Because of the limited effectiveness of HSCT and the potential risk of late effects, alternative strategies with novel targeted therapies should be explored for infants with MLL-r ALL.[4,18,19] Recent research has demonstrated that the aberrant epigenetic status, induced by a reciprocal *MLL* translocation via the H3K79 methyltransferase DOT1L, has a central role in MLL-r leukemogenesis.[20–22] The clinical development of epigenetic modifiers, such as DNA methyltransferase inhibitors and/or histone deacetylase inhibitors, is currently in progress. A small-molecule inhibitor of DOT1L is also in clinical development. Meanwhile, HSCT should be restricted to patients at higher risk of relapse, who are likely to benefit from this treatment modality.[23] This stratification is currently being evaluated in our ongoing JPLSG MLL-10 study.

In conclusion, short-course chemotherapy and the early use of HSCT in our study was feasible for infants with MLL-r ALL. However, given the limited effects of HSCT and the potential risk of late effects, the indication for HSCT should be restricted to specific subgroups with poor risk factors, and an alternative approach incorporating molecular-targeted drugs should be established in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank all JPLSG investigators and the following investigators who contributed to the central diagnosis in this study: H Nakamura and S Yamagata (Chiba University, Chiba) for the BU pharmacokinetic study; Y Komada (Mie University Graduate School of Medicine, Mie) and H Ohta (Osaka University, Osaka) for immunophenotypic diagnostics; T Taki (Kyoto Prefectural University of Medicine, Kyoto) for the molecular biological analyses; and J Fujimoto (National Center for Child Health and Development, Tokyo) for the preservation of the diagnostic specimens. This work was supported by a Grant for Clinical Cancer Research and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

AUTHOR CONTRIBUTIONS

K Koh, DT, TM, MH, Y Takahashi, AO, K Kato, KS and EI (principal investigator) participated actively in the study conception and design; K Koh, DT and EI reviewed the data analysis and interpretation and were the main authors of the manuscript; AMS and TW conducted the statistical analysis; TS was responsible for the busulfan pharmacokinetic study; TD and MT were responsible for the immunophenotyping diagnostics; YH was responsible for coordinating the molecular biology analyses; K Koh, K Kato, JT and Y Takeshita recruited patients; MT, KH and SM contributed to the financial and administrative support of the study; and all authors contributed to the conduct of the trial and were involved in the review of the results and the final approval of the manuscript.

REFERENCES

1 Pui CH, Kane JR, Crist WM. Biology and treatment of infant leukemias. *Leukemia* 1995; **9**: 762–769.
2 Armstrong SA, Staunton JE, Silverman LB, Pieters R, den Boer ML, Minden MD *et al.* MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat Genet* 2002; **30**: 41–47.
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**: 240–250.
4 Tomizawa D, Koh K, Sato T, Kinukawa N, Isoyama K, Kosaka Y *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.
5 Hilden JM, Dinndorf PA, 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**: 441–451.
6 Silverman LB, McLean TW, Gelber RD, Donnelly MJ, Gilliland DG, Tarbell NJ *et al.* Intensified therapy for infants with acute lymphoblastic leukemia: results from the Dana-Farber Cancer Institute Consortium. *Cancer* 1997; **80**: 2285–2295.
7 Biondi A, Rizzari C, Valsecchi MG, de Lorenzo P, Aricò M, Basso G *et al.* Role of treatment intensification in infants with acute lymphoblastic leukemia: results of two consecutive AIEOP studies. *Haematologica* 2006; **91**: 534–537.
8 Dördelmann M, Reiter A, Borkhardt A, Ludwig WD, Götz N, Viehmann S *et al.* Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. *Blood* 1999; **94**: 1209–1217.
9 Ferster A, Benoit Y, Francotte N, Dresse MF, Uyttebroeck A, Plouvier E *et al.* Treatment outcome in infant acute lymphoblastic leukemia. Children Leukemia Cooperative Group--EORTC. European Organization for Research and Treatment of Cancer. *Blood* 2000; **95**: 2729–2731.
10 Chessells JM, Harrison CJ, Watson SL, Vora AJ, Richards SM. Treatment of infants with lymphoblastic leukaemia: results of the UK Infant Protocols 1987-1999. *Br J Haematol* 2002; **117**: 306–314.
11 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**: 3527–3534.
12 Isoyama K, Eguchi M, Hibi S, Kinukawa N, Ohkawa H, Kawasaki H *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.
13 Pieters R, den Boer ML, Durian M, Janka G, Schmiegelow K, Kaspers GJL *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.
14 Nakamura H, Sato T, Okada K, Miura G, Ariyoshi N, Nakazawa K *et al.* Population pharmacokinetics of oral busulfan in young Japanese children before hematopoietic stem cell transplantation. *Ther Drug Monit* 2008; **30**: 75–83.
15 Salzer WL, Jones TL, Devidas M, Hilden JM, Winick N, Hunger S *et al.* Modifications to induction therapy decrease risk of early death in infants with acute lymphoblastic leukemia treated on Children's Oncology Group P9407. *Pediatr Blood Cancer* 2012; **59**: 834–839.
16 Tomizawa D, Koh K, Hirayama M, Miyamura T, Hatanaka M, Saikawa Y *et al.* Outcome of recurrent or refractory acute lymphoblastic leukemia in infants with MLL gene rearrangements: a report from the Japan Infant Leukemia Study Group. *Pediatr Blood Cancer* 2009; **52**: 808–813.
17 Schechter T, Finkelstein Y, Doyle J, Verjee Z, Moretti M, Koren G *et al.* Pharmacokinetic disposition and clinical outcomes in infants and children receiving intravenous busulfan for allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2007; **13**: 307–314.
18 Pui C-H, Gaynon PS, Boyett JM, Chessells JM, Baruchel A, Kamps W *et al.* Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. *Lancet* 2002; **359**: 1909–1915.
19 Dreyer ZE, Dinndorf PA, Camitta B, Sather H, La MK, Devidas M *et al.* Analysis of the role of hematopoietic stem-cell transplantation in infants with acute lymphoblastic leukemia in first remission and MLL gene rearrangements: a report from the Children's Oncology Group. *J Clin Oncol* 2011; **29**: 214–222.
20 Stumpel DJPM Schneider P, van Roon EHJ, Boer JM, de Lorenzo P, Valsecchi MG *et al.* Specific promoter methylation identifies different subgroups of MLL-rearranged infant acute lymphoblastic leukemia, influences clinical outcome, and provides therapeutic options. *Blood* 2009; **114**: 5490–5498.
21 Bernt KM, Zhu N, Sinha AU, Vempati S, Faber J, Krivtsov AV *et al.* MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. *Cancer Cell* 2011; **20**: 66–78.
22 Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J *et al.* Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer Cell* 2011; **20**: 53–65.
23 Mann G, Attarbaschi A, Schrappe M, De Lorenzo P, Peters C, Hann I *et al.* Improved outcome with hematopoietic stem cell transplantation in a poor prognostic subgroup of infants with mixed-lineage-leukemia (MLL)-rearranged acute lymphoblastic leukemia: results from the Interfant-99 Study. *Blood* 2010; **116**: 2644–2650.

Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

Prognostic impact of gained chromosomes in high-hyperdiploid childhood acute lymphoblastic leukaemia: a collaborative retrospective study of the Tokyo Children's Cancer Study Group and Japan Association of Childhood Leukaemia Study

Although the prognosis of high-hyperdiploid (HHD) acute lymphoblastic leukaemia (ALL) is excellent, relapse occurs in 10–15% of cases (Look *et al*, 1985). A gained chromosome is commonly found (Heerema *et al*, 2000; Kawamata *et al*, 2008), and previous studies reported a correlation between other chromosome combinations and outcomes. The Pediatric Oncology Group (POG) and Children's Oncology Group (COG) demonstrated that the combined gain of chromosomes 4, 10 and 17 (termed as triple trisomy) was associated with a better prognosis (Sutcliffe *et al*, 2005), and POG data suggested that +4 and +10 (termed as double trisomy) patients had a very low risk of relapse (Harris *et al*, 1992).

In this study, we performed a retrospective analysis with the Tokyo Children's Cancer Study Group (TCCSG) cohort as a test set to investigate the relationship between a combination of specific chromosome gains and disease outcomes, and used patients included in the Japan Association of Childhood Leukaemia Study (JACLS) as a validation set.

Paediatric ALL patients (aged 1–18 years old) enrolled in the TCCSG L95-14 (Igarashi *et al*, 2005) (n = 597, 1995–99), L99-15 (Manabe *et al*, 2008; Hasegawa *et al*, 2012; Kato *et al*, 2014) (n = 770, 1999–2003), and JACLS ALL97 (Suzuki *et al*, 2010) (n = 674, 1997–2002) trials were analysed. Based on cytogenetic data obtained with the G-banding test, HHD was defined as a modal chromosome number of 51 or more (Heerema *et al*, 2000), and 186 and 75 HHD patients were analysed from the TCCSG and JACLS cohorts, respectively.

Associations between the gained chromosome pattern and outcomes were investigated with all two combinations of each chromosome in the TCCSG cohort. Combinations with a small number of patients (<25% of all patients, i.e. 47 patients) were excluded. Fifteen combinations were found to have a significant impact on outcome; the association of these combinations and outcomes in the JACLS cohort was analysed for validation (Table SI and Figure S1).

Event-free survival (EFS) was calculated using Kaplan–Meier estimates, and the log-rank test was used to detect significant differences. Multivariate analysis was performed using the Cox proportional hazard regression model. All statistical analyses were performed with the statistical software R (version 2.13.0; The R Foundation for Statistical Computing, Vienna, Austria). A two-sided P-level of <0·05 was considered significant for all analyses.

The characteristics of the paediatric patients with HHD ALL are shown in Table I. The median follow-up period was 2160 d in the TCCSG cohorts. The 6-year EFS and overall

Table I. Characteristics and outcomes of patients with high-hyperdiploidy acute lymphoblastic leukaemia.

	n	Median age at diagnosis, years (range)	P	Median WBC at diagnosis, $\times 10^9$/l (range)	P	6-year EFS	P*
TCCSG cohort							
All	186	4 (1–14)		5·8 (0·3–16·5)		79·7 ± 3·2%	
+11 or +17							
Yes	135	4 (1–14)	0·85	6·2 (0·3–16·5)	0·29	83·2 ± 3·5%	0·027
No	51	3 (1–12)		4·7 (0·8–46·4)		70·8 ± 6·7%	
JACLS cohort							
All	75	3 (1–15)		5·6 (1·6–970)		86·6 ± 4%	
+11 or +17							
Yes	56	3 (1–15)	0·63	5·3 (1·6–970)	0·52	91·1 ± 3·8%	0·045
No	19	4 (1–9)		6·3 (1·9–60·1)		73·0 ± 10·4%	

TCCSG, Tokyo Children's Cancer Study Group; JACLS, Japan Association of Childhood Leukaemia study; WBC, white blood cell count.
*Log-rank test.

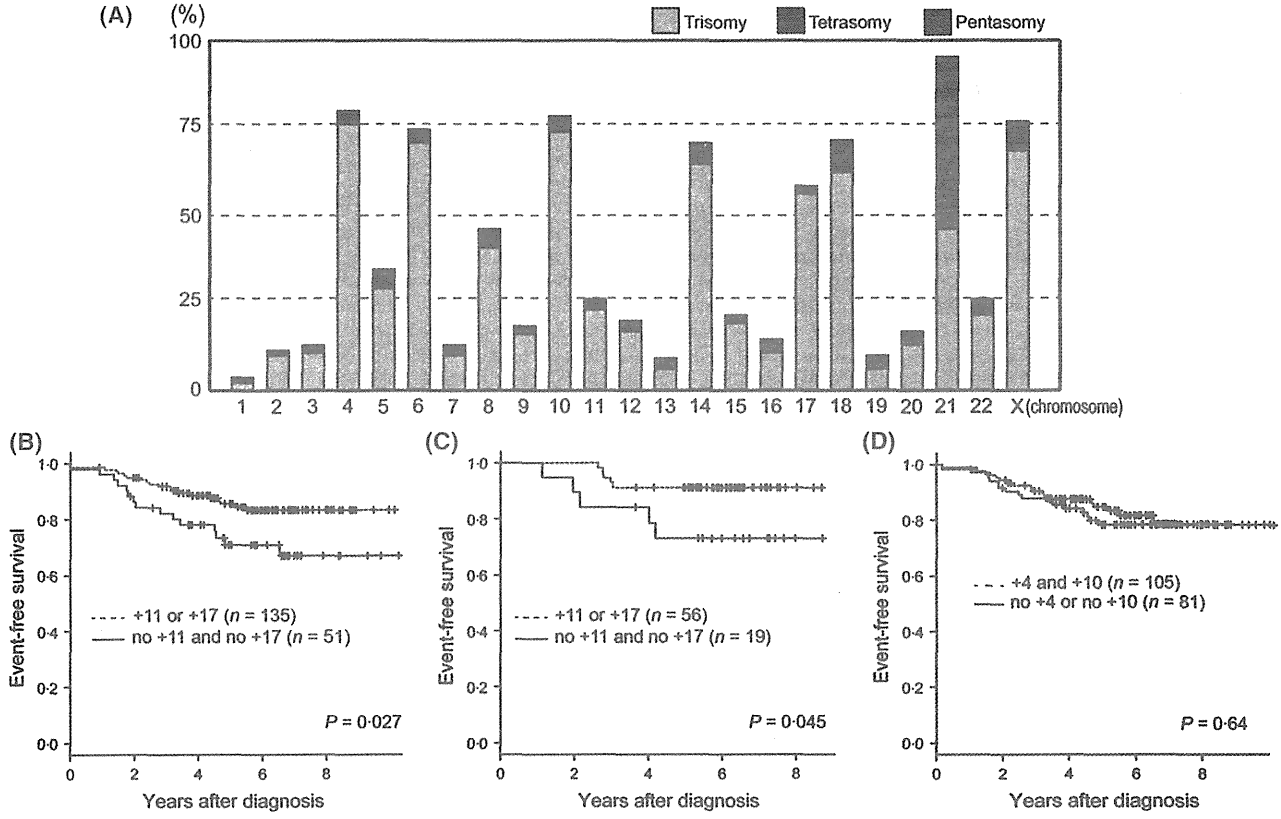

Fig 1. Gained chromosomes in HHD-ALL and their prognostic impact. (A) Frequency of gained chromosomes in 186 HHD-ALL patients in the TCCSG cohort. Event-free survival in the TCCSG and JACLS cohorts is shown. Concurrent absence of a gain of both chromosomes 11 and 17 was associated with poor outcome in the TCCSG cohort (B) and JACLS cohort (C). (D) Event-free survival of ALL patients with or without double trisomy (+4 and +10) in the TCCSG cohort. HHD, high-hyperdiploidy; ALL, acute lymphoblastic leukaemia; TCCSG, Tokyo Children's Cancer Study Group; JACLS, Japan Association of Childhood Leukaemia study.

survival (OS) for HHD patients in the TCCSG cohort was 79·7 ± 3·2% and 91·2 ± 2·3% respectively, compared to 74·4 ± 1·3% and 83·4 ± 1·1%, respectively, for non-HDD patients. The distribution of gained chromosomes in the TCCSG cohort is shown in Fig 1A. In the JACLS cohort, the median follow-up period was 2791 d, and the 6-year EFS and OS was 86·6 ± 4·0% and 97·2 ± 2·0%, respectively.

The absence of +11 and +17 was associated with a poorer outcome in the TCCSG cohort (Table I and Fig 1B). Fifty-one of 186 (27·4%) patients with HHD-ALL and no extra copies of these two chromosomes had a significantly poorer prognosis, with a 6-year EFS of 70·8 ± 6·7% compared to HHD patients with either +11 or +17 (83·2 ± 3·5%, $P = 0·027$). However, no significant difference was observed in OS between the two groups (83·5 ± 5·9% with no +11 and no +17, and 94·3 ± 2·1% with +11 or +17, $P = 0·09$). Mulitvariate analysis failed to identify these chromosome gains as statistically significant due to small sample size (Table SII).

The correlation was concordant with that found in the JACLS cohort. Nineteen of 75 (25·3%) HHD patients had neither +11 nor +17, and EFS was inferior to that of patients with +11 or +17 (73·0 ± 10·4% and 91·1 ± 3·8%, $P = 0·045$) (Fig 1C). No significant difference was observed in age, leucocyte count at diagnosis, or gender between the two groups (Table I).

In contrast to the findings of previous studies by the POG and COG (Harris *et al*, 1992; Sutcliffe *et al*, 2005), double/triple trisomy was not correlated with outcome in our cohort. In the TCCSG cohort, 105 of 186 (56·5%) patients had both +4 and +10, and EFS at 6 years was 81·2 ± 4·2%, whereas EFS of 81 patients without +4 or +10 was 77·8 ± 4·8%, which was not significantly different ($P = 0·64$) (Fig 1D).

Based on the finding that the gain of chromosomes is non-random, it is assumed that the gain of specific chromosomes contributes to leukaemogenesis, while the gain of other chromosomes is a "passenger" event, which is a by-product of leukaemic cell development.

This study showed an association between +11 and +17 and EFS probability in two independent patient cohorts, each of which received different treatments. Data from the POG and COG showed that +4, +10, and +17 was associated with outcome (Heerema *et al*, 2000; Sutcliffe *et al*, 2005), and another report on the Berlin-Frankfürt-Münster study cohort demonstrated that patients with neither +17 nor +18 had

poor outcomes (Kawamata *et al*, 2008). Thus, the gain of chromosome 17 may be the most important 'driver' abnormality in HHD pathogenesis and provide a favourable phenotype. However, it should be noted that no relapse was observed in 21 patients with +11 and +17 of the TCCSG cohort, which suggests that +11 is still associated with better prognosis even in patients with +17.

Some of our findings were inconsistent with those of previous studies. This suggests that the association between the gained chromosome combination and outcome may be influenced by the treatment regimen or ethnicity because most of the enrolled patients in the present study were Asian. The significance of the gain of specific chromosomes in HHD ALL on prognosis should be considered carefully.

Acknowledgement

All authors have no conflict of interest to disclose. We thank Kaori Itagaki for preparing and refining the protocol data for ALL in the TCCSG. We also thank Ms. Noriko Sato for the data management of JACLS ALL97. We thank Dr. Ryo Inuzuka for technical assistance. The TCCSG study was supported in part by the Children's Cancer Association of Japan.

Author contributions

A.M., K.K., H.T., T.T., Y.H., M.T. and A.O. designed the TCCSG study and collected the data. T.I., Y.H., A.S., H.H., M.I., K.H., S.K. and M.O. designed the JACLS study and collected the data. M.K. and T.I. analysed the data. M.K., T.I. and A.M wrote the paper. All authors discussed the results and commented on the manuscript.

Motohiro Kato[1,2]*
Toshihiko Imamura[3]*
Atsushi Manabe[4]
Yoshiko Hashii[5]
Katsuyoshi Koh[6]
Atsushi Sato[7]
Hiroyuki Takahashi[8]
Hiroki Hori[9]
Tomohiko Taki[10]
Masami Inoue[11]
Yasuhide Hayashi[12]
Keizo Horibe[13]
Masahiro Tsuchida[14]
Seiji Kojima[15]
Megumi Oda[16]
Akira Ohara[8]

[1]Department of Cell Therapy and Transplantation Medicine, University of Tokyo, [2]Department of Paediatrics, University of Tokyo, Tokyo, [3]Department of Paediatrics, Kyoto Prefectural University of Medicine, Kyoto, [4]Department of Paediatrics, St. Luke's International Hospital, Tokyo, [5]Department of Developmental Medicine, Osaka University, Osaka, [6]Department of Haematology/Oncology, Saitama Children's Medical Centre, Saitama, [7]Department of Haematology/Oncology, Miyagi Children's Hospital, Sendai, [8]Department of Paediatrics, Toho University, Tokyo, [9]Department of Paediatrics, Mie University, Tsu, [10]Department of Molecular Diagnostics and Therapeutics, Kyoto Prefectural University of Medicine, Kyoto, [11]Department of Haematology/Oncology, Osaka Medical Centre and Research Institute for Maternal of Child Health, Osaka, [12]Department of Haematology/Oncology, Gunma Children's Medical Centre, Maebashi, [13]Clinical Research Centre, National Hospital Organization Nagoya Medical Centre, Nagoya, [14]Department of Haematology/Oncology, Ibaraki Children's Medical Centre, Mito, [15]Department of Paediatrics, Nagoya University, Nagoya, and [16]Department of Paediatrics, Okayama University, Okayama, Japan
E-mail: katom-tky@umin.ac.jp

*These authors contributed equally to this study.

Keywords: high-hyperdiploid, acute lymphoblastic leukaemia, children, prognostic factor, chromosome

First published online 17 March 2014
doi: 10.1111/bjh.12836

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table SI. Association of gained chromosome combination and outcome.

Table SII. Multivariate analysis of risk factors on the events.

Fig S1. Flow diagram.

References

Harris, M.B., Shuster, J.J., Carroll, A., Look, A.T., Borowitz, M.J., Crist, W.M., Nitschke, R., Pullen, J., Steuber, C.P. & Land, V.J. (1992) Trisomy of leukemic cell chromosomes 4 and 10 identifies children with B-progenitor cell acute lymphoblastic leukemia with a very low risk of treatment failure: a Pediatric Oncology Group study. *Blood*, **79**, 3316–3324.

Hasegawa, D., Manabe, A., Ohara, A., Kikuchi, A., Koh, K., Kiyokawa, N., Fukushima, T., Ishida, Y., Saito, T., Hanada, R. & Tsuchida, M. (2012) The utility of performing the initial lumbar puncture on day 8 in remission induction therapy for childhood acute lymphoblastic leukemia: TCCSG L99-15 study. *Pediatric Blood & Cancer*, **58**, 23–30.

Heerema, N.A., Sather, H.N., Sensel, M.G., Zhang, T., Hutchinson, R.J., Nachman, J.B., Lange, B.J., Steinherz, P.G., Bostrom, B.C., Reaman, G.H., Gaynon, P.S. & Uckun, F.M. (2000) Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (> 50 chromosomes). *Journal of Clinical Oncology*, **18**, 1876–1887.

Igarashi, S., Manabe, A., Ohara, A., Kumagai, M., Saito, T., Okimoto, Y., Kamijo, T., Isoyama, K., Kajiwara, M., Sotomatsu, M., Sugita, K., Sugita, K., Maeda, M., Yabe, H., Kinoshita, A., Kaneko, T., Hayashi, Y., Ikuta, K., Hanada, R. & Tsuchida, M. (2005) No advantage of dexamethasone over prednisolone for the outcome of standard-

and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group L95-14 protocol. *Journal of Clinical Oncology*, **23**, 6489–6498.

Kato, M., Koh, K., Manabe, A., Saito, T., Hasegawa, D., Isoyama, K., Kinoshita, A., Maeda, M., Okimoto, Y., Kajiwara, M., Kaneko, T., Sugita, K., Kikuchi, A., Tsuchida, M. & Ohara, A. (2014) No impact of enhanced early intensification with intermediate-risk pediatric acute lymphoblastic leukemia: Results of Randomized Trial TCCSG study L99-15. *British Journal of Haematology*, **164**, 376–383.

Kawamata, N., Ogawa, S., Zimmermann, M., Kato, M., Sanada, M., Hemminki, K., Yamatomo, G., Nannya, Y., Koehler, R., Flohr, T., Miller, C.W., Harbott, J., Ludwig, W.D., Stanulla, M., Schrappe, M., Bartram, C.R. & Koeffler, H.P. (2008) Molecular allelokaryotyping of pediatric acute lymphoblastic leukemias by high-resolution single nucleotide polymorphism oligonucleotide genomic microarray. *Blood*, **111**, 776–784.

Look, A.T., Roberson, P.K., Williams, D.L., Rivera, G., Bowman, W.P., Pui, C.H., Ochs, J., Abromowitch, M., Kalwinsky, D. & Dahl, G.V. (1985) Prognostic importance of blast cell DNA content in childhood acute lymphoblastic leukemia. *Blood*, **65**, 1079–1086.

Manabe, A., Ohara, A., Hasegawa, D., Koh, K., Saito, T., Kiyokawa, N., Kikuchi, A., Takahashi, H., Ikuta, K., Hayashi, Y., Hanada, R. & Tsuchida, M. (2008) 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*, **93**, 1155–1160.

Sutcliffe, M.J., Shuster, J.J., Sather, H.N., Camitta, B.M., Pullen, J., Schultz, K.R., Borowitz, M.J., Gaynon, P.S., Carroll, A.J. & Heerema, N.A. (2005) High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology Group (COG) initiative. *Leukemia*, **19**, 734–740.

Suzuki, N., Yumura-Yagi, K., Yoshida, M., Hara, J., Nishimura, S., Kudoh, T., Tawa, A., Usami, I., Tanizawa, A., Hori, H., Ito, Y., Miyaji, R., Oda, M., Kato, K., Hamamoto, K., Osugi, Y., Hashii, Y., Nakahata, T. & Horibe, K. & Japan Association of Childhood Leukemia Study (2010) Outcome of childhood acute lymphoblastic leukemia with induction failure treated by the Japan Association of Childhood Leukemia study (JACLS) ALL F-protocol. *Pediatric Blood & Cancer*, **54**, 71–78.

Could a citrus keep the haematologist away?

Anaemia has long been recognized as one of the most important prognostic factors in chronic lymphocytic leukaemia (CLL) (Binet *et al*, 1981). Many mechanisms can cause anaemia in CLL including bone marrow infiltration, auto-immunity, cytotoxic therapy, hypersplenism, inflammation, iron deficiency and poor nutritional status (Mauro *et al*, 2002). Among nutritional causes of anaemia, vitamin B9 and vitamin B12 deficiencies are often sought, whereas vitamin C deficiency (hypovitaminosis C) is underestimated (Fain, 2004). Vitamin C, also known as ascorbic acid (AA), is one of the four main antioxidants (AA, vitamin E, selenium and β-carotene) available in human alimentation (Fain, 2004). The pathophysiology of CLL may involve oxidative stress (Sarmento-Ribeiro *et al*, 2012). Because of its role in preventing anaemia and its ability to degrade free radicals and oxidants, we hypothesized that hypovitaminosis C level could be associated with Binet stage C. Herein, we report a single-centre study comparing the blood level of vitamin C in patients with Binet stage A and Binet stage C CLL.

Between June 2012 and November 2012, we performed a prospective exploratory study of the vitamin C plasma level in patients followed for CLL at the Department of Hematology of University Hospital of Tours. In order to compare vitamin C plasma level between low and high burden of disease, we randomly selected 40 patients with Binet stage A CLL and 40 patients with Binet stage C CLL (Binet *et al*, 1981). The only exclusion criterion was ongoing vitamin C supplementation. The following baseline demographical and clinical data were recorded for all of the study patients: age, sex, weight, size, performance status and date of CLL diagnosis. The following blood tests were performed in patients who provided an informed consent: complete blood cell count, reticulocytes, aspartate aminotransferase, serum creatinine level, thyroid-stimulating hormone (TSH), C-reactive protein (CRP), serum iron level, transferrin saturation, ferritin, albumin, folic acid, vitamin B12 and vitamin C serum levels. To protect AA from light and air alteration, blood samples were collected in tubes encased in foil. Results are expressed as mean and confidence intervals. Wilcoxon's test was used to test the comparisons between the two groups. A P value of less than 0·05 was considered significant.

Baseline data and biological test results of the patients are presented in Table I. Stage C patients had lower haemoglobin level than stage A patients (107 g/l, vs. 134 g/l, respectively, $P < 0{\cdot}00005$). Anaemia was mostly normocytic, normochromic and hyporegenerative. Platelet count was also lower in stage C patients than in stage A patients (111×10^9/l, vs. 200×10^9/l, respectively, $P < 0{\cdot}00005$). Thyroid, liver and renal functions, as well as vitamin B9 and serum iron levels, were normal in both groups. CRP and ferritin levels were significantly higher in patients with Stage C (10·0 mg/l, vs. 3·0 mg/l, and 431 μg/l, vs. 117 μg/l, respectively, $P < 0{\cdot}05$ for both comparisons). There was no statistical difference in Vitamin B12 level between the two groups. Vitamin C level was significantly higher in stage A patients than in stage C patients (58·7 μmol/l, vs. 37·7 μmol/l, $P < 0{\cdot}00005$). In total, 22 CLL patients (27·5%) had hypovitaminosis C including three stage A patients (7·5%) and 19 stage C patients (47·5%).

bjh research paper

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

Motohiro Kato,[1,2]* Katsuyoshi Koh,[1]* Atsushi Manabe,[3] Tomohiro Saito,[4] Daisuke Hasegawa,[3] Keiichi Isoyama,[5] Akitoshi Kinoshita,[6] Miho Maeda,[7] Yuri Okimoto,[8] Michiko Kajiwara,[9] Takashi Kaneko,[10] Kanji Sugita,[11] Akira Kikuchi,[12] Masahiro Tsuchida[13] and Akira Ohara[14]

[1]Department of Haematology/Oncology, Saitama Children's Medical Centre, Saitama, [2]Department of Paediatrics, the University of Tokyo, [3]Department of Paediatrics, St. Luke's International Hospital, [4]Department of Health Policy, National Research Institute for Child Health and Development, Tokyo, [5]Department of Paediatrics, Showa University Fujigaoka Hospital, Yokohama, [6]Department of Paediatrics, St. Marianna University School of Medicine, Kawasaki, [7]Department of Paediatrics, Nippon Medical School, Tokyo, [8]Department of Haematology/Oncology, Chiba Children's Hospital, Chiba, [9]Department of Paediatrics, Tokyo Medical and Dental University, [10]Department of Haematology/Oncology, Tokyo Metropolitan Children's Medical Centre, Tokyo, [11]Department of Paediatrics, University of Yamanashi, Yamanashi, [12]Department of Paediatrics, Teikyo University, Tokyo, [13]Department of Paediatrics, Ibaraki Children's Hospital, Ibaraki, and [14]Department of Paediatrics, Toho University, Tokyo, Japan

Received 7 August 2013; accepted for publication 18 September 2013

Correspondence: Motohiro Kato, Department of Haematology/Oncology, Saitama Children's Medical Centre. 2100, Magome, Iwatsuki-ku, Saitama, 339-8551, Japan.
E-mail: katom-tky@umin.ac.jp
*These two authors equally contributed to this study.

Summary

The Tokyo Children's Cancer Study Group conducted a randomized controlled study to evaluate the effect of experimental early intensification using high-dose cytarabine and L-asparaginase in paediatric intermediate-risk (IR) acute lymphoblastic leukaemia (ALL). A total of 310 IR ALL patients were randomized to receive either experimental early intensification (n = 156) or standard early intensification including standard-dose cytarabine arm (n = 154) after induction therapy. The experimental arm consisted of high-dose cytarabine and L-asparaginase, while the standard arm consisted of standard-dose cytarabine, oral 6-mercaptopurine and cyclophosphamide. The probabilities of event-free survival at 8 years in the experimental and standard arms were 72·3 ± 3·7% and 77·5 ± 3·5%, respectively (P = 0·32). The 8-year overall survival rates for these two arms were 85·0 ± 3·0% and 86·9 ± 2·8%, respectively (P = 0·72). The frequency of infectious events was significantly higher in the experimental arm (66·4%) than in the standard arm (24·6%) (P < 0·001). In conclusion, experimental early intensification including high-dose cytarabine followed by L-asparaginase had no advantage over standard early intensification in paediatric IR ALL patients.

Keywords: acute lymphoblastic leukaemia, child, randomized trial, early intensification, high-dose cytarabine.

First published online 26 October 2013
doi: 10.1111/bjh.12632

Introduction

Chemotherapy for paediatric acute lymphoblastic leukaemia (ALL) typically comprises three phases: remission induction, consolidation and maintenance(Inaba *et al*, 2013). The importance of each component has been confirmed by several clinical trials and provided dramatic improvement of treatment outcome; however, a certain fraction of ALL patients still suffer from relapse or severe adverse events, thus optimal drug doses and administration schedule need to be determined.

Based on the Goldie-Coldman hypothesis(Goldie & Coldman, 1979), early reduction of leukaemic cells by multiple and non-cross resistant agents is essential to prevent relapse and improve treatment outcome. Consequently, we hypothesized that optimization of early intensification could improve the survival probability of paediatric ALL. To confirm this hypothesis, we performed a randomized controlled trial to compare our experimental early intensification with standard early intensification in the Tokyo Children's Cancer Study Group (TCCSG) L99-15 study.

As a standard early intensification, the combination of 6-mercaptopurine (6MP) and standard doses of cytarabine (AraC) and cyclophosphamide has been widely adopted in several study groups, including TCCSG. To optimize this essential component, we amended the standard early intensification to an experimental regimen, which included high-dose AraC (HD-AraC) followed by L-asparaginase (L-ASP), based on the 'Capizzi regimen', which showed a synergic effect for relapsed acute myeloid leukaemia (AML) (Capizzi *et al*, 1988).

To date, no studies have investigated the effectiveness of early intensification using HD-AraC in intermediate-risk (IR) ALL, but this drug has become widely employed in the treatment of children with AML (Mayer *et al*, 1994; Lie *et al*, 2005; Ravindranath *et al*, 2005; Becton *et al*, 2006; Tomizawa *et al*, 2007; Creutzig *et al*, 2011) and relapsed ALL(Wells *et al*, 1985; Bernstein *et al*, 1997; Einsiedel *et al*, 2005). Previous studies showed that the addition of HD-AraC to regimens based on the Berlin-Frankfurt-Muenster (BFM) protocol failed to improve overall treatment outcomes of paediatric ALL(Schrappe *et al*, 2000; Millot *et al*, 2001); however, some studies showed that it demonstrated excellent outcomes in high-risk ALL patients(Arico *et al*, 2002; Pui *et al*, 2009).

Here we report the results of the TCCSG L99-15 randomized controlled trial to investigate effectiveness of experimental early intensification with HD-AraC/asparaginase for children with IR ALL.

Patients and methods

Patients

Between April 1999 and June 2003, 770 children (aged 1–18 years) with previously untreated ALL were consecutively enrolled in the TCCSG L99-15 study (Fig 1). The protocol was approved by the institutional review boards of the participating institutions or equivalent organizations. Written informed consent was obtained from the parents or guardians, and from the patients when appropriate for their age and conceptual ability. Sixteen patients were excluded due to severe complications before starting the treatment ($n = 8$) or a lack of data ($n = 8$). Thus, 754 patients were evaluable for analysis.

The diagnosis of ALL was based on the following; morphological, biochemical and flow cytometric features of leukaemic cells, including lymphoblast morphology on May- or Wright-Giemsa-stained bone marrow smears, negative staining for myeloperoxidase and reactivity with monoclonal antibodies to B-lineage-associated or T-lineage-associated lymphoid differentiation antigens, as described elsewhere (Toyoda *et al*, 2000; Manabe *et al*, 2001; Igarashi *et al*, 2005). Remission was defined as the presence of <5% blasts with the recovery of normal haematopoiesis.

The overall outcome of children registered on TCCSG L99-15 has been published(Manabe *et al*, 2008; Hasegawa *et al*, 2011), and the event-free survival of all 754 patients at 4 years was 87·6 ± 1·2%.

Risk classification

At diagnosis, patients were tentatively assigned to a standard-risk (SR), intermediate-risk (IR) or high-risk (HR) group according to patient age and peripheral blood leucocyte count (Fig S1). The initial risk grouping was modified after identification of the prednisolone (PSL) response, cytogenetic analysis and other clinical features, as previously described (Manabe *et al*, 2008; Hasegawa *et al*, 2011). The PSL response was defined by the blast cell count in the peripheral blood on day 8 after a 7-day exposure to PSL: PSL very good response (PVGR) with undetectable blasts, PSL good response (PGR) with a blast cell count of 0·001–0·999 $\times$ 10^9/l, and prednisolone poor response (PPR) with a blast cell count >1·0 x 10^9/l.

For B-cell precursor ALL, SR was initially defined as age 1–6 years and a leucocyte count < 20 $\times$ 10^9/l at diagnosis. HR was initially age 9 years or older, or a leucocyte count >100 $\times$ 10^9/l. Patients who were neither SR nor HR were initially defined as IR. Initial SR patients with PPR and initial HR patients with VGPR were reclassified into the IR group. Initial IR patients with VGPR/PGR were stratified into the IR group. IR patients also included T-lineage ALL patients with PVGR and SR patients with t(1;19) or the *TCF3-PBX1* chimeric gene. Patients with the Philadelphia chromosome (Ph1) or 11q23 were excluded from the IR group and included in the haematopoietic stem cell transplantation group.

Treatment protocol

Remission induction therapy for IR patients consisted of prednisolone, vincristine, *E. coli* L-asparaginase(L-ASP), daunorubicin, cyclophosphamide and triple intrathecal (IT) injections. IR patients who achieved complete remission after

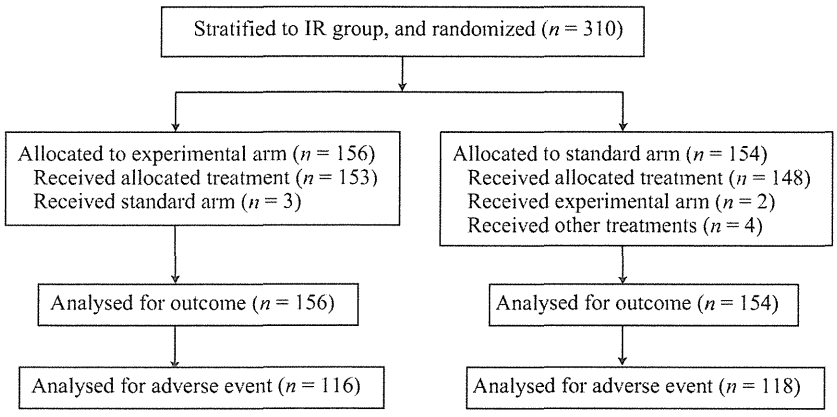

Fig 1. CONSORT diagram of the TCCSG L99-15 study. IR: intermediate risk.

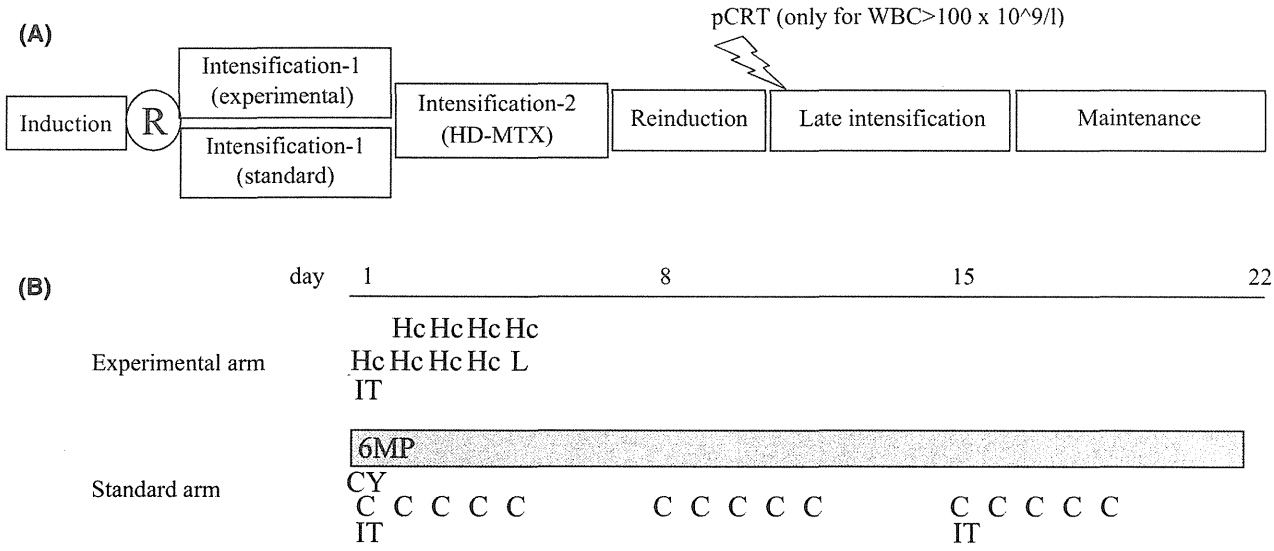

Fig 2. Treatment schema for the IR group in the TCCSG L99-15 study. (A) The treatment outline and (B) detailed schedule of the randomized arm are shown. Abbreviations: R, randomization; pCRT, prophylactic cranial irradiation; WBC, white blood cell count; HD-MTX, high dose methotrexate; Hc, high dose cytarabine; L, L-asparaginase; C, cytarabine; CY, cyclophosphamide; IT, intrathecal injection.

induction therapy were randomly assigned into either the experimental arm or standard arm (Fig 1). In the experimental arm, 8 doses of HD-AraC (2 g/m^2) were given twice a day (every 12 h) beginning on the evening of day 1, followed by L-ASP 3 h after the last dose of HD-AraC. Triple-IT injections were administered on day 1. In the standard arm, 5 doses of standard-dose AraC (75 mg/m^2) were given by an intravenous push once a day from days 1–5, days 8–12 and days 15–18. Oral 6MP 60 mg/m^2 was given from day 1 to day 21. Cyclophosphamide 1 g/m^2 was given on day 1, and two doses of IT-methotrexate were administered on days 1 and 15. The subsequent treatment protocol was identical for both of the arms (Fig 2, Table S1). Ten patients whose initial leucocyte count was more than 100 x 10^9/l were allocated to undergo prophylactic cranial irradiation.

Statistical analysis

The data were analysed as of October 2012. Event-free survival (EFS) was defined as the time from the initiation of therapy to either treatment failure (relapse, death or the diagnosis of secondary cancer) or to the time of the last follow-up. Overall survival (OS) was defined as the time from the initiation of therapy to death from any cause or the time of the last follow-up. The probability of EFS and OS was estimated by the Kaplan-Meier method and was tested for significant differences using the log-rank test. A 2-sided *P*-value <0·05 was considered statistically significant. Patients who were inadvertently or intentionally treated by the different regimens were analysed according to their randomly assigned arm (intent-to-treat analysis).

Results

Patient characteristics

In total, 310 (41·1%) of 754 evaluable patients were allocated to the IR group and randomized, 156 patients to the experimental arm and 154 to the standard arm. The laboratory and clinical characteristics of these IR patients are shown in

Table I. No feature was significantly different between the two arms.

Treatment outcomes

The median follow-up period of survivors was 8·6 years. The estimated 8-year probabilities (± standard error) of EFS for patients in the experimental and standard arms were 72·3 ± 3·7% and 77·5 ± 3·5%, respectively, and were not significantly different ($P = 0·32$) (Fig 3A). The estimated 8-year OS rate was 85·0 ± 3·0% for the experimental arm and 86·9 ± 2·8% for the standard arm, and the difference was not statistically significant ($P = 0·72$) (Fig 3B).

No significant difference of EFS was observed between the two arms in patients with hyperdiploidy (77·3 ± 8·2% in the experimental group and 72·3 ± 7·9% in the standard group, $P = 0·74$), *ETV6-RUNX1* (84·6 ± 10·0% in the experimental group and 87·5 ± 8·3% in the standard group, $P = 0·82$) or *TCF3-PBX1* (69·5 ± 13·1% in the experimental group and 90·0 ± 8·7% in the standard group, $P = 0·22$).

The distribution of the relapse site was similar in the two arms ($P = 0·30$) (Table II). Isolated bone marrow relapse was the main cause of treatment failure in both arms. The incidences of central nervous system (CNS) relapse, whether isolated or combined, was similar in both arms.

Table I. Clinical characteristics of randomized patients.

Patient characteristics	Experimental arm ($n = 156$)	Standard arm ($n = 154$)	P-value
Median age, years (range)	8 (1–16)	8 (1–15)	0·96
male:female, n	88:68	85:69	0·83
WBC at diagnosis, n (%)			
<20 x 10^9/l	92 (59·0)	92 (59·7)	0·18
20–<50 x 10^9/l	45 (28·8)	43 (27·9)	
≥50–<100 x 10^9/l	17 (10·9)	11 (7·1)	
≥100 x 10^9/l	2 (1·3)	8 (5·2)	
Immunophenotype, n (%)			
non-T	145	143	1·00
T	11	11	
CNS			
CNS-1	151 (96·8)	150 (97·4)	0·41
CNS-2	0 (0·0)	1 (0·6)	
CNS-3	4 (2·6)	1 (0·6)	
No data	1 (0·6)	2 (1·3)	
Blast in PB on day 8			
<1000	143 (91·7)	143 (92·9)	0·83
≥1000	13 (8·3)	11 (7·1)	
Cytogenetics, n (%)			
Hyperdiploidy	27 (17·3)	33 (21·4)	0·47
ETV6-RUNX1	13 (8·3)	17 (11·0)	
TCF3-PBX1	17 (10·9)	11 (7·1)	

WBC, white blood cell count; CNS, central nervous system; PB, peripheral blood.

Toxicity

Detailed information regarding toxic adverse effects was available in 116 patients in the standard arm and 118 in the standard arm. The frequency of toxicity during early intensification is shown in Fig 4 and Table III. No patient in either arm died in this phase. The standard arm patients required more red blood cell transfusions ($P < 0·001$) (Fig 4A), though the frequency of platelet transfusions was not significantly different (Fig 4B). The experimental arm showed a trend toward a longer duration of severe neutropenia (<0·005 x10^9/l) (median of 11 days), compared to the standard arm (median of 8 days), although this did not reach statistical significance ($P = 0·08$) (Fig 4C). However, the period before starting the next treatment block, intensification-2, was shorter in the experimental arm than in the standard arm, with a median of 33 and 39 days, respectively ($P < 0·001$) (Fig 4D).

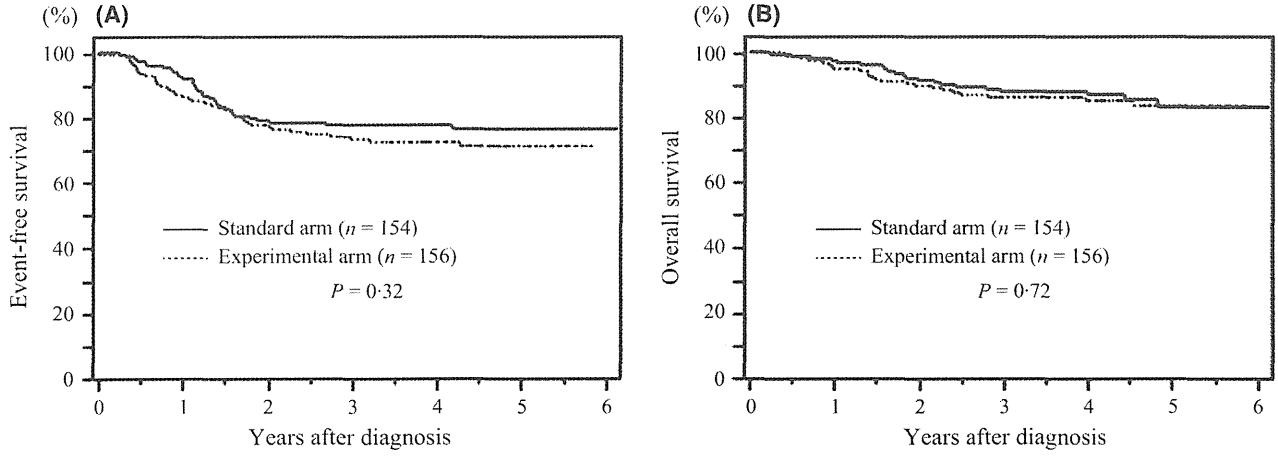

Fig 3. Event-free survival and overall survival of intermediate-risk patients in the TCCSG L99-15 study. (A) The event-free survival curve and (B) overall survival curve are shown for the experimental and standard arms.

Table II. Distribution of relapse sites according to treatment arms.

Relapse	Experimental arm	Standard arm
Total, n	36	30
Bone marrow	25	20
CNS	4	3
Testis	2	2
Bone marrow and CNS	3	3
Bone marrow and testis	0	1
Others	2	1

The distribution of relapse sites was not significantly different ($P = 0{\cdot}30$, paired t-test). CNS, central nervous system.

The frequency of infectious events was significantly higher in the experimental arm (77 of 116, 66·4%) than in the standard arm (29 of 118, 24·6%) ($P < 0{\cdot}001$), and most of the infectious events were febrile neutropenia. Of note, the incidence of bacterial sepsis was significantly higher in the experimental arm (23 of 116, 19·8%) than in the standard arm (5 of 118, 4·2%) ($P < 0{\cdot}001$). The infections were caused by streptococci in 16 of the 23 experimental patients, most of which were identified as viridans streptococci. The coagulation disorder, disseminated intravascular coagulation, was seen in one patient assigned to the experimental arm. Four patients had low fibrinogen levels due to the administration of L-ASP, but thrombotic and haemorrhagic events were not seen.

Discussion

Although the survival probability of paediatric ALL has improved to around 90%, further optimization of chemotherapy is still required. Previous studies showed that augmentation of standard BFM-backbone intensification chemotherapy with vincristine and L-ASP could provide an advantage in high-risk ALL(Nachman *et al*, 1998). In the L99-15 study, the TCCSG performed a randomized trial to investigate the effect of experimental early intensification with HD-AraC and L-ASP, which showed a synergic combination(Capizzi *et al*, 1988), but this combination failed to improve survival outcome in spite of more frequent infectious adverse events.

The addition of HD-AraC in early consolidation therapy for paediatric ALL was implemented in some clinical trials (Schrappe *et al*, 2000; Millot *et al*, 2001); however, the significance of HD-AraC administered shortly after remission induction therapy has never been evaluated. The St. Jude

Table III. Non-haematological toxicity according to treatment arms.

Measure	Experimental arm (n = 116)	Standard arm (n = 118)
Toxicity		
Infection	77	29
Febrile neutropenia	51	24
Sepsis	23	5
Meningitis	1	0
Pneumonia	2	0
Liver enzyme	22	26
Coagulation	5	0
Gastrointestinal	7	4
Neurology	2	1
Allergy	0	2

Grade 3/4 adverse events were counted.

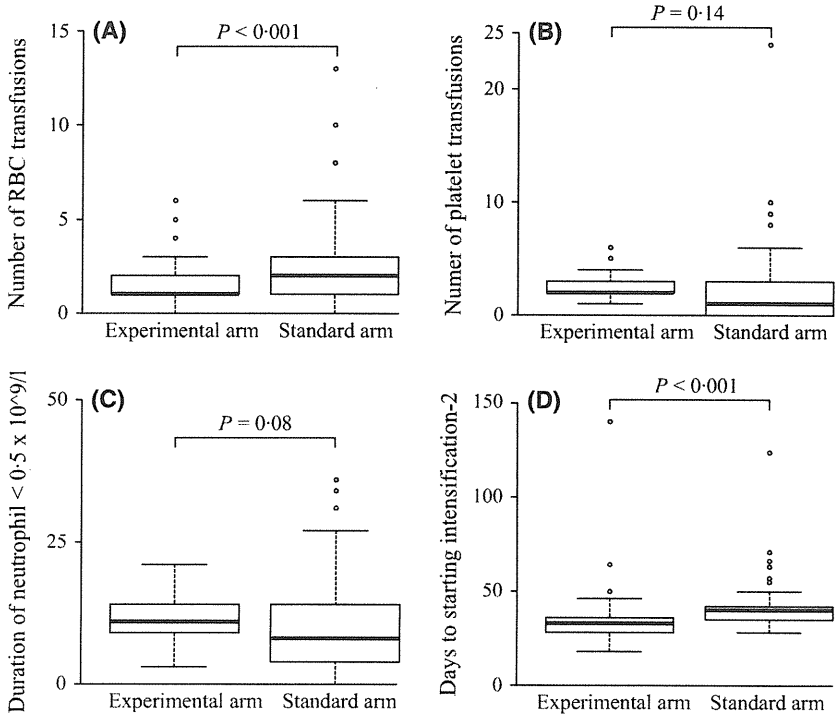

Fig 4. Haematological toxicity in the randomized phase. (A) The number of red blood cell (RBC) transfusions and (B) platelet transfusions for each of the treatment arms are shown. (C) The duration of a neutrophil count <0·05 × 10^9/l and (D) the time (days) before starting the next treatment block (intensification-2). The P-value was calculated by a non-paired t-test.

total XV(Pui *et al*, 2009) and Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIEOP)-ALL95(Arico *et al*, 2002) trials employed HD-AraC in the late consolidation phase and showed excellent treatment outcomes for HR patients, whereas the European Organization for Research and Treatment of Cancer (EORTC) Children's Leukaemia Cooperative Group (CLCG) failed to show an advantage of adding HD-AraC to high-dose methotrexate (HD-MTX) after early consolidation in a BFM-based protocol, in increased-risk ALL patients(Millot *et al*, 2001).

Our study was different from the EORTC-CLCG study in the timing and dosage of HD-AraC, where HD-AraC (1 g/m^2 × 2) was added to HD-MTX as CNS prophylaxis(Millot *et al*, 2001). In our study, HD-AraC concurrent with L-ASP was administered in the earlier phase of treatment and the dosage was more intensive (2 g/m^2 ×8), as we hypothesized that the synergistic effect of HD-AraC followed by L-ASP would reduce systemic and CNS relapse(Capizzi & Cheng, 1982; Capizzi *et al*, 1984). However, similar to the results of the EORTC-CLCG study, our study showed that the treatment outcome of HD-AraC was not superior to that of standard-dose AraC, while patients in HD-AraC arm had a higher frequency of infections including bacterial sepsis. A pharmacokinetic study by showed that the cellular retention of arabinoside triphosphate, the active metabolite of AraC, in leukaemic blasts was shorter in blasts from patients with T-cell ALL and AML compared to non-T ALL(Boos *et al*, 1996); therefore, the intensive administration of AraC such as HD-AraC and continuous infusion may not be important in the treatment of non-high risk ALL.

Although our study could not show survival advantage for IR ALL, it should be noted that our late intensification phase was more intensive than that given for similar risk patients in other study groups. The late intensification, which included HD-AraC for both arms, may minimize the benefit of our experimental early intensification. Our results do not address whether the experimental early intensification would or would not be beneficial if it was added to a less intensive treatment backbone.

An advantage of HD-AraC is its ability to reduce the incidence of CNS relapse because of its high penetration into CNS regions(Morra *et al*, 1993; Ritchey *et al*, 1999). Contrary to our expectation, however, the frequency of CNS relapses in the HD-AraC arm was not lower than that in the SD-AraC arm. The EORTC-CLCG studies showed that HD-AraC failed to reduce the incidence of CNS relapses (Millot *et al*, 2001), suggesting that their treatment protocol including HD-MTX combined with IT injections was intensive enough to protect against CNS relapses. Our treatment protocol also contained HD-MTX, intermediate dose -MTX, IT injections, and HD-AraC in late consolidation therapy, all of which could serve as CNS prophylaxis(Abromowitch *et al*, 1988; Morra *et al*, 1993; Conter *et al*, 1995), while the number of patients who received cranial irradiation in our study was low (3·2% of IR patients). We conclude that augmentation of early intensification with HD-AraC and L-ASP has little effect in reducing CNS relapse if sufficient CNS prophylaxis, consisting of intravenous MTX and IT injections, is given in the subsequent chemotherapy.

HD-AraC in early intensification was well-tolerated by most patients without any treatment-related death. However, patients in the HD-AraC/L-ASP arm had deeper myelosuppression and more serious infections than those in the standard arm, although the period of recovery was shorter than that in the standard arm. Earlier start of the next treatment block failed to reduce the rate of relapse. Previous trials for paediatric AML showed that the intensive timed Capizzi regimen was more effective than standard time Capizzi regimen, which was similar to our experimental regimen(Smith *et al*, 2005). From our results, the intensive timed Capizzi regimen would result in a higher frequency of infectious events, although it might provide better disease control in paediatric ALL.

The failure to improve overall outcomes and the occurrence of relatively more severe complications prompted us to abort experimental early intensification. In the subsequent TCCSG studies, L99-1502 and L04-16, treatment protocols for IR patients have contained only standard BFM-based early intensification. Although our experimental early intensification could not improve survival outcome, our study provides useful information to establish an optimal treatment regimen for paediatric ALL.

Acknowledgments

We thank Kaori Itagaki for preparing and refining the protocol data for ALL in the TCCSG. We also thank the paediatricians and nurses who participated in the treatment and follow-up of the patients in this study. This study was supported in part by the Children's Cancer Association of Japan.

Author contributions

K.K., A.M, T.S, K.I, A.Kinoshita, M.M, Y.O, M.Kajiwara, T.K, K.S, A.Kikuchi, M.T, and A.O designed the research. M.Kato, A.M, and A.O collected the data. M.Kato and A.O analysed the data, and M.Kato, K.K, A.M, D.H and A.O wrote the manuscript. All the authors discussed the results and commented on the manuscript.

Conflict of interests

None of the authors have any conflicts of interest to disclose.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Treatment Schedule of IR group for L99-15.

References

Abromowitch, M., Ochs, J., Pui, C.H., Fairclough, D., Murphy, S.B. & Rivera, G.K. (1988) Efficacy of high-dose methotrexate in childhood acute lymphocytic leukemia: analysis by contemporary risk classifications. *Blood*, **71**, 866–869.

Arico, M., Valsecchi, M.G., Conter, V., Rizzari, C., Pession, A., Messina, C., Barisone, E., Poggi, V., De Rossi, G., Locatelli, F., Micalizzi, M.C., Basso, G. & Masera, G. (2002) Improved outcome in high-risk childhood acute lymphoblastic leukemia defined by prednisone-poor response treated with double Berlin-Frankfurt-Muenster protocol II. *Blood*, **100**, 420–426.

Becton, D., Dahl, G.V., Ravindranath, Y., Chang, M.N., Behm, F.G., Raimondi, S.C., Head, D.R., Stine, K.C., Lacayo, N.J., Sikic, B.I., Arceci, R.J., Weinstein, H. & Pediatric Oncology, G. (2006) Randomized use of cyclosporin A (CsA) to modulate P-glycoprotein in children with AML in remission: Pediatric Oncology Group Study 9421. *Blood*, **107**, 1315–1324.

Bernstein, M.L., Abshire, T.C., Pollock, B.H., Devine, S., Toledano, S., Steuber, C.P., Bowman, W.P. & Buchanan, G.R. (1997) Idarubicin and cytosine arabinoside reinduction therapy for children with multiple recurrent or refractory acute lymphoblastic leukemia: a Pediatric Oncology Group study. *Journal of Pediatric Hematology/oncology*, **19**, 68–72.

Boos, J., Hohenlochter, B., Schulze-Westhoff, P., Schiller, M., Zimmermann, M., Creutzig, U., Ritter, J. & Jurgens, H. (1996) Intracellular retention of cytosine arabinoside triphosphate in blast cells from children with acute myelogenous and lymphoblastic leukemia. *Medical and Pediatric Oncology*, **26**, 397–404.

Capizzi, R.L. & Cheng, Y.C. (1982) Sequential high-dose cytosine arabinoside and asparaginase in refractory acute leukemia. *Medical and Pediatric Oncology*, **10**, 221–228.

Capizzi, R.L., Poole, M., Cooper, M.R., Richards, F. 2nd, Stuart, J.J., Jackson, D.V. Jr, White, D.R., Spurr, C.L., Hopkins, J.O. & Muss, H.B. (1984) Treatment of poor risk acute leukemia with sequential high-dose ARA-C and asparaginase. *Blood*, **63**, 694–700.

Capizzi, R.L., Davis, R., Powell, B., Cuttner, J., Ellison, R.R., Cooper, M.R., Dillman, R., Major, W.B., Dupre, E. & McIntyre, O.R. (1988) Synergy between high-dose cytarabine and asparaginase in the treatment of adults with refractory and relapsed acute myelogenous leukemia–a Cancer and Leukemia Group B Study. *Journal of Clinical Oncology*, **6**, 499–508.

Conter, V., Arico, M., Valsecchi, M.G., Rizzari, C., Testi, A.M., Messina, C., Mori, P.G., Miniero, R., Colella, R. & Basso, G. (1995) Extended intrathecal methotrexate may replace cranial irradiation for prevention of CNS relapse in children with intermediate-risk acute lymphoblastic leukemia treated with Berlin-Frankfurt-Munster-based intensive chemotherapy. The Associazione Italiana di Ematologia ed Oncologia Pediatrica. *Journal of Clinical Oncology*, **13**, 2497–2502.

Creutzig, U., Zimmermann, M., Bourquin, J.P., Dworzak, M.N., von Neuhoff, C., Sander, A., Schrauder, A., Teigler-Schlegel, A., Stary, J., Corbacioglu, S. & Reinhardt, D. (2011) Second induction with high-dose cytarabine and mitoxantrone: different impact on pediatric AML patients with t(8;21) and with inv(16). *Blood*, **118**, 5409–5415.

Einsiedel, H.G., von Stackelberg, A., Hartmann, R., Fengler, R., Schrappe, M., Janka-Schaub, G., Mann, G., Hahlen, K., Gobel, U., Klingebiel, T., Ludwig, W.D. & Henze, G. (2005) 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. *Journal of Clinical Oncology*, **23**, 7942–7950.

Goldie, J.H. & Coldman, A.J. (1979) A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. *Cancer Treatment Reports*, **63**, 1727–1733.

Hasegawa, D., Manabe, A., Ohara, A., Kikuchi, A., Koh, K., Kiyokawa, N., Fukushima, T., Ishida, Y., Saito, T., Hanada, R. & Tsuchida, M. (2011) The utility of performing the initial lumbar puncture on day 8 in remission induction therapy for childhood acute lymphoblastic leukemia: TCCSG L99-15 study. *Pediatric Blood & Cancer*, **58**, 23–31.

Igarashi, S., Manabe, A., Ohara, A., Kumagai, M., Saito, T., Okimoto, Y., Kamijo, T., Isoyama, K., Kajiwara, M., Sotomatsu, M., Sugita, K., Maeda, M., Yabe, H., Kinoshita, A., Kaneko, T., Hayashi, Y., Ikuta, K., Hanada, R. & Tsuchida, M. (2005) No advantage of dexamethasone over prednisolone for the outcome of standard- and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group L95-14 protocol. *Journal of Clinical Oncology*, **23**, 6489–6498.

Inaba, H., Greaves, M. & Mullighan, C.G. (2013) Acute lymphoblastic leukaemia. *Lancet*, **381**, 1943–1955.

Lie, S.O., Abrahamsson, J., Clausen, N., Forestier, E., Hasle, H., Hovi, L., Jonmundsson, G., Mellander, L., Siimes, M.A., Yssing, M., Zeller, B. & Gustafsson, G. (2005) Long-term results in children with AML: NOPHO-AML Study Group–report of three consecutive trials. *Leukemia*, **19**, 2090–2100.

Manabe, A., Tsuchida, M., Hanada, R., Ikuta, K., Toyoda, Y., Okimoto, Y., Ishimoto, K., Okawa, H., Ohara, A., Kaneko, T., Koike, K., Sato, T., Sugita, K., Bessho, F., Hoshi, Y., Maeda, M., Kinoshita, A., Saito, T., Tsunematsu, Y. & Nakazawa, S. (2001) Delay of the diagnostic lumbar puncture and intrathecal chemotherapy in children with acute lymphoblastic leukemia who undergo routine corticosteroid testing: Tokyo Children's Cancer Study Group study L89-12. *Journal of Clinical Oncology*, **19**, 3182–3187.

Manabe, A., Ohara, A., Hasegawa, D., Koh, K., Saito, T., Kiyokawa, N., Kikuchi, A., Takahashi, H., Ikuta, K., Hayashi, Y., Hanada, R. & Tsuchida, M. (2008) 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*, **93**, 1155–1160.

Mayer, R.J., Davis, R.B., Schiffer, C.A., Berg, D.T., Powell, B.L., Schulman, P., Omura, G.A., Moore, J.O., McIntyre, O.R. & Frei, E. 3rd (1994) Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. *New England Journal of Medicine*, **331**, 896–903.

Millot, F., Suciu, S., Philippe, N., Benoit, Y., Mazingue, F., Uyttebroeck, A., Lutz, P., Mechinaud, F., Robert, A., Boutard, P., Marguerite, G., Ferster, A., Plouvier, E., Rialland, X., Behard, C., Plantaz, D., Dresse, M.F., Philippet, P., Norton, L., Thyss, A., Dastugue, N., Waterkeyn, C., Vilmer, E. & Otten, J. (2001) Value of high-dose cytarabine during interval therapy of a Berlin-Frankfurt-Munster-based protocol in increased-risk children with acute lymphoblastic leukemia and lymphoblastic lymphoma: results of the European Organization for Research and Treatment of Cancer 58881 randomized phase III trial. *Journal of Clinical Oncology*, **19**, 1935–1942.

Morra, E., Lazzarino, M., Brusamolino, E., Pagnucco, G., Castagnola, C., Bernasconi, P., Orlandi, E., Corso, A., Santagostino, A. & Bernasconi, C. (1993) The role of systemic high-dose cytarabine in the treatment of central nervous system leukemia. Clinical results in 46 patients. *Cancer*, **72**, 439–445.

Nachman, J.B., Sather, H.N., Sensel, M.G., Trigg, M.E., Cherlow, J.M., Lukens, J.N., Wolff, L., Uckun, F.M. & Gaynon, P.S. (1998) Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. *New England Journal of Medicine*, **338**, 1663–1671.

Pui, C.H., Campana, D., Pei, D., Bowman, W.P., Sandlund, J.T., Kaste, S.C., Ribeiro, R.C., Rubnitz, J.E., Raimondi, S.C., Onciu, M., Coustan-Smith, E., Kun, L.E., Jeha, S., Cheng, C., Howard, S.C., Simmons, V., Bayles, A., Metzger, M.L., Boyett, J.M., Leung, W., Handgretinger, R., Downing, J.R., Evans, W.E. & Relling, M.V. (2009) Treating childhood acute lymphoblastic leukemia without cranial irradiation. *New England Journal of Medicine*, **360**, 2730–2741.

Ravindranath, Y., Chang, M., Steuber, C.P., Becton, D., Dahl, G., Civin, C., Camitta, B., Carroll, A., Raimondi, S.C. & Weinstein, H.J. (2005) Pediatric Oncology Group (POG) studies of acute myeloid leukemia (AML): a review of four consecutive childhood AML trials conducted between 1981 and 2000. *Leukemia*, **19**, 2101–2116.

Ritchey, A.K., Pollock, B.H., Lauer, S.J., Andejeski, Y., Barredo, J. & Buchanan, G.R. (1999) Improved survival of children with isolated CNS relapse of acute lymphoblastic leukemia: a

pediatric oncology group study. *Journal of Clinical Oncology*, **17**, 3745–3752.

Schrappe, M., Reiter, A., Ludwig, W.D., Harbott, J., Zimmermann, M., Hiddemann, W., Niemeyer, C., Henze, G., Feldges, A., Zintl, F., Kornhuber, B., Ritter, J., Welte, K., Gadner, H. & Riehm, H. (2000) Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. *German-Austrian-Swiss ALL-BFM Study Group. Blood*, **95**, 3310–3322.

Smith, F.O., Alonzo, T.A., Gerbing, R.B., Woods, W.G. & Arceci, R.J. (2005) Long-term results of children with acute myeloid leukemia: a report of three consecutive Phase III trials by the Children's Cancer Group: CCG 251, CCG 213 and CCG 2891. *Leukemia*, **19**, 2054–2062.

Tomizawa, D., Tabuchi, K., Kinoshita, A., Hanada, R., Kigasawa, H., Tsukimoto, I. & Tsuchida, M. (2007) Repetitive cycles of high-dose cytarabine are effective for childhood acute myeloid leukemia: long-term outcome of the children with AML treated on two consecutive trials of Tokyo Children's Cancer Study Group. *Pediatric Blood & Cancer*, **49**, 127–132.

Toyoda, Y., Manabe, A., Tsuchida, M., Hanada, R., Ikuta, K., Okimoto, Y., Ohara, A., Ohkawa, Y., Mori, T., Ishimoto, K., Sato, T., Kaneko, T., Maeda, M., Koike, K., Shitara, T., Hoshi, Y., Hosoya, R., Tsunematsu, Y., Bessho, F., Nakazawa, S. & Saito, T. (2000) Six months of maintenance chemotherapy after intensified treatment for acute lymphoblastic leukemia of childhood. *Journal of Clinical Oncology*, **18**, 1508–1516.

Wells, R.J., Feusner, J., Devney, R., Woods, W.G., Provisor, A.J., Cairo, M.S., Odom, L.F., Nachman, J., Jones, G.R. & Ettinger, L.J. (1985) Sequential high-dose cytosine arabinoside-asparaginase treatment in advanced childhood leukemia. *Journal of Clinical Oncology*, **3**, 998–1004.

第76回日本血液学会学術集会
小児疾患
EL-64 疾患

小児ALLの治療の現状

康 勝好

Key words : Acute lymphoblastic leukemia (ALL), Prognostic factor, Minimal residual disease (MRD), Randomized controlled trial (RCT)

諸 言

小児急性リンパ性白血病（acute lymphoblastic leukemia, ALL）の治療成績は飛躍的に向上し，現在では約90%の長期生存率が達成されつつある[1,2]。今後，短期，長期の毒性を軽減しつつ，さらに治療成績を向上させるためには，新たなbiology研究の成果を取り入れたより精密な層別化と，新薬を含む新たな治療戦略の開発が必要である。本稿では小児ALLの治療の現状について，最近の知見や国内の新たな臨床試験の動向を含めて概説する。

1. 小児ALLの治療成績と国内の状況

小児ALLの治療成績は過去40年間に飛躍的に向上し，約70～85%の無イベント生存率（EFS），約80～90%の長期生存率（OS）が達成されている[1,2]。Table 1に最近の世界の治療研究グループの治療成績，Fig. 1に米国St. Jude小児病院における治療成績の変遷を示す。これらの治療成績の進歩は，ランダム化比較試験（randomized controlled trial, RCT）を含む多数の臨床試験の積み重ねと，予後因子に基づく層別化の改善によって達成されてきた。

国内においても，日本小児白血病研究会（JACLS），東京小児がん研究グループ（TCCSG），小児癌・白血病研究グループ（CCLSG），九州山口小児がん研究グループ（KYCCSG）の4グループがそれぞれ臨床研究を行い，重要な成果を挙げてきた。近年の治療成績は欧米とほぼ同等である[3～7]。

JACLSはALL-02研究において1,000例を越える国内最大規模の臨床試験を実施し，B前駆細胞性ALLにおいて予防的頭蓋照射の全廃に成功した[3]。TCCSGはL95-14研究において標準危険群，中間危険群を対象にprednisone（PSL）とdexamethasone（DEX）のRCTを行い，両群に差が無かったことを報告した[4]。L99-15研究においては，中間危険群を対象に早期強化療法における大量cytarabine群と通常量cytarabine群のRCTを行い，大量cytarabine療法の有用性を認めなかった[5]。CCLSGはALL2000研究において国内で初めてPCR法を用いた微小残存病変（MRD）量を治療介入に用いることによって成績の向上をめざした。301例にMRD解析が実施され234例にMRDによる第2層別化が行われた。このMRD層別化群の5年EFSは82.5% ±2.6%で前回のALL941研究の成績やMRD非層別化群の成績に較べて有意に優れていた[6]。KYCCSGはALL96研究において維持療法におけるLSA2L2 typeの強力な治療と，経口の6-mercaptopurine（6-MP）/methotrexate（MTX）を主体とする標準的な治療の比較試験を行い，5年EFSに差を認めなかった[7]。

これらの4グループは，大規模な比較試験の実施による更なる治療成績の向上とbiology研究の活性化を目的として国内統一研究の実施に合意し，日本小児白血病リンパ腫研究グループ（JPLSG）においてT細胞性ALLを対象とするJPLSG ALL-T11研究（以下T11研究）とB前駆細胞性ALLを対象とするJPLSG ALL-B12研究（以下B12研究）を開始した。

2. 予後因子

上記したように既に良好な治療成績を挙げている小児ALLにおいて，短期，長期の毒性を軽減しつつ，治療成績を向上させるためには，予後因子に基づく精密な層別化治療がこれまで以上に重要である。

埼玉県立小児医療センター 血液・腫瘍科

Table 1 Results of recently completed clinical trials for acute lymphoblastic leukemia[1)]

Study	Years of study	No. of patients	Age range (yr)	Event-free survival at 5 yr (%±SE)	Survival at 5 yr (%±SE)	Reference
AIEOP-95	1995–2000	1743	0–17	75.9±1.0	85.5±0.8	Conter et al
BFM-95	1995–2000	2169	0–18	79.6±0.9	86.3±0.6	Möricke et al
CCG-1900	1996–2002	4464	0–21	76.0±0.7	86.3±0.6	Gaynon et al
COALL-7	1997–2003	667	0–18	76.7±1.7	85.4±1.4	Escherich et al
CPH-95	1996–2002	380	0–18	72.1±2.3	83.0±1.9	Stary et al
DCOG-9	1997–2004	859	1–18	80.6±1.4	86.4±1.2	Kamps et al
DFCI 00-01	2000–2004	492	0–18	80.0±2.0	91.0±1.0	Vrooman et al
INS 98	1998–2003	315	0–18	78.7±2.3	83.8±2.1	Stark et al
NOPHO-2000	2002–2007	1023	1–15	79.4±1.5	89.1±1.1	Schmiegelow et al
SJCRH-13B	1994–1998	247	0–18	80.1±2.6	85.7±2.2	Pui et al
SJCRH-15	2000–2007	498	1–18	85.6±2.9	93.5±1.9	Pui et al
TCCSG-95-14	1995–1999	597	1–15	76.8±1.8	84.9±1.5	Tsuchida et al
TPOG-2002	2002–2007	788	0–18	77.4±1.7	83.5±1.6	Liang et al
UKALL-97/99	1999–2002	938	1–18	80.0±1.2	88.0±1.1	Mitchell et al

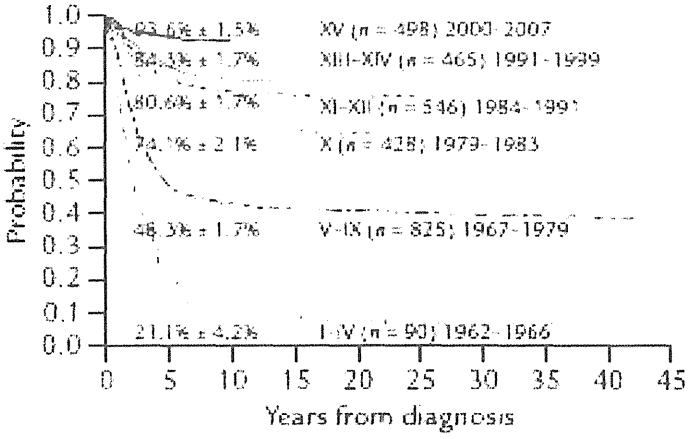

Fig. 1 Kaplan–Meier analysis of survival for 2,852 children with newly diagnosed acute lymphoblastic leukemia treated in 15 consecutive studies at St. Jude Children's Research Hospital from 1962 to 2007[1)]

1) 従来の予後因子

簡便な予後因子として従来から，年齢（1歳未満と10歳以上が不良），診断時白血球数（多いほど不良），免疫学的分類（T細胞性がやや不良）が採用されてきたが，治療成績の向上に伴ってこれらの因子の重要性は相対的に小さくなってきた。現在においては染色体・遺伝子異常といったbiologyと，治療開始後一定の時期に評価する治療反応性が重視されている。染色体・遺伝子異常については，予後不良の病型として，Philadelphia染色体陽性（Ph-ALL），*MLL*遺伝子再構成陽性，染色体数44本以下の低2倍体，(17;19) 転座（*E2A-HLF*）などが，予後良好の病型として，(12;21) 転座（*TEL-AML1*，最近は*ETV6-RUNX1*と表記される）や，染色体数51本以上の高2倍体などが挙げられてきた[1,2)]。

2) biology研究の最近の進歩

最近では，次世代シークエンサーによる全ゲノムの網羅的解析など様々な分子生物学的解析手法の進歩により，小児ALLは事実上ほぼすべての症例が何らかの特異的細胞遺伝学的異常を有することが明らかになってきた。Fig. 2に最近の知見も含めた小児ALLの細胞遺伝学的異常の頻度を示す[2)]。これらの最近明らかになったbiologyのうち，予後との関連で重要なsubtypeについていくつか紹介する。

まずT細胞性ALLでは，予後不良のsubtypeとしてearly T-cell precursor ALL（ETP-ALL）が注目されている[8,9)]。ETP-ALLは，特徴的な細胞表面マーカー（CD1a

陰性，CD5 弱陽性，CD8 陰性，骨髄性抗原または幹細胞性抗原陽性）を有し，microarray でも特徴的な遺伝子発現パターンを有する。全 genome シークエンスでは，これらの blast は急性骨髄性白血病と共通する遺伝子変異が見いだされ，更に正常造血幹細胞と共通した転写 profile が認められた[9]。これらより，ETP-ALL は造血幹細胞に近く，骨髄性細胞への分化能も有する未熟な前駆細胞の腫瘍化と考えられる。以前から言われていた myeloid/T-cell leukemia に類縁する概念であり，一部は急性骨髄性白血病 M0 とも overlap している。このような ETP-ALL は小児 T 細胞性 ALL の約 10～15％を占め，治療に対する初期反応性不良，微小残存病変（MRD）高値と強く相関し，化学療法による予後は極めて不良とされている[8]。ただし，最近報告された AIEOP-BFM2000 研究では，高危険群以外の ETP-ALL の予後は必ずしも不良ではなかったことから，他の予後不良因子を持たない場合の ETP-ALL の予後については，未だ不確定な部分が多い[10]。

次に B 前駆細胞性 ALL における新たな subtype として，*IKZF1* 変異，*CRLF2* 過剰発現，Ph-like ALL，iAMP21 について紹介する。

IKZF1（Ikaros family zing finger 1）はリンパ球の分化に必要な転写因子であり，この遺伝子の変異や部分欠失は dominant negative な効果で分化を抑制し，腫瘍化に関与していると考えられている。*IKZF1* 変異は小児 B 前駆細胞性 ALL の約 12％，Ph（*bcr-abl*）陽性 ALL の 80％以上，後述する Ph-like ALL の約 3 分の 1 に見られ，その予後は極めて不良とされている[11, 14]。この *IKZF1* 変異と MRD を組み合わせることで，再発を高率に予測できるという報告もある[12]。

CRLF2（cytokine receptor-like factor 2）は，リガンドと結合して JAK-STAT 経路を活性化するレセプターであり，この遺伝子の過剰発現は JAK-STAT 経路の活性化により細胞増殖に関与する。*CRLF2* 遺伝子過剰発現は，小児前駆細胞性 ALL の約 5～7％にみられるが，特に Down 症に合併した ALL では 50％以上で認められる[13, 14]。*CRLF2* 遺伝子の過剰発現が特に高危険群において予後不良因子であるという報告がいくつかある[14]。特に *CRLF2* 過剰発現は *IKZF1* 変異と異なり，治療反応性や MRD などの既知の予後因子との相関がなく，独立した予後因子となる可能性があるため重要である。しかしながら，過剰発現群全体が予後不良なのではなく，*CRLF2-P2RY8* 転座を有する群のみが非高危険群において予後不良であるという報告がある[15] など，過剰発現とその基盤となるゲノム異常，予後との相関についてはまだ十分なコンセンサスが得られていない。また

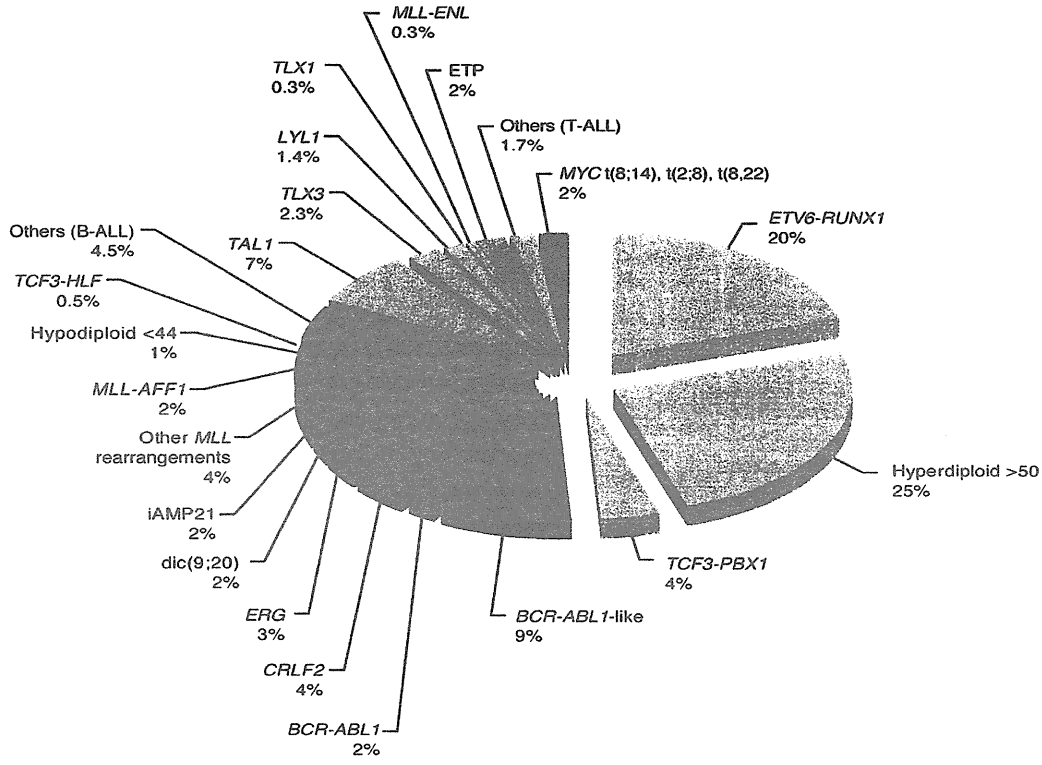

Fig. 2 Estimated frequency of specific genotypes in childhood ALL including recently identified genotypes[2]

CRLF2 異常を同定する手法についても flow cytometry や PCR 法，FISH 法などがあり，至適な評価方法や cut off の設定など技術的な問題についても国際的なコンセンサスはこれから形成されていくと考えられる。なお Down 症に合併した ALL については今のところ予後との相関は報告されていない。

次に Ph-like または *bcr-abl* like ALL である。この subtype は当初 microarray による発現解析において，*bcr-abl* 転座が陰性であるのに遺伝子発現のパターンが *bcr-abl* 陽性例に類似し，しかも *bcr-abl* 同様に予後不良である一群が存在することから見出された[16]。小児 B 前駆細胞性 ALL の約 10％を占めるこの subtype はその後，米国のグループから *bcr-abl* 陽性例と同様に高率に *IKZF1* 変異を伴うこと，約半数で *CRLF2* 過剰発現を伴うこと等が報告された[17]。更に全 genome/transcriptome シークエンスの結果，CRLF2 過剰発現を伴わない群の多くは，*abl* や *PDGFR* などチロシンキナーゼの関与する異常やサイトカインレセプターの活性化変異が見られることも明らかになった[18]。Ph-like ALL に対するチロシンキナーゼインヒビター（TKI）治療については治療の項で後述する。

最後に iAMP21（intra-chromosomal amplification of choromosome 21）である。UK のグループは *ETV6-RUNX1* 転座の検出を目的とした FISH 法によるスクリーニングの過程で，21 番染色体の一部が増幅している iAMP21 の症例を同定し，その予後が不良であることを報告した[19]。UK のグループはその後の治療研究 UKALL2003 において iAMP21 の症例を高危険群に層別化し，強化した治療を行うことで予後が改善したことを報告した[20]。予後不良な biology を有する一群を同定し治療強化を行うことで，治療成績が改善する可能性を示した貴重な実例である。

B 前駆細胞性 ALL にみられるこれらの新たに同定された遺伝子異常は未だ臨床試験における層別化因子として広く採用されてはいないが，iAMP21 については上記のとおり UK のグループが高危険群に層別化しており，また *IKZF1* 変異についてはオランダのグループが治療強化の対象にしようとしている。今後更なる生物学的メカニズムの解明と相まって，後述する Ph-like ALL に対する TKI など分子標的薬剤のターゲットとなる可能性もあり，前方視的な解析を行っていくことが重要である。

3）MRD

治療反応性の指標として，従来は治療開始 1 週間後の末梢血芽球数で判定する PSL 反応性や，1 または 2 週間後の骨髄芽球比率などが用いられてきたが，現在では治療開始後一定の時期（寛解導入療法後，早期強化療法後など）の MRD のレベルがもっとも強力な予後因子であることが確立されてきている[10, 21, 22]。

MRD の評価方法としては，PCR 法による IgH/TCR 再構成の検出（PCR-MRD），flow cytometry による白血病特異的表面マーカーパターンの検出（Flow-MRD），白血病特異的染色体転座により生じるキメラ遺伝子の PCR 法による検出（キメラ MRD）などがある。キメラ MRD は特定の転座のある症例でしか測定できない。PCR-MRD と Flow-MRD を比較すると，保存検体で検査可能な点，評価が客観的である点などから，現時点では PCR-MRD の方が多施設共同研究の層別化に採用しやすいが，将来的には Flow-MRD に置き換わっていくことも期待される。特に治療開始後早期の評価は Flow-MRD が有利かもしれない。

MRD を層別化に組み込んだ大規模な臨床試験の結果も既に公表されている[10, 22, 23]。MRD 陽性群に対する治療強化が ALL 全体の予後を改善するかどうかは確立されておらず，ドイツ，イタリアを中心として行われた AIEOP-BFM2000 研究においても MRD 陽性群の予後は不良であった[10, 22]。MRD を層別化に組み込んだとしても，MRD 陽性群に対する適切な治療を開発しなければ治療成績の向上は得られないことを銘記する必要がある。一方，UK のグループは UKALL2003 研究において，臨床的な予後不良因子をもたない MRD 陰性群に対する治療軽減をランダム化比較試験で検証し，治療軽減に成功した[23]。MRD は既に研究段階ではなく，実用化段階に達していると言える。国内では愛知医大を中心として PCR 法による MRD 研究が精力的に進められ，前述したように MRD を層別化に用いた CCLSG の臨床試験の結果も報告されている[6]。PCR-MRD は愛知医大，国立名古屋医療センターの努力により先進医療として認められ，今後実績を積むことにより将来的な保険収載の道が展望されるようになった。JPLSG の ALL 研究では，T11 研究においては全例で PCR-MRD による層別化を行うとともに Flow-MRD との比較も行うことで，将来的に PCR-MRD を Flow-MRD に置き換えたり，PCR-MRD で評価不能な症例の層別化を Flow-MRD で補完したりする可能性も模索している。B12 研究においては，全例で PCR-MRD のリアルタイムな測定を行って層別化に用いることは検査施設の capacity から現時点では不可能であるため，高危険群の造血幹細胞移植適応の判定にのみ用いることとした。標準危険群，中間危険群においても事後的な評価を行って予後因子としての検証を行う予定であり，B12 研究の後継試験においては全例で PCR-MRD を層別化に用いることが可能になると展望される。

3. 治療骨格の基本とその改良の試み

小児 ALL に対する治療プロトコールは，各研究グループによって多くのバリエーションがあるが，その基本的な骨格は寛解導入療法，再寛解導入療法を含む強化療法，中枢神経（CNS）再発予防療法，維持療法からなる[1,2]。

1）寛解導入療法

寛解導入療法は，steroid＋vincristine＋L-asparaginase＋anthracycline の 4 剤の組み合わせが世界的標準であり，95～98％の寛解導入率が期待できる。寛解導入療法における改良の試みは steroid と anthracycline について行われてきた。

従来使用されてきた steroid 剤の多くは PSL であったが，近年長い半減期と良好な中枢神経（CNS）移行に着目して DEX の使用が試みられてきた。DEX の優位性を示した複数の無作為割付試験が報告されている[24～27]が，上述したとおり両者に差を認めなかったという TCCSG からの報告もある[4]。DEX は抗白血病効果に優れる一方，精神症状や大腿骨頭壊死，感染症といった合併症の頻度が高く，また至適な投与量，投与スケジュールについても確立していない。DEX と PSL との優劣については更なる研究が必要とされている。T11 研究では，10 歳未満では 3 週間連続で DEX を投与する一方，10 歳以上では中間の 1 週間を休薬することで，抗腫瘍効果を維持しつつ，合併症を軽減することに挑戦している。

anthracycline については，米国 Children's Oncology Group（COG）や UK のグループは，標準危険群の寛解導入においては anthracycline を使用せず，治療後半で anthracycline を含む再寛解導入を行っている[24]。ドイツ BFM（Berlin-Frankfurt-Munster）グループの寛解導入療法は daunorubicin（DNR）4 回投与が基本であるが，彼らも標準危険群においては DNR を 2 回に減量する試みを行い，治療効果を落とすことなく減量に成功している[28]。寛解導入における anthracycline の減量や省略は，短期的な合併症の減少，晩期合併症の軽減を期待できる。B12 研究においては，標準危険群については DNR を 2 回に減量しているが，day 8 末梢血芽球数，day 15 骨髄芽球比率で初期反応性不良群については DNR を 4 回とする中間または高危険群に shift up することで治療成績の低下を回避しようと試みている。

2）強化療法

強化療法は，寛解導入療法で用いた薬剤と交叉耐性の無い薬剤の組み合わせによる治療と，寛解導入療法と同様の薬剤を再び用いる再寛解導入療法からなる。再寛解導入療法はドイツ BFM グループが導入し，すべての危険群において予後を改善させた[29]必須の治療要素となっている。高危険群の中で初期反応性不良群は予後不良であるが，再寛解導入を 2 回繰り返す治療も試みられている。

ALL の治療薬剤のうち L-asparaginase は，骨髄抑制の副作用が軽く，治療成績向上を図るために強化しやすい key drug の 1 つである。T-ALL については，米国の Dana Faber Cancer Institute（DFCI）が良好な治療成績を報告しているが[30]，その特徴は L-asparaginase の多用にある。L-asparaginase を一定量以上投与できた群では，有害事象のため投与量が不十分であった群と比較して有意に予後良好であったとされている。L-asparaginase 強化による治療成績向上の試みは現在でも多くの臨床試験で行われており，B12 研究では中間危険群を対象に L-asparaginase 強化の有無による第 III 相無作為割り付け比較試験を行っている。また B12，T11 研究ともに高危険群においては L-asparaginase を強化している。L-asparaginase 投与に当たってはアレルギー反応により治療継続できなくなる症例が一定の割合（10～40％前後）で生じることが明らかになっており，これらの症例においては代替 L-asparaginase 製剤が必要である。欧米では標準薬である *E-coli* 由来 asparaginase が使用できなくなった場合には *Erwinia* 菌由来 asparaginase が使用されており，また投与回数が少なくアレルギー反応が少ない PEG 化 asparaginase 製剤が使用可能である。国内においては，これらのいずれも使用できないことが小児 ALL 治療における難点の 1 つであったが，*Erwinia* 菌由来 asparaginase については既に治験が終了し，早期の承認が期待される。L-asparaginase については，最近 DFCI から重要な報告が発表された[27]。彼らは L-asparaginase の投与量を固定する Fixed 群と L-asparaginase 活性を monitoring して投与量を決定する individualized 群の RCT を行い，individualized 群の予後が有意に良好であったと報告した。これは individualized 群においては，臨床的なアレルギー反応は無いにも関わらず asparaginase に対する抗体が産生されて asparaginase 活性が低下する，いわゆる silent inactivation を生じた症例を同定し，これらの症例に対しては *Erwinia* 菌由来 asparaginase に切り替えることで，asparaginase の効果減弱を防ぐことができたためであるとされている。今後は国内においても *Erwinia* 菌由来 asparaginase の使用が可能になるため，asparaginase 活性を monitoring できる体制の構築が求められる。

次に MTX 大量療法（2～5 g/m^2）は多くのグループで採用されており，中枢神経，睾丸への移行が良好であることから有用な治療要素と考えられる。米国 Children's Oncology Group（COG）は高危険群 B 前駆細胞

性 ALL を対象に，MTX 大量療法（5 g/m²）と漸増式通常量 MTX 療法（capizzi MTX 療法）の比較試験を行い，大量療法群の予後が有意に良好であったと報告した[26]。今後少なくとも高危険群においては大量 MTX 療法が標準治療となると考えられるが，標準危険群における有用性や，MTX の投与量については検討の余地がある。B12 研究では標準危険群においては 2 g/m²，中間・高危険群においては 5 g/m² の投与を行っている。

3）CNS 再発予防療法

中枢神経系（CNS）には血液脳関門が存在し薬剤が到達しにくいため，CNS 再発予防を行なわなければ，50%以上に CNS 再発が生じる。CNS 再発予防において，もっとも確実な手段は全脳への放射線照射（CRT）である。しかしながら CRT は，成長障害，内分泌障害，2 次がん等の重篤な晩期障害を高頻度にもたらすことが明らかになっており，各グループは髄注や MTX 大量療法の導入によって CRT の対象症例を減少させてきている[31]。最近では，CRT の対象症例は全症例の 10～20%，照射量も当初の 24 Gy から 18 Gy，さらに 12 Gy まで減量することが可能になってきている[28, 29, 31]。更に髄注や全身治療の強化により CRT の全廃に成功した臨床試験も報告されてきている[23, 31, 32]。B12，T11 研究においては，DEX の使用（T11），髄注の強化，L-asparaginase の強化によって，予防的 CRT の全廃に挑戦しており，その成果が期待される。

4）維持療法

もっともシンプルな維持療法は，経口の MTX と 6-MP の組み合わせによる 1～2 年の治療である。MTX/6-MP を静注にしたり，この組み合わせに steroid＋vicristine の pulse（以下 pulse 療法）を加えたり，といった多くの試みが行われてきたが，現在までのところ明らかな利益は証明されていない。維持療法中の pulse 療法については，1970～80 年代に行われた RCT のメタアナリシスでは pulse 療法の有用性が示されたが[33]，1990 年代に中間危険群を対象にヨーロッパで行われた国際共同 RCT では有用性が示されなかった[34]。全体としての治療強度が比較的弱い場合には pulse 療法が有用であっても，全体としての治療強度が強くなった現代の治療では pulse 療法の意義はないという解釈が可能である。しかしながら，上記の国際共同 RCT と同時期に行われた EORTC 58951 研究では，pulse 療法群の予後が有意に良好であったと報告されている[35]。米国や UK では現在でも維持療法中に pulse 療法を行っており，pulse 療法の意義は未だ不確定であると言える。B12 研究では標準危険群を対象に維持療法中の pulse 療法の有無で RCT を行っており，その結果が注目される。

治療期間については，18 ヶ月（BFM グループによる RCT[29]）や 1 年（TCCSG L92-13 研究[36]）に短縮する試みが行なわれたが，いずれも再発が増加し，現時点のコンセンサスでは 2～3 年の総治療期間が必要である。

4. 新薬の導入

これまでの小児 ALL の治療成績は主として 30 年以上前に開発された古典的薬剤の組み合わせの最適化によって達成されてきた。しかし，一部の予後不良な高危険群や再発例の治療成績の向上，既存の抗がん剤による毒性の軽減のためには分子標的薬剤などの新薬の導入が必要になってきている。

Ph 陽性 ALL を対象として，COG が行った imatinib 併用化学療法の臨床試験では，imatinib を連続的に併用した cohort 5 の患者群の 3 年 EFS は 80%と移植群と同等であったことを報告した[37]。この結果は追跡期間を延長したその後の報告でも同様であった[38]。またヨーロッパを中心とする国際共同臨床試験 EsPhALL での imatinib 併用化学療法の治療成績も good risk 群と poor risk 群の 4 年 DFS がそれぞれ 75.2%，53.5%と従来と比較して大きく向上した[39]。EsPhALL 試験では good risk 群では imatinib 併用の有無で RCT が実施されており，RCT で新薬の有用性が証明された意義は極めて大きい。

小児 ALL においても新薬の導入が予後を改善させる可能性があることを示した画期的な試験であった。

T 細胞性 ALL に対しては COG が高危険群を対象に neralabine の有無による無作為割り付け比較試験を実施している。T11 研究においても中間・高危険群を対象に neralabine の有用性を検証している。

Biology 研究の最近の進歩の項で言及した Ph-like ALL のうち，*ABL* や *PDGFRB* といったチロシンキナーゼ遺伝子の転座を有する例では TKI の有用性が報告されている[40]。予後不良の病型の biology の解明が治療開発につながりうる可能性を示した貴重な例となっている。Ph-like ALL については国内でも 4 グループの過去の症例を対象とした後方視的な検討や，前方視的な TKI による介入試験の可能性が模索されている。

再発例に対しては海外で anti CD3/19 antibody である blinatumomab やプロテアーゼ阻害剤 bortezomib, purine nucleoside phosphorylase 阻害剤 forodesine などの開発が進行してきている[2]。今後は国内においてもこれらの未承認薬や適応外薬を用いた臨床試験が可能となるよう，JPLSG 再発 ALL 委員会などを中心として様々な模索が行われている。

5. おわりに

小児 ALL の治療の最近の知見について概説した。小児 ALL の治療の進歩は今後も新薬の導入も含む絶え間

ない臨床試験の実施によって達成されていくであろう。国内における全国統一試験，JPLSG ALL T11/B12 研究の成果が期待される。

著者の COI（conflicts of interest）開示：本論文発表内容に関連して特に申告なし

文 献

1) Pui CH. Recent research advances in childhood acute lymphoblastic leukemia. J Formos Med Assoc. 2010; 109: 777-787.
2) Pui CH, Mullighan CG, Evans ME, Relling MV. Pediatric acute lymphoblastic leukemia: where are we going and how do we get there? Blood. 2012; 120: 1165-1174.
3) Hasegawa D, Hara J, Suenobu S, et al. Successful abolition of prophylactic cranial irradiation in children with non-T Acute Lymphoblastic Leukemia (ALL) in the Japan Association of Childhood Leukemia Study (JACLS) ALL-02 Trial [abstract]. Blood. 2011; 118: Abstract1506.
4) Igarashi S, Manabe A, Ohara A, et al. No advantage of dexamethasone over prednisolone for the outcome of standard- and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group L95-14 protocol. J Clin Oncol. 2005; 23: 6489-6498.
5) Hasegawa D, Manabe A, Ohara A, et al. Tokyo Children's Cancer Study Group. The utility of performing the initial lumbar puncture on day 8 in remission induction therapy for childhood acute lymphoblastic leukemia: TCCSG L99-15 study. Pediatr Blood Cancer. 2012; 58: 23-30.
6) Yamaji K, Okamoto T, Yokota S, et al.; Japanese Childhood Cancer Leukemia Study Group. Minimal residual disease-based augmented therapy in childhood acute lymphoblastic leukemia: a report from the Japanese Childhood Cancer and Leukemia Study Group. Pediatr Blood Cancer. 2010; 55: 1287-1295.
7) Nagatoshi Y, Matsuzaki A, Suminoe A, et al. Randomized trial to compare LSA2L2-type maintenance therapy to daily 6-mercaptopurine and weekly methotrexate with vincristine and dexamethasone pulse for children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2010; 55: 239-247.
8) Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009; 10: 147-156.
9) Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature. 2012; 481: 157-163.
10) Schrappe M, Valsecchi MG, Bartram CR, et al. Late MRD response determines relapse risk overall and in subsets of childhood T-cell ALL: results of the AIEOP-BFM-ALL 2000 study. Blood. 2011; 118: 2077-2084.
11) Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007; 446: 758-764.
12) Waanders E, van der Velden VH, van der Schoot CE, et al. Integrated use of minimal residual disease classification and IKZF1 alteration status accurately predicts 79% of relapses in pediatric acute lymphoblastic leukemia. Leukemia. 2011; 25: 254-258.
13) Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009; 114: 2688-2698.
14) Chen IM, Harvey RC, Mullighan CG, et al. Outcome modeling with CRLF2, IKZF1, JAK, and minimal residual disease in pediatric acute lymphoblastic leukemia: a Children's Oncology Group Study. Blood. 2012; 119: 3512-3522.
15) Cario G, Zimmermann M, Romey R, et al. Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. Blood. 2010; 115: 5393-5397.
16) Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009; 10: 125-134.
17) Mullighan CG, Su X, Zhang J, et al. Children's Oncology Group. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009; 360: 470-480.
18) Roberts KG, Morin RD, Zhang J, et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell. 2012; 22: 153-166.
19) Moorman AV, Richards SM, Robinson HM, et al. UK Medical Research Council (MRC) /National Cancer Research Institute (NCRI) Childhood Leukaemia Working Party (CLWP). Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). Blood. 2007; 109: 2327-2330.
20) Moorman AV, Robinson H, Schwab C, et al. Risk-directed treatment intensification significantly reduces the risk of relapse among children and adolescents with acute lymphoblastic leukemia and intrachromosomal amplification of chromosome 21: a comparison of the MRC ALL97/99 and UKALL2003 trials. J Clin Oncol. 2013; 31: 3389-3396.
21) Campana D. Minimal residual disease monitoring in childhood acute lymphoblastic leukemia. Curr Opin Hematol. 2012; 19: 313-318.
22) Conter V, Bartram CR, Valsecchi MG, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood. 2010; 115: 3206-3214.
23) Vora A, Goulden N, Wade R, et al. Treatment reduction for