We previously reported that the presence of hyposegmented mature neutrophils (Pelger), degranulation of neutrophils (agranular or hypogranular neutrophils; Hypo-Gr), and micromegakaryocytes (mMgk) in BM or peripheral blood (PB) were found in 76%, 30%, and 74% of MDS cases, respectively, whereas there was no AA case with these dysplasias.[8] We confirmed the specificity of these dysplasias in a different case series showing that Pelger ≥ 10% or mMgk ≥ 10% in BM was not found in the AA group (presented at the Ninth International Symposium on MDS). We also showed that, among patients with refractory anemia (RA) according to the French-American-British (FAB) classification[9] (FAB-RA), excluding MDS associated with isolated deletion of chromosome 5q (5q-syndrome), the presence of Pelger ≥ 10% or mMgk ≥ 10% in BM (15% and 14% of RA cases, respectively) were significantly related to the shorter overall survival (OS) and leukemia-free survival (LFS). The median OS and LFS of cases with Pelger ≥ 10% were 29 months and 36 months, respectively, and were significantly shorter than those without Pelger ≥ 10% (158 months and not reached, respectively; $P < .001$ in both). Micromegakaryocytes ≥ 10% showed similar effect on OS and LFS (23 months vs. 153 months for OS [$P < .001$] and 51 months vs. not reached for LFS [$P < .001$]).[10] The concordance rates of Pelger and mMgk were reasonably high among observers.[11] These dysplasias are much easier to detect, not only for expert morphologists but also for clinical hematologists in general. We considered that misdiagnosis and discordance would be avoided by enumerating these MDS-specific dysplasias.

Idiopathic cytopenia of uncertain significance (ICUS) was first proposed by Mufti et al at the Eighth International Symposium on MDS in Nagasaki, Japan, in 2005. If patients with normal karyotype and < 5% BM blasts do not show morphologic dysplasia (ie, < 10% of any cell lineage) and all other diseases have been ruled out as a cause of cytopenia, the patients are diagnosed with ICUS. The cytopenia(s) should persist for ≥ 6 months without any other cause identified. The criteria for ICUS was proposed in a recent publication by Valent et al.[12] Idiopathic cytopenia of uncertain significance might be a useful category for patients with unexplained cytopenia who do not fulfill the criteria of MDS (either of the FAB classification or the World Health Organization [WHO] classification[13]). Extensive study for this category in terms of MDS pathophysiology, particularly a molecular aspect, will clarify the clinical and pathophysiologic features of the ICUS category.

We previously compared the morphologic features between FAB-RA, excluding 5q-syndrome AA at the Ninth International Symposium on MDS, held in Florence, Italy, in 2007. One hundred patients with FAB-RA, excluding 5q-syndrome, were diagnosed by a joint review of a Japanese and German collaboratory study.[10,14] Forty patients with AA who registered to the Japanese AA and MDS Registration System of the National Research Group on Idiopathic Bone Marrow Failure Syndromes, Japan were diagnosed by the Central Review Working Group. In all patients with FAB-RA, the frequency of dysplasia was ≥ 10% in ≥ 1 lineage. Some (17%) patients with AA showed Dys E ≥ 10% in BM; Hypo-Gr ≥ 10%, Pelger ≥ 10%, or mMgk ≥ 10% were found only in the FAB-RA group. In addition, dysplasia ≥ 10% in ≥ 2 lineages was found only in the FAB-RA group. The number of megakaryocytes was markedly decreased in all patients with AA. The presence (≥ 5%) of blasts in BM was never found in the patients with AA.

Table 1 Prerequisite Criteria

Criteria
A. Constant cytopenia (≥ 6 months) in ≥ 1 of the following lineages:
Hemoglobin < 11 g/dL
Absolute neutrophil count < 1.5×10^9/L
Platelet count < 100×10^9/L
B. Less than 20% blasts in PB or BM and absence of cytogenetic findings related with acute myeloid leukemia with recurrent cytogenetic abnormalities*
C. Less than 1×10^9/L monocytes in PB
D. Exclusion of all other hematopoietic or nonhematopoietic disorders as primary reason for cytopenia
E. Exclusion of aplastic anemia. In case of hypoplastic BM, exclusion of aplastic anemia needs to be considered using morphologic findings and cytogenetic data.

A-E must be fulfilled.
*t(8;21)(q22;q22); (AML1/ETO), t(15;17)(q22;q12); (PML/RARα), and inv(16)(p13;q22) or (t16;16)(p13;q22); (CBFβ/MYH11).

Recently, minimal diagnostic criteria for MDS have been proposed by Valent et al.[12] They did not show a list of dysplastic cells in their criteria. We think a clear and definite list of dysplastic cells is necessary for diagnostic criteria. We propose a grading system for diagnostic accuracy of MDS by combining the results of our morphologic study presented at the Ninth International Symposium on MDS with the criteria proposed by Valent et al.

A Grading System for Diagnostic Accuracy of Myelodysplastic Syndrome

Exclusion of disorders with constant cytopenia(s) and some morphologic dysplasia(s) other than MDS is a prerequisite for diagnosing MDS. We propose that dysplasia(s) be divided into category A (high specificity) and category B (low specificity) for assessment of the frequency of dysplasia(s). A quantitative morphologic evaluation of category A or A + B is essential to start diagnosis of MDS. We then suggest a grading of dysplasia based on the enumeration and a division of cytogenetic findings. The criteria for grading of diagnostic accuracy are a combination of the frequency of blasts in BM, grade of dysplasia, and divisions of cytogenetics. The grades of diagnostic accuracy are divided into "definite," "probable," or "possible" in addition to "ICUS." Patients who are diagnosed as "definite," "probable," or "possible" should be classified according to the WHO classification for MDS.

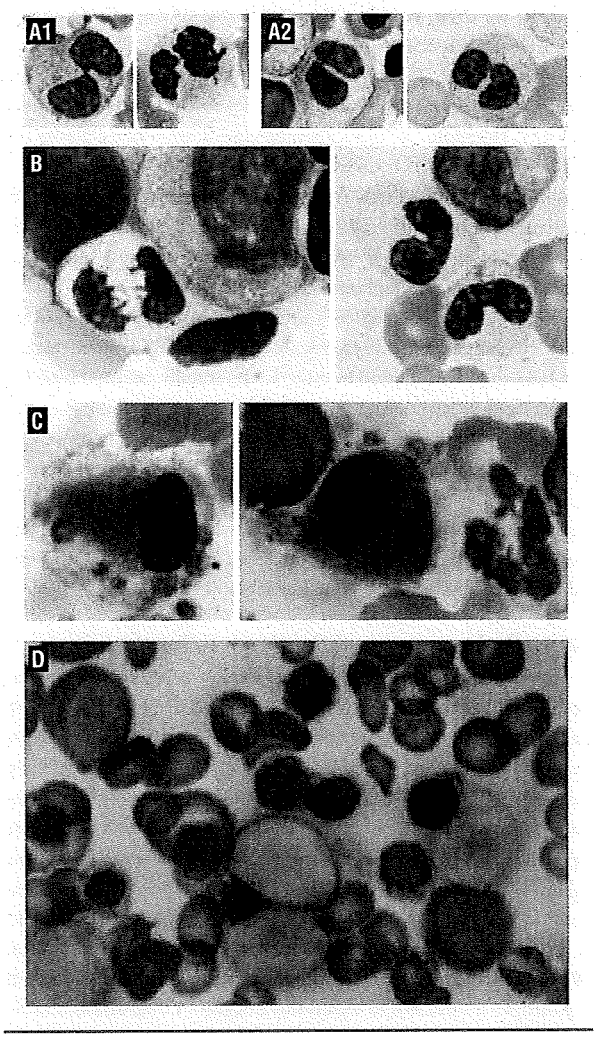
Step I: Exclusion Diagnosis of Disorders Other Than Myelodysplastic Syndrome

We modified the excellent prerequisite criteria proposed by Valent et al.[12] Table 1 shows our prerequisite criteria, consisting of the definition of constant cytopenias (≥ 6 months) and exclusion of disorders with constant cytopenias or some myelodysplasia. Acute myeloid leukemia (AML) should be excluded by frequency of blasts and cytogenetic findings. Bone marrow differential counts should be performed on 500 cells. Counting the number of monocytes in PB is necessary for the exclusion of chronic myelomonocytic

Table 2 Classification of Dysplasia

- **Category A**
  - Granulocytic series
    - Hyposegmented mature neutrophils (Pelger)
    - Degranulation (agranular or hypogranular neutrophils; Hypo-Gr)
  - Megakaryocytic series
    - Micromegakaryocytes
  - Erythroid series
    - Ringed sideroblasts
- **Category B**
  - Granulocytic series
    - Small size
    - Hypersegmentation
    - Pseudo–Chediak-Higashi granule
  - Megakaryocytic series
    - Nonlobulated nuclei
    - Multiple, widely separated nuclei
  - Erythroid series
    - Nucleus
      - Budding
      - Internuclear bridging
      - Karyorrhexis
      - Multinuclearity
      - Megaloblastoid change
    - Cytoplasm
      - Vacuolization

Figure 1 Dysplasia of Category A



(A) Hyposegmented mature neutrophils (Pelger). Two lobes are connected with a fine (1) or thin (2) filament. **(B)** Degranulation of neutrophils (agranular or hypogranular neutrophils; Hypo-Gr). **(C)** Micromegakaryocytes. **(D)** Ringed sideroblasts. **(A-D)** Provided by the National Research Group on Idiopathic Bone Marrow Failure Syndromes, Japan.

leukemia. To exclude nonclonal disorders (Table 1D), laboratory studies (eg, serum iron, ferritin, cobalamin, and folic acid levels; Coombs test; anti-nuclear antibody; thyroid function tests; etc) and abdominal ultrasonography are necessary.

For evaluations of BM cellularity, specimens of BM trephine biopsy must be examined. A BM biopsy of good quality and adequate length (≥ 1.5 cm) is necessary. Often, repeat BM examination is required to confirm the diagnosis when there is doubt about initial BM examination or if an inadequate sample was taken.[15] Because BM cellularity is highly age-dependent, hypocellularity is defined as < 30% in patients aged < 60 years or < 20% in patients aged ≥ 60 years.[16,17] In hypocellular BM, microscopic examinations for the assessment of dysplasias should be performed with ≥ 2 BM films, if necessary.

In patients with hypocellular BM, it is absolutely necessary to exclude AA to diagnose hypoplastic MDS (Table 1E). Dysplasia of BM cells, the percentage of blasts in BM, and abnormal localization of immature precursors (ALIP) are useful markers for this differentiation. As mentioned earlier, significant dysplasia in ≥ 2 lineages and > 10% of Hypo-G, Pelger, or mMgk strongly suggest MDS rather than AA. Abnormal localization of immature precursors is usually not found in AA but is found in MDS, and blasts in BM are usually < 5% in AA cases. In this regard, it is very difficult to diagnose hypoplastic RA cases that show dysplasia only in erythroid lineage. Morphologic evaluation alone might not be enough for the diagnosis, and other data such as cytogenetics will provide further useful information when they show MDS-specific karyotype (see Step VI). However, in some cases with hypoplastic BM, in particular those having dysplasia in a single lineage or at low percentage, careful course observation is necessary to make a diagnosis. On the other hand, the presence of paroxysmal nocturnal hemoglobinuria–type cells[18] or dysplasia in erythroid lineage alone does not support the diagnosis of MDS alone.

Step II: Classification of Dysplasia

Table 2 shows a classification of dysplasias into category A (highly specific) and category B (less specific), which is the thrust of our proposal. Dysplasias in Table 2 are modified from those described in the WHO classification, except for the periodic acid-Schiff (PAS) reaction for erythroid cells because the PAS reaction is no longer used routinely

in hematologic laboratories. As described earlier, Pelger, Hypo-Gr, and mMgk are highly specific to MDS when present at levels ≥ 10%. In addition, we think that the diagnostic value of ringed sideroblasts (RS) is similarly specific when present at a level of ≥ 15%. Dysplasias other than Pelger, Hypo-Gr, mMgk, and RS are less specific for MDS but, if present at ≥ 10%, are sufficient to suggest a diagnosis of MDS. Therefore, we think that the classification of dysplasias for the diagnosis of MDS is necessary and helpful for clinical hematologists in general. Quantitative assessment of category A or category B dysplasias is a basis for grading the accuracy of diagnosis of MDS. Four types of category A dysplasias are shown in Figure 1 (A-D) and Table 2. Category B dysplasias are shown in Table 2.

Pelger are hyposegmented (dumbbell-shaped) mature neutrophils. Two lobes are connected with a fine or thin filament (Figure 1A), and their chromatin structure is abnormally coarse. Hypo-Gr are neutrophils with a total or > 80% loss of neutrophilic granules in the cytoplasm (Figure 1B). Micromegakaryocytes are mono- or bi-nulceated megakaryocytes with a size less than that of normal promyelocytes and abundant platelet granule formation (Figure 1C). Ringed sideroblasts are erythroid cells with perinuclear siderotic granules occupying > one third of the nuclear margin or > 5 distinct siderotic granules in the perinuclear region (Figure 1D).

Step III: Assessment of Category-A Dysplasias

For the assessment of Pelger and Hypo-Gr, ≥ 100 mature neutrophils should be examined on BM films. The frequencies of Pelger and Hypo-Gr should be evaluated individually, not the sum of Pelger or Hypo-Gr. Because BM films frequently fail to stain optimally for neutrophil granules, observation of PB films is very helpful in confirming degranulation. In particular, when Hypo-Gr is the sole dysplasia in the absence of other dysplastic features, the assessment of Hypo-Gr should not be evaluated as a positive finding unless confirmed as mentioned earlier. Concerning the frequency of mMgk, ≥ 25 megakaryocytes should be examined on multiple BM films. When the megakaryocyte number is markedly reduced, detection of ≥ 3 mMgks is sufficient to regard this category-A dysplasia as ≥ 10%. In almost all patients with AA, megakaryocytes are absent or very few in number. For RS, ≥ 100 erythroblasts of all stages should be examined. Independent assessment of category-A dysplasias is necessary for grading of diagnostic accuracy of MDS.

Step IV: Assessment of Dysplasia A + B in Each Lineage

Concerning the frequencies of dysplasia A + B in each lineage, we suggest the microscopic methods as follows: ≥ 100 mature neutrophils, ≥ 25 megakaryocytes, and ≥ 100 erythroblasts in BM should be examined. The frequency of dysplasia in each lineage is evaluated by total dysplastic cells (%) showing category A or B. The frequency of Dys E is evaluated by the sum of frequency of RS on iron-stained films and that of category B on May-Giemsa–stained films. For example, when the frequency of RS and that of category B in erythroid lineage are 5% and 10%, respectively, the frequency of Dys E is calculated as 15%. The microscopic examinations for the assessment of dysplasia should be performed with multiple BM films if necessary. If the megakaryocyte number is markedly reduced, detection of ≥ 3 dysplastic megakaryocytes is sufficient to regard dysplasia A + B as ≥ 10%.

Table 3 Grade of Dysplasia

Dysplasia Grade
High (Defined as 1 or 2)
1. Pelger ≥ 10% or Hypo-Gr ≥ 10% plus mMgk ≥ 10%
2. RS ≥ 15%
Intermediate
Dysplasia (category A or B) ≥ 10% in 2-3 lineages
Low
Dysplasia (category A or B) ≥ 10% in 1 lineage
Minimal
Dysplasia (category A or B) 1%-9% in 1-3 lineages

Step V: Grade of Dysplasia

As shown in Table 3, the grade of dysplasia is divided into high, intermediate, low, or minimal. High is defined as follows: (1) when Pelger ≥ 10% or Hypo-Gr ≥ 10% plus mMgk ≥ 10% in granulocytic and megakaryocytic lineages or (2) when RS ≥ 15% in erythroid series. In order to classify a case as high by the existence of RS ≥ 15% alone, other sideroblastic anemias such as alcoholic anemia must be excluded. Intermediate is defined as dysplasia A + B ≥ 10% in 2-3 lineages. Low is defined as dysplasia A + B ≥ 10% in a single lineage. Minimal is defined as 1%-9% of dysplasia A + B in 1-3 lineages.

Step VI: Division of Cytogenetic Findings

The divisions of cytogenetic findings are abnormal, normal, or unknown. Abnormal is defined as typical clonal abnormal karyotypes recurrently found in MDS (del[5q], –7/7q–, +8, del[20q], complex, and others) with high frequency as reported by Haase et al.[19] This definition is similar to that of typical chromosome abnormalities proposed by Valent et al.[12] t(8;21)(q22;q22), t(15;17)(q22;q12), inv(16)(p13;q22), and t(16;16)(p13;q22) are not included in the abnormal division even when the blast percentage is < 20%. Patients with these cytogenetic abnormalities are diagnosed with AML with recurrent cytogenetic abnormalities according to the WHO classification. Normal is defined as normal karyotype by analyzing > 10 metaphases. When cytogenetic findings are not available because of poor samples or an absence of metaphases, cases are labeled unknown.

Step VII: Grade of Diagnostic Accuracy

Table 4 shows the criteria for grading the diagnostic accuracy. These criteria are a combination of the frequency of blasts in BM, grade of dysplasia, and division of cytogenetics. The grade of diagnostic accuracy is divided into definite, probable, possible, and ICUS. The reliability of the diagnosis as MDS is high in the following order: definite, probable, and possible. In patients diagnosed as possible or ICUS, the diagnostic accuracy is low; thus, re-examination at suitable intervals is required to confirm the diagnosis. In such cases, the diagnosis might become more accurate when re-examination provides a result of definite or probable or remains possible or ICUS for a long period. The observation of the clinical course of patients with possible or ICUS will provide important information on the pathophysiologic similarity or dissimilarity between these diagnostic groups based on diagnostic grading.

Table 4 Grade of Diagnostic Accuracy for Myelodysplastic Syndromes

Grade	Blasts in BM (%)	Grade of Dysplasia	Division of Cytogenetics
	5-19	High, intermediate, low	Any
MDS Definite	0-4	High, intermediate, low	Abnormal
	0-4	High	Any
MDS Probable	0-4	Intermediate	Normal or unknown
MDS Possible	0-4	Low	Normal or unknown
ICUS	0-4	Minimal or none	Normal or unknown

Step VIII: Subtyping According to the World Health Organization Classification

Patients who are diagnosed as definite, probable, or possible should be classified according to the WHO classification. Patients classified in the possible category in our system are diagnosed as RA- or MDS-unclassified (refractory neutropenia or refractory thrombocytopenia) according to the WHO classification. However, the diagnosis of these patients should be tentative. The diagnosis according to the WHO classification of these patients must be decided by re-examination of BM at suitable intervals.

Discussion

Diagnosis of MDS must be as accurate and consistent as that of acute leukemia. However, the judgment of morphologic dysplasias has the inherent problem of the subjective nature of the morphology, and the objectivity of the evaluation has long been problematic. For the elimination or reduction of these problems, we propose a grading system for diagnostic accuracy of MDS. Category-A dysplasias are much easier for clinical hematologists to detect on routine BM diagnosis. Category-B dysplasias are sufficiently reliable when observed along with category A. Therefore, quantitative, morphologic evaluation by using this system will facilitate the routine diagnosis of MDS.

Exclusion of nonclonal disorders with minimal or no morphologic dysplasia is extremely important for the differential diagnosis of MDS as described in Step I. We believe our system is also useful in this respect. If there is no certain evidence for this exclusion diagnosis of non-MDS disorders despite the careful performance of other laboratory examinations, the possibility of misdiagnosis would likely be markedly reduced by using this grading system for diagnosis. Repeat BM examination at suitable intervals for patients graded as possible or ICUS will make clearer the still-vague margin of MDS as a clinical entity. It is also important to identify differences if present in responses to new drugs such as lenalidomide and hypomethylating agents.

Of course, our diagnostic schema still requires validation and demonstration of reliability, hopefully in 2 populations or a split-sample cohort. Long-term observation of MDS cases diagnosed with our proposal is also necessary for the evaluation of this proposal. Recent techniques in the detection of genetic abnormalities such as fluorescence in situ hybridization and single nucleotide polymorphism arrays[20] expand cytogenetic data of MDS. Although universal biomarkers for the diagnosis of MDS are still lacking, new data on genetic abnormality of MDS will be quite useful for accurate diagnosis and understanding the biology of MDS. In conclusion, until the discovery of universal biomarkers for entire MDS or subtypes of MDS, this diagnostic grading system could be useful for clinical routine work.

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Brief Report

Karyotype at diagnosis is the major prognostic factor predicting relapse-free survival for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia treated with imatinib-combined chemotherapy

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ABSTRACT

To identify factors associated with relapse-free survival (RFS), 80 patients with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia, enrolled in a phase II study of imatinib-combined chemotherapy, were analyzed. The median follow-up of surviving patients was 26.7 months (maximum, 52.5 months). Twenty-eight out of 77 patients who had achieved CR relapsed. The probability of RFS was 50.5% at 2 years. Multivariate analysis revealed that the presence of secondary chromosome aberrations in addition to t(9;22) at diagnosis constitute an independent predictive value for RFS (p=0.027), and increase the risk of treatment failure by 2.8-fold.

Key words: acute lymphoblastic leukemia, Philadelphia chromosome, BCR-ABL, imatinib, karyotype.

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Introduction

The treatment for Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph$^+$ ALL) has changed dramatically since imatinib, a selective inhibitor of the ABL tyrosine kinase, was introduced.[1,2] Combined with chemotherapy, or even as a single agent, it can produce complete remission (CR) rates of 90% or higher in newly diagnosed patients.[3-9] We previously reported the results of a phase II study by the Japan Adult Leukemia Study Group (JALSG) to test the efficacy and feasibility of imatinib-combined chemotherapy for newly diagnosed Ph$^+$ ALL.[6] The rate of CR reached 96%, and that of BCR-ABL negativity in bone marrow 71%. However, despite a relatively short follow-up period, relapse occurred in a subset of the patients who had achieved CR.

On the other hand, remarkable progress is being made with the development of novel tyrosine kinase inhibitors with more potent *in vitro* and *in vivo* activities than imatinib.[10,11] Given this, we investigated factors associated with relapse-free survival (RFS).

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Design and Methods

Patients and treatments

Eligibility criteria included newly diagnosed Ph+ ALL, age between 15 and 64 years, an Eastern Co-operative Oncology Group performance status between 0 and 3, and adequate liver, kidney and heart function. Written informed consent was obtained from all patients prior to registration.

For remission induction therapy, imatinib was administered from day 8 to day 63 in combination with daunorubicin, cyclophosphamide, vincristine (VCR) and prednisolone (PSL). Consolidation therapy consisted of an odd course (C1) comprising high-dose methotrexate, high-dose cytarabine and methylprednisolone, and an even course (C2) with single-agent imatinib for 28 days. C1 and C2 were alternated for 4 cycles each. After completion of the consolidation therapy, patients received maintenance therapy consisting of VCR, PSL and imatinib for up to 2 years from the date CR had been achieved.[6] The daily dose of imatinib used in this study was 600 mg. Allogeneic hematopoietic stem cell transplantation (HSCT) was recommended if a matched sibling donor was available, and was allowed from an alternative donor.

The protocol was reviewed and approved by the institutional review board of each of the participating centers and was conducted in accordance with the Declaration of Helsinki.

Cytogenetic and molecular analysis

At diagnosis, bone marrow samples were examined for cytogenetic abnormalities with standard banding techniques. Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature.[12] The number of BCR-ABL copies in bone marrow was determined at a central laboratory with the real-time quantitative RT-PCR test according to the previously described method.[13]

Statistical analysis

Kaplan-Meier survival analysis was performed to estimate the probabilities of RFS, event-free survival (EFS), and overall survival (OS), with differences between the groups compared by the log-rank test. Cumulative incidences of relapse were calculated with non-relapse mortality considered as a competing risk, and differences between the groups were compared with the Gray's test. For risk factor analysis, a Cox proportional hazards model was constructed. In multivariate analysis, variables with p values of <0.10 determined by univariate analysis were included in the final model. A hazard ratio (HR) was calculated in conjunction with a 95% confidence interval (CI).

Results and Discussion

A total of 80 patients were recruited between September 2002 and January 2005. The median age was 48 years (range 15-63), with 49 males and 31 females. CR was achieved by 77 (96.2%) patients. During a median follow-up of 26.7 months (maximum 52.5 months), 28 patients relapsed. Of the 17 relapses observed during the consolidation therapy, 13 occurred during the imatinib course. The probabilities of EFS and OS were 48.5±5.7% and 58.1±5.7% at 2 years (Figure 1). For patients who had achieved CR, the probability of RFS was 50.5±5.9% at 2 years. Allogeneic HSCT was performed for 60 patients, including 24 from a sibling donor, 1 from a related donor other than a sibling, 25 from an unrelated donor, and 10 from unrelated cord blood. Disease status at the time of transplantation was first CR for 44 patients, second CR for 4 and non-CR for 12. The 2-year RFS for those who had undergone allogeneic HSCT during first CR was 62.6±7.5% and 62.1±12.3% for those who had not undergone allogeneic HSCT. When allogeneic HSCT was considered as a time-dependent covariate, it was shown to have no significant effect on RFS (HR, 1.03; 95% CI, 0.51-2.09; p=0.934). Major and minor BCR-ABLs were detected in 23 and 56 patients respectively. The transcript type of the remaining patient could not be determined because fluorescent *in situ* hybridization analysis was used instead of the PCR test. Neither transcript types nor copy numbers at diagnosis were associated with RFS (p=0.763 and 0.912). Pre-treatment cytogenetic results were not available for 4 patients because analysis was not performed (n=2) or was not successful (n=2). Of the remaining 76 patients, 22 showed only t(9;22) or variant translocations, 51 showed additional chromosome aberrations, and 3 showed normal karyotype. Additional aberrations exceeding a frequency of 10% comprised +der(22)t(9;22) in 17 patients, abnormalities involving the short arm of chromosome 9 [abn(9p)] in 17, monosomy 7 in 10, and trisomy 8 in 10. Figure 2 compares RFS for patients with and without additional chromosome aberrations. The presence of additional aberrations was significantly associated with shorter RFS (p=0.003). The relapse rate was also higher in patients with additional aberrations (41% *vs.* 20% at 2 years, p=0.0414). Analyses of the 4 recurrent abnormalities mentioned above demonstrated a statistically significant negative impact on RFS for +der(22)t(9;22) and abn(9p) (p<0.001 and p=0.005). Even after allogeneic HSCT, patients with additional aberrations appeared to have a trend for shorter RFS than those without (p=0.080), but this might reflect a larger proportion of transplantation beyond first CR in the former (31% *vs.* 17%). In patients allografted during first CR, there was no difference in cumulative incidences of relapse dated from the day of transplantation between the 2 groups

(16.5% *vs.* 12.5% at 2 years, p=0.546). Variables that showed a significant effect on RFS in the univariate Cox model included additional chromosome aberrations (p=0.005), peripheral blood blasts % (p=0.024) and sex (p=0.03). Results of multivariate analysis are shown in Table 1. The presence of additional chromosome aberrations was identified as the only independent prognostic factor for RFS (p=0.027). These updated data strongly support recent reports showing the feasibility and remarkable efficacy of imatinib-combined chemotherapy for newly diagnosed Ph+ ALL.[3-9,14,15] The main objective of this report was to identify factors affecting RFS, an issue of rapidly increasing importance given the development of novel tyrosine kinase inhibitors which are expected to further expand the treatment options for this disease. Our data indicated that additional chromosome aberrations, particularly +der(22)t(9;22) and abn(9p), were associated with shorter RFS. It is well known that additional chromosome aberrations are seen frequently in Ph+ ALL. Before the imatinib era, some groups reported the prognostic relevance of additional aberrations.[16-18] By contrast, from a large series of 204 patients, Moorman *et al.*[19] recently showed no significant effect of specific additional aberrations, including +der(22)t(9;22) and del(9p), on survival. In this study, analyzing patients treated with imatinib-combined chemotherapy, the 2-year RFS rate exceeded 80% for those without additional aberrations, whereas outcomes for those with additional aberrations were relatively unfavorable.

Acquisition of resistance to imatinib is an emerging problem in the treatment of chronic myeloid leukemia. One of the most common mechanisms of resistance is the mutation involving the ABL kinase domain. Although it has not been confirmed whether such mutations compromise the clinical outcome of Ph+ ALL patients treated with imatinib-combined chemotherapy, our observation that most of the early relapses occurred during the consolidation courses consisting of imatinib alone implies possible imatinib resistance. If that is the case, switching from imatinib to other novel tyrosine kinase inhibitors based on the pre-treatment cytogenetic results soon after achieving CR or even earlier could be an alternative treatment approach for further improving outcome in Ph+ ALL. Lack of mutation analysis is a major limitation of this study. Recently, Pfeifer *et al.*[20] studied the ABL kinase domain mutation status in newly diagnosed Ph+ ALL patients who were treated with imatinib-combined chemotherapy, and showed that even before exposure to imatinib, mutations were detected in 38% of patients. Importantly, the frequency of the mutant allele was low in such patients. However, at the time of relapse, the same mutation was present as the dominant clone in 90% of the relapsing cases.[20] Altogether, further insights will be provided by investigating the association between karyotype and mutation status at diagnosis.

Despite such limitations, the analysis of 80 patients entered into a single trial identified karyotype at diagnosis as a significant prognostic factor for RFS in newly diagnosed Ph+ ALL patients treated with imatinib-combined chemotherapy. Although our results need to be confirmed regarding kinase domain mutation status, these findings may play a critical role in the future treatment of Ph+ ALL.

Table 1. Multivariate analysis of factors associated with relapse-free survival.

P-value	*HR (95% CI)**	*Factors*	
Additional [chromosome aberrations	0.027	2.84 (1.12-7.19) 1.00	Present Absent
Peripheral blood blasts%	0.051	1.12 (1.00-1.22)	Per 10% increase
Sex	0.148 1.00	1.73 (0.82-3.64) Female	Male

*HR, hazard ratio; 95% CI, 95% confidence interval. *Values higher than unity indicate higher risk for failure.*

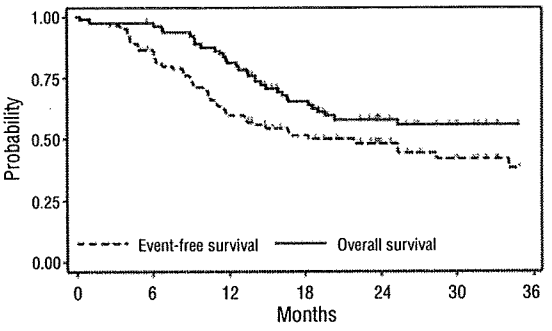

Figure 1. Kaplan-Meier curves for event-free and overall survival. The probabilities of event-free and overall survival at 2 years were 48.5% and 58.1% respectively (n=80).

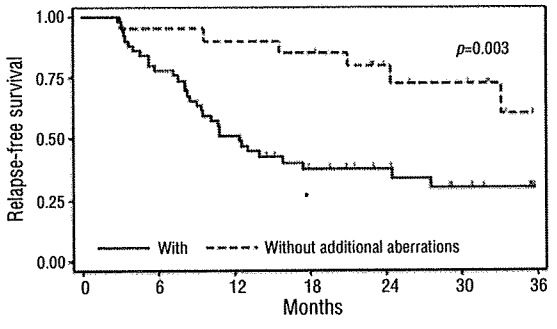

Figure 2. Relapse-free survival for patients with and without additional cytogenetic aberrations. Patients with additional cytogenetic aberrations (n=50) had significantly shorter relapse-free survival than those without (n=20).

Authorship and Disclosures

MY designed and co-ordinated the study, analyzed the data, and wrote the paper; JT, NU, FY, SM, and IJ designed the study, and provided patient sample and clinical data; IS, HA, KN, YU, MT, and AM provided patient sample and clinical data; HN co-ordinated the study, and revised the paper. YM provided patient sample and clinical data, and engaged in data management. SO designed the study, provided patient sample and clinical data, and engaged in data management; KM designed the study, and analyzed the data; TN chaired the study group, co-ordinated the study, and revised the paper; RO served as the principal investigator, chaired the study group, and revised the paper. All authors reviewed the paper, interpreted the results, and approved the final version. The authors reported no potential conflicts of interest.

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CASE REPORT

Successful cord blood transplantation for mycosis fungoides

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Abstract A 26-year-old female diagnosed as mycosis fungoides (MF, clinical stage IV) was treated with single-agent chemotherapy, multi-drug chemotherapy and unrelated bone marrow transplantation with reduced-intensity conditioning (engraftment failure), resulting in failure. Unrelated cord blood transplantation (CBT) as second transplantation following myeloablative conditioning brought complete remission (CR), but relapse of MF occurred 3 months after transplantation. However, discontinuation of immune suppressant led to the regression of MF regions and to second CR that continued for more than 23 months. This is the first report of successful CBT for MF, suggesting the graft-versus-MF effect in a setting of CBT.

Keywords Mycosis fungoides · Cord blood transplantation · Graft-versus-lymphoma effect

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Mycosis fungoides (MF) is a cutaneous T-cell lymphoma associated with the invasion of transformed mature T-cells into the skin demonstrating polymorphic atrophic patches, plaques, to generalized erythrodermia. In general, the prognosis for advanced MF patients with metastasis to other sites has been reported to be poor even when treated with systemic therapies [1, 2]. Several reports of allogeneic hematopoietic stem cell transplantation (allo-HSCT) with both myeloablative and reduced-intensity conditioning regimens [3–5] suggest the efficacy of allo-HSCT for MF through a graft-versus-lymphoma (GVL) effect [6].

In June 2004, a 26-year-old woman was admitted to our hospital because of generalized erythrodermia, a skin tumor of the head, and multiple lymphadenopathy. Her medical history started from 1996 with itchy erythema diagnosed as parapsoriasis in January 2001. Skin tumors developed 3 years later on the head, diagnosed as MF on biopsy. In June 2004, her disease status advanced with generalized multiple skin tumors and lymphadenopathy, eosinophilia (20% of WBC), and the elevation of LDH (367 IU/L, normal range 119–229). Lymph node biopsy and bone marrow analysis revealed the invasion of abnormal T cells, leading to a diagnosis of stage IV MF. Since systemic combination (biweekly CHOP, 8 cycles) or low-dose chemotherapy did not elicit any clinical response, allo-HSCT was considered appropriate for the treatment of this patient. In April 2005, allogeneic bone marrow transplantation from an unrelated donor was performed after reduced-intensity conditioning (fludarabine at 25 mg/m^2 day^{-1} for 5 days and melphalan at 70 mg/m^2 day^{-1} for 2 days) infusing 2.9 × 10^8 cells/kg of bone marrow cells, which resulted in the rejection of donor cells. MF lesions that showed temporal regression after conditioning recurred within 5 weeks after transplantation. Another chemotherapy regimen with cladribine and etoposide did not lead to any

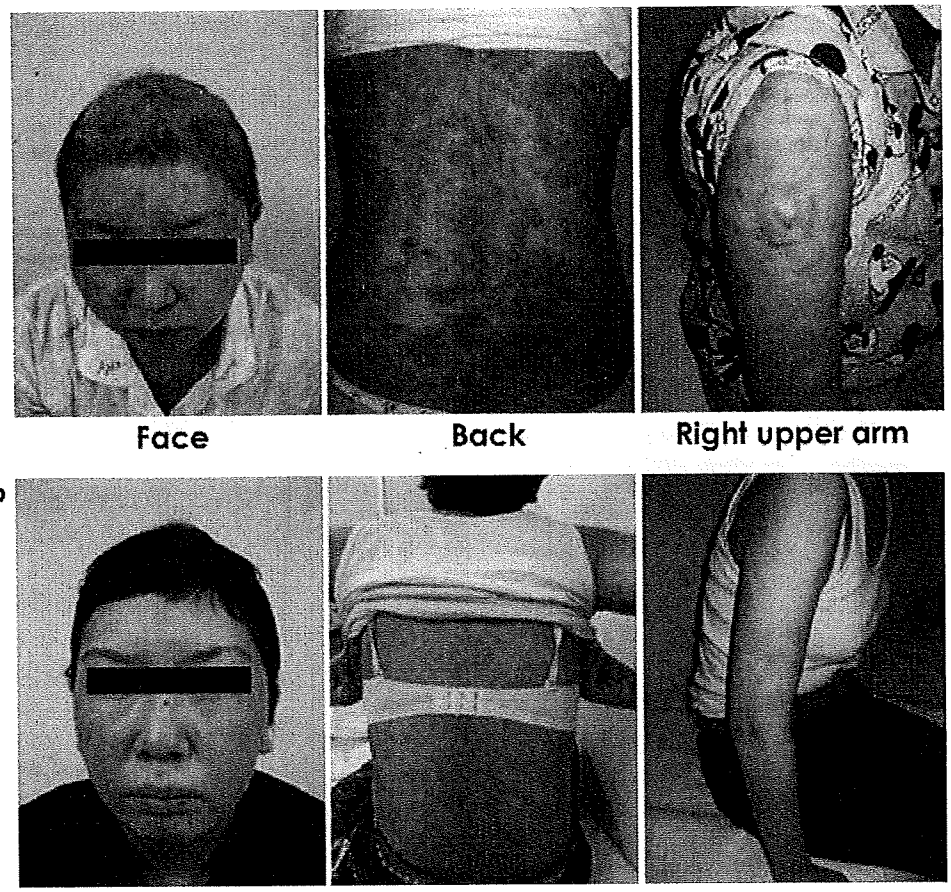

Fig. 1 Skin lesions of MF before CBT (**a**), and those after the discontinuation of tacrolimus (**b**). Skin tumors (*on the head, right eyelid, back,* and *upper arm*) and erythroderma markedly improved (**b**)

improvement of MF after the first transplantation. She had multiple skin tumors with generalized erythrodermia and lymphadenopathy.

Considering the refractory nature of MF in this patient, we decided to perform a second allo-HSCT. In August 2005, after total body irradiation (12 Gy, 6 fractions) and cyclophosphamide (60 mg/kg day^{-1}, 2 days), cord blood (2.2×10^7 cells/kg, HLA 2 loci mismatched, from a male donor) from the Japanese Cord Blood Bank Network was transplanted. For prophylaxis for graft-versus-host disease (GVHD), tacrolimus (0.03 mg/kg, continuous infusion) was used as a single agent. Neutrophils recovered on day 14, and engraftment was confirmed in bone marrow by FISH analysis of sex chromosomes. Platelet recovery (>50,000 per mm^3 without transfusion) was observed on day 41. In terms of MF regions, skin tumors, erythrodermia, and lymphadenopathy began to diminish during conditioning, and disappeared by the time of engraftment, achieving clinical complete remission. Around day 85 after transplantation, skin tumors appeared again on both her legs with itchy skin regions, along with multiple duodenal ulcers (by endoscopic examination) and multiple areas of lymph node swelling (neck, axilla, mediastinum, and para-aorta by CT scan). Skin tumor biopsy confirmed the relapse of MF, and histological analysis of the duodenal ulcer strongly suggested EB virus-associated lymphoproliferative disease. Tacrolimus was reduced and discontinued within 2 weeks; then, skin tumors and skin lesions showed a gradual decrease in size and completely diminished by day 140 (Fig. 1). No chemotherapy was added. There was no clear sign of acute or chronic GVHD even after the discontinuation of tacrolimus. There was no sign of MF on her skin and no lymphadenopathy on CT scan at more than 23 months after the second CR, with a Karnofsky score of 90%.

Several groups described that neither conventional chemotherapy nor high-dose chemotherapy with autologous stem cell support was sufficient for the long-term remission of MF [7, 8]. Based on the successful reports of allo-HSCT for MF and the efficacy of the withdrawal of immunosuppressants for some relapsed MF cases, the important role of the GVL effect for the control of MF is suggested [9, 10]. This is the first report of successful CBT for advanced MF with the graft-versus-MF effect. Since cord blood is available for many patients through cord blood banks and the waiting period is relatively short, CBT could be a therapeutic option for MF patients who are candidates for allo-HSCT but lack suitable related or unrelated donors.

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bjh research paper

Prospective monitoring of *BCR-ABL1* transcript levels in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia undergoing imatinib-combined chemotherapy

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Summary

The clinical significance of minimal residual disease (MRD) is uncertain in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL) treated with imatinib-combined chemotherapy. Here we report the results of prospective MRD monitoring in 100 adult patients. Three hundred and sixty-seven follow-up bone marrow samples, collected at predefined time points during a uniform treatment protocol, were analysed for *BCR-ABL1* transcripts by quantitative reverse transcription polymerase chain reaction. Ninety-seven patients (97%) achieved complete remission (CR), and the relapse-free survival (RFS) rate was 46% at 3 years. Negative MRD at the end of induction therapy was not associated with longer RFS or a lower relapse rate ($P = 0{\cdot}800$ and $P = 0{\cdot}964$ respectively). Twenty-nine patients showed MRD elevation during haematological CR. Of these, 10 of the 16 who had undergone allogeneic haematopoietic stem cell transplantation (HSCT) in first CR were alive without relapse at a median of 2·9 years after transplantation, whereas 12 of the 13 who had not undergone allogeneic HSCT experienced a relapse. These results demonstrate that, in Ph+ ALL patients treated with imatinib-combined chemotherapy, rapid molecular response is not associated with a favourable prognosis, and that a single observation of elevated MRD is predictive of subsequent relapse, but allogeneic HSCT can override its adverse effect.

Keywords: acute lymphoblastic leukaemia, Philadelphia chromosome, *BCR-ABL1*, imatinib, minimal residual disease.

The recent development of imatinib-combined chemotherapy has drastically improved overall treatment results in Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL) (Ottmann & Wassmann, 2005; Yanada & Naoe, 2006; Thomas, 2007). Nearly 95% of newly diagnosed patients now achieve complete remission (CR) (Thomas *et al*, 2004;

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Lee *et al*, 2005; Wassmann *et al*, 2006; Yanada *et al*, 2006). However, outcome after CR depends on the individual patient and is not predictable. Young patients generally undergo allogeneic haematopoietic stem cell transplantation (HSCT) after achieving CR if a suitable donor is available, based on the concept that it is the established treatment with curative potential for this disease (Cornelissen *et al*, 2001; Dombret *et al*, 2002; Stirewalt *et al*, 2003; Yanada *et al*, 2005). Nevertheless, a fraction of patients experience a relapse even prior to transplantation, whereas some remain alive in remission for years without undergoing HSCT.

Minimal residual disease (MRD), as measured by reverse transcription-polymerase chain reaction (RT-PCR) or flow cytometry, has been shown to be useful for predicting prognosis in paediatric (Brisco *et al*, 1994; Cave *et al*, 1998; Coustan-Smith *et al*, 1998; van Dongen *et al*, 1998; Dworzak *et al*, 2002; Nyvold *et al*, 2002; Zhou *et al*, 2007) and adult ALL patients (Brisco *et al*, 1996; Mortuza *et al*, 2002; Vidriales *et al*, 2003; Bruggemann *et al*, 2006; Raff *et al*, 2007). However, the utility of MRD as a prognostic indicator has been established on the basis of data from patients treated with chemotherapy alone, and it remains to be determined whether it is useful in patients treated with chemotherapy in combination with imatinib. The Japan Adult Leukemia Study Group (JALSG) recently conducted a phase II trial of imatinib-combined chemotherapy in newly diagnosed Ph+ ALL patients (Towatari *et al*, 2004; Yanada *et al*, 2006, 2008). In that trial, *BCR-ABL1* transcript levels in bone marrow were prospectively monitored at predetermined time points using quantitative real-time RT-PCR (RQ-PCR). The results are presented here, with particular emphasis on the prognostic significance of rapid MRD clearance and MRD kinetics.

Patients and methods

Patients

The patient eligibility requirements of the phase II trial were as follows: newly diagnosed with Ph+ ALL, aged 15–64 years, an Eastern Cooperative Oncology Group performance status of 0–3, and adequate liver, kidney and heart function. Written informed consent was obtained from all patients prior to registration. The protocol was reviewed and approved by the institutional review boards of all of participating centres and was conducted in accordance with the Declaration of Helsinki. This trial was registered at http://www.clinicaltrials.gov as #NCT00130195.

The treatment schedule is summarized in Table I. Allogenic HSCT was allowed after achieving CR if the patient had a suitable donor. The original target sample size was 77 patients (Yanada *et al*, 2006), with the CR rate defined as the primary endpoint. Eighty patients had been enrolled by January 2005, when enrolment was extended to 100 patients to attain a more precise point estimate of the overall survival (OS) rate. This sample size enabled the lower limit of the 95% confidence interval (CI) of OS rate (expected to be 70% at 1 year) to be higher than 60%.

MRD evaluation

Molecular monitoring was performed with use of the RQ-PCR assay in a single independent laboratory. Bone marrow samples were collected at diagnosis; at days 28 and 63 of the induction course; after the first, second, fifth and sixth consolidation courses; after 1 year of treatment; and at the end of therapy (2 years from the date of CR).

Total RNA was extracted from mononuclear cells using the QIAamp RNA blood mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of RNA were measured by spectrophotometric determination of the A260/A280 ratio. Total RNA (1·5 μg) was transcribed to cDNA in a 22·5-μl reaction mixture containing 500 ng of random hexamer (Invitrogen, Carlsbad, CA, USA), 50 units of reverse transcriptase (Invitrogen), 40 units of RNase inhibitor (Invitrogen) and 500 μmol/l dNTP. The reaction mixture (total volume: 50 μl) contained 7·5 μl of a 22·5-μl RNA mixture (corresponding to 500 ng of RNA), 15 pmol of forward and reverse primers, 10 pmol of TaqMan probe, and 25 μl of 2× TaqMan universal PCR master mix (Applied Biosystems, Foster City, CA, USA). The primer and probe sequences have been described elsewhere (Towatari *et al*, 2004). Amplification was carried out with an initial activation of the polymerase at 50°C for 2 min and 95°C for 10 min, followed by 50 cycles consisting of two steps: 95°C for 15 s and 60°C for 1 min. Fluorescent emission spectra were monitored every 7 s and analysed using the PRISM 7700 system with SEQUENCE DETECTION SYSTEM software (version 1.7; Applied Biosystems). Amplified cDNA fragments were cloned into the pCRII vector (Invitrogen) and used as the reference standard. The copy number of each plasmid was calculated from the DNA concentration (determined by measuring A260) and the molecular weight of the plasmid. The copy number of the *BCR-ABL1* transcripts was calculated by comparing the C_t values of samples with those of the standard and converted to molecules per microgram RNA after being normalized by means of *GAPDH*. The threshold for quantification was 50 copies/μg RNA, which corresponded to a minimal sensitivity of 10^{-5}. Detectable MRD levels below this threshold were referred to as '<50 copies/μg' to distinguish from undetectable MRD. Nested PCR was not performed in this study. Samples with *GAPDH* levels below $5{\cdot}7 \times 10^5$ copies/μg RNA were not eligible for MRD evaluation.

Statistical analysis

Relapse-free survival (RFS) was defined as the time from CR to relapse, death, or last follow-up, and OS was defined as the time from registration to death or last follow-up. A Kaplan–Meier survival analysis was performed to estimate the probabilities of RFS and OS, with differences between the curves

Table I. Treatment schedule.

Drug	Dose	Route	Days
Induction			
Cyclophosphamide	1200 mg/m^2 (800 mg/m^2)*	IV (3 h)	1
Daunorubicin	60 mg/m^2 (30 mg/m^2)*	IV (1 h)	1–3
Vincristine	1·3 mg/m^2†	IV (bolus)	1, 8, 15, 22
Prednisolone	60 mg/m^2	PO	1–21 (1–7)*
Imatinib	600 mg	PO	8–63
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	29
Consolidation #1			
Methotrexate	1 g/m^2	IV (24 h)	1
Cytarabine	2 g/m^2 (1 g/m^2)* twice a day	IV (3 h)	2, 3
Methylprednisolone	50 mg twice a day	IV (bolus)	1–3
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	1
Consolidation #2			
Imatinib	600 mg	PO	1–28
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	1
Consolidation #3	Repeat #1		
Consolidation #4	Repeat #2		
Consolidation #5	Repeat #1		
Consolidation #6	Repeat #2		
Consolidation #7	Repeat #1		
Consolidation #8	Repeat #2		
Maintenance‡			
Vincristine	1·3 mg/m^2†	IV (bolus)	1
Prednisolone	60 mg/m^2	PO	1–5
Imatinib	600 mg	PO	1–28

IV, intravenously; PO, orally; IT, intrathecally.
*For patients aged 60 and older.
†Maximum 2·0 mg.
‡Repeated every 4 weeks up to 2 years from the date of complete remission.

qualified with the log-rank test. The cumulative incidence of relapse was calculated with death during CR considered as a competing risk, and differences between the curves were qualified with Gray's test. STATA version 8 software (StataCorp, College Station, TX, USA) and R software version 2.4.0 (The R Foundation for Statistical Computing, http://www.r-project.org) were used for statistical analyses. P values ≤0·05 were considered to be statistically significant.

Results

Patients and treatment results

The median patient age was 45 years (range 15–64 years); 55 were male and 45 were female. Twenty-five patients were positive for major *BCR-ABL1*, and 75 for minor *BCR-ABL1*. Ninety-seven patients (97%) achieved CR. The median and maximum follow-up periods were 3·2 and 5·1 years respectively. The outcomes of 100 patients are detailed in Fig 1. Relapse occurred in 38 patients after a median CR duration of 7·3 months (range 2·1–37·4). Allogeneic HSCT was performed in 60 patients during first CR, and in 19 patients beyond first CR. For patients allografted in first CR, the median time to HSCT was 5·3 months (range 2·2–17·1). No patient underwent autologous HSCT. The probability of OS for the entire cohort was 55% at 3 years. The 1-year OS rate, the endpoint for the study extension, was 83% (95% CI 74–89%). Among the 97 patients who achieved CR, the probability of RFS was 46% at 3 years. Neither transcript types nor copy numbers at diagnosis were associated with RFS (P = 0·709 and P = 0·851 respectively).

MRD kinetics

The number of patients who underwent MRD monitoring decreased with time because of prior relapse, death, or transfer to allogeneic HSCT. Thus, the total number of follow-up samples was 367 (77% of all possible samples at all time points): 86 of 98 (88%) at day 28, 85 of 97 (88%) at day 63, 75 of 90 (83%) after the first consolidation (C#1), 55 of 73 (75%) after C#2, 31 of 38 (82%) after C#5, 22 of 32 (69%) after C#6, 11 of 15 (73%) at 1 year, and 2 of 9 (22%) at 2 years.

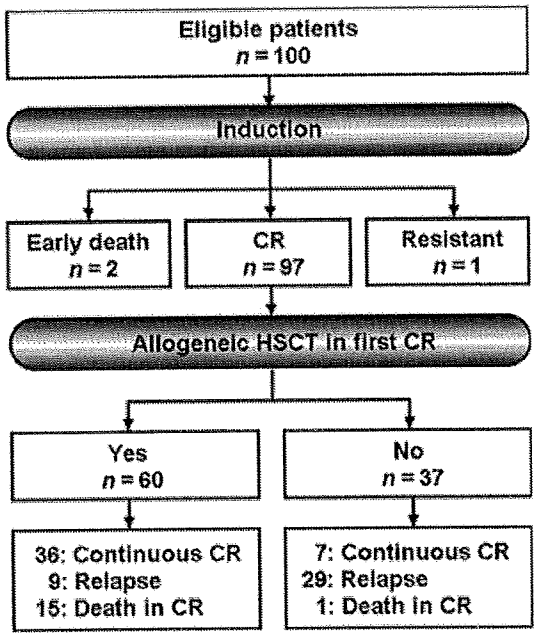

Fig 1. Flow diagram showing patient outcomes. CR, complete remission; HSCT, haematopoietic stem cell transplantation.

Figure 2 shows the percentages of patients with negative and low (<50 copies/μg) MRD levels at each time point. There was a progressive increase in the percentage of patients with negative MRD during the early treatment courses, with 24% at day 28, 48% at day 63, 68% after C#1, and 67% after C#2. Nearly all samples measured at 1 year and at 2 years were negative for MRD, although only a small number of samples were analysed at these time points. The only patient whose MRD was positive (87 copies/μg) at 1 year experienced a relapse 8 months later. All of the three patients who experienced a relapse during maintenance therapy had showed MRD elevation prior to haematological relapse.

Rapid MRD clearance and outcome

RQ-PCR results at the end of induction therapy (day 63) were available for 85 patients. One patient with a *BCR-ABL1* level of 160 000 copies/μg failed to achieve CR. Figure 3 shows the RFS rates and cumulative incidences of relapse in 84 CR patients according to MRD detection at day 63. PCR negativity was not associated with a higher RFS rate (46% vs. 42% at 3 years, $P = 0{\cdot}800$; Fig 3A) or a lower relapse rate (40% vs. 41% at 3 years, $P = 0{\cdot}964$; Fig 3B). A relatively small number of patients ($n = 11$) whose MRD levels exceeded 1000 copies/μg at day 63 had trends toward lower RFS ($P = 0{\cdot}092$, Fig 4A) and higher relapse rate ($P = 0{\cdot}070$, Fig 4B). Neither PCR negativity at day 28 nor after C#1 was associated with higher RFS ($P = 0{\cdot}867$ and $P = 0{\cdot}549$) or lower relapse rates ($P = 0{\cdot}796$ and $P = 0{\cdot}667$).

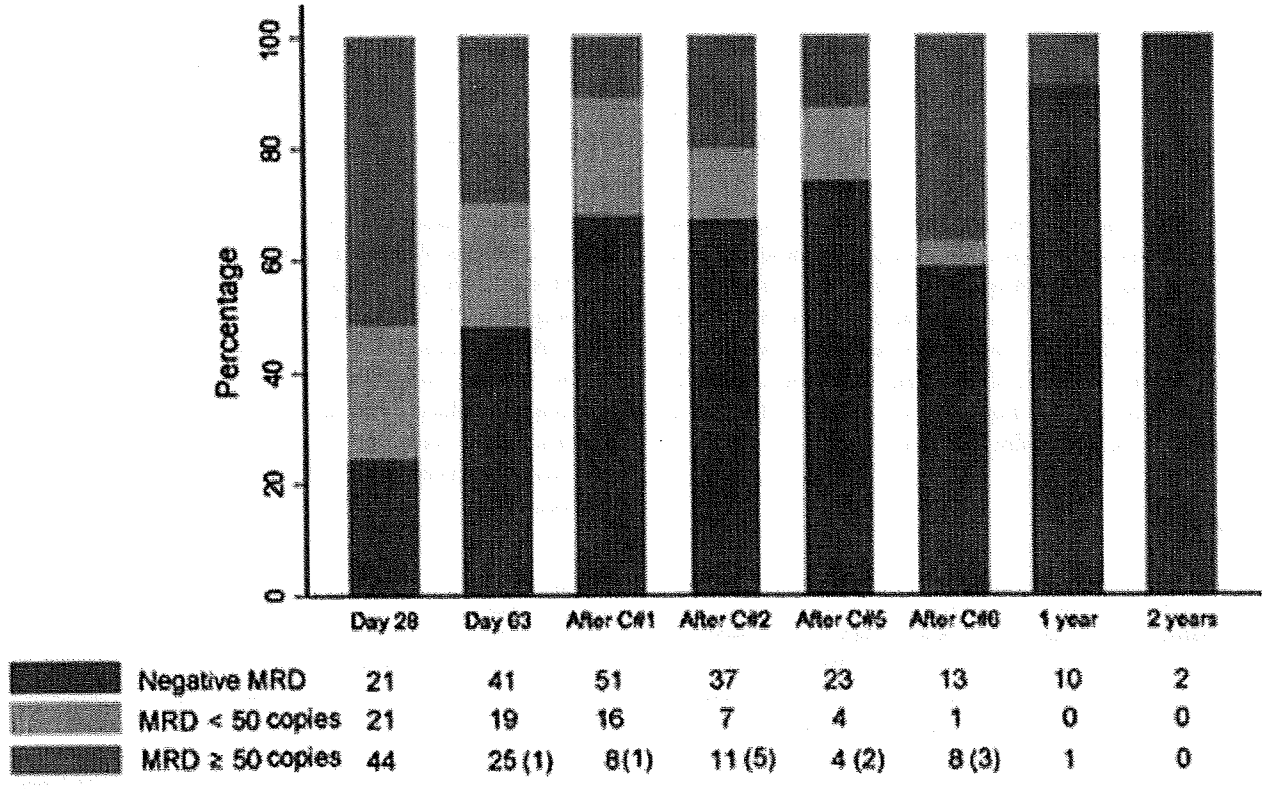

Fig 2. Frequencies of negative and low (<50 copies/μg RNA) *BCR-ABL1* transcript levels at each time point. Figures in parentheses represent the number of patients who developed haematological relapse at that time point.

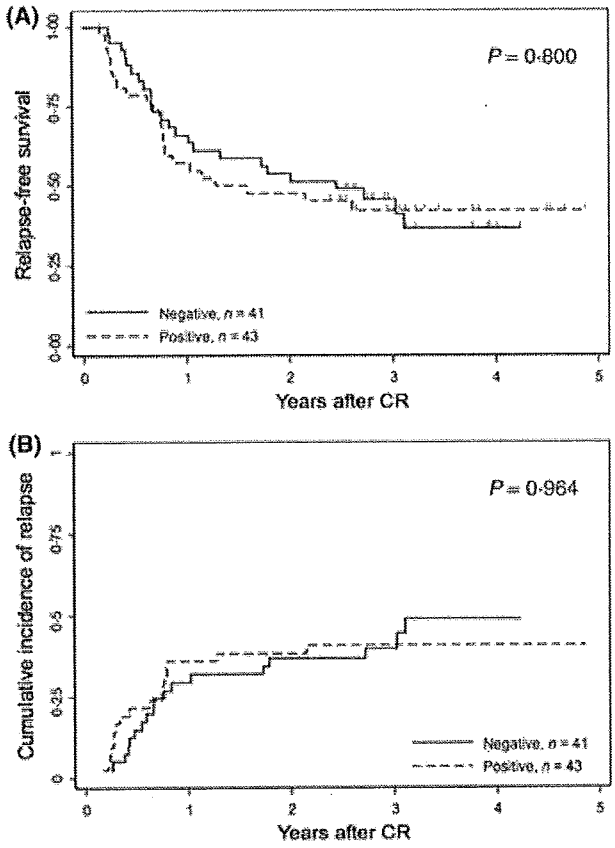

Fig 3. Relapse-free survival (A) and cumulative incidence of relapse (B) in patients with negative and positive *BCR-ABL1* transcript levels at the end of induction therapy.

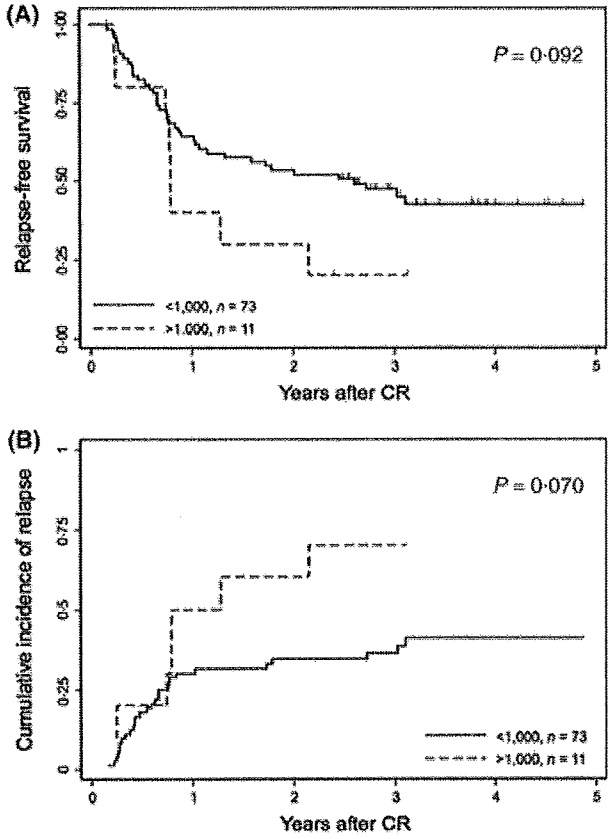

Fig 4. Relapse-free survival (A) and cumulative incidence of relapse (B) in patients with *BCR-ABL1* transcript levels below or above 1000 copies/μg RNA at the end of induction therapy.

MRD elevation during haematological remission

Elevated MRD levels during CR were documented in 29 patients. Of these, six patients experienced MRD elevation twice, and the second elevation was accompanied by simultaneous haematological relapse in five patients. The outcome and duration from the first observation of MRD elevation to relapse or allogeneic HSCT, whichever came first, in each patient are presented in Table II. Sixteen underwent allogeneic HSCT in first CR. The median duration from the first documentation of elevated MRD to allogeneic HSCT was 2·3 months (range 0·4–5·6). Death during first CR and relapse after transplantation occurred in three patients each, and 10 remained in first CR at a median of 2·9 years (range 2·0–4·6 months) after transplantation. In contrast, among the 13 non-transplantation patients, 12 had a relapse at a median of 2·0 months (range 0·5–35·0) after the first MRD elevation. Another patient once achieved PCR negativity after C#2, but showed detectable MRD below the threshold (<50 copies/μg) after C#5. However, MRD became negative after C#6, and the patient remained alive without relapse at 2·8 years after MRD elevation. The conversion from negative MRD to '<50 copies/μg' was observed in another six patients. Four remained in first CR after undergoing allogeneic HSCT, and the remaining two who had not undergone HSCT experienced a relapse.

Discussion

Minimal residual disease levels at various time points in CR, especially at the end of induction therapy, are considered an important prognostic factor in ALL (Pui *et al*, 2008). Although there were few studies that focused on Ph+ ALL with a relatively large number of patients (Dombret *et al*, 2002; Pane *et al*, 2005), Pane *et al* (2005) reported that significant reductions in *BCR-ABL1* levels after induction and consolidation therapy were associated with better outcomes. Most published studies on imatinib-combined chemotherapy include MRD findings (Thomas *et al*, 2004; Towatari *et al*, 2004; Lee *et al*, 2005; Rea *et al*, 2006; Wassmann *et al*, 2006; Yanada *et al*, 2006; de Labarthe *et al*, 2007; Ottmann *et al*, 2007a), but the prognostic significance of early treatment response remains to be determined. Our data remarkably demonstrated that the RFS rate for the patients with negative MRD at the end of induction therapy was similar to that for patients with positive MRD. We considered the possibility that this lack of difference was influenced by the confounding effect of allogeneic HSCT.

Table II. Outcome of patients who experienced an MRD elevation during haematological CR.

UPN	Outcome	Months from MRD elevation to relapse	UPN	Outcome	Months from MRD elevation to HSCT	Outcome after HSCT
63	CCR without HSCT	–	36	HSCT	1·0	CCR
17	Relapse	0·5	72	HSCT	1·0	CCR
43	Relapse	0·9	12	HSCT	2·1	CCR
50	Relapse	1·5	77	HSCT	2·2	CCR
58	Relapse	1·5	10	HSCT	2·6	CCR
82	Relapse	1·6	1	HSCT	2·9	CCR
62	Relapse	1·9	81	HSCT	3·3	CCR
14	Relapse	2·0	94	HSCT	3·6	CCR
85	Relapse	3·6	55	HSCT	4·8	CCR
56	Relapse	4·3	8	HSCT	5·1	CCR
51	Relapse	7·9	34	HSCT	0·4	Relapse
60	Relapse	8·4	87	HSCT	2·0	Relapse
18	Relapse	35·0	47	HSCT	2·4	Relapse
			48	HSCT	1·9	NRM
			16	HSCT	2·0	NRM
			49	HSCT	5·6	NRM

UPN, unique patient number; MRD, minimal residual disease; HSCT, haematopoietic stem cell transplantation; CCR, continuous complete remission; NRM, non-relapse mortality.

However, MRD negativity was not beneficial in terms of relapse rate ($P = 0{\cdot}964$) or even in terms of RFS after we censored patients who underwent allogeneic HSCT at the time of transplantation ($P = 0{\cdot}470$). A trend toward a higher relapse rate in the 11 patients (13%) with MRD levels of ≥1000 copies/μg suggests that MRD levels at the end of induction therapy may be helpful in identifying a small subgroup of patients at high risk for relapse. However, the finding that negative MRD was not associated with a favourable outcome precludes prognostication of the remaining majority of patients, and indicates that relapse risk in these patients depends on factors unrelated to initial treatment response. Acquisition of resistance during treatment may explain why rapid molecular response is not prognostically relevant.

Another important finding of this study was the significant relationship between MRD elevation and relapse. This finding is in accordance with those of several studies published in the 1990s in which the conversion from negative to positive RT-PCR results was associated with subsequent relapse in Ph+ ALL patients (Miyamura *et al*, 1992; Preudhomme *et al*, 1997; Radich *et al*, 1997; Mitterbauer *et al*, 1999). Our results suggest that an increase in the MRD level at a single time point is predictive of subsequent relapse, but such patients can be successfully treated with allogeneic HSCT. Given a median duration of only 2 months from MRD elevation to haematological relapse, an alternative therapeutic intervention should be considered immediately after MRD elevation. Because of its rapid availability, cord blood transplantation may be a practical treatment option for patients without a related donor, if they are fit for the procedure. Switching from imatinib to other novel tyrosine kinase inhibitors, such as dasatinib (Talpaz *et al*, 2006; Ottmann *et al*, 2007b) and nilotinib (Kantarjian *et al*, 2006), may also be a reasonable option for patients without a mutation resistant to these agents. Additionally, frequent MRD monitoring increases the chances of detecting MRD elevation during CR, prolonging the duration prior to haematological relapse, and enabling the use of alternative therapies in patients who would otherwise experience an overt relapse.

When MRD data are analysed in relation to outcome, differences in conditions such as treatment and sampling time points can affect results. In this regard, strength of this study is that all samples were collected at scheduled time points during a uniform treatment protocol. On the other hand, one limitation of our study is that samples were not obtained from all patients at all time points. Nevertheless, the percentage of available samples collected at the end of induction therapy was 86% (84 of the 97 CR patients). Furthermore, sample availability did not seem to be a significant source of selection bias: we found no difference in RFS between patients whose samples were available or not at the end of induction therapy ($P = 0{\cdot}345$). Also the utility of MRD elevation in predicting subsequent relapse would have been strengthened if the proportion of missing samples had been smaller. Finally, it may be disputed that our detection method was partly different from those used in other countries, specifically in that results were reported as a copy number normalized by the control gene and in that PCR negativity was not confirmed by nested PCR. Nevertheless, we believe this point would not impair our main results.

In summary, our prospective MRD monitoring of Ph+ ALL patients treated with imatinib-combined chemotherapy revealed that rapid molecular response is not associated with a superior prognosis and that a single observation of elevated

MRD is strongly predictive of subsequent relapse but allogeneic HSCT can override its adverse effect. Such patients may also benefit from novel tyrosine kinase inhibitors. We conclude that frequent MRD monitoring is beneficial in clinical decision making for Ph+ ALL patients treated with imatinib-combined chemotherapy. Incorporating MRD data into a treatment protocol will be necessary in future clinical trials of Ph+ ALL.

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Japanese epidemiological survey with consensus statement on Japanese guidelines for treatment of iron overload in bone marrow failure syndromes

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Abstract Many patients with bone marrow failure syndromes need frequent transfusions of red blood cells, and most of them eventually suffer from organ dysfunction induced by excessively accumulated iron. The only way to treat transfusion-induced iron overload is iron chelating therapy. However, most patients have not been treated effectively because daily/continuous administration of deferoxamine is difficult for outpatients. Recently, a novel oral iron chelator, deferasirox, has been developed, and introduction of the drug may help many patients benefit from iron chelation therapy. In this review, we will discuss the current status of iron overload in transfusion-dependent patients, and the development of Japanese guidelines for the treatment of iron overload in Japan, which were established by the National Research Group on Idiopathic Bone Marrow Failure Syndromes in Japan.

Keywords Bone marrow failure syndrome · Iron overload · Iron chelation · Guidelines

1 Introduction

Many patients with aplastic anemia (AA) or myelodysplastic syndromes (MDS) need frequent transfusions of red blood cells (RBCs). One unit (derived from 200 mL of whole blood) of RBC transfusion in Japan contains about 100 mg of iron. Because there is no physiological mechanism for iron excretion in humans, and daily iron excretion is no more than 1 mg in a healthy man, repeated RBC transfusions will soon result in iron overload. Excess iron is mainly deposited in the liver, heart and pancreas, and causes organ dysfunction [1, 2].

As phlebotomy is not an option because of the underlying bone marrow failure, the only way to treat iron overload is by iron chelation therapy. However, difficulty in optimal administration of deferoxamine (DFO, Desferal®) in Japan has hampered effective chelation, and currently most patients are not treated effectively [3].

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