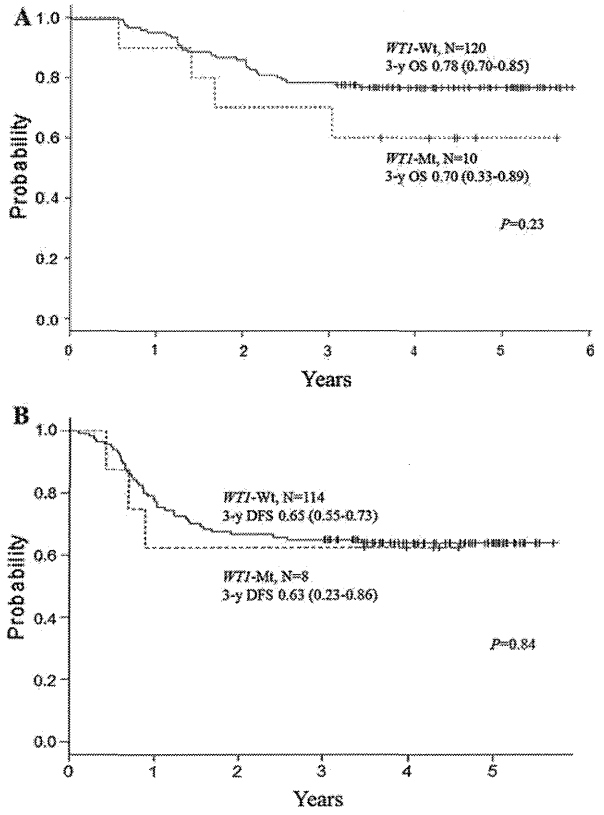


Fig. 1 Probability of 3-year OS (**a**) and DFS (**b**) in 130 AML patients, excluding those with FAB-M3 and Down syndrome. Kaplan–Meier estimates for patients with and without *WT1* mutation are shown

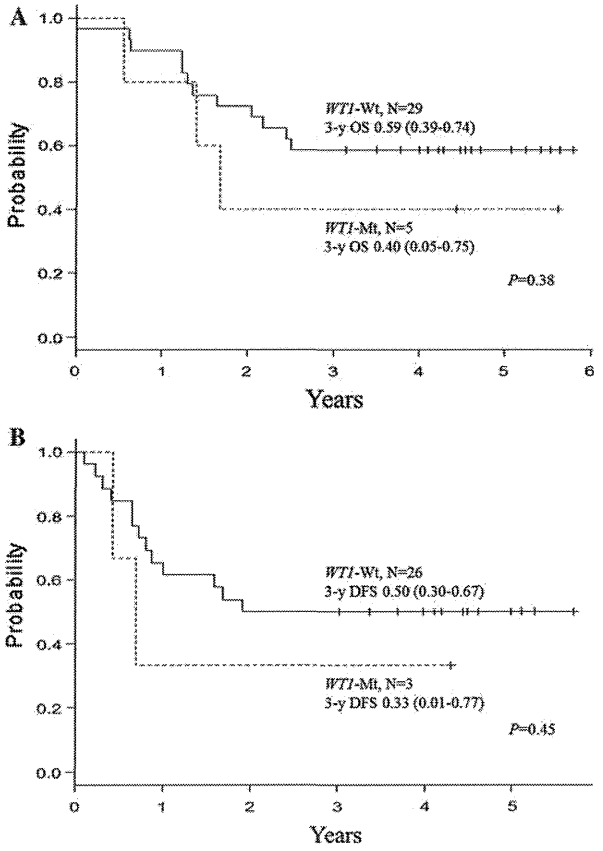


Fig. 2 Probability of 3-year OS in 34 patients (**a**) and DFS in 29 patients (**b**) with CN-AML, excluding those with FAB-M3 and Down syndrome. Kaplan–Meier estimates for patients with and without *WT1* mutation are shown

mutations had no independent effects on the outcome when the *FLT3*-ITD status was taken into account [18, 21, 22]. These results suggest an effect on the clinical outcome due to the different treatment regimens used in each study. For example, when comparing the treatment protocols, the cumulative doses of high-dose cytarabine given for consolidation treatment were markedly different. The current patients were treated on the Japanese Childhood AML Cooperative Study Group Protocol, AML99, in which the intensive use of cytarabine, including high-dose cytarabine, was considered to improve the outcome. Improvement of the clinical outcome of patients with *WT1* mutation in this study might have decreased the differences in the 3-year OS and DFS between patients with and without *WT1* mutation, although *WT1* mutation was not a significant risk factor on 3-year OS and DFS by multivariate analyses. *WT1* mutations have been reported to be an adverse prognostic factor in some studies because of the high frequency of coexisting *FLT3*-ITD [18, 21, 22]; however, only 2 cases had both *WT1* mutation and *FLT3*-ITD in our study. Because of the small number of cases, prognostic analysis for each status of *WT1* mutation and *FLT3*-ITD was not performed. On the other hand, *WT1* mutations were associated with a poor prognosis in patients with CN-AML excluding those with *FLT3*-ITD and less than 3 years old. There has been no similar report of this result. Although further validation of the present results is required, *WT1* mutation might be a prognostic factor in patients with CN-AML excluding those with *FLT3*-ITD and <3 years old.

The frequencies of *WT1* mutations (6.5 % of total AML and 15.2 % of CN-AML) tended to be low compared with previous pediatric reports (8.2–11.7 and 14.3–22.3 %, respectively) [19, 21, 22], although the differences were not significant. This might be due to racial differences or the samples used. Previous reports used genomic DNA for analyzing *WT1* mutation; however, we could use only cDNA. We could not amplify *WT1* genes by RT-PCR in 4 cases (2.5 %). In a previous study, Hollink et al. [19] described 2 cases (1 %) with homozygous deletion out of 298 patients. If homozygous deletions occur in tumor suppressor genes, expressions will be lost and analyses

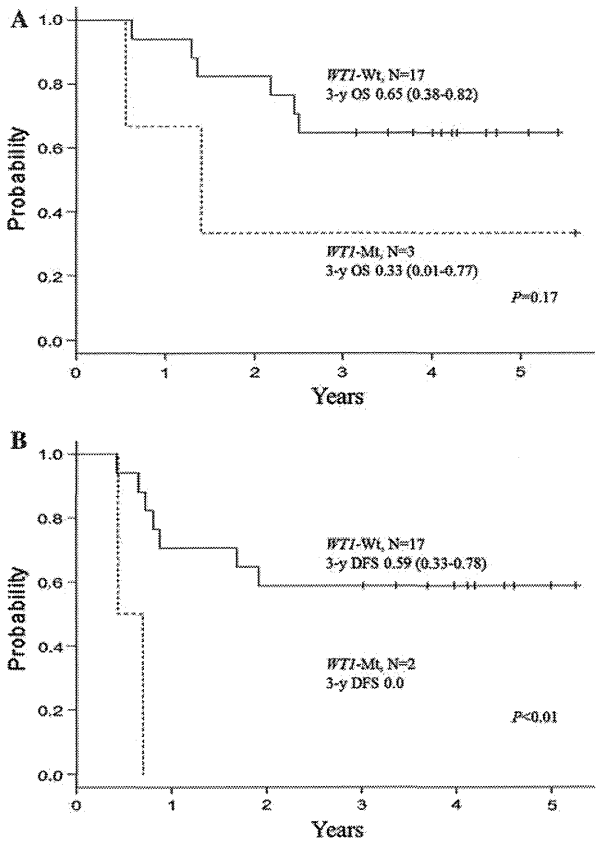


Fig. 3 Probability of 3-year OS in 20 patients (**a**) and DFS in 19 patients (**b**) aged 3 years or older with CN-AML, excluding those with *FLT3*-ITD, FAB-M3, and Down syndrome. Kaplan–Meier estimates for patients with and without *WT1* mutation are shown

Table 4 Multivariate model of prognostic risk factors (overall survival, $N = 130$)

Variables	Hazard ratio	*P* value	95 % CI
FLT3-ITD	5.62	<0.01	2.41–13.12
MLL-PTD	1.96	0.11	0.87–4.46
RAS mutation	1.89	0.15	0.80–4.47
WT1 mutation	1.36	0.62	0.40–4.57
KIT mutation	0.75	0.64	0.22–2.50
FLT3 D835 mutation	<0.01	1.00	0.00–∞

CI confidence intervals

using cDNA will be impossible. As a result, the rate of detecting mutations in these genes is generally higher in genomic DNA than in cDNA [31–33]. Therefore, genomic DNA is usually used for the analysis of these genes. Loss of *WT1* amplification in our study might be partially explained by homozygous deletions of *WT1* genes; however, the frequency of homozygous deletion based on a previous report [19] seemed to be low. Thus, its influence on our research might be limited.

Table 5 Multivariate model of prognostic risk factors (disease-free survival, $N = 122$)

Variables	Hazard ratio	*P* value	95 % CI
MLL-PTD	2.37	0.21	1.14–4.96
FLT3-ITD	2.29	0.10	0.86–6.09
KIT mutation	1.70	0.26	0.68–4.22
RAS mutation	1.27	0.50	0.63–2.57
WT1 mutation	0.57	0.41	0.15–2.20
FLT3 D835 mutation	0.47	0.45	0.06–3.45

CI confidence intervals

When combined with three previous pediatric reports and our data, *WT1* mutations were identified in 50 out of 286 (17.5 %) patients with CN-AML [19, 21, 22]. On the other hand, they were identified in 146 out of 1,283 (11.4 %) adult patients when combined with three large-scale reports [16–18]. The frequency of *WT1* mutation in patients with CN-AML was significantly higher in pediatric compared with adult patients ($P < 0.01$). It was impossible to compare the frequencies of *WT1* mutations in AML patients other than CN-AML because the analyses were usually performed focusing on patients with CN-AML in adult reports.

In 70 % of cases with *WT1* mutations, two or more mutations were detected in the *WT1* gene (Table 1). This frequency was higher than those in previous reports by Hollink et al. [19] (16/35, 46 %, $P = 0.28$), and Ho et al. [21] (15/70, 21 %, $P < 0.01$). It became clear that the existence of multiple mutations of *WT1* genes was not rare. Meanwhile, 90 % of cases with *WT1* mutations were accompanied by other mutations, including *FLT3*-ITD, *MLL*-PTD, and mutations of *FLT3* D835, *KIT*, or *RAS* (Table 1). This frequency was higher than in patients without *WT1* mutation (67/143, 47 %, $P < 0.01$). From these results, there is a possibility that mutations of *WT1* and other genes collaborate and participate in the development of AML. The traditional model of molecular-genetic cooperativity in myeloid leukemogenesis states that "class II" events, which impair differentiation, must be coupled with "class I" events, which confer a proliferative advantage [34]. In our study, *WT1* mutations showed significant overlap with class I mutation, such as *FLT3*-ITD, *MLL*-PTD, and mutations of *FLT3* D835 and *KIT*, so the role of *WT1* mutation in the stepwise evolution might be associated with the arrest of differentiation.

WT1 mRNA expression at diagnosis tended to be higher in patients with compared with those without *WT1* mutation, although the difference was not significant, probably due to the low number of patients with *WT1* mutation. Overexpression of wild-type *WT1* is a common finding in AML [35–37], although *WT1* mutations in AML appear to

result in a loss of *WT1* function. This contradiction, in which a single gene might function as both an oncogene as well as a tumor suppressor, may stem from the ability of the WT1 protein to function either as a transcriptional activator or repressor, depending on a multitude of factors [11]. There is still much to be learned about the biology of *WT1* in AML.

In conclusion, *WT1* mutations were the most common in patients with normal karyotype AML, and showed no correlation with the 3-year OS and DFS. However, these mutations were associated with a poor prognosis in patients with CN-AML excluding those with *FLT3*-ITD and <3 years old.

Acknowledgments This work was supported by a grant for Cancer Research and a grant for Research on Children and Families from the Ministry of Health, Labor, and Welfare of Japan, a Grant-in-Aid for Scientific Research (B, C) and Exploratory Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by a Research grant for Gunma Prefectural Hospitals.

Conflict of interest There is no conflict of interest.

Appendix

Committee members of the Japanese Childhood AML Cooperative Study Group who contributed data to this study include Akira Morimoto, Department of Pediatrics, Kyoto Prefectural University of Medicine; Hiromasa Yabe, Department of Pediatrics, Tokai University School of Medicine; Kazuko Hamamoto, Department of Pediatrics, Hiroshima Red Cross Hospital; Shigeru Tsuchiya, Department of Pediatric Oncology, Institute of Development, Aging and Cancer, Tohoku University; Yuichi Akiyama, Department of Pediatrics, National Hospital Organization Kyoto Medical Center; Hisato Kigasawa, Department of Hematology, Kanagawa Children's Medical Center; Akira Ohara, First Department of Pediatrics, Toho University School of Medicine; Hideki Nakayama, Department of Pediatrics, Hamanomachi Hospital; Kazuko Kudo, Department of Pediatrics, Nagoya University Graduate School of Medicine; and Masue Imaizumi, Department of Hematology and Oncology, Miyagi Children's Hospital.

References

1. Gibson BE, Wheatley K, Hann IM, Stevens RF, Webb D, Hills RK, et al. Treatment strategy and long-term results in paediatric patients treated in consecutive UK AML trials. Leukemia. 2005;19:2130–8.
2. Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, et al. Long term results in children with AML: NOPHO-AML Study Group—report of three consecutive trials. Leukemia. 2005;19:2090–100.
3. Creutzig U, Zimmermann M, Lehrnbecher T, Graf N, Hermann J, Niemeyer CM, et al. Less toxicity by optimizing chemotherapy, but not by addition of granulocyte colony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98. J Clin Oncol. 2006;24:4499–506.
4. Lange BJ, Smith FO, Feusner J, Barnard DR, Dinndorf P, Feig S, et al. Outcomes in CCG-2961, a children's oncology group phase 3 trial for untreated pediatric acute myeloid leukemia: a report from the children's oncology group. Blood. 2008;111:1044–53.
5. Tsukimoto I, Tawa A, Horibe K, Tabuchi K, Kigasawa H, Tsuchida M, et al. Risk-stratified therapy and the intensive use of cytarabine improves the outcome in childhood acute myeloid leukemia: The AML99 trial from the Japanese Childhood AML Cooperative Study Group. J Clin Oncol. 2009;27:4007–13.
6. Rubnitz JE, Inaba H, Dahl G, Ribeiro RC, Bowman WP, Taub J, et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML 02 multicentre trial. Lancet Oncol. 2010;11:543–52.
7. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome of AML: analysis of 1612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood. 1998;92:2322–33.
8. Mrózek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood. 2007;109:431–48.
9. Marcucci G, Maharry K, Whitman SP, Vukosavljevic T, Paschka P, Langer C, et al. High expression levels of the ETS-related gene, ERG, predict adverse outcome and improve molecular risk-based classification of cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B Study. J Clin Oncol. 2007;25:3337–43.
10. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell. 1990;60:509–20.
11. Yang L, Han Y, Suarez Saiz F, Minden MD. A tumor suppressor and oncogene: the WT1 story. Leukemia. 2007;21:868–76.
12. Haber DA, Buckler AJ, Glaser T, Call KM, Pelletier J, Sohn RL, et al. An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor. Cell. 1990;61:1257–69.
13. Ariyaratana S, Loeb DM. The role of the Wilms tumour gene (WT1) in normal and malignant haematopoiesis. Expert Rev Mol Med. 2007;9:1–17.
14. Yamagami T, Sugiyama H, Inoue K, Ogawa H, Tatekawa T, Hirata M, et al. Growth inhibition of human leukemic cells by WT1 (Wilms tumor gene) antisense oligodeoxynucleotides: implications for the involvement of WT1 in leukemogenesis. Blood. 1996;87:2878–84.
15. Nishida S, Hosen N, Shirakata T, Kanato K, Yanagihara M, Nakatsuka S, et al. AML1-ETO rapidly induces acute myeloblastic leukemia in cooperation with the Wilms tumor gene, WT1. Blood. 2006;107:3303–12.
16. Paschka P, Marcucci G, Ruppert AS, Whitman SP, Mrozek K, Maharry K, et al. Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. J Clin Oncol. 2008;26:4595–602.

17. Virappane P, Gale R, Hills R, Kakkas I, Summers K, Stevens J, et al. Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. J Clin Oncol. 2008;26:5429–35.
18. Gaidzik VI, Schlenk RF, Moschny S, Becker A, Bullinger L, Corbacioglu A, et al. Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. Blood. 2009;113:4505–11.
19. Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, Balgobind BV, Arentsen-Peters ST, Alders M, et al. Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia. Blood. 2009;113:5951–60.
20. Summers K, Stevens J, Kakkas I, Smith M, Smith LL, Macdougall F, et al. Wilms' tumour 1 mutations are associated with FLT3-ITD and failure of standard induction chemotherapy in patients with normal karyotype AML. Leukemia. 2007;21:550–1.
21. Ho PA, Zeng R, Alonzo TA, Gerbing RB, Miller KL, Pollard JA, et al. Prevalence and prognostic implications of WT1 mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. Blood. 2010;116:702–10.
22. Staffas A, Kanduri M, Hovland R, Rosenquist R, Ommen HB, Abrahamsson J, et al. Presence of FLT3-ITD and high BAALC expression are independent prognostic markers in childhood acute myeloid leukemia. Blood. 2011;118:5905–13.
23. Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M, et al. KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): A study of the Japanese Childhood AML Cooperative Study Group. Blood. 2006;107:1806–9.
24. Kobayashi R, Tawa A, Hanada R, Horibe K, Tsuchida M, Tsukimoto I. Extramedullary infiltration at diagnosis and prognosis in children with acute myeloid leukemia. Pediatr Blood Cancer. 2007;48:393–8.
25. Shimada A, Taki T, Tabuchi K, Taketani T, Hanada R, Tawa A, et al. Tandem duplications of MLL and FLT3 are correlated with poor prognoses in pediatric acute myeloid leukemia: a study of the Japanese Childhood AML Cooperative Study Group. Pediatr Blood Cancer. 2008;50:264–9.
26. Xu F, Taki T, Yang HW, Hanada R, Hongo T, Ohnishi H, et al. Tandem duplication of the FLT3 gene is found in acute lymphoblastic leukaemia as well as acute myeloid leukaemia but not in myelodysplastic syndrome or juvenile chronic myelogenous leukaemia in children. Br J Haematol. 1999;105:155–62.
27. Taketani T, Taki T, Sugita K, Furuichi Y, Ishii E, Hanada R, et al. FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with MLL rearrangements and pediatric ALL with hyperdiploidy. Blood. 2004;103:1085–8.
28. Sano H, Shimada A, Taki T, Murata C, Park MJ, Sotomatsu M, et al. RAS mutations are frequent in FAB type M4 and M5 of acute myeloid leukemia, and related to late relapse: a study of the Japanese Childhood AML Cooperative Study Group. Int J Hematol. 2012;95:509–15.
29. Shimada A, Taki T, Koga D, Tabuchi K, Tawa A, Hanada R, et al. High WT1 mRNA expression after induction chemotherapy and FLT3-ITD have prognostic impact in pediatric acute myeloid leukemia: a study of the Japanese Childhood AML Cooperative Study Group. Int J Hematol. 2012;96:469–76.
30. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1957;53:457–81.
31. Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, et al. A cell cycle regulator potentially involved in genesis of many tumor types. Science. 1994;264:436–40.
32. Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K, Carson DA. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature. 1994;368:753–6.
33. Okamoto A, Demetrick DJ, Spillare EA, Hagiwara K, Hussain SP, Bennett WP, et al. Mutations and altered expression of p16INK4 in human cancer. Proc Natl Acad Sci USA. 1994;91:11045–9.
34. Renneville A, Roumier C, Biggio V, Nibourel O, Boissel N, Fenaux P, et al. Cooperating gene mutations in acute myeloid leukemia: a review of the literature. Leukemia. 2008;22:915–31.
35. Willasch AM, Gruhn B, Coliva T, Kalinova M, Schneider G, Kreyenberg H, et al. Standardization of WT1 mRNA quantitation for minimal residual disease monitoring in childhood AML and implications of WT1 gene mutations: a European multicenter study. Leukemia. 2009;23:1472–9.
36. Østergaard M, Olesen LH, Hasle H, Kjeldsen E, Hokland P. WT1 gene expression: an excellent tool for monitoring minimal residual disease in 70% of acute myeloid leukaemia patients—results from a single-centre study. Br J Haematol. 2004;125:590–600.
37. Noronha SA, Farrar JE, Alonzo TA, Gerbing RB, Lacayo NJ, Dahl GV, et al. WT1 expression at diagnosis does not predict survival in pediatric AML: a report from the Children's Oncology Group. Pediatr Blood Cancer. 2009;53:1136–9.

REFERENCES

1 Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J *et al.* Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol* 2009; **27**: 6041–6051.
2 National Comprehensive Cancer Network clinical practice guidelines in oncology: chronic myelogenous leukemia version 2, 2011. http://www.nccn.org/NCCN Guidelines™ & Clinical Resources/ (accessed 20 June 2011).
3 Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 2002; **100**: 2292–2302.
4 Huang XJ, Xu LP, Liu KY, Liu DH, Chen H, Liu YR *et al.* Individualized intervention guided by BCR-ABL transcript levels after HLA-identical sibling donor transplantation improves HSCT outcomes for subjects with chronic myeloid leukemia. *Biol Blood Marrow Transplant* 2010; **17**: 649–656.
5 Jiang Q, Xu LP, Liu DH, Liu KY, Chen SS, Jiang B *et al.* Imatinib mesylate versus allogeneic hematopoietic stem cell transplantation for subjects with chronic myelogenous leukemia in the accelerated phase. *Blood* 2011; **117**: 3032–3040.
6 Guilhot J, Baccarani M, Clark RE, Cervantes F, Guilhot F, Hochhaus A *et al.* Definitions, methodological and statistical issues for phase 3 clinical trials in chronic myeloid leukemia: a proposal by the European LeukemiaNet. *Blood* 2012; **119**: 5963–5971.
7 Klein JP, Zhang MJ. Statistical challenges in comparing chemotherapy and bone marrow transplantation as a treatment for leukemia. In: Jewell, Kimber, Lee, Whitmore (eds). *Lifetime data: models in reliability and survival analysis*. Kluwer Academic Publishers: Boston, MA, 1996, pp 175–185.
8 Hochhaus A, O'Brien SG, Guilhot F, Druker BJ, Branford S, Foroni L *et al.* IRIS Investigators. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia* 2009; **23**: 1054–1061.
9 Deininger M, O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP *et al.* International Randomized Study of Interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. *Blood* 2009; **114**: Abstract #1126.
10 Pavlu J, Szydlo RM, Goldman JM, Apperley JF. Three decades of transplantation for chronic myeloid leukemia: what have we learned? *Blood* 2011; **117**: 755–763.
11 Radich J. Stem cell transplant for chronic myeloid leukemia in the imatinib era. *Semin Hematol* 2010; **47**: 354–361.
12 National Marrow Donor Program. Disease and transplant outcome data, NMDP-facilitated transplant outcomes. National Marrow Donor Program, 2004. Available from URL http://www.marrow.org/MEDICAL/disease outcome data.html (accessed 20 June 2011).
13 Robin M, Guardiola P, Devergie A, Yeshurun M, Shapiro S, Esperou H *et al.* A 10-year median follow-up study after allogeneic stem cell transplantation for chronic myeloid leukemia in chronic phase from HLA-identical sibling donors. *Leukemia* 2005; **19**: 1613–1620.
14 de Lavallade H, Apperley JF, Khorashad JS, Milojkovic D, Reid AG, Bua M *et al.* Imatinib for newly diagnosed patients with chronic myeloid leukemia: incidence of sustained responses in an intention-to-treat analysis. *J Clin Oncol* 2008; **26**: 3358–3363.
15 Jabbour E, Kantarjian H, O'Brien S, Shan J, Quintas-Cardama A, Faderl S *et al.* The achievement of an early complete cytogenetic response is a major determinant for outcome in patients with early chronic phase chronic myeloid leukemia treated with tyrosine kinase inhibitors. *Blood* 2011; **118**: 4541–4546.

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

Excess treatment reduction including anthracyclines results in higher incidence of relapse in core binding factor acute myeloid leukemia in children

Leukemia (2013) **27,** 2413–2416; doi:10.1038/leu.2013.153

Patients with core binding factor acute myeloid leukemia (CBF-AML) with translocation (8;21)(q22;q22) [t(8;21)] and inversion of chromosome 16(p13.1q22), or its variant t(16;16)(p13.1;q22) [inv(16)], generally have favorable outcomes, as the probabilities of event-free survival (pEFS) and overall survival (pOS) were around 80% and 90%, respectively, in recent clinical trials of pediatric AML.[1–3] It is well recognized that intensive post remission chemotherapy with high-dose cytarabine (HDCA) has contributed to the improved survival of CBF-AML.[4,5] Although there is evidence that high doses of anthracyclines—another key component of AML chemotherapy—improves the outcomes in children and adults with AML,[6,7] its effects in CBF-AML are not well established, unlike those of HDCA. Moreover, higher doses of anthracyclines are associated with increased risk of late cardiotoxicity, which could occur at a lower cumulative dose (for example, $>300\,mg/m^2$) in children than in adults.[8] Therefore, it needs to be evaluated whether intensive use of HDCA could compensate for a reduction in the other treatment components, especially the cumulative anthracycline dose, by maintaining high pEFS and pOS and reducing the risk of late complications such as anthracycline-induced cardiotoxicity in particular.

Following the excellent outcomes of the AML99 study line conducted by the Japanese Childhood AML Cooperative Study Group,[2] a nationwide multicenter study (termed the AML-05 study) was conducted by a new national study group established in 2003, the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG), to optimize risk-stratified therapies for childhood AML. This trial is registered with UMIN Clinical Trials Registry (UMIN-CTR, URL: http://www.umin.ac.jp/ctr/index.htm), number UMIN000000511. The main objective for the low-risk (LR) group, which included most of the patients with CBF-AML, was to evaluate the efficacy and safety of a chemotherapy regimen comprising a reduced cumulative dose of anthracyclines and etoposide. Between November 2006 and December 2010, 485 consecutive patients aged <18 years with suspected AML diagnosed at 118 centers and hospitals in Japan were registered in AML-05. Patients with acute promyelocytic leukemia, Down syndrome, secondary AML, myeloid/natural killer cell leukemia and myeloid sarcoma were not eligible. AML was diagnosed using the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues (3rd edition).[9] Among the 443 eligible patients, 122 (27.5%) presented with t(8;21) and 32 (7.2%) with inv(16). Their data were compared with historical controls consisting of 89 CBF-AML children in the previous AML99 study. Written informed consent, provided according to the Declaration of Helsinki, was obtained from the guardians of the patients. All aspects of the study were approved by the Institutional Review Boards at all the participating institutions. The therapeutic regimens used in the AML-05 and AML99 studies are presented in Figure 1. Several changes were introduced in AML-05

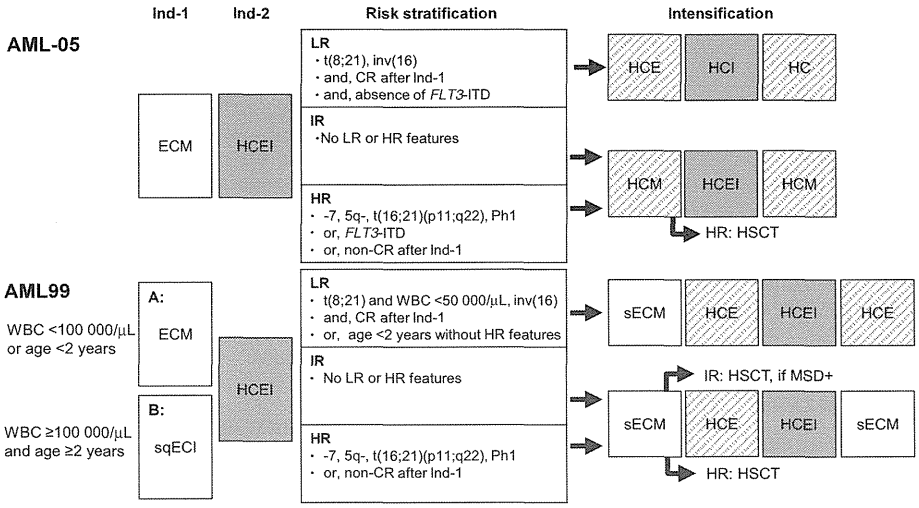
Accepted article preview online 16 May 2013; advance online publication, 4 June 2013

compared with AML99. First, the initial induction course was unified to the ECM (consisted of etoposide, cytarabine and mitoxantrone, with triple intrathecal chemotherapy). Second, the risk groups were redefined as follows: all of the CBF-AML patients with good initial responses after the initial induction course were included in the LR group, without considering the patient's age and white blood cell (WBC) count, while patients with Fms-like tyrosine kinase 3 internal tandem duplications (*FLT3*-ITD) were included in the high-risk (HR) group. We also reduced the total number of chemotherapy courses from six to five. Finally, the indication for allogeneic hematopoietic stem cell transplantation (HSCT) was limited to the HR group. Therefore, most of the patients with CBF-AML (87.6% (135/154)) were included in the LR group and received five courses of intensive chemotherapy, of which four included HDCA, but the cumulative anthracycline dose was limited to 225 mg/m^2 daunorubicin-equivalents (the cumulative anthracycline dose was calculated relative to the amount of daunorubicin using a conversion rate of 5:1 for daunorubicin to mitoxantrone/idarubicin). A small subset of patients with HR CBF-AML (5.1% (8/154)), those with residual leukemia in any site after the initial induction course or those with *FLT3*-ITD, received HSCT at the first remission (1CR).

The characteristics of patients at diagnosis in the AML-05 and AML99 studies are reported in Table 1. Sex, age and WBC count were comparable in both studies, but the distribution of French–American–British classification categories differed. This is because the patients were diagnosed according to the WHO classification (3rd edition) in AML-05, which included patients with low bone marrow blast percentages of 20–30% who are classified as having refractory anemia with excess blasts in transformation (RAEB-T). Additionally, while *FLT3*-ITD status was prospectively examined in all patients in AML-05, it was examined retrospectively in approximately 60% of patients in AML99, although the difference was not significant.

Induction responses in children with CBF-AML were not significantly different between AML-05 and AML99. Among the 147 CBF-AML patients who achieved 1CR in AML-05, 135 patients were assigned to the LR group, while the other 8 patients were assigned to the HR group. Four patients with CBF-AML discontinued the study because of incorrect risk assignment in three and the guardian's decision in one. Among 87 CBF-AML patients who achieved 1CR in the AML99 study, 77 were assigned to the LR group, 7 to the intermediate-risk group and 3 to the HR group.

The 3-year pEFS, pOS and cumulative incidence of relapse (CIR) for all CBF-AML, t(8;21), inv(16), and CBF-AML without unfavorable factors (t(8;21) and WBC >50 000/μl, RAEB-T and *FLT3*-ITD) in AML-05 and AML99 are reported in Table 1. The median follow-up was 3.08 years (range, 0.04 to 5.28 years) and 4.78 years (range, 0.17 to 6.08 years) in AML-05 and AML99, respectively. Among all CBF-AML patients, the CIR was significantly higher in AML-05 (29.9% (95% CI, 23.1–38.1%) vs 17.1% (95% CI, 10.7–26.8%); $P=0.041$), although relapsed patients are likely to be salvaged by variable second-line chemotherapy followed by HSCT (37/38 relapsed LR patients received HSCT after the first relapse). The 3-year pEFS, pOS and CIR for RAEB-T patients were 84.0% (95% CI, 62.8–93.6%), 85.3% (60.6–95.1%) and 16.0% (6.3–37.1%), respectively, which were comparable to the other CBF-AML patients in AML-05. Predictive factors for pEFS, treatment



Study	Risk group	Cytarabine (g/m^2)	Anthracyclines (mg/m^2)*			Etoposide (mg/m^2)
			Total*	Mitoxantrone	Idarubicin	
AML-05	LR	77.4	225	25	20	1750
	IR (HR)	77.4	375	55	20	1750
AML99	LR; A/B	78.4 / 80.0	300 / 295	40 / 15	20 / 44	3 200 / 3 350
	IR (HR); A/B	59.4 / 61.0	375 / 370	55 / 30	20 / 44	3 150 / 3 300

Figure 1. Treatment schedules, risk stratification and cumulative doses of cytarabine/anthracyclines/etoposide in the AML-05 and AML99 studies. ECM consisted of etoposide (150 mg/m^2 per day on days 1–5), cytarabine (200 mg/m^2 per day via 12 h continuous intravenous infusion (CIV) on days 6–12), mitoxantrone (5 mg/m^2 per day on days 6–10) and age-adjusted dose of triple intrathecal chemotherapy (TIT) on day 6. sqECI consisted of etoposide (100 mg/m^2 per day on days 1–3 and 200 mg/m^2 per day on days 11–13), cytarabine (500 mg/m^2 per day via 24 h CIV on days 4–6 and 500 mg/m^2 per day via 20 h CIV on days 11–13), idarubicin (8 mg/m^2 per day on days 4–6), and TIT on day 4. HCEI consisted of HDCA(3 g/m^2 every 12 h on days 1–3), etoposide (100 mg/m^2 per day on days 1–5), idarubicin (10 mg/m^2 on day 1), and TIT on day 1. HCE consisted of HDCA (2 g/m^2 every 12 h on days 1–5), etoposide (100 mg/m^2 per day on days 1–5) and TIT on day 1. HCI consisted of HCEI without etoposide. HC consisted of HCE without etoposide. HCM consisted of HDCA (2 g/m^2 every 12 h on days 1–5), mitoxantrone (5 mg/m^2 per day on days 1–3), and TIT on day 1. sECM consisted of etoposide (150 mg/m^2 per day on days 1–3), cytarabine (200 mg/m^2 per day via 24 h CIV on days 4–8), mitoxantrone (5 mg/m^2 per day on days 4–6), and TIT on day 1. Ind-1, induction course 1; Ind-2, induction course 2; HSCT, allogeneic HSCT; MSD, matched sibling donor. *The cumulative anthracycline dose was calculated relative to the amount of daunorubicin using a conversion rate of 5:1 for daunorubicin to mitoxantrone/idarubicin.

Table 1. Characteristics and treatment response of patients with CBF-AML in the AML-05 and AML99 studies

	AML-05 (n = *154*)	*AML99* (n = *89*)	P-*value*
Sex, n (%)			
Male	92 (59.7)	53 (59.5)	
Female	62 (40.2)	36 (40.4)	0.976
Age at diagnosis (years)			
Median	8.5	8	
Range	0–17	0–15	0.655
WBC at diagnosis (/μl)			
Median	15 540	14 800	
Range	617–266 900	1 340–365 000	0.250
FAB classification, n *(%)*			
M1	16 (10.3)	10 (11.2)	
M2	82 (53.2)	63 (70.7)	
M4	14 (9.0)	9 (10.1)	
M4Eo	14 (9.0)	4 (4.4)	
M5	2 (1.2)	3 (3.3)	
RAEB-T*	25 (16.2)	0 (0.0)	
ND	1 (0.6)	0 (0.0)	<0.001
Cytogenetics, n *(%)*			
t(8;21)	122 (79.2)	77 (86.5)	
inv(16)	32 (20.7)	12 (13.4)	0.154
FLT3-ITD status, n *(%)*			
Positive	5 (3.2)	2 (2.2)	
Negative	149 (96.7)	51 (57.3)	
NE	0 (0.0)	36 (40.4)	0.796
Induction response, n *(%)*			
CR after induction 1	144 (93.5)	84 (94.3)	0.785
CR after induction 2	147 (95.4)	87 (97.7)	0.361
Early death	1 (0.6)	0 (0.0)	0.446
Non-response (after induction 2)	3 (1.9)	2 (2.2)	0.185
Survival of all CBF-AML patients, % (s.e.)			
3-year pEFS	68.3 (3.9)	80.9 (4.1)	0.058
3-year pOS	92.1 (2.4)	92.1 (2.8)	0.953
3-year CIR	29.9 (3.8)	17.1 (4.0)	0.041
Survival of t(8;21) patients, % (s.e.)			
3-year pEFS	67.0 (4.4)	79.2 (4.6)	0.112
3-year pOS	91.0 (2.8)	90.9 (3.2)	0.912
3-year CIR	31.5 (4.3)	18.5 (4.4)	0.068
Survival of inv(16) patients, % (s.e.)			
3-year pEFS	74.1 (7.9)	91.6 (7.9)	0.218
3-year pOS	96.8 (3.0)	100	0.540
3-year CIR	23.2 (7.7)	8.3 (7.9)	0.278
Survival of CBF-AML patients without t(8;21) and WBC > 50 000/μl, RAEB-T or FLT3-ITD, % (s.e.)			
3-year pEFS	68.6 (4.6)	82.6 (4.3)	0.051
3-year pOS	95.0 (2.4)	94.6 (2.5)	0.953
3-year CIR	31.3 (4.6)	16.2 (4.2)	0.031

Abbreviations: CBF-AML, core binding factor acute myeloid leukemia; CIR, cumulative incidence of relapse; CR, complete remission; FAB, French–American–British; *FLT3*-ITD, Fms-like tyrosine kinase 3 internal tandem duplications; ND, not determined; NE, not evaluated; pEFS, probability of event-free survival; pOS, probability of overall survival; RAEB-T, refractory anemia with excess blasts in transformation; s.e., standard error; WBC, white blood cell.

(AML-05 vs AML99), age at initial diagnosis (≥10 vs <10 years), sex (female vs male), WBC count at initial diagnosis (≥50 000/μl vs <50 000/μl), and cytogenetics [inv(16) vs t(8;21)], were evaluated by univariate and multivariate analyses (patients receiving HSCT at 1CR were excluded). In Cox regression analysis, the adjusted hazard ratio for pEFS was significantly worse for patients treated in AML-05 than in patients treated in AML99 (hazard ratio, 2.088 (95% CI, 1.125–3.875); $P = 0.020$), and was significantly better in patients aged ≥10 years at diagnosis compared with those aged <10 years at diagnosis (hazard ratio, 0.494 (95% CI, 0.271–0.899); $P = 0.021$).

Thus, our attempt to reduce the cumulative dose of anthracyclines to <300 mg/m^2 for CBF-AML patients in AML-05, while maintaining the treatment intensity by using intensive HDAC in 4/5 treatment courses, resulted in the decrease of pEFS by ~10% because of the higher CIR. Interestingly, this effect was prominent in patients aged <10 years at diagnosis, as the pEFS in children aged <10 years vs ≥10 years was 57.4 vs 82.6% in AML-05, while it was 82.0 vs 82.0% in AML99. Of course, it is not only the cumulative dose of anthracycline, but also the number of treatment courses and the cumulative dose of etoposide that were changed in AML-05 relative to those in AML99 (Figure 1). However, high pEFS and pOS for CBF-AML were achieved with four to five treatment courses and a similar dose of etoposide in other studies[1,3,10] In addition, decrease in pEFS was not observed for non CBF-AML cases in AML-05 with similar changes in number of treatment courses and cumulative etoposide dose, while cumulative anthracycline dose was not changed from AML99 (to be reported in detail in a separate manuscript). Considering these facts, it is likely that the reduced cumulative anthracycline dose was responsible for the higher CIR in the current study than in AML99. Despite higher CIR among CBF-AML patients in AML-05, pOS was identical to that in AML99. However, the overall transplantation rate was higher in AML-05 than in AML99 (33% (51/154) vs 19% (17/89)), because most of the patients who experienced relapse underwent HSCT as second-line treatment, which is unlikely to be accepted by patients with CBF-AML. Therefore, we reintroduced the AML99-based post remission chemotherapeutic regimen for CBF-AML patients included in our subsequent study, AML-12. Nevertheless, the finding that nearly 70% of the CBF-AML patients were cured with very low doses of anthracyclines suggests that other underlying biological factors, such as *KIT* mutations,[11] should be identified for future stratification of CBF-AML in children.

In conclusion, the results of this study indicate that caution is needed when reducing the cumulative anthracycline dose to <300 mg/m^2 as part of conventional combination chemotherapy for treating CBF-AML in children. Several novel agents, including gemtuzumab ozogamicin, had clinical benefits for CBF-AML patients as part of induction chemotherapy.[12,13] The introduction of such agents into combination chemotherapy might be required before considering further reduction of the anthracycline dose.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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.

D Tomizawa[1], A Tawa[2], T Watanabe[3], AM Saito[4], K Kudo[5], T Taga[6], S Iwamoto[7], A Shimada[8], K Terui[9], H Moritake[10], A Kinoshita[11], H Takahashi[12], H Nakayama[13], K Koh[14], H Kigasawa[15], Y Kosaka[16], H Miyachi[17], K Horibe[4], T Nakahata[18] and S Adachi[19]

[1]*Department of Pediatrics, Tokyo Medical and Dental University, Tokyo, Japan;*
[2]*Department of Pediatrics, National Hospital Organization, Osaka Medical Center, Osaka, Japan;*
[3]*Department of Nutritional Science, Faculty of Psychological and Physical Science, Aichi Gakuin University, Nisshin, Japan;*
[4]*Clinical Research Center, National Hospital Organization, Nagoya Medical Center, Nagoya, Japan;*

[5]Division of Hematology and Oncology, Shizuoka Children's Hospital, Shizuoka, Japan; [6]Department of Pediatrics, Shiga University of Medical Science, Otsu, Japan; [7]Department of Pediatrics, Mie University School of Medicine, Tsu, Japan; [8]Department of Pediatrics, Okayama University, Okayama, Japan; [9]Department of Pediatrics, Hirosaki University Graduate School of Medicine, Hirosaki, Japan; [10]Division of Pediatrics, Department of Reproductive and Developmental Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan; [11]Department of Pediatrics, St Marianna University School of Medicine, Kawasaki, Japan; [12]Department of Pediatrics, Saiseikai Yokohama City Southern Hospital, Yokohama, Japan; [13]Department of Pediatrics, Fukuoka-Higashi Medical Center, National Hospital Organization, Koga, Japan; [14]Department of Hematology/Oncology, Saitama Children's Medical Center, Saitama, Japan; [15]Department of Hematology/Oncology, Kanagawa Children's Medical Center, Yokohama, Japan; [16]Department of Hematology and Oncology, Hyogo Children's Hospital, Kobe, Japan; [17]Department of Laboratory Medicine, Tokai University School of Medicine, Isehara, Japan; [18]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan and [19]Human Health Sciences, Kyoto University, Kyoto, Japan
E-mail: dtomizawa.ped@tmd.ac.jp

REFERENCES

1 Rubnitz JE, Inaba H, Dahl G, Ribeiro RC, Bowman WP, Taub J *et al.* Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. *Lancet Oncol* 2010; **11**: 543–552.
2 Tsukimoto I, Tawa A, Horibe K, Tabuchi K, Kigasawa H, Tsuchida M *et al.* Risk-stratified therapy and the intensive use of cytarabine improves the outcome in childhood acute myeloid leukemia: the AML99 trial from the Japanese Childhood AML Cooperative Study Group. *J Clin Oncol* 2009; **27**: 4007–4013.
3 Creutzig U, Zimmermann M, Bourquin JP, Dworzak MN, von Neuhoff C, Sander A *et al.* Second induction with high-dose cytarabine and mitoxantrone: different impact on pediatric AML patients with t(8;21) and with inv(16). *Blood* 2011; **118**: 5409–5415.
4 Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P *et al.* Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. *N Engl J Med* 1994; **331**: 896–903.
5 Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, Tantravahi R *et al.* Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer research* 1998; **58**: 4173–4179.
6 Lowenberg B, Ossenkoppele GJ, van Putten W, Schouten HC, Graux C, Ferrant A *et al.* High-dose daunorubicin in older patients with acute myeloid leukemia. *N Engl J Med* 2009; **361**: 1235–1248.
7 Fernandez HF, Sun Z, Yao X, Litzow MR, Luger SM, Paietta EM *et al.* Anthracycline dose intensification in acute myeloid leukemia. *N Engl J Med* 2009; **361**: 1249–1259.
8 Kremer LC, van der Pal HJ, Offringa M, van Dalen EC, Voute PA. Frequency and risk factors of subclinical cardiotoxicity after anthracycline therapy in children: a systematic review. *Ann Oncol* 2002; **13**: 819–829.
9 Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 2002; **100**: 2292–2302.
10 Gibson BE, Wheatley K, Hann IM, Stevens RF, Webb D, Hills RK *et al.* Treatment strategy and long-term results in paediatric patients treated in consecutive UK AML trials. *Leukemia* 2005; **19**: 2130–2138.
11 Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M *et al.* KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. *Blood* 2006; **107**: 1806–1809.
12 Burnett AK, Hills RK, Milligan D, Kjeldsen L, Kell J, Russell NH *et al.* Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 Trial. *J Clin Oncol* 2011; **29**: 369–377.
13 Castaigne S, Pautas C, Terre C, Raffoux E, Bordessoule D, Bastie JN *et al.* Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet* 2012; **379**: 1508–1516.

Associations between genome-wide Native American ancestry, known risk alleles and B-cell ALL risk in Hispanic children

Leukemia (2013) **27,** 2416–2419; doi:10.1038/leu.2013.130

Hispanic children have a 10–30% greater incidence rate of ALL than non-Hispanic whites, and nearly double the rate observed in African-Americans.[1] Ethnic differences in ALL incidence may be explained by population-level differences in the frequency of genetic risk factors, including those first discovered in genome-wide association studies of European-ancestry populations.[2–5] As Hispanics are an admixed population with European, African and Native American ancestry, differences in ALL incidence observed in Hispanics may be attributable to genetic risk factors associated with Native American ancestry.

Increased Native American ancestry has been linked to increased risk of relapse among Hispanic children with ALL,[6] but no study has yet investigated the contribution of genome-wide Native American ancestry to ALL incidence. Using genome-wide SNP data from 298 Hispanic children with B cell ALL and 456 matched controls from the California Childhood Leukemia Study (CCLS), we investigated whether genome-wide Native-American ancestry was associated with increased risk of B-cell ALL. Additionally, we assessed whether the risk alleles at loci identified in genome-wide association studies of European-ancestry populations (*IKZF1*, *CDKN2A*, *PIP4K2A*, *ARID5B*, *CEBPE*) were more common in individuals with greater levels of Native American ancestry. Finally, we quantified the contribution of these validated risk loci to the increased ALL incidence observed in Hispanics relative to populations of European or African ancestry.

Study participants were Hispanic children from the CCLS, whose recruitment and enrollment procedures have been described in detail previously (Supplementary Table S1).[7,8] Cytogenetic characteristics of included cases are shown in Supplementary Table S1. DNA was isolated from dried bloodspots collected at birth and archived by the California Department of Public Health. Samples were genotyped using the Illumina OmniExpress platform, assaying 730 525 single-nucleotide polymorphism (SNPs). Samples with genotyping call rates $<98\%$, with discordant sex information (reported versus genotyped sex), or showing evidence of cryptic relatedness were excluded from analyses. To exclude poorly genotyped SNPs, SNPs with genotyping call rates $<98\%$ or Hardy–Weinberg Equilibrium P-value $<1\times10^{-5}$ in controls were removed from analyses.

A linkage-reduced set of 63 303 autosomal SNPs, evenly distributed across the genome, was extracted from the

Accepted article preview online 25 April 2013; advance online publication, 17 May 2013

Int J Hematol (2013) 98:74–88
DOI 10.1007/s12185-013-1364-2

ORIGINAL ARTICLE

Incidence and survival rates of hematological malignancies in Japanese children and adolescents (2006–2010): based on registry data from the Japanese Society of Pediatric Hematology

Keizo Horibe · Akiko M. Saito · Tetsuya Takimoto · Masahiro Tsuchida · Atsushi Manabe · Midori Shima · Akira Ohara · Shuki Mizutani

Received: 18 December 2012 / Revised: 7 May 2013 / Accepted: 7 May 2013 / Published online: 24 May 2013

Abstract Neither accurate incidence nor survival data for pediatric patients with hematological malignancies (HM) have been available in Japan to date. Incidence of patients under 20 years of age, who were diagnosed with HM from 2006 to 2010, and their two-year survival rate (2y-OS) were obtained from disease registry data maintained by the Japan Society of Pediatric Hematology (JSPH). A total of 5,287 cases of HM were identified during this period. Acute lymphoblastic leukemia (ALL, 46.6 %) showed the highest incidence, followed by acute myeloid leukemia (AML, 16.7 %), non-Hodgkin lymphoma (NHL, 11.9 %), and histiocytosis (11.8 %). ALL, AML and histiocytosis were common in younger patients aged 1–4, while NHL tended to occur more frequently in older patients aged 5–14. The 2y-OS of HM was 91.6 %, with that for the most common B-precursor ALL rising to 96.2 %. The 2y-OS for M3 AML, lymphoblastic-B-precursor or diffuse large B cell NHL, Hodgkin lymphoma, myeloproliferative disorders, and Langerhans cell histiocytosis was >95 %. There were no gender differences in prognosis, while infants (88.0 %) and adolescents aged 15–19 (90.6 %) tended toward a poorer prognosis. This is the first report to describe incidence and survival times from the nationwide JSPH disease registry. More precise data with longer follow-up is needed.

Keywords Hematological malignancies · Children · Registry data · Survival

K. Horibe (✉) · A. M. Saito
Clinical Research Center, National Hospital Organization Nagoya Medical Center, 4-1-1 Sannomaru, Naka-ku, Nagoya, Aichi 460-0001, Japan
e-mail: horibek@nnh.hosp.go.jp

T. Takimoto
Clinical Research Center, National Center for Child Health and Development, Tokyo, Japan

M. Tsuchida
Ibaraki Children's Hospital, Ibaraki, Japan

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

M. Shima
Department of Pediatrics, Nara Medical University, Nara, Japan

A. Ohara
Toho University Omori Medical Center, Tokyo, Japan

S. Mizutani
Department of Pediatrics, Tokyo Medical and Dental University, Tokyo, Japan

Introduction

Until now, knowledge of the incidence of pediatric hematological malignancies in Japan has relied on registration at the Research Program for the Treatment of Chronic Pediatric Diseases of Specified Categories [1, 2] which is the epidemiological research done by the research and investigation section of the Ministry of Health, Labour and Welfare, and population-based cancer registries [3–6]. Because of the quality problems, all these available data is far from a comprehensive, systematic investigation of pediatric hematological diseases across the country, and the precision of the data gained thereby is limited. Furthermore, while the progress in treatment and supportive care for the last several decades have led to improve treatment outcome [7–14], the absence of nationwide data not only for the incidence but also for survival prognoses of

pediatric hematological malignancies has made it difficult to judge whether these advances in medical science have contributed to the welfare of children across the country.

In order to resolve these issues, the Japanese Society of Pediatric Hematology (JSPH), which unified with the Japan Society of Pediatric Oncology to the Japanese Society of Pediatric Hematology/Oncology since January, 2012, began a registry of newly diagnosed hematological diseases including non-malignant diseases partly in conjunction with the Japanese Society of Hematology in 2006, and planned complementary research into the prognoses (outcomes regarding dead or alive) as part of a research project intended to grasp the total number of pediatric patients with hematological diseases. This is the first report to describe survival times for the nationwide patients with pediatric hematological malignancies as well as the incidence of them from the JSPH disease registry [15, 16].

Materials and methods

The disease registry survey was conducted on the treatment facilities, where JSPH members are working and also preregistered to JSPH Disease Registry Project. Using electronic or paper-based survey forms, the participating facilities voluntarily and continuously registered the cases of patients below the age of 20, who were diagnosed as hematological malignancies or benign hematological disorders after 2006. As for the electronic registration, E-DMS online by the e-Trial Co., Ltd. was used until December 2011 when it was replaced by Patient Data Organizing System (Ptosh) developed by the National Hospital Organization Nagoya Medical Center Clinical Research Center in collaboration with a non-profit organization, Organization for Supporting Clinical Research (NPO-OSCR). For fax registrations, the disease registry data were sent to the Data Management Department of NPO-OSCR. The database prepared for the registry was coordinated with the registry for epidemiological researches/clinical trials organized by Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) in an attempt to "unify the registration" so as to provide more convenience to the participating facilities and to prevent non-registration.

In order to maintain the uniformity of the diagnoses concerning diseases to be registered, JSPH Disease Registry Committee prepared a guideline for diagnosis, to which the participants were requested to conform [17]. Acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), rare leukemia, myelodysplastic syndromes and/or myeloproliferative neoplasms (MDS and/or MPN), or transient abnormal myelopoiesis associated with Down syndrome (Down-TAM), non-Hodgkin (NHL) and Hodgkin lymphomas (HL), histiocytosis including Langerhans cell histiocytosis (LCH) and hemophagocytic lymphohistiocytosis (HLH), other lymphoproliferative disorders (LPD) and other hematological malignancies were defined as the hematological malignancies to be registered. Underlying diseases, pathological/immunological/cytogenetic characteristics, pathogenetic forms (primary/secondary), and other natures of diseases were also recorded.

When a patient is affected by multiple diseases, each disease was registered as one entry. Patients' genders, places of residence at the initial diagnosis, dates of birth, dates of diagnosis, etc., were registered as the basic patient information. The outcomes of the respective diseases (alive or death), along with the diagnosed disease information, were recorded up to the end of May and were registered for every calendar year.

The registered data were compiled according to diagnoses, diagnosed years, genders, age categories (0, 1–4, 5–9, 10–14, and 15–19) and residential areas at diagnosis (Hokkaido, Tohoku, Kanto-Koshinetsu, Tokai-Hokuriku, Kinki, Chugoku-Shikoku, and Kyushu-Okinawa) to indicate the numbers of cases, respectively. A crude incidence rate is the number of new cases by diagnoses in a gender/age-specified populations under age 20 as of each diagnosed year, expressed as the number of hematological malignancies per 100,000 population at risk. Overall survival (OS) was defined as the length of time from the diagnosis of hematological malignancies to death from any cause. Patients were censored at the time of loss of follow-up or June 15, 2011. OS was estimated using the Kaplan–Meier method, and 2-year survival rate (2y-OS) was measured with a 95 % confidence interval (95 % CI) using Greenwood's formula. All statistical analyses were carried out using the SAS software Release 9.1 (SAS Institute Inc., Cary, NC, USA).

This registry project is operated upon obtaining the approval of JSPH Clinical Research Review Committee, followed by consents from the head of each participating institute.

Results

Numbers of registered institutions and cases

The number of institutions registered had increased by 16 from 223 institutions of the 2007 survey to 239 by the time of the 2011 survey (including the 4 institutions which had withdrawn during this period), with participation from 47 prefectures throughout Japan. Registration of cases with hematological malignancies was conducted by 187 (78.2 %) among the 239 institutions. Since retrospective registration was allowed for cases diagnosed since 2006, increases in the number of registered cases were found up

to 3 years in plateau after the diagnoses. A total of 5,287 cases were registered as hematological malignancies from 2006 to 2010, and the numbers by year were 2006: 967 cases, 2007: 1,053 cases, 2008: 1,116 cases, 2009: 1,081 cases and 2010: 1,070 cases.

Incidences

The results of broadly classifying hematological malignancies into the disease groups of ALL, AML, MDS and/or MPN, NHL, HL, histiocytosis, LPD, other hematological malignancies and Down-TAM, and tabulating by the year of diagnosis are shown in Table 1. A total of 5,287 cases were registered in 5 years, which was an annual incidence of hematologic malignancies of 4.5 cases per 100,000 people. The greatest number of cases was ALL with 2,464 cases (46.6 %), followed by AML with 891 cases (16.9 %), NHL with 628 cases (11.9 %) and histiocytosis with 624 cases (11.8 %), and this trend remained nearly constant without any dependence on the year of diagnosis. On the other hand, the number of cases reported as Down-TAM in 2010 had increased by about 1.7 times from the average number of cases reported in previous years. In addition, there were a large number of cases reported as other LPD in 2007, about two times more than in other years. The number of registered cases including rare leukemia (36 cases), other LPD (51 cases) and other hematological malignancies (6 cases) were small.

Table 2 shows the number of registrations by gender, age category, and residential areas at diagnosis for each disease group classification.

In the tabulation of ALL by the immunophenotypic classification, the most part was accounted for by B-precursor ALL with 2,110 cases (85.6 %), followed by T-ALL with 1,269 cases (10.9 %) and mature B-ALL with 60 cases (2.4 %). The peak incidence of ALL occurs between 1 and 4 years of age. In addition, the incidence of ALL is slightly higher among male children than female children, and this difference is consistent regardless of the classification by immunophenotype. Genetic abnormalities in 2,464 cases with ALL included 281 cases (11.4 %) with hyperdiploid karyotype over 50 chromosomes, 247 cases (10.0 %) with ETV6-RUNX1, 135 cases (5.5 %) with MLL rearrangement, 113 cases (4.6 %) with E2A-PBX1, 106 cases (4.3 %) with BCR-ABL1 gene rearrangement, 29 cases (1.2 %) with t(v;8q24) and 583 cases (23.7 %) with other abnormalities.

In the tabulation of AML by FAB classification, overall the greatest number was M2 with 218 cases (24.5 %), followed by M7 with 212 cases (21.5 %), M5 with 124 cases (13.9 %) and M4 with 112 cases (12.6 %). The distribution by age category showed the greatest numbers of M2 for ages 5–9 and 10–14 years (the peak of incidence during age 10–14 years), but for ages 0 and 1–4 years the incidence of M7 was extremely high (the peak of incidence during age 1–4 years), making up almost half of the incidences. Half of patients diagnosed with M7 AML were associated with Down syndrome ($n = 114$), corresponding to 94.2 % of 121 AML patients with Down syndrome. No clear difference in the number of cases of disease was found between the genders.

Table 1 Numbers of cases and incidence rates of hematological malignancies in Japanese children and adolescents, diagnosed between 2006 and 2010

Disease	Total (%)	Crude incidence rate[a]	Year of diagnosis				
			2006	2007	2008	2009	2010
Acute lymphoblastic leukemia	2,464 (46.6)	2.1	444	506	532	504	478
Acute myeloid leukemia	891 (16.9)	0.8	167	165	184	193	182
Rare leukemia	36 (0.7)	0.0	9	7	4	10	6
Myelodysplastic syndrome and/or myeloproliferative neoplasms	296 (5.6)	0.3	61	60	46	54	75
Non-Hodgkin lymphoma	628 (11.9)	0.5	118	129	138	137	106
Hodgkin lymphoma	107 (2.0)	0.1	19	21	24	14	29
Histiocytosis	624 (11.8)	0.5	114	108	138	128	136
Transient abnormal myelopoiesis associated with Down syndrome	184 (3.5)	0.2	26	37	37	31	53
Other hematological malignancies	6 (0.1)	0.0	0	0	5	1	0
Other lymphoproliferative disorders	51 (1.0)	0.0	9	20	8	9	5
Hematological malignancies, Total	5,287 (100.0)	4.5	967	1,053	1,116	1,081	1,070

[a] Crude incidence rate is the number of new cases by diagnoses in a gender/age-specified populations under age 20 as of each diagnosed year, expressed as the number of hematological malignancies per 100,000 population at risk

Table 2 Numbers of incidences of hematological malignancies according to gender, age category, and residential areas at diagnosis in Japanese children and adolescents, diagnosed between 2006 and 2010

Disease	Subtype	*n* (%)	*n* (%)	Gender, *n* (%)		Age, *n* (%)					Residential areas at diagnosis, *n* (%)						
				Male	Female	0	1–4	5–9	10–14	15–19	Hokkaido	Tohoku	Kanto-Koshinetsu	Tokai-Hokuriku	Kinki	Chugoku-Shikoku	Kyushu-Okinawa
Acute lymphoblastic leukemia		2,464 (46.6)		1,411 (57.3)	1,053 (42.7)	108 (4.4)	1044 (42.4)	711 (28.9)	499 (20.3)	102 (4.1)	105 (4.3)	192 (7.8)	971 (39.4)	347 (14.1)	415 (16.8)	190 (7.7)	244 (9.9)
	B-precursor		2,110 (85.6)	1,151	959	102	979	580	372	77	83	168	829	306	350	164	210
	Mature B		60 (2.4)	38	22	5	9	24	20	2	4	2	22	6	11	7	8
	T cell		269 (10.9)	206	63	1	46	101	99	22	15	19	112	34	46	18	25
	Unknown		25 (1.0)	16	9	0	10	6	8	1	3	3	8	1	8	1	1
Acute myeloid leukemia		891 (16.9)		451 (50.6)	440 (49.4)	109 (12.2)	306 (34.3)	168 (18.9)	240 (26.9)	68 (7.6)	32 (3.6)	69 (7.7)	348 (39.1)	121 (13.6)	134 (15.0)	78 (8.8)	109 (12.2)
	M0		33 (3.7)	15	18	4	7	10	7	5	1	4	15	4	4	0	5
	M1		73 (8.2)	35	38	2	17	19	28	7	4	4	30	12	10	6	7
	M2		218 (24.5)	109	109	3	37	73	88	17	11	17	78	28	35	20	29
	M3, M3v		70 (7.9)	37	33	2	12	17	29	10	1	2	22	11	17	7	10
	M4, M4Eo		112 (12.6)	57	55	12	29	20	38	13	3	12	37	19	21	8	12
	M5a, M5b		124 (13.9)	63	61	28	33	16	39	8	6	9	47	15	20	13	14
	M6a, M6b		14 (1.6)	8	6	0	7	3	3	1	0	1	6	1	2	2	2
	M7		212 (23.8)	104	108	55	150	2	3	2	6	17	99	28	19	16	27
	Unknown		35 (3.9)	23	12	3	14	8	5	5	0	3	14	3	6	6	3
Rare leukemia		36 (0.7)		24 (66.7)	12 (33.3)	5 (13.9)	8 (22.2)	6 (16.7)	14 (38.9)	3 (8.3)	2 (5.6)	2 (5.6)	14 (38.9)	4 (11.1)	4 (11.1)	5 (13.9)	5 (13.9)
Myelodysplastic syndrome (MDS) and/or Myeloproliferative neoplasms (MPN)		296 (5.6)		171 (57.8)	125 (42.2)	42 (14.2)	73 (24.7)	66 (22.3)	92 (31.1)	23 (7.8)	13 (4.4)	10 (3.4)	127 (42.9)	40 (13.5)	43 (14.5)	34 (11.5)	29 (9.8)
	MPN		111 (37.5)	62	49	2	13	33	57	6	4	3	46	17	19	13	9
	MDS/MPN		49 (16.6)	32	17	27	18	1	1	2	2	2	23	7	5	3	7
	MDS		136 (45.9)	77	59	13	42	32	34	15	7	5	58	16	19	18	13
Non-Hodgkin lymphoma		628 (11.9)		446 (71.0)	182 (29.0)	7 (1.1)	96 (15.3)	237 (37.7)	240 (38.2)	48 (7.6)	26 (4.1)	45 (7.2)	218 (34.7)	107 (17.0)	107 (17.0)	56 (8.9)	69 (11.0)
	Lymphoblastic-T-precursor		136 (21.7)	100	36	0	14	48	63	11	8	14	51	21	24	8	10
	Lymphoblastic-B-precursor		71 (11.3)	42	29	5	20	31	12	3	5	4	25	14	7	9	7
	Burkitt		154 (24.5)	128	26	0	30	75	43	6	3	9	52	28	30	14	18
	Diffuse large B cell		121 (19.3)	80	41	0	12	42	50	17	4	9	35	17	22	17	17
	Anaplastic large cell		100 (15.9)	71	29	0	13	31	49	7	4	5	35	21	17	7	11

Table 2 continued

Disease	Subtype	*n* (%)	*n* (%)	Gender, *n* (%)		Age, *n* (%)					Residential areas at diagnosis, *n* (%)						
				Male	Female	0	1–4	5–9	10–14	15–19	Hokkaido	Tohoku	Kanto-Koshinetsu	Tokai-Hokuriku	Kinki	Chugoku-Shikoku	Kyushu-Okinawa
	Other		46 (7.3)	25	21	2	7	10	23	4	2	4	20	6	7	1	6
Hodgkin lymphoma		107 (2.0)		61 (57.0)	46 (43.0)	0 (0.0)	6 (5.6)	28 (26.2)	55 (51.4)	18 (16.8)	4 (3.7)	9 (8.4)	37 (34.6)	19 (17.8)	15 (14.0)	7 (6.5)	16 (15.0)
Histiocytosis		624 (11.8)		329 (52.7)	295 (47.3)	113 (18.1)	259 (41.5)	139 (22.3)	95 (15.2)	18 (2.9)	27 (4.3)	36 (5.8)	213 (34.1)	96 (15.4)	125 (20.0)	55 (8.8)	72 (11.5)
	Langerhans cell histiocytosis		345 (55.3)	199	146	66	143	81	50	5	20	20	110	50	66	39	40
	Hemophagocytic lymphohistiocytosis		265 (42.5)	123	142	44	108	56	44	13	6	15	97	44	55	16	32
	Other		14 (2.2)	7	7	3	8	2	1	0	1	1	6	2	4	0	0
Transient abnormal myelopoiesis associated with Down syndrome		184 (3.5)		100 (54.4)	84 (45.7)	182 (98.9)	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.2)	3 (1.6)	86 (46.7)	23 (12.5)	28 (15.2)	13 (7.1)	27 (14.7)
Other	lymphoproliferative disorders		51 (1.0)		24 (47.1)	27 (52.9)	1 (2.0)	9 (17.7)	19 (37.3)	18 (35.3)	4 (7.8)	0 (0.0)	4 (7.8)	13 (25.5)	8 (15.7)	9 (17.7)	11 (21.6)
6 (11.8)																	
Other hematologic malignancies		6 (0.1)		2 (33.3)	4 (66.7)	5 (83.3)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	2 (33.3)	0 (0.0)	2 (33.3)	1 (16.7)	0 (0.0)	0 (0.0)	1 (16.7)
Hematological malignancies	Total	5,287 (100.0)		3,019 (57.1)	2,268 (42.9)	572 (10.8)	1,803 (34.1)	1,375 (26.0)	1,253 (23.7)	284 (5.4)	215 (4.1)	370 (7.0)	2,029 (38.4)	766 (14.5)	880 (16.6)	449 (8.5)	578 (10.9)

Among NHL patients, those with Burkitt lymphoma (BL, 24.5 %), precursor T-lymphoblastic lymphoma (21.7 %), or diffuse large B cell lymphoma (DLBCL, 19.3 %), respectively, accounted for more than 20 %. When combined with those with anaplastic large cell lymphoma (ALCL, 15.9 %) and those with precursor B-lymphoblastic lymphoma (11.3 %), patients with these types of lymphoma accounted for 92.7 % of all NHL patients. Three subtypes, i.e., nodular sclerosis (37 cases), nodular lymphocyte predominance (29 cases), and mixed cellularity (31 cases), accounted for 90.7 % of all HL patients. While NHL patients were predominantly male, accounting for 2.5 times the number of female patients, no significant gender difference was observed for HL, with only 1.3 times male predominance. Peak incidences of both NHL and HL occurred at 5 years of age or older. While the incidence of BL peaked between ages 5 and 9, the incidence of precursor T-lymphoblastic lymphoma, DLBCL, and ALCL increased with age and peaked between ages 10 and 14.

The majority of histiocytosis included LCH, at 345 cases (55.3 %), followed by HLH at 265 cases (42.5 %). Incidences of both HLH and LCH mainly occurred in children aged four and under.

Among patients with MDS and/or MPN, those with MDS made up the majority (136 cases, 45.9 %), followed by MPN (111 cases, 37.5 %), including chronic myeloid leukemia (CML), and MDS/MPN (49 cases, 16.6 %), including juvenile myelomonocytic leukemia (JMML) and chronic myelomonocytic leukemia (CMML). The breakdown of registered cases of MDS by the JSPH guideline for diagnosis showed that refractory anemia (33 cases) account for 22 %, refractory cytopenia with multi-lineage dysplasia (25 cases) for 18 %, refractory anemia with excess blasts (RAEB)-1 (22 cases) for 16 %, and RAEB2 (19 cases) for 14 %, respectively.

Down-TAM accounted for 3.5 % of all cases, with the incidence rate being slightly higher in males (1.2 times higher than females).

No significant regional difference was observed when looking at age-specific incidences (data not shown).

Survival

Table 3 and Figs. 1, 2, 3, 4, 5 and 6 show disease-specific prognostic information for 5,287 cases of patients who were diagnosed with pediatric hematological malignancies between 2006 and 2010. The median observation period (range) was 1.7 (0.0–5.3) years. The point estimate (95 %CI) of 2y-OS for all pediatric patients with hematological malignancies was 91.6 (90.7–92.5) %. No difference was observed in survival rates in terms of gender (2y-OS 91.5 % for male, 91.9 % for female; log-rank test p value = 0.76) or residential areas at diagnosis (2y-OS 88.0 % for Hokkaido, 93.3 % for Tohoku, 91.5 % for Kanto-Koshinetsu, 91.7 % for Tokai-Hokuriku, 94.2 % for Kinki, 92.2 % for Chugoku-Shikoku, and 88.1 % for Kyushu-Okinawa; p value = 0.11). Survival rates in different age categories showed that children aged 5–9 years had the best prognosis (94.4 %). This was followed by children aged 1–4 years (93.8 %) and those aged 10–14 years (90.8 %), indicating a 2y-OS of more than 90 %. On the other hand, the 2y-OS for patients aged 15–19 years was about 10 % points lower at 80.5 %. The 2y-OS for infants less than 1 year old did not reach 90 % either at 85.6 % (log-rank test p value < 0.0001).

The comparison of 2y-OS among different diseases indicates that patients with HL (95.2 %) had the best prognosis.

Table 3 Survival for Japanese children and adolescents diagnosed with hematological malignancies

Disease	n	1 year		2 year		3 year		4 year		5 year	
		1-yr OS	95 % CI	2-yr OS	95 % CI	3-yr OS	95 % CI	4-yr OS	95 % CI	5-yr OS	95 % CI
Acute lymphoblastic leukemia	2,464	97.3	96.5–97.9	94.2	93.0–95.2	91.1	89.4–92.5	89.1	87.0–90.9	88.7	86.4–90.6
B-precursor	2,110	98.0	97.3–98.6	96.2	95.1–97.0	93.6	92.0–94.9	91.8	89.7–93.5	91.3	88.9–93.2
Mature B	60	95.6	83.4–98.9	84.7	68.8–92.9						
T cell	269	92.4	87.9–95.2	81.3	74.3–86.5	71.0	61.8–78.3	66.9	56.3–75.4		
Unknown	25	90.5	67.0–97.5	75.4	33.3–93.0						
Acute myeloid leukemia	891	91.2	88.9–93.0	83.3	80.1–86.1	77.4	73.3–80.9	76.3	71.9–80.1	75.2	70.3–79.4
M0	33	81.2	60.5–91.8	71.8	49.3–85.7	59.9	30.0–80.3				
M1	73	87.7	75.8–94.0	80.5	66.3–89.2	76.8	61.0–86.9				
M2	218	93.5	88.8–96.3	85.9	79.2–90.6	78.9	70.2–85.3	76.4	66.4–83.8	72.2	58.8–81.9
M3, M3v	70	95.6	87.0–98.6	95.6	87.0–98.6						
M4, M4Eo	112	91.7	84.0–95.8	79.0	68.1–86.6	72.4	59.5–81.7				

Table 3 continued

Disease	*n*	1 year		2 year		3 year		4 year		5 year	
		1-yr OS	95 % CI	2-yr OS	95 % CI	3-yr OS	95 % CI	4-yr OS	95 % CI	5-yr OS	95 % CI
M5a, M5b	124	83.7	74.9–89.6	76.7	66.4–84.2	70.5	58.3–79.7	67.4	54.1–77.6		
M6a, M6b	14	90.9	50.8–98.7	90.9	50.8–98.7						
M7	212	94.0	89.1–96.8	84.8	77.0–90.1	80.2	70.8–86.9				
Unknown	35	90.7	73.9–96.9	82.9	63.2–92.6	56.8	21.9–81.0				
Rare leukemia	36	90.6	73.5–96.9	75.2	51.5–88.5	68.9	43.9–84.5				
Myelodysplastic syndrome (MDS) and/or myeloproliferative neoplasms (MPN)	296	96.2	93.1–98.0	92.8	88.4–95.6	87.9	81.8–92.1	85.3	76.6–91.0		
MPN	111	100.0	–	98.6	90.2–99.8	96.7	87.4–99.2				
MDS/MPN	49	90.6	76.5–96.4	86.6	69.9–94.4	80.0	57.2–91.4				
MDS	136	95.0	89.2–97.7	90.0	81.9–94.6	82.6	71.3–89.7	76.2	57.8–87.4		
Non-Hodgkin lymphoma	628	94.6	92.3–96.2	92.1	89.3–94.2	90.5	87.0–93.1				
Lymphoblastic-T-precursor	136	96.4	90.6–98.6	90.4	82.2–95.0	86.9	77.0–92.7	83.1	69.8–90.9		
Lymphoblastic-B-precursor	71	98.4	89.3–99.8	96.2	85.3–99.1						
Burkitt	154	93.3	87.4–96.4	91.1	84.4–95.0						
Diffuse large B cell	121	97.1	91.3–99.1	97.1	91.3–99.1						
Anaplastic large cell	100	91.0	82.7–95.4	91.0	82.7–95.4						
Other	46	90.7	76.9–96.4	83.8	66.7–92.6						
Hodgkin lymphoma	107	98.7	91.2–99.8	95.2	85.5–98.4						
Histiocytosis	624	94.9	92.8–96.5	93.9	91.4–95.6						
Langerhans cell histiocytosis	345	99.3	97.3–99.8	98.7	95.8–99.6						
Hemophagocytic lymphohistiocytosis	265	88.9	84.3–92.3	87.7	82.8–91.4						
Other	14	100.0	–	90.0	47.3–98.5						
Transient abnormal myelopoiesis associated with Down syndrome	184	89.8	84.3–93.4	89.8	84.3–93.4						
Other lymphoproliferative disorders	51	89.5	76.6–95.5	86.3	71.6–93.7						
Other hematologic malignancies	6	83.3	27.3–97.5	83.3	27.3–97.5						
Hematological malignancies, Total	5,281	95.2	94.6–95.8	91.6	90.7–92.5	88.8	87.6–89.8	87.5	86.2–88.8	87.0	85.4–88.3
Gender											
Female	2,268	95.3	94.2–96.1	91.9	90.5–93.1	89.3	87.4–90.8	87.6	85.5–89.5	86.8	84.3–88.9
Male	3,019	95.2	94.3–96.0	91.5	90.2–92.6	88.4	86.8–89.8	87.5	85.7–89.0	87.2	85.3–88.8
Age											
0	572	88.0	84.8–90.5	85.6	82.0–88.5	83.7	79.7–87.0				
1–4	1,803	96.7	95.7–97.5	93.8	92.4–95.0	91.4	89.5–93.0	90.9	88.8–92.6		
5–9	1,375	97.1	96.0–97.9	94.4	92.7–95.7	91.0	88.7–92.9	89.5	86.7–91.7		
10–14	1,253	95.4	93.9–96.5	90.8	88.6–92.5	88.1	85.5–90.3	85.9	82.7–88.6	84.0	79.6–87.5
15–19	284	90.6	86.2–93.7	80.5	74.2–85.5	73.7	65.8–80.1			69.1	56.6–78.6
Residential areas											
Hokkaido	215	92.8	87.9–95.8	88.0	81.7–92.3	85.9	78.8–90.8			82.6	72.1–89.4
Tohoku	370	96.6	93.9–98.1	93.3	89.4–95.8	89.1	83.7–92.7	86.9	80.5–91.3		
Kanto-Koshinetsu	2,029	95.0	93.9–96.0	91.5	89.9–92.8	87.8	85.6–89.6	86.5	84.0–88.6		
Tokai-Hokuriku	766	95.5	93.6–96.8	91.7	89.1–93.7	90.8	88.0–93.0	90.3	87.3–92.7	89.0	84.7–92.2
Kinki	880	97.1	95.6–98.0	94.2	92.1–95.7	90.7	87.7–93.0	89.0	85.3–91.8	88.0	83.7–91.2
Chugoku-Shikoku	449	94.0	91.1–96.0	92.2	88.8–94.6	88.1	83.4–91.6				
Kyushu-Okinawa	578	93.6	91.1–95.4	88.1	84.6–90.9	87.3	83.7–90.9	87.3	83.7–90.2	85.1	80.5–88.7

yr year, *OS* overall survival

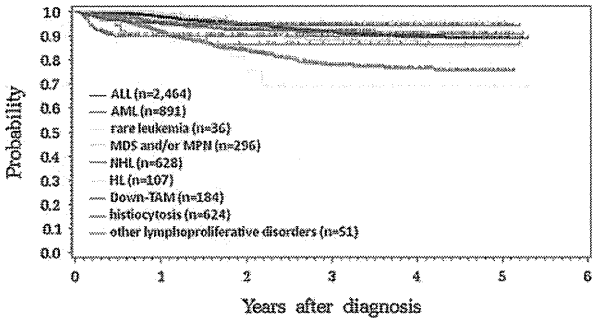


Fig. 1 Overall survival for patients diagnosed with hematological malignancies ($n = 5{,}287$). *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *MDS and/or MPN* myelodysplastic syndrome and/or myeloproliferative neoplasms, *NHL* non-Hodgkin lymphoma, *HL* Hodgkin lymphoma, *Down-TAM* transient abnormal myelopoiesis associated with Down syndrome

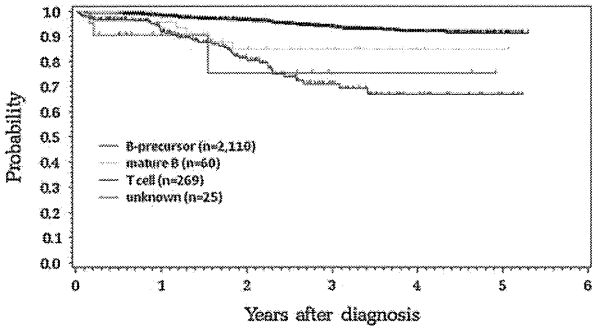


Fig. 2 Overall survival for patients diagnosed with acute lymphoblastic leukemia

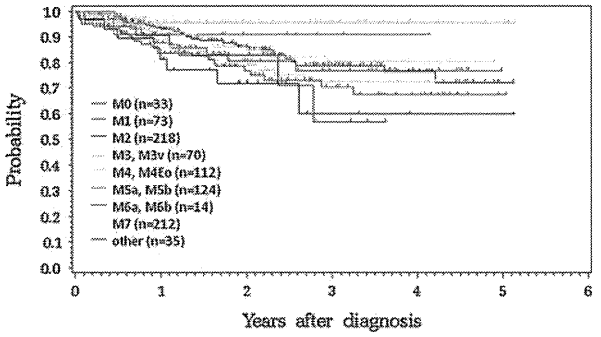


Fig. 3 Overall survival for patients diagnosed with acute myeloid leukemia

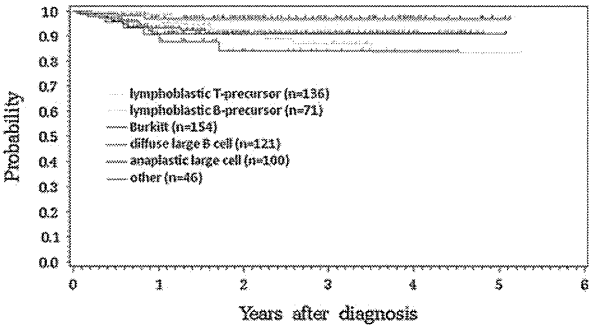


Fig. 4 Overall survival for patients diagnosed with non-Hodgkin lymphoma

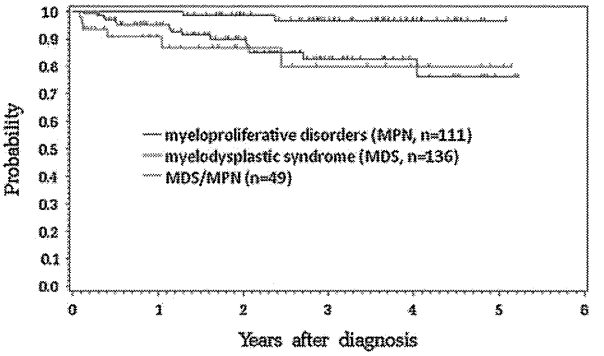


Fig. 5 Overall survival for patients diagnosed with myelodysplastic syndrome and/or myeloproliferative. *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasms

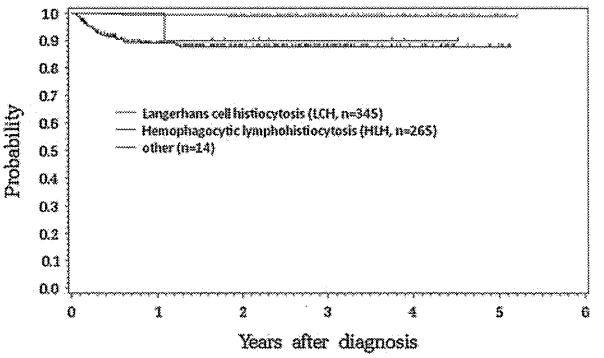


Fig. 6 Overall survival for patients diagnosed with histiocytosis

This was followed by patients with ALL (94.2 %), histiocytosis (93.9 %), MDS/MPN (92.4 %), and NHL (92.1 %). All patients had a survival rate of 90 % or more within 2 years after their disease was diagnosed. The 2y-OS for patients with Down-TAM (89.8 %) and those with other LPD (86.3 %) was estimated more than 85 %, while that for AML (83.3 %) and rare leukemia (75.2 %) was inferior to it.

Examination based on the immunophenotypic classification of ALL shows that patients with B-precursor ALL had the highest 2y-OS at 96.2 %, surpassing the 2y-OS for patients with mature B cell ALL (84.7 %) or T cell ALL (81.3 %).

Examination based on the FAB classification of AML indicates that M3 had the best prognosis (2y-OS 95.6 %),

followed by M6 (90.6 %). The 2y-OS accounted for more than 80 % with the exception of M0 (71.8 %), M5 (76.7 %), and M4 (79.0 %) whose survival rates did not reach 80 %.

Any type of NHL, without being based on the immunological classification, indicated a survival rate of more than 90 % within 2 years after diagnosis; especially, DLBCL (97.1 %) and precursor B-lymphoblastic lymphoma (96.2 %) had excellent prognoses.

Among histiocytosis, LCH indicated an extremely excellent 2y-OS of 98.7 % while that of HLH was 87.7 %. As for MDS and/or MPN, the 2y-OS of MPN was 98.6 % and the best, followed by MDS (90.0 %) and MDS/MPN (86.6 %).

Discussion

This study, which includes the largest childhood cohorts of hematological malignancies ever reported in Japan, documented progressive improvements in survival for children enrolled onto the disease registry project of the JSPH between 2007 and 2011. Considering the number of participating institutions, we estimated that our patient sample collected through the system represented about 80 % of all the cases of hematological malignancies in Japan. As cases newly diagnosed during the 5 years from January 1, 2006 to December 31, 2010, it has reported 5,287 cases of hematological malignancies from 187 institutions (diagnosis and treatment departments) across 47 prefectures nationwide; this result is equivalent to the prevalence of 4.5 cases per 100,000 people per year. The number of registered cases for age 15–19 years is much lower than those at age 14 or younger, which may be reflecting the fact that patients over the age of 16 usually visit internists rather than pediatrician. In order to figure out the exact trends in disease incidence for this age category in Japan, it is necessary to establish a registration system that can be accessed both by internists and pediatricians.

Regarding the incidence by disease group, ALL accounted for approximately half of hematological malignancies and more than 80 % when combined with AML, NHL and histiocytosis, which accounted for 10–15 %, respectively. The incidence by disease was nearly constant regardless of the diagnosis year. The reason why reports of Down-TAM almost doubled in 2010 is inferred that such increase accompanied wider recognition and utilization of the TAM central diagnosis system realized through a nationwide clinical observational study, JPLSG TAM-10, started in the same year (UMIN # 000005418).

According to the immunophenotypic classification counting of ALL, although the percentage of B-precursor ALL in Japan is higher than that in the US (85.6 vs. 63 %), T-ALL and mature B-ALL showed almost the same ratios [18]. It was also consistent with the findings in the US that the age of peak incidence was under 4 years old and that the incidence of ALL is slightly higher among male children than female children [19]. It is reported that introduction of risk-stratified treatment and improvement in supportive care have helped to achieve better treatment results of ALL with its 5y-OS higher than 85 % [14]. Our data showed that the 2y-OS of ALL was 94.2 % while its 5y-OS also exceeded 80 %. This indicates an improvement compared to the results of the European disease registry data during the second half of the twentieth century (1978–1997) [20], suggesting that prognoses as good as those in the results of recent foreign clinical studies have been achieved nationwide [14].

A dramatic improvement in the success rate of the treatment of AML has been seen [21], from about 20 % during the 1970s to 55 % in the decade following 2000. Although there are differences in thinking among different groups studying the treatment, such as those with regard to chemotherapy as well as hematopoietic stem cell transplantation depending on the disease risk, the overall survival in clinical trial has also been improved to have reached 42 $\sim$ 62 % [22–24]. In the AML99 clinical trials (2000–2002) [25–28] conducted in Japan, good results of a 5y-OS of over 76 % were obtained. In the present study also, it was found that by and large a good 2y-OS of higher than 80 % has been obtained even when M3 (2y-OS: 95.6 %), with the best prognosis, is excluded.

The incidence of HL in our data was very low compared to that from other countries. There are reports, both domestic and from overseas, that the incidences of both NHL and HL are relatively high in adolescents, and that the ratio of male to female children is high [29–33]. In our data, NHL is uncommon in infant, and the incidence of NHL increases throughout life. Although NHL is more common than HL in children younger than 15 years, the relative incidence of HL increases in children older than 10 years, making the incidence of HL in children aged between 15 and 19, almost twice that of NHL. In addition, higher incidence of NHL in male children was observed, while there was a slight male predominance in the incidence of HL, with an incidence ratio of 1.3 in male and female children. For NHL, favorable outcomes of treatment were obtained in more than 90 % of the cases within 2 years after diagnosis regardless of the immunological classification, which were similar to reports of clinical trials from inside and outside of the country [34–37].

Similarly, there was a high incidence of LCH, which accounts for the majority of cases of histiocytosis, in the age group of 1–4 years as in the overseas reports [38], and the prognosis was also good [39]. About half of the patients (42.5 %) with histiocytosis were diagnosed as having

HLH. In accordance with the previous literature in Japan, our data showed that the incidence of HLH cases per year was about 50 (mean 53, range 43–64) [40]. And clinical outcomes of HLH were considerably improved compared to the results of HLH-94 study [41].

With regard to MDS and/or MPN, hematopoietic stem cell transplantation, rather than conventional chemotherapy, has come to be a good indication [42] in cases in which there is an HLA-matched sibling donor. Children with low-risk MDS, including refractory anemia and refractory anemia with ring sideroblasts, were not candidates for hematopoietic stem cell transplantation [43]. Although the 5y-OS of children under the age of 16 was, respectively, 50–67 % for MDS and 51–75 % for MPN, depending on the type of transplant, in the national survey results (2011 report) from 1991 to 2010 by the Japan Society for Hematopoietic Cell Transplantation (JSHCT), our data showed that there was an improvement to 86.6 % for MDS/MPN including JMML, for which the prognoses are the worst [44], although the follow-up period was not sufficient.

In treatments for hematological malignancies during childhood and adolescents, long-term toxicity, including treatment-related deaths and secondary neoplasms, still remain as important issues. Therefore, we will continuously evaluate the trends in the national levels of diagnosis and treatment through the JSPH disease registry project and will show data concerning trends in disease incidence and deaths accompanied by prognostic information. Continuous activity to monitor the level of medical care is considered quite important in aiming at the development of more effective treatments which maintain long-term safety.

Acknowledgments The survey on hematological malignancies incidence in Japan was conducted with contributions from the 187 institutions, described in Appendix 1. The authors thank deeply the members, especially Kaori Nagai, Kazumi Takeuchi, Maki Nishimura, and Midori Otomo of the Data Management Department of the NPO-OSCR, for their support in the management of the electronic or paper-based survey system and in the cleaning and tabulation of the registered data.

Conflict of interest The authors have no financial relationship to declare.

Appendix

See appendix Table 4.

Table 4 Institutions with registered cases of hematological malignancies

S. no.	District	Institutions
1	Hokkaido	Oji General Hospital
2	Hokkaido	Sapporo Medical University Hospital
3	Hokkaido	Hokkaido Medical Center for Child Health and Rehabilitation
4	Hokkaido	Sapporo Hokuyu Hospital
5	Hokkaido	Hokkaido University Hospital
6	Hokkaido	KKR Sapporo Medical Center
7	Hokkaido	Asahikawa Medical University Hospital
8	Hokkaido	Hospital Hakodate Hokkaido
9	Hokkaido	Kushiro city General Hospital
10	Hokkaido	National Hospital Organization Hokkaido Cancer Center
11	Tohoku	Hirosaki University school of Medicine and Hospital
12	Tohoku	Nakadori General Hospital
13	Tohoku	Akita University Hospital
14	Tohoku	Iwate Medical University Hospital
15	Tohoku	Iwate Prefectural Chubu Hospital
16	Tohoku	Iwaki Kyoritsu Hospital
17	Tohoku	Fukushima Medical University Hospital
18	Tohoku	Tohoku University Hospital
19	Tohoku	Miyagi Children's Hospital
20	Tohoku	Yamagata University Hospital
21	Tohoku	Sendai City Hospital
22	Kanto and Koshinetsu	Ibaraki Children's Hospital
23	Kanto and Koshinetsu	Tsukuba University Hospital

Table 4 continued

S. no.	District	Institutions
24	Kanto and Koshinetsu	Yokohama City University Hospital
25	Kanto and Koshinetsu	Saiseikai Yokohama City Nanbu Hospital
26	Kanto and Koshinetsu	Kitasato University Hospital
27	Kanto and Koshinetsu	Tokai University Hospital
28	Kanto and Koshinetsu	Showa University Fujigaoka Hospital
29	Kanto and Koshinetsu	Kanagawa Children's Medical Center
30	Kanto and Koshinetsu	St. Marianna University School of Medicine Hospital
31	Kanto and Koshinetsu	Gunma Children's Medical Center
32	Kanto and Koshinetsu	Gunma University Hospital
33	Kanto and Koshinetsu	Saitama Medical Center
34	Kanto and Koshinetsu	Saitama Children's Medical Center
35	Kanto and Koshinetsu	National Defense Medical College Hospital
36	Kanto and Koshinetsu	Teikyo University Chiba Medical Center
37	Kanto and Koshinetsu	Kameda Medical Center
38	Kanto and Koshinetsu	Nippon Medical School Chiba Hokusoh Hospital
39	Kanto and Koshinetsu	Kokuho Asahi General Hospital
40	Kanto and Koshinetsu	Japanese Red Cross Narita Hospital
41	Kanto and Koshinetsu	Chiba University Hospital
42	Kanto and Koshinetsu	Chiba Children's Hospital
43	Kanto and Koshinetsu	Matsudo City Hospital
44	Kanto and Koshinetsu	National Center for Global Health and Medicine
45	Kanto and Koshinetsu	Nihon University Itabashi Hospital
46	Kanto and Koshinetsu	Japanese Red Cross Musashino Hospital
47	Kanto and Koshinetsu	Teikyo University Hospital
48	Kanto and Koshinetsu	Tokyo Medical And Dental University Hospital Faculty of Medicine
49	Kanto and Koshinetsu	The Jikei University Daisan Hospital
50	Kanto and Koshinetsu	Tokyo Metropolitan Children's Medical Center
51	Kanto and Koshinetsu	The Jikei University Hospital
52	Kanto and Koshinetsu	Nippon medical School Hospital
53	Kanto and Koshinetsu	Tokyo Women's Medical University Medical Center East
54	Kanto and Koshinetsu	The University of Tokyo Hospital
55	Kanto and Koshinetsu	Keio University Hospital
56	Kanto and Koshinetsu	Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital
57	Kanto and Koshinetsu	Toho University Omori Medical Center
58	Kanto and Koshinetsu	Showa University Hospital
59	Kanto and Koshinetsu	Juntendo University Hospital
60	Kanto and Koshinetsu	National Center for Child Health and Development
61	Kanto and Koshinetsu	St. Luke's International Hospital
62	Kanto and Koshinetsu	Kyorin University Hospital
63	Kanto and Koshinetsu	Tokyo Dental College Ichikawa General Hospital
64	Kanto and Koshinetsu	Dokkyo Medical University Hospital
65	Kanto and Koshinetsu	Jichi Medical University Hospital
66	Kanto and Koshinetsu	Shinshu University Hospital
67	Kanto and Koshinetsu	Nagano Children's Hospital
68	Kanto and Koshinetsu	Niigata University Medical and Dental Hospital
69	Kanto and Koshinetsu	Niigata Cancer Center Hospital
70	Kanto and Koshinetsu	University of Yamanashi Hospital
71	Kanto and Koshinetsu	Japanese Red Cross Maebashi Hospital

Table 4 continued

S. no.	District	Institutions
72	Kanto and Koshinetsu	Saitama Medical University International Medical Center
73	Kanto and Koshinetsu	Yokosuka Kyosai Hospital
74	Kanto and Koshinetsu	Kofu Municipal Hospital
75	Kanto and Koshinetsu	Teikyo University School of medicine University Hospital, Mizonokuchi
76	Tokai and Hokuriku	Fujita Health University
77	Tokai and Hokuriku	Aichi Medical University Hospital
78	Tokai and Hokuriku	Komaki City Hospital
79	Tokai and Hokuriku	National Hospital Organization Nagoya Medical Center
80	Tokai and Hokuriku	Nagoya Daini Red Cross Hospital
81	Tokai and Hokuriku	Anjo Kosei Hospital
82	Tokai and Hokuriku	Japanese Red Cross Nagoya Daiichi Hospital
83	Tokai and Hokuriku	Nagoya University Hospital
84	Tokai and Hokuriku	Kasugai Municipal Hospital
85	Tokai and Hokuriku	Nagoya City University Hospital
86	Tokai and Hokuriku	Toyohashi Municipal Hospital
87	Tokai and Hokuriku	Ichinomiya Municipal Hospital
88	Tokai and Hokuriku	Okazaki City Hospital
89	Tokai and Hokuriku	Kanazawa University Hospital
90	Tokai and Hokuriku	Ishikawa Prefectural Central Hospital
91	Tokai and Hokuriku	Kanazawa Medical University Hospital
92	Tokai and Hokuriku	Gifu Municipal Hospital
93	Tokai and Hokuriku	Toki Municipal General Hospital
94	Tokai and Hokuriku	Gifu University Hospital
95	Tokai and Hokuriku	Hamamatsu Medical Center
96	Tokai and Hokuriku	Hamamatsu University School of Medicine, University Hospital
97	Tokai and Hokuriku	Shizuoka Children's Hospital
98	Tokai and Hokuriku	Iwata City Hospital
99	Tokai and Hokuriku	Seirei Hamamatsu General Hospital
100	Tokai and Hokuriku	Toyama University Hospital
101	Tokai and Hokuriku	Fukui Red Cross Hospital
102	Tokai and Hokuriku	University of Fukui Hospital
103	Tokai and Hokuriku	Mie University Hospital
104	Tokai and Hokuriku	National Mie Hospital
105	Tokai and Hokuriku	Nagoya City East Medical Center
106	Kinki	National Hospital Organization Osaka National Hospital
107	Kinki	Osaka City University Hospital
108	Kinki	Kinki University Hospital
109	Kinki	Yao Municipal Hospital
110	Kinki	Matsushita Memorial Hospital
111	Kinki	Osaka Medical Center and Research Institute for Maternal and Child Health
112	Kinki	Toyonaka Municipal Hospital
113	Kinki	Osaka University Hospital
114	Kinki	Sakai Hospital Kinki University Faculty of Medicine
115	Kinki	Osaka Medical College Hospital
116	Kinki	Kansai Medical University Hirakata Hospital
117	Kinki	Kitano Hospital, The Tazuke Kofukai Medical Research Institute
118	Kinki	Osaka City General Hospital
119	Kinki	Osaka Red Cross Hospital