. Both of the patients who were not evaluable for response

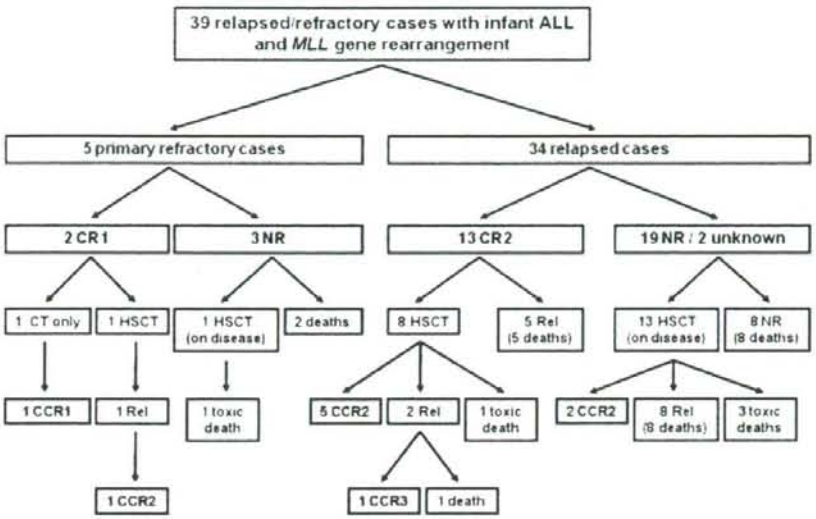

Fig. 1. Summary of outcome in patients with recurrent/refractory infant ALL and *MLL* gene rearrangements. CCR, continuous complete remission; CR, complete remission; CT, chemotherapy; HSCT, hematopoietic stem cell transplantation; NR, no response; Rel, relapse.

underwent HSCT but relapsed and died, one of progressive disease and one of bronchiolitis obliterans (BO).

The overall induction rate by second-line chemotherapy was 40.5% (15/37), and the 5-year OS rate in the whole cohort was 25.6% ± SE 6.9% (Fig. 2).

Prognostic Factors

Univariate analysis revealed three factors associated with a higher risk of failure: age less than 3 months at initial diagnosis, central nervous system (CNS) involvement at initial diagnosis (defined as more than 5 cells/mm^3 with recognizable blasts in cerebrospinal fluid), and induction failure by second-line chemotherapy (Table III). Differences in CR rate by second-line chemotherapy or survival rate were not observed between infants that

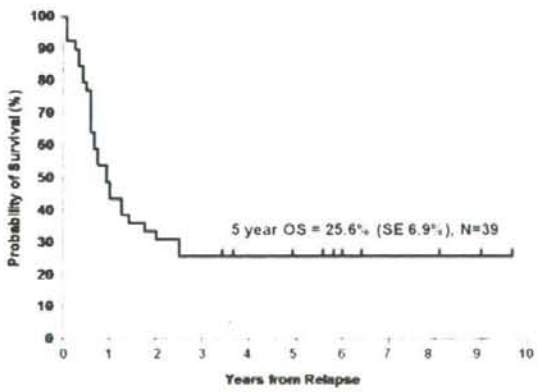

Fig. 2. Overall survival for patients with recurrent/refractory infant ALL and *MLL* gene rearrangements.

TABLE III. Five-Year Survival Rates by Selected Prognostic Features for 39 Cases of Recurrent/Refractory Infant ALL

	No. of patients	5-year OS, % (SE)	*P* value
Age at onset, months			
<3	12	16.7 (10.7)	0.03
≥3	27	29.6 (8.7)	
WBC count at onset, $\times 10^9$/L			
<300	25	24.0 (8.5)	0.54
≥300	14	28.5 (12.0)	
CNS disease at onset			
Positive	8	12.5 (11.6)	0.04
Negative	25	36.0 (9.6)	
Karyotype			
t(4;11)(q21;q23)	22	22.7 (8.9)	0.54
Others	17	29.4 (11.1)	
Duration of CR1[a]			
<12 months	25	16.0 (7.3)	0.06
≥12 months	9	44.4 (16.5)	
Relapse site[a]			
Bone marrow involved	31	28.1 (7.9)	0.55
Isolated extramedullary	3	0.0 (0.0)	
History of prior HSCT[a]			
Yes	13	21.4 (11.9)	0.47
No	21	25.0 (9.6)	
Response to second-line therapy			
CR	15	53.3 (12.8)	0.001
No CR	22	9.0 (6.1)	

BU, busulfan; CNS, central nervous system; CR, complete remission; HSCT, hematopoietic stem cell transplantation; OS, overall survival; SE, standard error; TBI, total-body irradiation; WBC, white blood cells.
[a]Relapsed patients only (five refractory patients excluded).

TABLE IV. Multivariate Analysis of Prognostic Factors in Infants With Recurrent/Refractory ALL

	Parameter estimates	Risk ratio (95% CI)	*P* value
Age at initial diagnosis, less than 3 months	0.453	1.573 (0.665–3.715)	0.301
CNS leukemia at initial diagnosis	0.584	1.794 (0.708–4.546)	0.217
Time to first event, less than 12 months	0.566	1.762 (0.621–5.002)	0.286
Failure to achieve CR after second-line therapy	1.230	3.422 (1.339–8.746)	0.010

CI, confidence interval; CNS, central nervous system; CR, complete remission.

relapsed before and after HSCT. Multivariate analysis revealed induction failure as the only significant prognostic factor (Table IV).

Long-Term Complications in the 10 Surviving Patients

Data regarding long-term complications were analyzed in 9 of the 10 survivors. The median age of the 9 patients at analysis was 6.5 years (range, 4.8–11.0 years). All had received HSCT and 4 were transplanted twice. Eight patients had received TBI-based conditioning regimen, while 1 had received BU-based conditioning regimen.

In these 9 patients, chronic GVHD was observed in 5 (extensive type in 4 and limited type in 1), hypothyroidism in 2, short stature (defined as a height standard deviation [SD] score below −2.0) in 8, skin abnormalities (alopecia, scleroderma, hypopigmentation) in 4, fasciitis in 1, ophthalmologic complications (dry eye, corneal opacity, retinal vasculitis) in 4, pulmonary complications (interstitial pneumonia, bronchiolitis obliterans) in 2, chronic diarrhea with malnutrition in 1, dental abnormalities in 4, and neurocognitive deficits (learning disability, intelligence impairment, autism) in 2. There were no patients with secondary malignancy or symptomatic chronic heart failure. Pubertal development could not be evaluated because all study patients were younger than 12 years old and had not entered puberty at the time of analysis.

DISCUSSION

Relapses are relatively frequent events in ALL infants with *MLL* gene rearrangement, despite intensive chemotherapy with or without HSCT [1–7]. Most previous studies report only a small difference between OS and EFS rates, indicating a dismal prognosis for relapsed or refractory cases in infant ALL. The present study found a response rate of 40.5% with second-line chemotherapy and a 5-year OS rate of 25.6% despite receiving HSCT, which is far from satisfactory.

The high chemotherapeutic-resistance of infant ALL cells is well described elsewhere. For example, Pieters et al. [8,9] demonstrated that infant ALL cells were more resistant in vitro to prednisone and L-asparaginase than those from older children. This result is supported by the finding that in comparison with older children, infants with ALL commonly show a poor response to prednisone [1]. In relapsed infant ALL patients, the high CR rate of 90–95% after induction therapy but high early relapse rate of 30–50% after remission also indicate the presence of a more resistant leukemic clone [5]. This might be explained by infant ALL cells rapidly acquiring additional resistance or by the selection of a more resistant leukemic clone that resided as a minor subclone at diagnosis, both presumably occurring during initial chemotherapy [10–12]. Although the exact mechanism associated with the resistance of ALL cells to chemotherapy remains unknown, there is no doubt that the recurrent or refractory infant ALL cells become highly resistant, thereby leading to the dismal outcome in these patients.

In the present study, we investigated various prognostic factors in recurrent or refractory infant ALL. Young age (<3 months of age) and CNS involvement at initial diagnosis confer a high risk of treatment failure in newly diagnosed ALL infants with *MLL* gene rearrangements, and can be regarded as surrogates of treatment resistance. Of note, univariate analysis revealed that infants with these factors harbor poorer prognosis compared with those without, even in recurrent or refractory infant ALL.

Two important prognostic factors have been established in recurrent or refractory childhood ALL [13–18]: duration of first remission and site of relapse. In the present study, however, we found that these factors had no prognostic impact among recurrent and refractory ALL infants. In the relapsed ALL studies of the BFM group (ALL-REZ studies), three factors (time point of relapse, site of relapse, and immunophenotype of leukemic cells) were used to classify each patient into four groups: from the S1 group (late and isolated medullary relapse) having the best prognosis, to the S4 group (very early and isolated BM relapse) having the worst prognosis [14]. When we classify the 34 relapsed patients in the present study according to this system, there are no patients in the S1 group, 6 in S2, 1 in S3, and 27 in S4. Thus, 79.4% (27/34) of the recurrent ALL infants are assigned to the group with the worst prognosis.

An important finding of the present study is that the achievement of further remission by second-line chemotherapy is clearly associated with an improved prognosis in recurrent or refractory ALL infants: a greater than 50% chance of survival can be obtained if CR is achieved. Therefore, to improve the outcome of recurrent or refractory infant ALL, it is necessary to find a means of improving the salvage therapy and increasing the CR rate.

In the present study, AML-oriented or AML/ALL-hybrid chemotherapy was successful in some infants with refractory ALL, with 6 of 16 patients in continuous CR (Table II). It has been demonstrated that infant ALL cells are more sensitive to cytarabine *in vitro* than those of older children, and that a subgroup may benefit from regimens that include intensive use of cytarabine, followed by HSCT [3,8,9]. Although some cases may benefit from this approach and obtain a chance of long-term survival, most would suffer from some kind of late complications as illustrated in the current report. Taking into account the vulnerability of infants and the highly intensive nature of therapy for *MLL*-rearranged ALL, there exists a clear need for the development of novel therapeutic modalities.

Gene expression profiling has led to the identification of several potential therapeutic targets based on the unique biology of *MLL*-rearranged infant ALL. Among these, inhibition of FLT3, a tyrosine kinase receptor, is currently being tested in a clinical trial [19–21].

Considering the poor outcome associated with relapsed/refractory infant ALL, these patients would benefit from the early clinical development of novel agents.

ACKNOWLEDGMENT

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REFERENCES

1. Dordelmann M, Reiter A, Borkhardt A, et al. Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. Blood 1999;94:1209–1217.
2. Hilden JM, Dinndorf PA, Meerbaum SO, et al. Analysis of prognostic factors of acute lymphoblastic leukemia in infants: Report on CCG 1953 from the Children's Oncology Group. Blood 2006;108:441–451.
3. Silverman LB, McLean TW, Gelber RD, et al. Intensified therapy for infants with acute lymphoblastic leukemia: Results from the Dana-Farber Cancer Institute Consortium. Cancer 1997;80:2285–2295.
4. Pieters R, Schrappe M, De Lorenzo P, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): An observational study and a multicentre randomised trial. Lancet 2007;370:240–250.
5. Isoyama K, Eguchi M, Hibi S, et al. Risk-directed treatment of infant acute lymphoblastic leukaemia based on early assessment of MLL gene status: Results of the Japan Infant Leukaemia Study (MLL96). Br J Haematol 2002;118:999–1010.
6. Kosaka Y, Koh K, Kinukawa N, et al. Infant acute lymphoblastic leukemia with MLL gene rearrangements: Outcome following intensive chemotherapy and hematopoietic stem cell transplantation. Blood 2004;104:3527–3534.
7. Tomizawa D, Koh K, Sato T, et al. Outcome of risk-based therapy for infant acute lymphoblastic leukemia with or without an MLL gene rearrangement, with emphasis on late effects: A final report of two consecutive studies, MLL96 and MLL98, of the Japan Infant Leukemia Study Group. Leukemia 2007;21:2258–2263.
8. Pieters R, den Boer ML, Durian M, et al. Relation between age, immunophenotype and in vitro drug resistance in 395 children with acute lymphoblastic leukemia–implications for treatment of infants. Leukemia 1998;12:1344–1348.
9. Ramakers-van Woerden NL, Beverloo HB, Veerman AJ, et al. In vitro drug-resistance profile in infant acute lymphoblastic leukemia in relation to age, MLL rearrangements and immunophenotype. Leukemia 2004;18:521–529.
10. Choi S, Henderson MJ, Kwan E, et al. Relapse in children with acute lymphoblastic leukemia involving selection of a preexisting drug-resistant subclone. Blood 2007;110:632–639.
11. Klumper E, Pieters R, Veerman AJ, et al. In vitro cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukemia. Blood 1995;86:3861–3868.
12. Li AH, Rosenquist R, Forestier E, et al. Detailed clonality analysis of relapsing precursor B acute lymphoblastic leukemia: Implications for minimal residual disease detection. Leuk Res 2001; 25:1033–1045.
13. Gaynon PS, Qu RP, Chappell RJ, et al. Survival after relapse in childhood acute lymphoblastic leukemia: Impact of site and time to first relapse–the Children's Cancer Group Experience. Cancer 1998;82:1387–1395.
14. Borgmann A, von Stackelberg A, Hartmann R, et al. Unrelated donor stem cell transplantation compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission: A matched-pair analysis. Blood 2003;101:3835–3839.
15. Chessells JM, Veys P, Kempski H, et al. Long-term follow-up of relapsed childhood acute lymphoblastic leukaemia. Br J Haematol 2003;123:396–405.
16. Einsiedel HG, von Stackelberg A, Hartmann R, et al. Long-term outcome in children with relapsed ALL by risk-stratified salvage therapy: Results of trial acute lymphoblastic leukemia-relapse study of the Berlin-Frankfurt-Munster Group 87. J Clin Oncol 2005;23:7942–7950.
17. Henze G, Fengler R, Hartmann R, et al. Six-year experience with a comprehensive approach to the treatment of recurrent childhood acute lymphoblastic leukemia (ALL-REZ BFM 85). A relapse study of the BFM group. Blood 1991;78:1166–1172.
18. Schroeder H, Garwicz S, Kristinsson J, et al. Outcome after first relapse in children with acute lymphoblastic leukemia: A population-based study of 315 patients from the Nordic Society of Pediatric Hematology and Oncology (NOPHO). Med Pediatr Oncol 1995;25:372–378.
19. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet 2002;30:41–47.
20. Brown P, Levis M, McIntyre E, et al. Combinations of the FLT3 inhibitor CEP-701 and chemotherapy synergistically kill infant and childhood MLL-rearranged ALL cells in a sequence-dependent manner. Leukemia 2006;20:1368–1376.
21. Stam RW, den Boer ML, Schneider P, et al. Targeting FLT3 in primary MLL-gene-rearranged infant acute lymphoblastic leukemia. Blood 2005;106:2484–2490.

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ORIGINAL ARTICLE: CLINICAL

Outcome of childhood B-cell non-Hodgkin lymphoma and B-cell acute lymphoblastic leukemia treated with the Tokyo Children's Cancer Study Group NHL B9604 protocol

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Abstract

From June 1996 to January 2001, 91 patients with B-cell non-Hodgkin lymphoma or B-cell acute lymphoblastic leukemia up to 18 years of age were enrolled in Tokyo Children's Cancer Study Group (TCCSG) NHL B9604 protocol study. Five-day intensive chemotherapy courses including high-dose methotrexate and high-dose cyclophosphamide were used for localized disease (Groups A and B). High-dose cytarabine was added for advanced disease (Groups C and D). Fifteen patients experienced an adverse event. There were three induction failures, eight relapses (three local, four bone marrow (BM), one BM + local), two toxic deaths and two second malignant neoplasm. Event-free survival at 6 years in Group D and in all patients was 82.4% ± 9.2% and 81.9% ± 4.4%, respectively. The TCCSG NHL B9604 protocol achieved an excellent treatment outcome especially in patients with the most advanced disease (Group D: high BM blast cell burden and/or central nervous system involvement).

Keywords: *B-NHL, B-ALL, intensified chemotherapy, TCCSG NHL B9604*

Introduction

The malignant cells of B-cell (surface immunoglobulin-positive [Ig+]) non-Hodgkin lymphoma (B-NHL) and B-cell acute lymphoblastic leukemia (B-ALL), which are classified as Burkitt lymphoma/leukemia in WHO classification [1], share morphologic, immunophenotypic, cytogenetic and clinical features and are considered to represent a continuum of the same disease. Diffuse large B-cell lymphoma is a distinct disease entity from Burkitt lymphoma/leukemia. However, the treatment for patients with large cell lymphoma is the same as that for patients with Burkitt histology. In early trials, children with advanced B-NHL and B-ALL had a worse outcome characterized by early recurrences despite a high initial complete remission rate [2,3]. After the introduction of short, intensive therapy courses primarily based on cyclophosphamide (CY), methotrexate (MTX), and intrathecal (i.t.) therapy, the prognosis of these mature B-cell neoplasms has improved significantly [4–11].

Most previous clinical experiences about childhood B-NHL and B-ALL were reported from European and North American study groups, however, there are few data on Japanese or Asian patients. We report here the treatment and results of 91 Japanese children with B-NHL or B-ALL registered to the Tokyo Children's Cancer Study Group (TCCSG) NHL B9604. The aim of this study

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was to evaluate the efficacy and safety of the short and intensive chemotherapy regimen designed by TCCSG for childhood B-NHL or B-ALL.

Patients and methods

Children and adolescents up to 18 years of age newly diagnosed with B-NHL or B-ALL were eligible for this study. From June 1996 to January 2001, 91 patients with B-NHL or B-ALL were enrolled in this study. Diagnosis was made by pathologists at a regional hospital and registration was made based on the regional diagnosis. A central review was performed retrospectively by the reference laboratory according to the WHO classification [1]. The St. Jude system was used for disease staging [12]. Patients were stratified into four groups according to stage, resection status, bone marrow (BM) blast cell burden and central nervous system (CNS) involvement as shown in Figure 1.

Five-day intensive chemotherapy courses including high-dose MTX (HD-MTX) and high-dose CY (HD-CY) were used for Groups A and B. High-dose cytarabine (HD-AraC) was added for Groups C and D. Patients in Groups B, C and D received a 5-day cytoreductive prephase before the first course was administered (Table I). Courses were administered at 3–4-week intervals. The number of treatment courses was three in Group A, six in B, six in C and seven in D, respectively. Treatment duration ranged from 10 to 27 weeks (Figure 1).

Events were defined as induction failure, relapse, death in induction, death in remission and second malignant neoplasm. Survival curves were calculated by the Kaplan–Meier method. Toxicities were graded according to National Cancer Institute Common Toxicity Criteria version 2. All treatments were performed with informed consent from the patients' guardians.

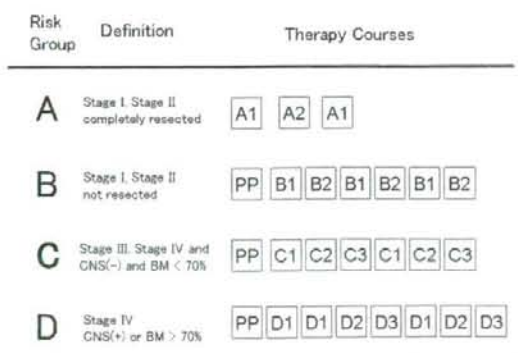

Figure 1. Treatment strategy. Patients were stratified into four risk groups; A, B, C and D. The composition of therapy courses is shown in Table I.

Results

The clinical characteristics of the 91 patients are shown in Table II. The percentage in Group A patients in our series was quite small (3.3%) compared with that of equal disease status patients in other studies (7–17%) [6,8,11]. A retrospective central review was performed in 64.8% (59/91) of the enrolled patients and the concordance rate was 88.1% (52/59). Fifteen patients experienced an adverse event. There were three induction failures, eight relapses (three local, four BM, one BM + local), two toxic deaths and two second malignant neoplasm. Nine patients died of induction failure in three, relapse in four and toxicity in two patients [intracranial hemorrhage (ICH) and sepsis]. Two patients developed a second malignant neoplasm. Grade 4 non-hematological toxicity was noted in 4 cases. Event-free survival (EFS) and overall survival (OS) were calculated as follows: EFS and OS in total were 81.9% ± 4.4% and 90.1% ± 3.1%, respectively. EFS was 66.7% ± 27.2% in Group A ($n=3$), 95.8% ± 4.1% in Group B ($n=25$), 77.6% ± 6.3% in Group C ($n=46$) and 82.4% ± 9.2% in Group D ($n=17$) (Figure 2). OS was in 100% in Groups A and B ($n=28$), 86.8% ± 5.0% in Group C and 82.4% ± 9.2% in Group D.

Discussion

In this article, we reported the outcome of 91 childhood B-NHL and B-ALL patients treated with TCCSG NHL B9604 protocol. OS and EFS at 6 years in total were 90.1% ± 3.1% and 81.9% ± 4.4%, respectively. OS of our study was comparable with SFOP study [8], BFM study [6] and UKCCSG study [7] and better than CCG study [9] or Venezuela study [11] whereas the observation period of our study was longer than other studies. Although the EFS of our study was worse than the SFOP study [8] and BFM study [6], it was comparable with the UKCCSG study [7] and better than the CCG study [9], POG study [4] and Venezuela study [11]. Especially in patients with the most advanced disease (Group D: high BM blast cell burden and/or CNS involvement), EFS was 82.4% ± 9.2%. This was comparable with the SFOP study [8] and BFM study [6]. The overall good prognosis of this study is thought to be mainly due to the relatively larger dose of chemotherapeutic drugs and intensified regimens resulting in a good outcome.

There were 15 event cases in this study, as shown in Table III. Among these cases, the diagnosis of two relapsed patients (UPN 132,146), whose diagnosis was DLBCL by the regional hospital, was corrected to B-LBL by retrospective central review. These

Table I. Therapeutic regimen of TCCSG NHL B9604 protocol

Group A	
Block A1:	
PDN 60 mg/m^2 P.O.	Days 1–5
MTX 3 g /m^2 6 h div with CFR	Days 1
CY 250 mg/m^2 1 h div	Days 2–5
VP16 100 mg/m^2 2 h div	Days 2–5
MH i.t.	Days 1
Block A2:	
PDN 60 mg/m^2 P.O.	Day 1–5
VCR 1.5 mg/m^2	Day 1
AraC150 mg/m^2 × 6 1 h div q12 h	Day 1–3
CY 1 g/m^2 2 h div	Day 2,3
MH i.t.	Day 1
Group B	
Block P(Prephase):	
PDN 60 mg/m^2 P.O.	Day 1–5
CY 200 mg/m^2 1 h div	Day 1, 2
MH i.t.	Day 1
Block B1:	
PDN 60 mg/m^2 P.O.	Day 1–5
MTX 3 g/m^2 6 h div with CFR	Day 1
CY 250 mg/m^2 1 h div	Day 2–5
VP16 100 mg/m^2 2 h div	Day 2–5
Epi 50 mg/m^2 1 h div	Day 5
MH i.t.	Day 1
Block B2:	
PDN 60 mg/m^2 P.O.	Day 1–5
VCR 1.5 mg/m^2	Day 1
AraC 150 mg/m^2 × 6 1 h div q12 h	Day 1–3
CY 1 g/m^2 2 h div	Day 2,3
Epi 50 mg/m^2 1 h div	Day 5
MH i.t.	Day 1
Group C	
Block P:	
PDN 60 mg/m^2 P.O.	Day 1–7
VP16 100 mg/m^2 1 h div	Day 5–7
MHC i.t.	Day 1,6
Block C1:	
Dex 10 mg/m^2 P.O.	Day 1–5
MTX 3 g/m^2 24 h div with CFR	Day 1
CY 250 mg/m^2 × 6 1 h div q12 h	Day 2–4
VP16 100 mg/m^2 2 h div	Day 2–5
(only day 2,3 in the first course)	
Epi 60 mg/m^2 1 h div	Day 5
MHC i.t.	Day 5 (in the first course)
MH i.t.	Day 1(in the second course)
Block C2:	
Dex 10 mg/m^2 P.O.	Days 1–5
VCR 1.5 mg/m^2	Days 1
AraC 150 mg/m^2 × 6 1 h div q12 h	Days 1–3
CY 1 g/m^2 2 h div	Days 2–4
Epi 60 mg/m^2 1 h div	Days 5
MHC i.t.	Day 1
MH i.t.	Day 8 (only in the first course)
Block C3:	
Dex 10 mg/m^2 P.O.	Day 1–5
VDS 3 mg/m^2	Day 1
VP16 100 mg/m^2 2 h div	Day 2–5
AraC 2 g/m^2 3 h div q12 h	Day 1–3
Epi 60 mg/m^2 1 h div	Day 5
MH i.t.	Day 1
MH i.t.	Day 8 (only in the first course)
Group D:	
Block P: the same as in Group C	
Block D1:	
Dex 10 mg/m^2 P.O.	Day 1–5
MTX 3 g/m^2 24 h div with CFR	Day 1
CY 300 mg/m^2 × 6 1 h div q12 h	Day 2–4
(only day 2,3 in the first course)	
Epi 80 mg/m^2 1 h div	Day 5
MHC i.t.	Day 5 (in the first course)
MH i.t.	Day 1 (in the second course)
MHC i.t.	Day 8 (in the second course)
MH i.t.	Day 1,8 (in the third course)
Block D2:	
Dex 10 mg/m^2 P.O.	Day 1–5
VCR 1.5 mg/m^2	Day 1
AraC 150 mg/m^2 × 6 1 h div q12 h	Day 1–3
CY 1 g/m^2 2 h div	Day 2–4
Epi 80 mg/m^2 1 h div	Day 5
MHC i.t.	Day 1 (in the first course)
MH i.t.	Day 8 (in the first course)
MH i.t.	Day 1,8 (in the second course)
Block D3:	
Dex 10 mg/m^2 P.O.	Day 1–5
VDS 3 mg/m^2	Day 1
VP16 100 mg/m^2 2 h div	Day 2–5
AraC 2 g/m^2 3 h div q12 h	Day 1–3
Epi 80 mg/m^2 1 h div	Day 5
MH i.t.	Day 1,8

Cranial irradiation for patients with CNS involvement at onset
24 Gy for older than 2 years old
18 Gy for 1 year old
0 Gy for less than 1 year old

Cumulative drug dosage in each group

Group	A	B	C	D
CY (g/m^2)	4	9.4	9	11.4
VP16 (mg/m^2)	800	1200	1700	2100
MTX (g/m^2)	6	9	6	9
CA (g/m^2)	0.9	2.7	25.8	28.8
Epi (mg/m^2)	0	300	360	560

PDN, prednisolone; CFR, citrovorum factor rescue; VP16, etoposide; MH, MTX and hydrocortisone; VCR, vincristine; AraC, cytarabine; q12h, every 12 h; Epi, epirubicine; MHC, MH and AraC; Dex, dexamethazone; VDS, vindesine.