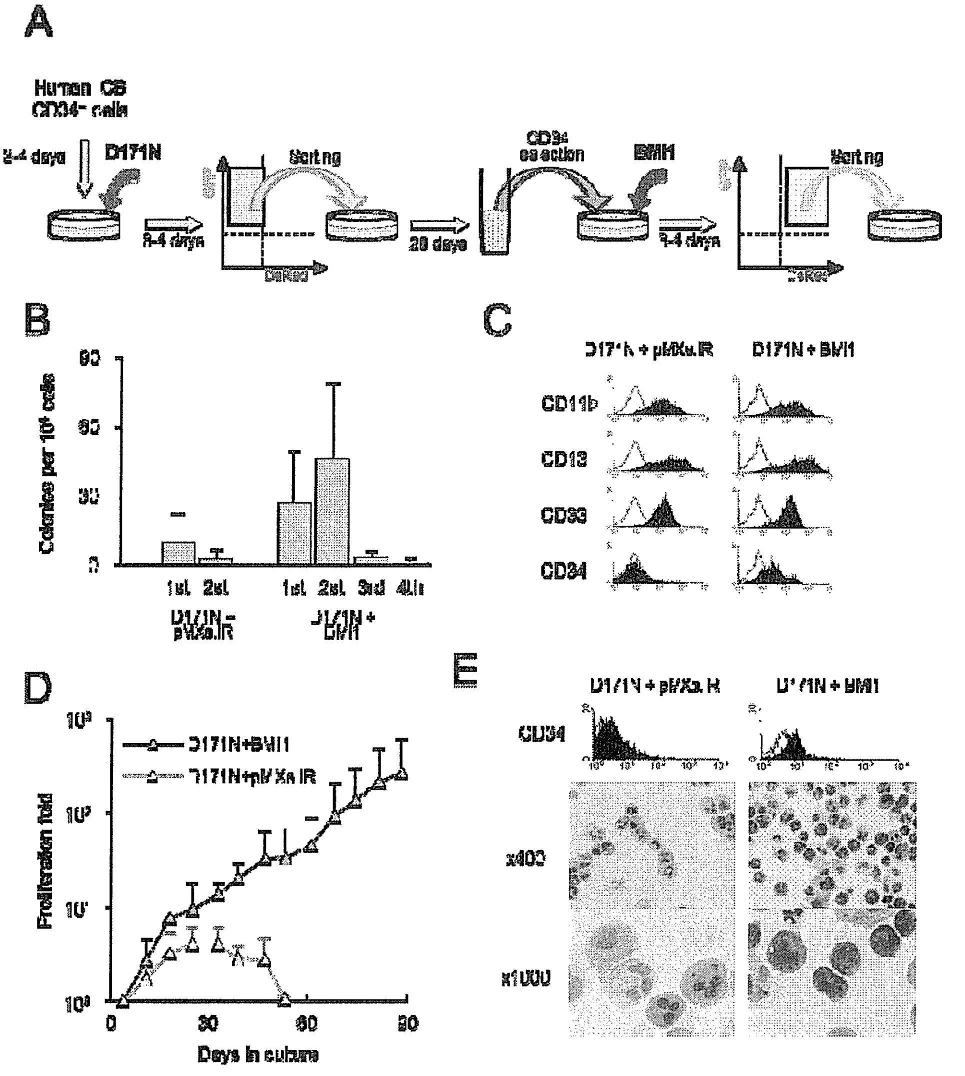
From bloodjournal.hematologylibrary.org at University of Tokyo on April 5, 2013. For personal use only.

Figure 7



Rutten, F.H. (2010) Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). *European Heart Journal*, **31**, 2369–2429.

Fogarty, P.F. (2009) Chronic immune thrombocytopenia in adults: epidemiology and clinical presentation. *Hematology/Oncology Clinics of North America*, **23**, 1213–1221.

Kuter, D.J., Bussel, J.B., Lyons, R.M., Pullarkat, V., Gernsheimer, T.B., Senecal, F.M., Aledort, L.M., George, J.N., Kessler, C.M., Sanz, M.A., Liebman, H.A., Slovick, F.T., de Wolf, J.T., Bourgeois, E., Guthrie, Jr, T.H., Newland, A., Wasser, J.S., Hamburg, S.I., Grande, C., Lefre're, F., Lichtin, A.E., Tarantino, M.D., Terebelo, H.R., Viallard, J.F., Cuevas, F.J., Go, R.S., Henry, D. H., Redner, R.L., Rice, L., Schipperus, M.R., Guo, D.M. & Nichol, J.L. (2008) Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomised controlled trial. *Lancet*, **37**, 95–403.

Severinsen, M.T., Engebjerg, M.C., Farkas, D.K., Jensen, A.Ø., Nørgaard, M., Zhao, S. & Sørensen, H.T. (2010) Risk of venous thromboembolism in patients with primary chronic immune thrombocytopenia: a Danish population-based cohort study. *British Journal of Haematology*, **152**, 360–362.

Stasi, R. (2011) Pathophysiology and therapeutic options in primary immune thrombocytopenia. *Blood Transfusion*, **9**, 262–273.

Tagariello, G., Sartori, R., Radossi, P., Maschio, N., Risato, R. & Stasi, R. (2011) Romiplostim for the early management of severe immune thrombocytopenia unresponsive to conventional treatment. *British Journal of Haematology*. Doi: 10.1111/j.1365-2141.2011.08950.x. [Epub ahead of print].

Expansion of $CD8^+$/perforin$^+$ T-cells predicts response to ciclosporin A therapy in patients with erythroid hypoplasia/aplasia

Erythroid hypoplasia or aplasia is a haematological condition observed in acquired pure red cell aplasia (PRCA) and aplastic anaemia. Myelodysplastic syndrome (MDS) with erythroid hypoplasia/aplasia is a rare form of MDS that is not included in existing classifications of MDS. Patients with erythroid hypoplasia/aplasia have common characteristics: transfusion dependence, immunological abnormalities, and successful immunosuppressive therapies, such as ciclosporin A (CsA) and/or antithymocyte globulin (ATG). Thus, we may regard erythroid hypoplasia/aplasia as an immunological disease entity. However, the pathogenic mechanisms of erythroid hypoplasia/aplasia have not been fully elucidated, although T-lymphocyte-mediated inhibition of erythropoiesis is considered the most likely mechanism of pathogenesis (Fisch *et al*, 2000). It has been reported that autoreactive $CD8^+$ T cells in patients with thymoma-associated PRCA suppress erythropoiesis *in vitro* (Mangan *et al*, 1986). Recently, we reported that oligoclonal expansion of $CD8^+$/perforin$^+$ T cells in the bone marrow was observed in patients with thymoma-associated PRCA, and the oligoclonality was exclusively detected in $CD8^+$ T cells, not in $CD4^+$ T cells (Nitta *et al*, 2010). To clarify the pathogenic role of the T cells, we analysed the T-cell subsets and therapeutic responses in patients with erythroid hypoplasia/aplasia.

A total of 22 patients with erythroid hypoplasia/aplasia, including eight MDS with erythroid hypoplasia/aplasia, three idiopathic PRCA, three thymoma-associated PRCA, and eight aplastic anaemia, were enrolled in this study. Patients with erythroid hypoplasia/aplasia that were possibly due to viral infection were excluded from this study. All patients were treated with CsA alone, and improvement of anaemia was evaluated in accordance with the International Working Group (IWG) 2006 criteria of MDS (Cheson *et al*, 2007) and guidelines of acquired aplastic anaemia and PRCA (Marsh *et al*, 2003; Sawada *et al*, 2007). Erythroid cellularity in all the patients was confirmed by performing bone marrow aspiration and/or biopsy. For T-cell subset analysis, mononuclear cells (MNCs) were purified from the bone marrow (BM) or peripheral blood (PB) of the patients. As controls, 30 patients without BM abnormalities and 30 patients with MDS without erythroid hypoplasia/aplasia were analysed. Patients gave written consent, in accordance with the Declaration of Helsinki. This study was approved by the Institutional Review Board of Hiroshima University.

For T-cell subset analysis, MNCs were stained with fluorescein isothiocyanate (FITC) [Becton Dickinson (BD), San Jose, CA, USA], phycoerythrin (PE) (BD), peridinin chlorophyll protein (PerCP) (BD), or allophycocyanin (APC) (BD)-conjugated antibodies for CD8 subpopulation analysis: FITC anti-CD8, PE anti-perforin or anti-CCR7 (CD197), PerCP anti-CD62L or anti-CD28, and APC anti-CD27 or anti-CD45RA or anti-CD45RO. In addition, to clarify the population of regulatory T cells (Tregs), CD4, CD25, and FoxP3 expressions were analysed in BM-MNCs by 3-colour flow cytometry using a PE-anti-human FoxP3 staining kit (BD); Th17 cells were identified by staining with anti-IL-17 antibody-PE and anti-CD4 antibody-PerCP. Intracellular analysis of perforin and FoxP3, and IL-17 staining were performed after fixation [BD Cytofix/Cytoperm fixatives (BD)]. The cells were washed with Perm/Wash buffer in accordance with the instruction manual. Data were analysed using a FACS Calibur flow cytometer (BD) with CellQuest software (BD).

© 2012 Blackwell Publishing Ltd

Table I. Characteristics of patients with erythroid hypoplasia/aplasia

Case no.	Age (years)/ sex	Disorder	Karyo type	Hb (g/l)	Ret (%)	Epo (iu/l)	Erythro blast% (BM)	NCC (x 10^9/l)	CD4/8 ratio (BM)	$CD8^+$/ $Perforin^+$% (BM)	*TRB@* rearrangement (BM)
CsA responders											
1	87/F	MDS (RA) with erythroid aplasia	46, XX	61	0·32	5220·0	2·4	15 8400	0·30	46·8	Rearranged
2	73/M	MDS (RA) with erythroid aplasia	46, XY	60	0·14	3360·0	0	48 050	0·32	64·8	Rearranged
3	70/M	MDS (RA) with erythroid hypoplasia	46, XY	72	0·70	25·9	11·0	25 000	0·42	50·2	Not rearranged
4	80/F	MDS (RA) with erythroid aplasia	46, XX	64	0·80	ND	3·0	24 000	0·78	41·9 (PB)	ND
5	67/F	Idiopathic PRCA	46, XX	47	0·19	3040·0	0·5	75 600	1·37 (PB)	45·2 (PB)	Rearranged
6	70/F	Thymoma-PRCA	46, XX	55	0·36	683·0	0	106 900	0·57	44·1	Rearranged
7	49/M	Thymoma-PRCA	46, XY	61	0·09	8590·0	0	329 200	0·36	34·8	Rearranged
8	58/F	Thymoma-PRCA	46, XX	43	0·13	ND	0	31 050	ND	51·8	Rearranged
9	81/F	Aplastic anaemia	46, XX	58	2·60	127·0	29·5	65 700	1·33	41·6 (PB)	Rearranged
10	57/M	Aplastic anaemia	46, XY	65	0·44	ND	6.5	57 150	0·88	36·1	ND
CsA non-responders											
11	76/F	MDS (RA) with erythroid aplasia	46, XX	68	0·32	3980.0	0.5	30 250	0·66	29·2	Rearranged
12	78/M	MDS (RA) with erythroid aplasia	46, XY	68	0.91	1810·0	1.0	150 200	1·15	25·3	Rearranged
13	73/M	MDS (RA) with erythroid aplasia	45, X, -Y [17/20]	57	0·19	346·0	3·0	102 750	1·03	11·9	Rearranged (PB)
14	56/F	MDS (RA) with erythroid hypoplasia	46, XX	74	0·72	ND	14·5	231 200	ND	13·6 (PB)	ND
15	33/F	Idiopathic PRCA	46, XX	53	0·38	ND	1·0	105 700	ND	20·8	Not rearranged
16	69/M	Idiopathic PRCA	46, XY	57	0·31	290·0	0·5	91 750	0·53	30·3	Not rearranged
17	52/M	Aplastic anaemia	46, XY	67	1·42	ND	26·0	21 100	0·24 (PB)	28·0	Rearranged (PB)
18	65/M	Aplastic anaemia	46, XY	53	1·47	ND	29·0	88 300	ND	19·0	ND
19	72/M	Aplastic anaemia	45, X, -Y [7/20]	59	1·33	ND	21·5	38 900	ND	6·3	ND
20	39/F	Aplastic anaemia	46, XX	85	2·18	ND	16·8	35 750	0·37	6·6	ND
21	71/M	Aplastic anaemia	46, XY	60	2·27	ND	37·5	11 900	ND	18·2	ND
22	66/M	Aplastic anaemia	46, XY	72	1·40	ND	16·4	40 400	ND	12·4	ND

CsA, ciclosporin A; Epo, erythropoietin; BM, bone marrow; PB, peripheral blood; NCC, nucleated cell count; MDS, myelodysplastic syndrome; RA, refractory anaemia; PRCA, pure red cell aplasia; M, male; F, female; ND, not done. [Correction added on 4 April 2012, after first online publication: Ages of patients in case numbers 6, 7, 8, 12 and 15 have been corrected.]

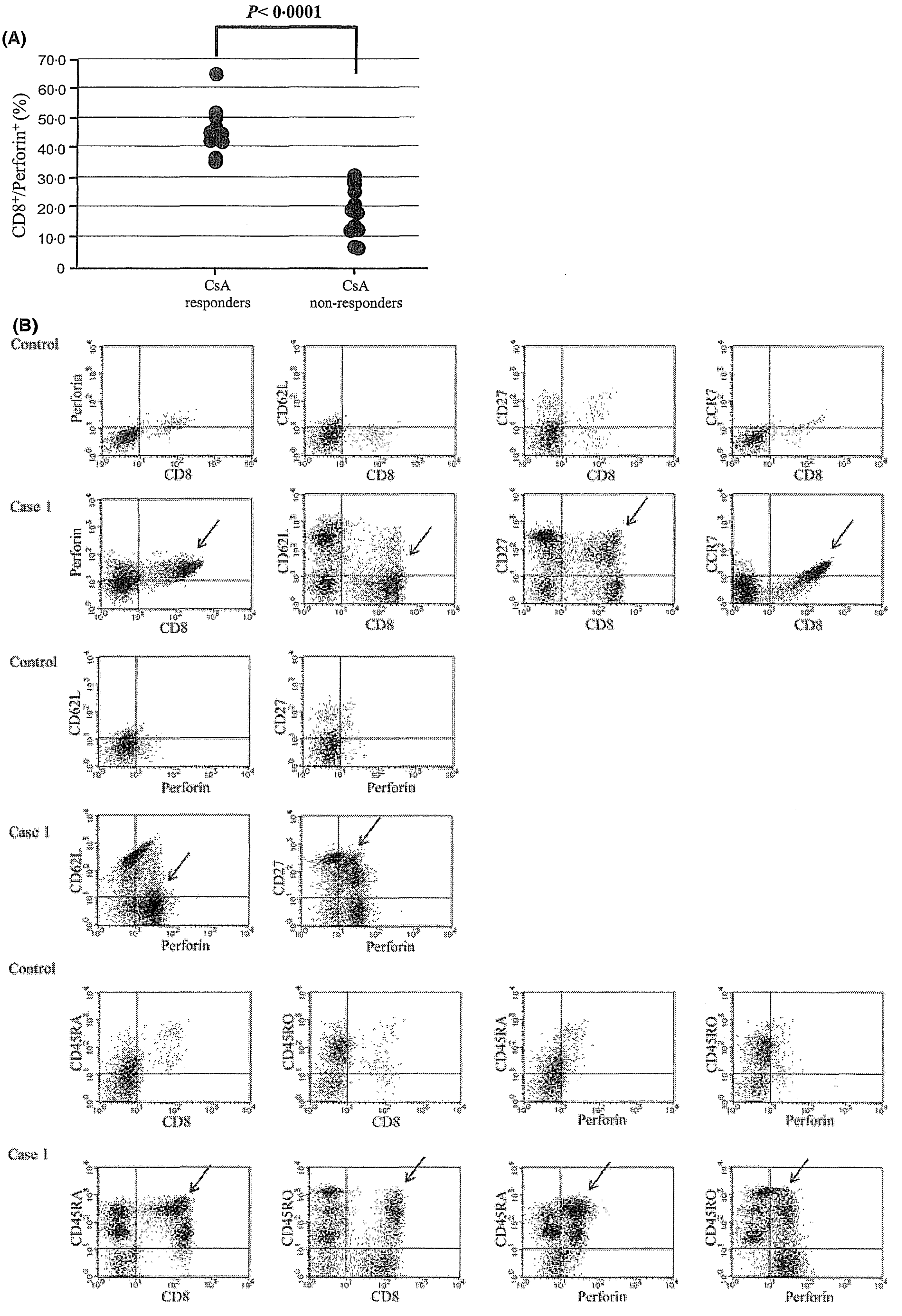
T-cell receptor beta gene (*TRB@*) rearrangements from BM-MNCs or PB-MNCs were assessed by polymerase chain reaction (PCR) assays using BIOMED-2 (Van Dongen *et al*, 2003) (InVivoScribe Technologies, San Diego, CA, USA).

Statistical significance of differences between independent groups was determined using Student's *t*-tests. *P* values <0·05 were considered statistically significant.

Among 22 patients with erythroid hypoplasia/aplasia, 10 patients (4 MDS with erythroid hypoplasia/aplasia, one idio-

Fig 1. (A) Comparison of $CD8^+$/$perforin^+$ T cells between ciclosporin A responders and non-responders. Patients in whom the proportion of $CD8^+$/$perforin^+$ T cells expanded (45·7 ± 8·6%, $n = 10$) in the bone marrow or peripheral blood were ciclosporin A (CsA) responders. In contrast, patients in whom the proportion of $CD8^+$/$perforin^+$ T cells did not expand (18·5 ± 8·5%, $n = 12$, $P < 0.0001$) were CsA non-responders. (B) Immunophenotypic analysis of proliferative T cells in bone marrow mononuclear cells (BM-MNCs) of a patient with MDS with erythroid aplasia showing good response to CsA therapy. $CD8^+$/$perforin^+$ T cells in BM-MNCs of MDS with erythroid aplasia showing good response to CsA therapy were significantly increased compared with those in the normal controls (Case 1: 46·8% vs. Control: 7·1%). The T-cell subpopulation expressing $CD8^+$ $perforin^+$ $CD62L^{low}$ $CD27^+$ $CCR7^{low}$ $CD45RA^{++}$ $CD45RO^+$, which was consistent with the $CD8^+$/$perforin^+$ T_{EM} subset, increased in the bone marrow.

© 2012 Blackwell Publishing Ltd



© 2012 Blackwell Publishing Ltd

pathic PRCA, three thymoma-associated PRCA, and 2 aplastic anaemia) responded to CsA therapy within 2–8 weeks (Table I). The median blood haemoglobin concentration increased from 65 g/l at the baseline to 93 g/l with treatment, with a median haemoglobin increase of 28 g/l from the baseline. We attempted to compare the T-cell subsets between CsA responders and non-responders. Given that $CD8^+$/perforin$^+$ T cells expanded in the bone marrow of 3 patients with thymoma-associated PRCA in our previous study, we focused on a T-cell subpopulation expressing $CD8^+$/perforin$^+$. Intriguingly, the $CD8^+$/perforin$^+$ T cells were significantly increased in the CsA responders ($45{\cdot}7 \pm 8{\cdot}6\%$, $n = 10$) compared with those in the non-responders ($18{\cdot}5 \pm 8{\cdot}5\%$, $n = 12$, $P < 0{\cdot}0001$, Fig 1A), normal BM controls ($16{\cdot}9 \pm 7{\cdot}0\%$, $n = 30$), and those with MDS without erythroid hypoplasia/aplasia ($15{\cdot}1 \pm 7{\cdot}0\%$, $n = 30$). Furthermore, we confirmed that the numbers and proportions of $CD8^+$/perforin$^+$ T cells in BM-MNCs or PB-MNCs decreased during remission following CsA therapy in 4 cases of CsA responders (data not shown). Among the $CD8^+$/perforin$^+$ T cells, the $CD62L^{low}$ $CD27^+$ $CCR7^{low}$ $CD45RA^{++}$ $CD45RO^+$ population was prominent (Fig 1B), which is consistent with an effector memory T (T_{EM}) cell subset (Decrion *et al*, 2007). In contrast, Treg ($CD4^+$ $CD25^{high}$ $FoxP3^+$) and Th17 ($CD4^+$ IL-17^+) cell populations were not associated with CsA responsiveness in patients with erythroid hypoplasia/aplasia (data not shown).

It has been reported that CsA therapy produced complete or partial remission in 19/20 (95%) thymoma-associated PRCA patients and all evaluable CsA responders became transfusion-independent within 2 weeks of the initiation of treatment (Hirokawa *et al*, 2008). However, most thymoma-associated PRCA patients, including our cases, required continuous CsA treatment (Hirokawa *et al*, 2008). These data suggest that CsA can predominantly suppress the cytotoxic function by $CD8^+$/perforin$^+$ T cells. Our study showed that the $CD8^+$/perforin$^+$ T-cell subset is a large population in patients with CsA-responsive erythroid hypoplasia/aplasia. It is suggested that the $CD8^+$/perforin$^+$ T-cell subset may have functions to reduce erythroid progenitors via immunological mechanisms. However, we could not confirm whether the $CD8^+$/perforin$^+$ T cells directly affect erythroid progenitors because the presence of $CD8^+$/perforin$^+$ T cells was identified by intracellular staining of perforin after fixation.

In conclusion, expansion of $CD8^+$/perforin$^+$ T cells predicts response to CsA therapy in patients with erythroid hypoplasia/aplasia. The disease entity of 'erythroid hypoplasia/aplasia with expansion of $CD8^+$/perforin$^+$ T-cell subset', including MDS, PRCA, and aplastic anaemia, may have common pathogenetic mechanisms. From our results, expansion of $CD8^+$/perforin$^+$ T cells in patients with erythroid hypoplasia/aplasia could be a new, useful, and simple marker in CsA therapy. We are convinced that CsA therapy should be actively applied for this disease entity.

Acknowledgements

The authors would like to thank Dr Kazuo Oshimi (ex-professor of Juntendo University School of Medicine, Tokyo) for critical comments on our manuscript, and Sachiko Fukumoto, for providing excellent technical support in carrying out these experiments.

Authorship and disclosures

H.N. performed research, analysed and interpreted data and wrote the manuscript; Y.H. interpreted data and revised the manuscript; H.H. provided patient samples; A.K. provided patient samples and revised the manuscript; and H.H designed the research and revised the manuscript.

Conflict of interest

The authors declare no competing financial interests.

Hideaki Nitta[1]
Yuka Harada[2]
Hideo Hyodo[1]
Akiro Kimura[1]
Hironori Harada[1]

[1]*Division of Clinical Research, Department of Haematology and Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, and* [2]*Division of Radiation Information Registry, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan*
E-mail: herf1@hiroshima-u.ac.jp

Keywords: erythroid hypoplasia/aplasia, myelodysplastic syndrome, $CD8^+$/perforin$^+$ T-cells, ciclosporin A

First published online 14 February 2012
doi: 10.1111/j.1365-2141.2012.09057.x

References

Cheson, B.D., Greenberg, P.L., Bennett, J.M., Lowenberg, B., Wijermans, P.W., Nimer, S.D., Pinto, A., Beran, M., de Witte, T.M., Stone, R. M., Mittelman, M., Sanz, G.F., Gore, S.D., Schiffer, C.A. & Kantarjian, H. (2007) Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood*, **108**, 419–425.

Decrion, A.Z., Varin, A., Drobacheff, C., Estavoyer, J.M. & Herbein, G. (2007) A subset of functional effector-memory CD8+ T lymphocytes in human immunodeficiency virus-infected patients. *Immunology*, **121**, 405–415.

Fisch, P., Handgretinger, R. & Schaefer, H.E. (2000) Pure red cell aplasia. *British Journal of Haematology*, **111**, 1010–1027.

Hirokawa, M., Sawada, K., Fujishima, N., Nakao, S., Urabe, A., Dan, K., Fujisawa, D., Yonemura, Y., Kawano, F., Omine, M. & Ozawa, K. (2008) Long-term response and outcome following immunosuppressive therapy in thymoma-associ-

© 2012 Blackwell Publishing Ltd

ated pure red cell aplasia: a nationwide cohort study in Japan by the PRCA collaborative study group. *Haematologica*, 93, 27–33.

Mangan, K.F., Volkin, R. & Winkelstein, A. (1986) Autoreactive erythroid progenitor-T suppressor cells in the pure red cell aplasia associated with thymoma and panhypogammaglobulinemia. *American Journal of Hematology*, 23, 167–173.

Marsh, J.C., Ball, S.E., Darbyshire, P., Gordon-Smith, E.C., Keidan, A.J., Martin, A., McCann, S.R., Mercieca, J., Oscier, D., Roques, A.W. & Yin, J.A.; British Committee for Standards in Haematology. (2003) Guidelines for the diagnosis and management of acquired aplastic anaemia. *British Journal Haematology*, 123, 782–801.

Nitta, H., Mihara, K., Sakai, A. & Akiro, K. (2010) Expansion of CD8+ /perforin+ effector memory T cells in the bone marrow of patients with thymoma-associated pure red cell aplasia. *British Journal of Haematology*, 150, 712–715.

Sawada, K., Hirokawa, M., Fujishima, N., Teramura, M., Bessho, M., Dan, K., Tsurumi, H., Nakao, S., Urabe, A., Omine, M. & Ozawa, K.; PRCA Collaborative Study Group (2007) Long-term outcome of patients with acquired primary idiopathic pure red cell aplasia receiving cyclosporine A. A nationwide cohort study in Japan for the PRCA Collaborative Study Group. *Haematologica*, 92, 1021–1028.

Van Dongen, J.J., Langerak, A.W., Brüggemann, M., Evans, P.A., Hummel, M., Lavender, F.L., Delabesse, E., Davi, F., Schuuring, E., García-Sanz, R., van Krieken, J.H., Droese, J., González, D., Bastard, C., White, H.E., Spaargaren, M., González, M., Parreira, A., Smith, J.L., Morgan, G.J., Kneba, M. & Macintyre, E.A. (2003) Design & standardization of PCR primers & protocols for detection of clonal immunoglobulin & T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 concerted action BMH4-CT98-3936. *Leukemia*, 17, 2257–2317.

Serum ferritin and total units transfused for assessing iron overload in adults with sickle cell disease

Blood transfusion therapy usage in sickle cell disease (SCD) is increasing (Drasar *et al*, 2011) and with this comes the potential for morbidity and mortality associated with the consequent iron overload. Whilst patients treated with regular blood transfusions have their iron indices monitored regularly and are under regular clinical review, patients receiving sporadic blood transfusions can accrue a substantial iron load relatively unnoticed. Serum ferritin (SF) is a widely available and cost-effective screening test for iron overload but can be unreliable in SCD due to the inflammatory nature of the condition, even in the steady state (Adamkiewicz *et al*, 2009). R2 magnetic resonance imaging (R2MRI) is a recognized non-invasive method of estimating liver iron concentration (LIC) (St Pierre *et al*, 2005) but has limited availability. A simple method of assessing iron load in patients having regular and intermittent transfusions is therefore needed to enable appropriate targeting of resources.

We present a retrospective review of the patients entered in our sickle cell database at King's College Hospital [KCH], London, covering data from a 20-year period, from 1st January 1990 to 31st January 2011. Six hundred and sixty adult SCD patients ranging from 16 to 80 years of age (mean 35 years, standard deviation [SD] ± 11·08) had steady-state SF levels and an accurate transfusion history. Fifty-seven percent of patients were female and patient genotypes consisted of HbSS 62·0%, HbSC 31·7%, HbSβ^0 1·8% and HbSβ^+ 4·5%. LIC was assessed non-invasively by R2MRI (Ferriscan®) in 52 patients and cardiac T2* was performed concurrently in 18 cases. R2MRI is a well-validated method of assessing liver iron load and therefore these patients formed the final study group. Clinical characteristics obtained were age, frequency of transfusion (regular *versus* sporadic), total top-up units transfused (TUT) and transfusion rate (TUT/top-up years). Exchange transfused units were not included in the analysis. Patient consent was formally obtained for the R2MRI scans under ethics number 08/H1101/123. Data were not normally distributed and therefore Spearman's rank test was used to compare the data ($P < 0{\cdot}05$ was used to define statistical significance).

Of the 660 patients, 317 (48%) had received at least 1 unit of blood, 238 (75%) of which were HbSS. The study group of 52 patients (35 female) consisted of 48 HbSS, two HbSβ^0 and two HbSC with age ranging from 19 to 63 years (mean 38 years).

We initially assessed which parameters most effectively predicted iron loading in the liver, as there is currently no consensus on this (Adamkiewicz *et al*, 2009; Inati *et al*, 2010). We found (in contrast to Inati *et al*, 2010) that TUT correlated more strongly with LIC rather than transfusion rate ($R = 0{\cdot}71$ $P < 0{\cdot}0001$ for TUT *versus* $R = 0{\cdot}62$ $P < 0{\cdot}0001$ for TUT/top-up years). A positive correlation was found between SF and TUT/top-up years, however this was weaker than other published data ($R = 0{\cdot}48$ $P < 0{\cdot}0001$). SF correlated significantly with LIC ($R = 0{\cdot}91$ $P < 0{\cdot}0001$) but in a non-linear manner. We subdivided our patients according to SF < or $\geq$1000 μg/l (as per National Institutes of Health guidelines (Adams *et al*, 2004)) and TUT < or $\geq$20 units TUT (see Fig 1).

Twenty-seven of the 52 patients had SF $\geq$ 1000 μg/l, with a mean of 3995 μg/l (range 1004–16 000 μg/l) and all 27 patients had LIC $\geq$ 2 mg/g dry-weight [g DW] (range 2·1–>43 mg/g DW, mean 22·6 mg/g DW). The normal range of LIC as estimated by R2MRI (Ferriscan®) was 0·7–

© 2012 Blackwell Publishing Ltd

From bloodjournal.hematologylibrary.org at University of Tokyo on April 5, 2013. For personal use only.

Clinical characteristics and outcome of refractory/relapsed myeloid leukemia in children with Down syndrome

Takashi Taga,[1] Akiko Moriya Saito,[2] Kazuko Kudo,[3] Daisuke Tomizawa,[4] Kiminori Terui,[5] Hiroshi Moritake,[6] Akitoshi Kinoshita,[7] Shotaro Iwamoto,[8] Hideki Nakayama,[9] Hiroyuki Takahashi,[10] Akio Tawa,[11] Akira Shimada,[12] Tomohiko Taki,[13] Hisato Kigasawa,[14] Katsuyoshi Koh,[15] and Souichi Adachi[16]

[1]Department of Pediatrics, Shiga University of Medical Science, Shiga, Japan; [2]Laboratory of Clinical, Epidemiological and Health Services Research, National Hospital Organization, Nagoya Medical Center Clinical Research Center, Aichi, Japan; [3]Department of Hematology/Oncology, Shizuoka Children's Hospital, Shizuoka, Japan; [4]Department of Pediatrics, Tokyo Medical and Dental University, Tokyo, Japan; [5]Department of Pediatrics, Hirosaki University Graduate School of Medicine, Aomori, Japan; [6]Department of Reproductive and Developmental Medicine, Division of Pediatrics, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan; [7]Department of Pediatrics, St Marianna University School of Medicine, Kanagawa, Japan; [8]Department of Pediatrics, Mie University Graduate School of Medicine, Mie, Japan; [9]Department of Pediatrics, National Hospital Organization, Fukuoka-Higashi Medical Center, Fukuoka, Japan; [10]Department of Pediatrics, Saiseikai Yokohama City Southern Hospital, Kanagawa, Japan; [11]Department of Pediatrics, National Hospital Organization, Osaka Medical Center, Osaka, Japan; [12]Department of Pediatrics, Nagoya University Graduate School of Medicine, Aichi, Japan; [13]Department of Molecular Diagnostics and Therapeutics, Kyoto Prefectural University of Medicine, Kyoto, Japan; [14]Department of Hematology/Oncology, Kanagawa Children's Medical Center, Kanagawa, Japan; [15]Department of Hematology/Oncology, Saitama Children's Medical Center, Saitama, Japan; and [16]Human Health Sciences, Kyoto University, Kyoto, Japan

Myeloid leukemia in Down syndrome (ML-DS) is associated with good response to chemotherapy and favorable prognosis. Because little research has been focused on refractory/relapsed (R/R) cases, we conducted a retrospective analysis for R/R ML-DS. Among ML-DS patients diagnosed between 2000 and 2010 in Japan, 26 relapsed (25 in the BM and 1 in the skin), and 3 refractory patients were enrolled. The male/female ratio was 18/11. The median age at initial diagnosis of ML-DS was 2 years, and the median time to relapse was 8.6 months. Each patient initially had been treated with ML-DS–specific protocols. Thirteen of the 26 patients achieved complete remission with various kinds of reinduction chemotherapies; 2 of 8 survived without further recurrence after receiving allogeneic hematopoietic stem cell transplantation, and 4 of 5 maintained complete remissions with chemotherapy alone. Treatment failures mostly were associated with disease progression rather than treatment-related toxicities. The 3-year OS rate was 25.9% ± 8.5%. A longer duration from initial diagnosis to relapse was a significant favorable prognostic factor ($P < .0001$). We conclude that clinical outcome for patients with R/R ML-DS generally are unfavorable, even in those receiving hematopoietic stem cell transplantation. Novel methods to identify poor prognostic factors for ML-DS are necessary. (*Blood*. 2012; 120(9):1810-1815)

Introduction

Down syndrome (DS) is one of the most common congenital disorders and is associated with an increased risk of acute leukemia.[1] Acute myeloid leukemia (AML) in patients with DS is categorized as myeloid leukemia associated with DS (ML-DS) in the 4th edition of the World Health Organization classification. Clinical and biologic features of ML-DS in children are quite different from those of AML in children without DS and include: younger age at onset, lower white blood cell (WBC) count at diagnosis, and greater incidence of acute megakaryoblastic leukemia.[2,3] ML-DS is known to exhibit good sensitivity against cytotoxic agents, especially cytarabine (Ara-C), and outcomes in recent clinical trials are favorable: long-term event-free survival has been reported in approximately 80% of patients.[4-11] However, little attention has focused on refractory or relapsed (R/R) cases because most treatment failures are the result of toxicities rather than to resistant or recurrent leukemia. We present is a nationwide retrospective analysis of patients with R/R ML-DS in Japan.

Methods

The present retrospective study was conducted on 120 institutions that belong to the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG), a Japanese nationwide collaborative study group for childhood hematologic malignancies, and data on 29 patients with R/R ML-DS treated in 26 hospitals were collected. Patients were either enrolled on one of the AML clinical trials or registered on patient database of the collaborative study group at initial diagnosis of ML-DS, of which the protocols and registrations were approved by the institutional review boards of each participating center with informed consent obtained in accordance with the Declaration of Helsinki. The present retrospective study was approved by the JPLSG steering committee and institutional review boards of Shiga University of Medical Science for all aspects of this investigation.

Submitted March 1, 2012; accepted June 24, 2012. Prepublished online as *Blood* First Edition paper, July 9, 2012; DOI 10.1182/blood-2012-03-414755.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2012 by The American Society of Hematology

From bloodjournal.hematologylibrary.org at University of Tokyo on April 5, 2013. For personal use only.

Inclusion criteria on current analyses were ML-DS patients initially treated with curative intent between January 1, 2000, and December 31, 2010, and age younger than 18 years at the onset of ML-DS. Patients with myelodysplastic syndrome with DS also were included because it is currently recognized that there are no biologic and clinical differences between myelodysplastic syndrome and overt AML in patients with DS. Clinical data at initial diagnosis of ML-DS, including sex, age, WBC count, extramedullary disease, French-American-British (FAB) morphology, therapy protocol given, and duration from initial diagnosis to relapse, were collected. In addition, treatment data, including achievement of further remission, HSCT, secondary cancer, outcome, and cause of death after diagnosis of R/R ML-DS, also were collected.

Statistical analyses

Descriptive statistical analyses to assess baseline characteristics and the clinical course of patients diagnosed with R/R ML-DS were performed by use of the χ^2 tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. Overall survival (OS) was defined as the length of time from the diagnosis of R/R ML-DS to death from any cause. OS percentages and standard errors were calculated with the Kaplan-Meier method, and log-rank tests were used for group comparisons. A Cox proportional hazards regression model was used to investigate risk factors that were associated with survival after diagnosis of R/R ML-DS. Variables including sex (female vs male), age at initial diagnosis ($\leq$ 2 years vs $>$ 2 years), WBC at initial diagnosis ($\geq$ 10 000/μL vs $<$ 10 000/μL), FAB morphology at initial diagnosis (M7 vs others), disease status of R/R ML-DS (induction failure or relapse $\leq$ 6 months vs relapse $>$ 6 months), achievement of further remission (yes vs no), and treatment of HSCT (yes vs no) that were significantly associated with survival in the univariate analyses were considered for inclusion in the model. Significant variables associated with survival were then identified. No statistical adjustment was made for performing multiple tests, but 2-sided *P* values greater than .05 were interpreted with caution. All data analyses were performed by the use of SAS Version 9.1.3 statistical software (SAS Institute). Follow-up data were actualized as of December 31, 2011.

Results

Patient characteristics and treatment at initial diagnosis

Relevant clinical data of the 29 patients at initial diagnosis are shown in Table 1; a slight predominance of male patients existed (male/female ratio was 18/11), median age at initial diagnosis for ML-DS was 2 years (range, 7 months to 16 years) at which 23 of 29 patients were younger than 4 years of age, median WBC count was 5600/μL (range, 900-143 600/μL), and only 1 patient had an extramedullary disease (at skin). Morphologically, 22 (75.8%) patients showed FAB M7 blasts. Karyotype analysis at initial diagnosis showed monosomy 7 in 2 patients, monosomy 7 associated with a ring or marker chromosome in 5 patients,[12] t(8;21)(q22;q22) with FAB M2 morphology in 1 patient, other various cytogenetic abnormalities in 13 patients, and 6 patients with normal karyotype and sole constitutional trisomy 21. Full karyotypes are listed in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

All patients initially were treated with one of the protocols specifically designed for ML-DS. Twenty patients were treated according to the AML99 Down protocol of the Japanese Childhood AML Cooperative Study[6]; 7 patients were officially enrolled in the study, and the rest were treated according to the institutional choice. Among the remaining 9 patients, 8 patients were treated with the JPLSG AML-D05 protocol (registered at http://www.umin.ac.jp/ctr/ as UMIN000000989) and 1 patient with the Japanese Children's Cancer and Leukemia Study Group AML9805 Down protocol.[7] The AML99 and AML-D05 protocol, with which 28 of 29 patients were treated, consists of 5 courses of pirarubicin

Table 1. Clinical characteristics and treatment of the 29 ML-DS patients at initial diagnosis

	No.	%
Age, y		
Median (range)	2 (0.6-16)	
0- < 1	2	6.9
1 ≤ - < 2	9	31.0
2 ≤ - < 3	6	20.7
3 ≤ - < 4	6	20.7
4 ≤	6	20.7
Sex		
Male	18	62.1
Female	11	37.9
WBC, × 10^9/L		
Median (range)	5.6 (0.9-143.6)	
FAB classification		
M1	1	3.5
M2	1	3.5
M5	1	3.5
M7	22	75.8
MDS/unclassified	4	13.7
Karyotype		
Constitutional trisomy 21	6	20.7
Monosomy 7	2	6.9
−7+ring/marker	5	17.2
t(8;21)(q22;q22)	1	3.5
Other abnormalities	13	44.8
Not available	2	6.9
Initial treatment for ML-DS		
AML99 Down protocol	20	69.0
JCCLSG AML9805 Down protocol	1	3.5
JPLSG AML-D05	8	27.5

FAB indicates French-American-British; JCCLSG, Japanese Children's Cancer and Leukemia Study Group; JPLSG, Japanese Pediatric Leukemia/Lymphoma Study Group; MDS, myelodysplastic syndrome; ML-DS, myeloid leukemia in Down syndrome; and WBC, white blood cell count.

(25 mg/m^2, 1-hour intravenous infusion on days 1 and 2), intermediate-dose Ara-C (100 mg/m^2, 1-hour intravenous infusion on days 1-7), with or without etoposide (150 mg/m^2, 2-hour intravenous infusion on days 3-5). Because of few incidences of CNS leukemia and CNS relapse among patients with ML-DS, cerebrospinal fluid was not routinely examined, and no CNS prophylaxis was delivered throughout the treatment on these patients. None of the patients received HSCT before the diagnosis of R/R ML-DS.

Patient characteristics at induction failure or relapse

Relevant clinical data of the patients at induction failure or at first relapse are shown in Table 2. There were 3 induction failures and 26 relapsed cases. Among the 26 relapsed cases, duration from initial diagnosis to relapse was 2.4 -71.8 months (median, 8.6 months). All patients who relapsed within 6 months (n = 8) were on chemotherapy for ML-DS. Twenty-four patients (92%) relapsed within 2 years after the initial chemotherapy for ML-DS. Twenty-five patients relapsed in the BM, and 1 relapsed with an isolated extramedullary mass in a skin lesion. The WBC count at relapse was between 1700 and 25 700/μL (median, 4100/μL). Twenty-three (88.5%) showed FAB M7 morphology. Ten patients had chromosomal abnormalities that were the same as at the initial diagnosis, and 12 patients had additional abnormalities.

GATA1 mutation status

Nine patients were examined for *GATA1* mutation of the leukemic blasts either at initial diagnosis of ML-DS or at relapse; 8 of them were confirmed to have the mutation (Table 3).

From bloodjournal.hematologylibrary.org at University of Tokyo on April 5, 2013. For personal use only.

Table 2. Clinical characteristics of the 29 ML-DS patients at induction failure or at relapse

	No	%
Disease status		
Refractory AML	3	10.3
Relapsed AML	26	89.7
Duration from initial diagnosis to relapse, mo (n = 26)		
Median (range)	8.6 (2.4-71.8)	
< 6	8	30.8
≤ 6 to < 12	13	50.0
≤ 12	5	19.2
Site of relapse (n = 26)		
Bone marrow	25	96.2
Extramedullary (skin)	1	3.8
FAB classification at relapse (n = 26)		
M1	1*	3.8
M7	23†	88.5
Not available	2‡	7.7
Karyotype at relapse (n = 26)		
Same as before	10	38.5
Additional abnormalities	12	46.1
Not available	4	15.4

AML indicates acute myeloid leukemia; FAB, French-American-British; and MDS, myelodysplastic syndrome.

*This patient was M7 at initial diagnosis.

†A total of 18 patients were M7, 1 was M1, 3 were MDS, and 1 unknown at initial diagnosis.

‡One patient was M5a, and 1 was M7 at initial diagnosis.

Treatment outcome for R/R ML-DS

The clinical data and outcome of all the 29 patients in this study are described in Table 3. Twenty-six of the 29 patients received various salvage chemotherapies with curative intent. Six patients were treated with an ML-DS–oriented induction regimen as previously reported[6,7]; 12 patients were treated with etoposide, mitoxantrone, and intermediate dose of Ara-C with continuous intravenous infusion[13]; and the other 8 patients were treated with various chemotherapy regimen, such as FLAG (fludarabine, high-dose Ara-C, and G-CSF), AVC (pirarubicin, vincristine, and Ara-C with continuous intravenous infusion), low-dose Ara-C + etoposide, and vincristine + asparaginase. No deaths because of toxicities were observed during reinduction therapy. Two patients only received palliative therapy and eventually died of disease progression (Table 3, no. 19 and 28). The details of the postrelapse clinical course could not be identified in one patient (no. 26), and this patient died of unknown cause after undergoing HSCT.

Among the 26 patients who were treated with curative intent, 13 patients (50%) achieved complete remission (CR). Eight of the 13 patients who achieved CR subsequently received allogeneic HSCT, and 2 survived without leukemia. The remaining 5 patients who achieved subsequent CR were treated with chemotherapy alone, and 4 were alive without any evidence of leukemia (no. 15, 16, 17, and 23). The 3-year OS rate of the patients who achieved CR was 57.7% ± 14.7%. All 13 patients who did not achieve CR eventually died because of disease progression. Six of those patients received allogeneic HSCT without attaining CR: 4 died because of disease progression, and 2 died because of transplantation-related toxicities. No secondary cancer was observed.

Preconditioning regimen varied among the 14 patients who received HSCT. We therefore categorized the conditioning regimen into 4 groups[14]: busulfan (BU)–based myeloablative conditioning (MAC) regimen (BU-MAC), when > 8 mg/kg of BU combination was used; BU-based reduced intensity conditioning (RIC) regimen (BU-RIC), when a lower dose of BU combination was used; total body irradiation (TBI)–based MAC (TBI-MAC), when ≥ 8 Gy of fractionated TBI combination was used; and TBI-based RIC (TBI-RIC), when a lower dose of the TBI combination was used. A total of 5 patients received BU-MAC, 3 received BU-RIC, 4 received TBI-MAC, and 2 received TBI-RIC. We did not find any difference in survival and toxic death among these 4 conditioning regimen (data not shown).

Finally, the 3-year OS rate of all patients was 25.9% ± 8.5% (Figure 1). The median follow-up period for all 29 patients was 0.9 years (range, 0.2-7.0 years). Among the 8 patients with *GATA1* mutation, 7 patients, including 1 patient with palliative therapy (no. 19), did not achieve subsequent remission and died of disease progression. In contrast, 11 of the 20 patients whose *GATA1* status was not examined attained subsequent remission, and 6 of 11 patients are alive without disease. The patient with wild-type *GATA1* (no. 10) died of transplantation-related toxicity although receiving HSCT in second CR.

Prognostic factors

Several predictive factors for OS were evaluated by univariate and multivariate analyses (Table 4). The unadjusted 3-year OS was found to be better for patients with a longer duration from initial diagnosis to relapse (patients with the duration from diagnosis to relapse > 12 months, 66.7% ± 27.2%; ≤ 12 to > 6 months, 23.1% ± 11.7%; and ≤ 6 months, 0%). In Cox regression analysis, the adjusted hazard ratios of patients who relapsed more than 6 months after the initial diagnosis were significantly better compared with those who did not achieve CR or who relapsed within 6 months after the diagnosis (hazard ratio 3.14; 95% confidence interval 1.28-7.67; $P = .012$), and this finding was still detected after we controlled for reinduction regimens (etoposide, mitoxantrone, and intermediate dose of Ara-C with continuous intravenous infusion vs others), HSCT (yes vs no), and biologic factors, including chromosomal abnormalities and *GATA1* status (hazard ratio 4.56; 95% confidence interval 1.07-19.41; $P = .04$). Two clinical factors were found to be associated with the duration from initial diagnosis to relapse. First, patients older than 2 years of age at initial diagnosis were more likely to relapse in a short period from initial diagnosis (≤ 6 months) compared with younger patients (trend test, $P = .02$). Second, patients who relapsed earlier were less likely to achieve CR when they received second-line chemotherapy ($P = .001$). Other factors, including sex, WBC at initial diagnosis, FAB morphologies (M7 vs others), chromosomal abnormalities, and *GATA1* status, were not significantly associated with survival. HSCT did not influence the prognosis even if performed after achieving further remission.

Discussion

Treatment strategy for ML-DS is based on reducing the intensity of chemotherapy protocol designed for non-DS AML patients, considering both the potential risk of treatment-related toxicities and greater sensitivity to cytotoxic agents. With this strategy, there have been successful reports on treating patients with ML-DS from several collaborative groups.[4-11] However, it is assumed that salvage of patients with R/R ML-DS is quite difficult because the OS and event-free survival rate are almost the same in these reports. Because little attention has been focused on these cases so far, a nationwide retrospective study was conducted. The present

From bloodjournal.hematologylibrary.org at University of Tokyo on April 5, 2013. For personal use only.

Table 3. Clinical data on 29 Down syndrome patients with refractory/relapsed myeloid leukemia

	Clinical characteristics at initial diagnosis of ML-DS											
No.	Sex	Age, y	WBC, /μL	FAB	Karyotype	*GATA1* status	IF/RL	Time to IF/RL, mo*	Subsequent CR	HSCT	Survival, mo†	Cause of death
1	F	3	3000	M7	Constitutional	NE	RL	4	No	No	5	Leukemia
2	M	3	51 700	M7	Constitutional	NE	IF	1	No	UCBT	8	Leukemia
3	M	2	10 000	M7	Other	NE	RL	2	No	No	3	Leukemia
4	F	2	5800	M7	Other	NE	RL	8	No	No	9	Leukemia
5	F	1	18 000	M7	−7+ring/marker	NE	RL	12	Yes	UCBT	16	Leukemia
6	M	0.7	19 200	M7	Other	NE	RL	8	Yes	RBMT	28	TRM
7	M	0.7	6500	M7	Other	mutated	RL	3	No	UCBT	7	TRM (non-CR)
8	F	5	25 800	M2	t(8;21)(q22;q22)	NE	IF	0	Yes	UCBT	> 54‡	
9	F	1	4800	M7	Monosomy 7	mutated	RL	3	No	RPBSCT	10	Leukemia
10	M	14	1900	M7	Na	WT	RL	17	Yes	RBMT	60	TRM
11§	M	1	11 600	M7	Monosomy 7	NE	RL	18	Yes	No	33	Leukemia
12	M	3	2600	M7	Constitutional	NE	RL	3	No	RBMT	19	Leukemia
13	M	4	2800	MDS	−7+ring/marker	NE	RL	12	Yes	UBMT	11	Leukemia
14	M	15	1800	RAEBt	Other	NE	IF	2	No	UCBT	11	Leukemia
15	F	10	900	M1	Other	NE	RL	39	Yes	No	> 33	
16	M	3	6700	M7	Constitutional	mutated	RL	72	Yes	No	> 28	
17	M	1	5100	M7	Other	NE	RL	10	Yes	No	> 46	
18	M	2	5600	MDS	−7+ring/marker	mutated	RL	9	No	No	10	Leukemia
19	M	1	7400	M7	Constitutional	mutated	RL	7	No	No	3	Leukemia
20	M	1	143 600	M7	Constitutional	NE	RL	11	Yes	RBMT	> 84	
21	M	2	8600	M7	Other	mutated	RL	5	No	No	4	Leukemia
22	F	3	5900	M7	Other	NE	RL	11	Yes	UBMT	> 32	
23	M	16	38 800	M5a	Other	NE	RL	20	Yes	No	> 23	
24	M	1	3480	M7	Other	NE	RL	9	Yes	UCBT	14	Leukemia
25	F	3	2100	MDS	Other	mutated	RL	7	No	No	3	Leukemia
26	F	2	2900	M7	−7+ring/marker	NE	RL	11	NA	Yes	11	unknown
27	F	2	2700	M7	Other	mutated	RL	4	No	No	4	Leukemia
28	M	1	2470	M7	−7+ring/marker	NE	RL	8	No	No	4	Leukemia
29	F	1	3680	M7	Na	NE	RL	4	No	UCBT	5	Leukemia

CR indicates complete response; F, female; IF, induction failure; M, male; NA, data not available; NE, not evaluated; other, other cytogenetic abnormalities; RBMT, related bone marrow transplantation; RPBSCT, related peripheral blood stem cell transplantation; RL, relapse; TRM, transplantation-related mortality; UBMT, unrelated bone marrow transplantation; and UCBT, unrelated cord blood transplantation

*Months from initial diagnosis to either induction failure or first relapse.

†Duration of survival from induction failure or first relapse.

‡Patient no. 8 is alive with disease.

§Patient no. 11 underwent HSCT in third CR on day 981 after relapse and died of transplantation-related toxicity on day 981.

study clearly showed that patients with R/R ML-DS are resistant to second-line chemotherapy and that the disease course rarely is salvaged by allogeneic HSCT.

It is well recognized that the outcome of ML-DS is much better than that of AML in non-DS patients; however, the OS rate of R/R ML-DS cases in the present study (26%) was no better than that of the reported survival rate of non-DS AML patients, which is 23%-33%.[15,16] Usually, non-DS AML patients are heavily pre-

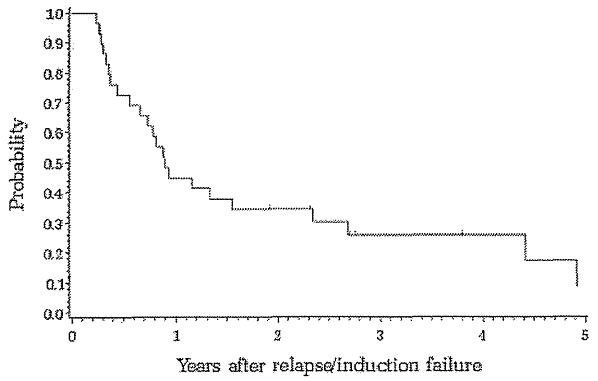


Figure 1. OS curve of R/R ML-DS. The 3-year OS rate was 25.9% ± 8.5%.

treated before relapse (eg, HSCT), and this explains one aspect of therapeutic resistance to second-line treatment that could lead to the low salvage rate in these patients. It is notable that the salvage rate of patients with ML-DS in the R/R setting also was very poor, even though these patients had initially received the low-intensive ML-DS–oriented chemotherapy. The initial therapies given for the present cases are even less intensive than the other ML-DS protocols used in developed countries and, of course, than that of the non-DS AML protocols.

Only 50% of patients with R/R ML-DS in the present study had achieved further remission with attempts of various reinduction therapies. It has been reported that the reinduction rate for non-DS patients with AML is 65%-77%, and achievement of subsequent CR was uniformly a good prognostic factor.[15,16] When we consider the results of the present study, which indicate that the achievement of further remission is a good prognostic factor, improvement of the reinduction rate for R/R ML-DS would be mandatory for a better prognosis.

The reported 5-year OS rate of patients with non-DS AML who attained second CR and subsequently received HSCT were 58%-62%.[17-19] In the present study, 8 of the 13 patients subsequently received allogeneic HSCT, but only 2 of those 8 patients (25%) survived. It is well known that the transplantation-related mortality

From bloodjournal.hematologylibrary.org at University of Tokyo on April 5, 2013. For personal use only.

Table 4. Univariate and multivariate analyses of prognostic factors for refractory/relapsed ML-DS patients

	Univariate analysis		Multivariate analyses			
			Model 1 (baseline)		Model 2 (relapsed phase)	
Variables	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex: male vs female	0.78 (0.33-1.83)	.56	0.71 (0.30-1.71)	.45	0.85 (0.36-2.05)	.72
Age at initial diagnosis: ≤ 2 y vs > 2 y	1.98 (0.83-4.72)	.12	2.73 (0.89-8.37)	.08	*	
WBC at initial diagnosis: ≥ 10 000/μL vs < 10 000/μL	0.57 (0.22-1.47)	.24	0.43 (0.15-1.19)	.10	0.60 (0.22-1.60)	.30
FAB classification: M7 vs others	1.63 (0.60-4.44)	.34	0.92 (0.27-3.09)	.89	1.88 (0.66-5.37)	.24
Disease status: IF or RL ≤ 6 mo vs RL > 6 mo	2.92 (1.24-6.88)	.01	–		3.14 (1.28-7.67)	.012
Response to r-induction therapy: non-CR vs CR	16.54 (4.44-61.59)	< .0001	–		*	

Reference groups: female > 2 years, < 10 000/μL, others (FAB), RL > 6 months, and CR.

CI indicates confidence interval; CR, complete response, HR, hazard ratio; IF, induction failure; mo, month(s); and RL, relapse.

*A variable of "patient age at initial diagnosis" was excluded from model 2 because it was a predictive factor for the variable of "type of relapse/induction failure." Patients who relapsed in the later phase (after 6 months) were more likely to achieve in CR, and therefore, a variable of "response to reinduction therapy" was also excluded from the model 2 in the multivariate analysis.

of patients with ML-DS is greater than in patients with non-DS AML[20]; however, the main cause of transplantation failure was disease progression, not transplantation-related complications, where only one death in remission was documented. Our result was consistent with the report by Meissner et al in which they described that relapse was the major cause of treatment failure in children with DS treated by HSCT for acute leukemia.[21]

However, 5 of the 13 R/R ML-DS patients with further remission subsequently were treated with chemotherapy only, and 4 of the 5 patients survived (duration after relapse, 22-45 months). Three of those 4 patients had relapsed more than 12 months from the initial diagnosis, which was found to be good prognostic factor in this study. Moreover, most of these patients were treated with continuous and/or high-dose Ara-C, which might be a key component of a salvage regimen for R/R ML-DS. Allogeneic HSCT might not be essential for these patients, especially for "late" relapsed patients, as for the non-DS cases[15,16]

The duration from initial diagnosis to R/R was shown as the strongest prognostic factor, but biologic factors (including chromosomal abnormalities and *GATA1* status) were not relevant in this R/R ML-DS study. Although more than 80% of patients with ML-DS could be cured with low-intensive chemotherapy, methods that can be used to identify the remaining poor subgroups with a poor prognosis and a treatment strategy distinct from "usual" ML-DS are urgently needed. This necessity is because of the fact that they are rarely salvageable once they experience morphologic induction failure or relapse, as was indicated in this retrospective study. Future treatment protocols in this patient population could include adherence to a very low-intensity chemotherapy for the majority of patients with ML-DS, identification of the subgroup with a poor prognosis using minimal residual disease, and stratification of these patients to receive a more intensive chemotherapy containing high-dose and/or continuous infusion of intermediate-dose Ara-C.

Acknowledgments

The authors are grateful to Dr I. Iguchi (Hokkaido University), Dr A. Sato (Miyagi Children Hospital), Dr T. Watanabe (Niigata Cancer Center), Dr M. Sotomatsu (Gunma Children Hospital), Dr K. Ida (Tokyo University), Dr M. Nagasawa (Tokyo Medical and Dental University), Dr T. Kaneko (Tokyo Metropolitan Children's Medical Center, Dr R. Ooyama (St Marianna Medical College), Dr M. Akiyama (Jikei Medical College), Dr M. Yabe (Tokai University), Dr D. Toyama (Showa University), Dr Y. Taneyama (Chiba Children's Hospital), Dr K. Matsumoto (Nagoya First Red Cross Hospital), Dr M. Inoue (Osaka Medical Center and Maternal and Child Research Institute), Dr D. Hasegawa (Hyogo Children's Hospital), Dr I. Usami (Kobe City Hospital), Dr Y. Kataoka (Matsuyama Red Cross Hospital), Dr S. Kawakami (Ehime University), Dr T. Anan (Kumamoto University), and the 120 institutions and all of the doctors in the Japanese Pediatric Leukemia/Lymphoma Study Group for their invaluable contributions to data collection. They also thank Dr T. Toki and Dr R. Wang (Hirosaki University Graduate School of Medicine), for performing *GATA1* mutation analysis.

This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan.

Authorship

Contribution: T. Taga and D.T. designed, organized, and performed research, analyzed data, and wrote the paper; A.M.S. designed research and collected and analyzed clinical data; K. Kudo, H.M., A.K., S.I., H.N., H.T., A.T., and A.S. designed, organized, and performed research and analyzed data; K.T. performed mutation screening and designed, organized, performed research and analyzed data; T. Taki designed, organized, and performed research and analyzed chromosomal data; K. Koh and H.K. provided clinical samples and data; and S.A. provided clinical data, designed and organized research, analyzed data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Takashi Taga, Department of Pediatrics, Shiga University of Medical Science, Seta-tsukinowa, Otsu, Shiga 520-2192, Japan; e-mail: ttaga@belle.shiga-med.ac.jp.

References

1. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. *Lancet.* 2000;355(9199):165-169.
2. Kojima S, Matsuyama T, Sato T, et al. Down's syndrome and acute leukemia in children: an analysis of phenotype by use of monoclonal antibodies and electron microscopic platelet peroxidase reaction. *Blood.* 1990;76(11):2348-2353.
3. Zipursky A, Thorner P, De Harven E, et al. Myelodysplasia and acute megakaryoblastic leukemia in Down's syndrome. *Leukemia Res.* 1994;18(3):163-171.
4. Gamis AS, Woods WG, Alonzo TA, et al: Children's Cancer Group Study 2891. Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. *J Clin Oncol.* 2003;21(18):3415-3422.

From bloodjournal.hematologylibrary.org at University of Tokyo on April 5, 2013. For personal use only.

5. Creutzig U, Reihardt D, Diekamp S, et al. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. *Leukemia.* 2005;19(8):1355-1360.
6. Kudo K, Kojima S, Tabuchi K, et al. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: the Japanese Childhood AML Cooperative Study Group. *J Clin Oncol.* 2007;25(34):5442-5447.
7. Taga T, Shimomura Y, Horikoshi Y, et al. Continuous and high-dose cytarabine combined chemotherapy in children with Down syndrome and acute myeloid leukemia: report from the Japanese Children's Cancer and Leukemia Study Group (JCCLSG) AML 9805 Down Study. *Pediatr Blood Cancer.* 2011;57(1):36-40.
8. Abildgaard L, Ellebaek E, Gustafsson G, et al. Optimal treatment intensity in children with Down syndrome and myeloid leukaemia: data from 56 children treated on NOPHO-AML protocols and review of the literature. *Ann Hematol.* 2006;85(5): 275-280.
9. Rao A, Hills RK, Stiller C, et al. Treatment for myeloid leukaemia of Down syndrome: population-based experience in the UK and results from the Medical Research Council AML 10 and AML 12 trials. *Br J Haematol.* 2006;132(5):576-583.
10. Raimondi SC, Chang MN, Ravindranath Y, et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative Pediatric Oncology Group study-POG8821. *Blood.* 1999; 94(11):3707-3716.
11. Sorrell AD, Alonzo TA, Hilden JM. Favorable survival maintained in children who have myeloid leukemia associated with Down syndrome using reduced-dose chemotherapy on Children's Oncology Group trial A2971: a report from the Children's Oncology Group [published online ahead of print March 5, 2012]. *Cancer.* doi:10.1002/cncr.27484.
12. Fujino H, Fujita N, Hamamoto K, et al. Ring/marker chromosome derived from chromosome 7 in childhood acute megakaryoblastic leukemia with monosomy 7. *Int J Hematol.* 2010;92(2):386-390.
13. Tsukimoto I, Tawa A, Horibe K, et al. Risk-stratified therapy and intensive use of cytarabine improves the outcome in childhood acute myeloid leukemia. *J Clin Oncol.* 2009;27(24):4007-4013.
14. Bacigalupo A, Ballen K, Rizzo D, et al. Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant.* 2009; 15(12):1628-1633.
15. Aladjidi N, Auvrignon A, Leblanc T, et al. Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of Pediatric Hematology and Immunology. *J Clin Oncol.* 2003;21(23):4377-4385.
16. Rubnitz JE, Razzouk B, Lensing S, et al. Prognostic factors and outcome of recurrence in childhood acute myeloid leukemias. *Cancer.* 2007; 109(1):157-163.
17. Nemecek ER, Gooley TA, Woolfrey AE, et al. Outcome of allogeneic bone marrow transplantation for children with advanced acute leukemia. *Bone Marrow Transplant.* 2004;34(9):799-806.
18. Abrahamsson J, Clausen N, Gustafsson G, et al. Improved outcome after relapse in children with acute myeloid leukaemia. *Br J Haematol.* 2007; 136:229-236.
19. Shenoy S, Smith FO. Hematopoietic stem cell transplantation for childhood malignancies of myeloid origin. *Bone Marrow Transplant.* 2008;41(2): 141-148.
20. Rubin CM, Mick R, Johnson FL. Bone marrow transplantation for the treatment of haematological disorders in Down's syndrome: toxicity and outcome. *Bone Marrow Transplantation.* 1996; 18(3):533-540.
21. Meissner B, Borkhardt A, Dilloo D, et al. Relapse, not regimen-related toxicity, was the major cause of treatment failure in 11 children with Down syndrome undergoing haematopoietic stem cell transplantation for acute leukemia. *Bone Marrow Transplant.* 2007;40(10):945-949.