protein phosphatase 2A activity, and higher proliferation rates than those expressing the wild-type protein (Piazza *et al*, 2012). Although our cohort is too small to arrive at conclusions regarding the prognosis of patients with *SETBP1* mutations, mutated *SETBP1* indeed plays an essential role in the pathogenic mechanism in haematological malignancies.

In summary, *SETBP1* mutations were found in 4·8% of patients with JMML in this study, similar to the frequency reported previously for patients with chronic myelomonocytic leukaemia [3·7% (3/82) and 6·2% (12/195)] (Piazza *et al*, 2012; Damm *et al*, 2013) and JMML [7·6% (7/92)] (Sakaguchi *et al*, 2013). Our analysis of 414 patients with JMML or other haematological malignancies suggests that mutations of *SETBP1* may have some role in the pathogenesis of JMML and MDS but not in AML or infant ALL, although further evaluations are required.

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## Authors contributions

Y.H. designed the study. K.K., M.Sotomatsu, M.Sako, and E.I. provided critical reagents and samples. N.S., K.O., and M.P. performed the experiments. E.I. and H.A. supervised the work. N.S., K.O., and M.P. analysed the results. N.S. and Y.H. wrote the paper and all the authors critically reviewed and revised it.

## Conflict of interest

The authors declare no conflict of interest.

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bjh research paper

# Clinical characteristics of 15 children with juvenile myelomonocytic leukaemia who developed blast crisis: MDS Committee of Japanese Society of Paediatric Haematology/Oncology

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## Summary

Juvenile myelomonocytic leukaemia (JMML) is a rare haematopoietic stem cell disease of early childhood, which can progress to blast crisis in some children. A total of 153 children diagnosed with JMML were reported to the Myelodysplastic Syndrome Committee in Japan between 1989 and 2007; 15 of them (9·8%) had 20% or more blasts in the bone marrow (blast crisis) during the disease course. Blast crisis occurred during observation without therapy ($n = 3$) or with oral 6-mercaptopurine treatment ($n = 9$) and in relapse after haematopoietic stem cell transplantation (HSCT; $n = 3$). Six patients had a complex karyotype (5 including monosomy 7) and an additional three patients had isolated monosomy 7 at blast crisis. Seven patients received HSCT after blast crisis and four of them achieved remission. Eleven out of the 15 patients died; the cause of death was disease progression in 10 patients and transplant-related complication in one patient. In summary, patients with blast crisis have poor prognosis and can be cured only by HSCT. The emergence of monosomy 7 and complex karyotype may be characteristic of blast crisis in a substantial subset of children.

Keywords: juvenile myelomonocytic leukaemia, blast crisis, monosomy 7, haematopoietic stem cell transplantation.

Juvenile myelomonocytic leukaemia (JMML) is a rare haematopoietic stem cell disease in infants with features characteristic of both myeloproliferative and myelodysplastic disorders (Aricò *et al*, 1997; Niemeyer *et al*, 1997; Emanuel, 1999; Koike & Matsuda, 2008). Its clinical course is diverse. Sometimes the disease can even spontaneously resolve without any treatment (Matsuda *et al*, 2007). However, most patients have a rapidly progressive disease and early death because of the organ enlargement or bone marrow failure. Many patients are resistant to essentially all chemotherapy regimens and it is thought that haematopoietic stem cell transplantation (HSCT) is the only curative treatment for children with JMML (Locatelli *et al*, 2005; Yabe *et al*, 2008).

According to a previous report (Aricò *et al*, 1997), approximately one-third of the patients present with a rapidly progressive disease and another third of the patients show a more indolent disease course. The latter patients are usually stable, even if they have persistent splenomegaly,

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moderate leucocytosis or monocytosis. However, they may occasionally suddenly experience blast crisis. The remaining patients have an intermediate prognosis.

Luna-Fineman *et al* (1999) reported that 8 (13%) of 60 of patients with JMML developed acute leukaemia. Thus, it is known that JMML can progress to blast crisis in a subset of children. However there are only a few reports accounting for this transformation and the molecular mechanism of acute transformation is unknown.

In order to clarify the characteristics of blast crisis of JMML, we retrospectively analysed in detail those JMML patients who had progressed to blast crisis.

## Patients and methods

Between 1989 and 2007, 153 JMML children had been reported to the myelodysplastic syndrome (MDS) committee of the Japanese Society of Paediatric Haematology/Oncology (JSPHO). The diagnosis was made according to the criteria of the International JMML Working Group (Niemeyer *et al*, 1998; Chan *et al*, 2009). Patients registered before 1999 were diagnosed with JMML at individual institutions. Since 1999, bone marrow (BM) and peripheral blood (PB) smears were reviewed by two reference investigators of the MDS Committee at diagnosis and patients were prospectively registered to the MDS Committee (Sasaki *et al*, 2001). Based on clinical information reported to the MDS Committee, 15 JMML patients with blast crisis were identified. Blast crisis was defined as having 20% or more blasts in the BM during the course of illness. Questionnaires inquiring about the detailed clinical and laboratory findings, treatment and clinical outcome were sent to the physicians who treated these patients. These retrospectively collected data were analysed in this study. The study was approved by the MDS Committee of the JSPHO. Informed consent was obtained from the guardians of patients after 1999; however, this was not possible for cases registered before 1999 because an appropriate guideline was not provided for retrospective observational studies in that period.

Complete remission (CR) of JMML after therapy was defined if all of the following criteria were fulfilled: (i) no evidence of circulating blasts in PB, BM with less than 5% blasts and trilineage haematological recovery and (ii) absence of chromosome abnormalities; and iii) disappearance of clinical symptoms of JMML, such as organomegaly.

## Results

Fifteen (9·8%) patients of the 153 JMML cases had progressed to blast crisis. The characteristics of these patients at diagnosis are shown in Table I.

There was a male predominance with a male: female rate of 2·8: 1. There was no congenital anomaly, such as Noonan

Table I. Characteristics of the patients at diagnosis.

| Patient | Year of Dx | Sex/age (years) | WBC ($\times 10^9$/l) | PB mono ($\times 10^9$/l) | PB blasts (%) | Hb (g/l) | Plt ($\times 10^9$/l) | HbF (%) | BM blasts (%) | Karyotype | Initial treatment | CR before BC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1991 | M/0·3 | 38·0 | 1·9 | 1·0 | 104 | 85 | 15·4 | 0·2 | 46, XY | 6MP | No |
| 2 | 1992 | M/1·4 | 115·9 | 90·4 | 0 | 106 | 65 | 13·0 | 6·2 | 46, XY | None | No |
| 3 | 1992 | M/2·6 | 45·2 | 3·2 | 1·5 | 97 | 17 | 31·9 | 3·7 | 46, XY | 6MP + AML type | No |
| 4 | 1993 | M/0·3 | 89·6 | 17·0 | 3·0 | 90 | 43 | 14·7 | 3·6 | 46, XY | 6MP | No |
| 5 | 1994 | M/0·3 | 29·0 | 4·2 | 0 | 117 | 77 | 53·8 | 5·8 | 46, XY | 6MP | No |
| 6* | 1994 | M/3·9 | 8·9 | 0·4 | 6·0 | 120 | 43 | 64·2 | 16·8 | 46, XY | 6MP | No |
| 7 | 1995 | F/1·5 | 35·1 | 5·3 | 7·0 | 110 | 13 | 24·0 | ND | 46, XX | 6MP + PSL + Etoposide | No |
| 8 | 1997 | M/7·6 | 29·3 | 5·3 | 7·0 | 69 | 24 | 35·9 | 0 | 46, XY | None | No |
| 9 | 1997 | F/2·8 | 15·6 | 1·4 | 1·0 | 113 | 66 | 47·0 | 0 | 46, XX | None | No |
| 10 | 1999 | M/3·7 | 72·0 | 33·5 | 0 | 97 | 94 | 1·1 | 14·9 | 46, XY, −2, +mar | 6MP | No |
| 11 | 1999 | M/4·7 | 98·5 | 28·1 | 2·0 | 70 | 16 | 69·5 | 5·1 | 46, XY | 6MP | No |
| 12 | 2000 | M/1·7 | 17·8 | 1·4 | 0 | 74 | 20 | 55·0 | ND | 46, XY, t(3;18) (q25;q21) | HSCT | Yes |
| 13 | 2001 | M/4·7 | 58·5 | 14·9 | 0·5 | 120 | 102 | 9·0 | 0 | 45, XY, −7 | 6MP | No |
| 14 | 2002 | F/0·4 | 376·2 | 26·3 | 0 | ND | ND | 19·5 | 0 | 46, XX | 6MP + HSCT | Yes |
| 15 | 2003 | F/3·2 | 27·6 | 3·0 | 0 | 104 | 32 | 41·8 | 18·3 | 46, XX | 6MP + AML type + HSCT | Yes |

Dx, diagnosis; WBC, white blood cell count; PB, peripheral blood; Hb, haemoglobulin concentration; Plt, platelet count; ND, data not available; BM, bone marrow; 6MP, 6-mercaptopurine; PSL, prednisolone; AML, acute myeloid leukaemia; HSCT, haematopoietic stem cell transplantation; CR, complete remission; BC, blast crisis.

*This patient did not have monocyte count exceeding $1 \times 10^9$/l at diagnosis, however, he had splenomegaly and bone marrow mononuclear cells showed hypersensitivity to GM-CS.

syndrome or neurofibromatosis type 1. We only had gene mutation analysis data for two patients, because most patients were diagnosed in the 1990's. Patient 1 had *NRAS* mutation and Patient 7 had *PTPN11*.

At initial diagnosis, chromosomal analyses were available in all the 15 children and showed a normal karyotype in 12 children, monosomy 7 in one child and other aberrations in two children.

Three patients had blast crisis during observation without treatment, nine patients were treated with 6-mercaptopurine (6MP) before blast crisis and three patients had blast crisis following relapse after HSCT. The pre-blast crisis status of 12 patients was non-CR, whereas all three patients who received HSCT had achieved CR.

Table II shows the characteristics of the patients at blast crisis. The median time interval between diagnosis and blast crisis was 15 months (range, 1–48 months). The median time interval between the time of initial treatment and blast crisis was 5 months (range, 1–44 months) in the 6MP treatment group, whereas the time interval between HSCT and blast crisis was 8, 19 and 23 months, respectively.

A karyotypic abnormality was detected in 10 out of the 15 children at the time of blast crisis. The majority of patients had a complex karyotype and/or monosomy 7. When the French-American-British (FAB) classification was applied to classify the leukaemic cells at blast crisis, 2 were M2, 1 was M4, 3 were M6 and 2 were unclassified.

The treatment and outcome of these patients after blast crisis are summarized in Table II. Only two of 12 patients given therapy other than HSCT (acute myeloid leukaemia (AML) type chemotherapy: $n = 10$, low dose chemotherapy: $n = 1$, donor leucocyte infusion: $n = 1$) achieved CR. Seven patients received HSCT after the blast crisis and four of them had long-term survival. Ultimately, 11 out of 15 patients died after blast crisis, the main cause of death was the progression of their disease ($n = 10$). One patient died due to transplant-related complication after HSCT. At the time this report was written, four patients were alive in CR at a median of 9 years after blast crisis.

Finally, we compared the characteristics of the patients who developed blast crisis ($n = 15$) and those who did not ($n = 138$; Table III). Among several risk factors, the median

Table II. Characteristics at blast crisis, treatment and outcome.

| Pt | BM blast (%) | Interval after Dx (months) | Interval after initial Tx (months) | Karyotype | Treatment after blast crisis: Induction Tx | Response to Tx | Donor source | Outcome (months after blast crisis) | Cause of death |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 75 | 35 | 17 months after 6MP | 44, XX, der(6) (6;15) (q1?;q1?), −7, −15 | AML type | No | UBMT | Dead (11) | TRM |
| 2 | >20 | 14 | – | 45, XY, −7 | Etoposide, mPSL, Ara-C | No | None | Dead (1) | Progression |
| 3 | 30 | 15 | 15 months after chemotherapy | 50, XY, 19, +21, +X, +Y | AML type + irradiation | No | None | Dead (5) | Progression |
| 4 | 83 | 44 | 44 months after 6MP | 45, XY, del(6) (q21), −7, del(p12) | AML type | Yes | None | Dead (8) | Relapse |
| 5 | 31 | 26 | 26 months after 6MP | 45, XY, −7 | AML type | No | None | Dead (17) | Progression |
| 6 | 36 | 3 | 2 months after 6MP | 46, XY, 5q−, 17p− | AML type | No | None | Dead (15) | Progression |
| 7 | 75 | 16 | 3 months after 6MP | 45, XX, t(4;15) (q2?; q2?), −7 | AML type | No | None | Dead (13) | Progression |
| 8 | 75 | 2 | – | 46, XY | AML type | No | CBT | Alive (>111) | |
| 9 | 47 | 1 | – | 46, XX | AML type | Yes | Related PBSCT | Alive (>182) | |
| 10 | 37 | 5 | 5 months after 6MP | 46, XY, −2, −7, +der (9) t(2;9) (q13; q22),+19 | – | – | CBT | Dead (5) | Progression |
| 11 | 40 | 1 | 1 months after 6MP | 46, XY | AML type | ND | UBMT | Alive (>83) | |
| 12 | 44 | 25 | 8 months after HSCT | −7 and complex karyotype* | AML type | No | None | Dead (31) | Progression |
| 13 | 26 | 2 | 1 months after 6MP | 45, XY, −7 | – | – | CBT | Dead (4) | Progression |
| 14 | 30 | 48 | 19 months after HSCT | 46, XX | DLI | No | None | Dead (4) | Progression |
| 15 | 20 | 29 | 23 months after HSCT | 46, XX | – | – | Related BMT | Alive (>74) | |

BM, bone marrow; Dx, diagnosis; Tx, therapy; 6MP, 6-mercaptopurine; AML, acute myeloid leukaemia; DLI, donor lymphocyte infusion; mPSL, methylprednisolone; Ara-C, cytarabine; ND, no data; UBMT, unrelated bone marrow transplant; HSCT, haematopoietic stem cell transplantation; CBT, cord blood transplant; PBSCT, peripheral blood stem cell transplant; BMT, bone marrow transplant; TRM, transplant-related mortality.
*Complex karyotype: 44, X, −Y, t(1;3) (p10;p10), del(6) (8;21), −7 and 45, Y, del(X) (q22), add(3) (p25), −6, del(6) (p21), −7, +mar.

Table III. Summary of the characteristics of the patients who progressed to blast crisis.

| | With blast crisis | No blast crisis |
|---|---|---|
| Number (%) | 15 (9·8) | 138 (90·2) |
| Median age at initial Dx, years | 2·6 (range: 0·3–7·6) | 1·1 (range: 0–7·4) |
| Median WBC at initial DX, $\times 10^9$/l | 38 (range: 8·9–376·2) | 30·250 (range: 5·5–185) |
| Median Plt at initial Dx, $\times 10^9$/l | 4·3 (range: 1·3–10·2) | 4·6 (range: 0·1–99·7) |
| Median HbF at initial Dx, % | 31·9* (range: 1·1–69·5) | 18·8* (range: 0–66·3) |
| Monosomy 7 at Dx | 1 out of 15 patients | 9 out of 131 patients |
| Monosomy7 and/or complex karyotype at blast crisis | 9 out of 15 patients (8 including monosomy 7) | No data |
| Alive | 4 out of 15 patients (median 9 years) | 75 out of 138 patients |

Dx, diagnosis; WBC, white blood cell count; Plt, platelet count; HbF, fetal haemoglobin.
*$P = 0·047$, $P$ values were calculated using the Mann–Whitney $U$ test for comparisons between medians and ranges.

level of fetal haemoglobin (HbF) was higher in the former group compared to that in the latter ($P = 0·047$, Mann–Whitney $U$ test).

## Discussion

In this retrospective study, 15 (9·8%) of the 153 JMML patients had progressed to blast crisis and this rate was similar to a previous report (Luna-Fineman *et al*, 1999). There have been only a few other reports regarding blast crisis in JMML. Blast crisis was not described in a retrospective analysis of 110 cases with JMML (Niemeyer *et al*, 1997). However, in the 1980's, Castro-Malaspina *et al* (1984) reported that one-third of children with JMML developed blastic transformation.

In the present study, the median time interval between diagnosis and blast crisis was 15 months (range, 1–48 months). Three patients had blast crisis during observation and nine patients were treated with 6MP before the blast crisis. furthermore, three patients underwent a transformation to blast crisis after HSCT.

According to the European Working Group of MDS in Childhood, older children, reduced platelet count and increased percentage of HbF correlate with a poor outcome (Niemeyer *et al*, 1997). Regarding the risk factors for blast crisis, a higher level of HbF was associated with the occurrence of blast crisis and this should be confirmed in a different cohort.

In this study, only two of the 11 patients who received intensive chemotherapy including AML type, entered remission. Therefore, we conclude that JMML blast crisis is very resistant to chemotherapy. Indeed, the main cause of death was disease progression.

It is important to note that monosomy 7 and/or complex karyotype were observed in the majority of patients at the time of blast crisis. It seems that these cytogenetic aberrations play a critical role in the progression. Five patients with a normal karyotype at blast crisis may have had underlying unknown genetic events. Matsuda *et al* (2006) reported that some JMML patients, who initially had a normal karyotype, acquired chromosomal abnormalities including monosomy 7 during treatment with 6MP. They hypothesized that a minor population with an aberrant karyotype already existed at the onset in these patients. In our study, nine patients were treated with 6MP before blast crisis and 7 of them acquired additional chromosomal abnormalities after 6MP treatment. It was possible that 6MP might select resistant clones. Accumulation of molecular changes may also contribute to disease progression in JMML. Kato *et al* (2013) reported a case where a JMML patient with a heterozygous *KRAS* mutation developed aggressive transformation during 6MP therapy after acquisition of homozygous *KRAS* mutation through uniparental disomy mechanism (Kato *et al*, 2013). Very recently, Sakaguchi *et al* (2013) reported that the coexistence of several gene mutations, including *SETBP1* and *JAK3*, in addition to RAS-pathway abnormalities was related with poorer outcome in patients with JMML. Mutations of such genes may be relevant to the occurrence of blast crisis. However, the mechanism underlying the blast crisis was uncertain in most JMML cases. It is possible that 6MP might have induced the second hit and accelerated the disease as 6MP maintenance therapy is reported to be correlated with secondary malignant neoplasm (Schmiegelow *et al*, 2009).

It was possible that some patients developed second malignancy rather than blast crisis. However, in this study, none of the patients who did not receive HSCT achieved remission before presentation of blast crisis. Therefore, we assume that those patients had progression of the disease or blast crisis rather than second malignancy. Of note, Patient 12 had completely different chromosomal abnormalities at diagnosis and blast crisis, which it may indicate a second malignancy.

A recent report suggested that the pathogenesis of JMML is the dysregulation of granulocyte-macrophage colony-stimulating factor (GM-CSF)/RAS signal transduction and mutations in *RAS*, *NF1*, *PTPN11* and *CBL* genes interfering with the downstream components of this pathway (Side *et al*, 1998; Tartaglia *et al*, 2003; Flotho *et al*, 2007; Loh *et al*, 2009). These mutations can be identified in approximately 80% of JMML cases and are associated with clinical features and prognosis in JMML (Yoshida *et al*, 2012). Yoshida *et al* (2009) evaluated 71 children with JMML and they concluded that JMML with *PTPN11* mutation might have a poor

outcome. On the other hand, some patients with *KRAS* or homozygous *CBL* mutation have improved spontaneously (Matsuda *et al*, 2007; Loh, 2011). In our report, gene mutation analysis was performed only in two cases, because most of the patients were diagnosed with JMML in the 1990's. A prospective study with mutational analyses of the above genes in the RAS pathway and search for second molecular events will be needed to elucidate the pathogenesis of blast crisis in JMML.

In the last 10 years HSCT has become the only curative treatment for JMML and, in Japan, it was also proposed as a treatment for JMML after 1999 (Manabe *et al*, 2002). In our present report, 11 (15%) out of 74 patients progressed to blast crisis before 1999; on the other hand, there were only four cases (5%) with blast crisis among 79 patients diagnosed after 2000. It is possible that the frequency of patients with blast crisis decreased because HSCT was administered early on in the course of treatment in these cases. In fact, the number of JMML patients who received HSCT before and after 1999 were 43 out of 74 (58%) patients and 60 out of 79 (76%) patients, respectively. On the other hand, recent advances in genotyping of JMML may lead to a decrease in the indication for HSCT in patients with JMML. For example, children with *RAS* or *CBL* mutations may not need HSCT. In addition, it has been reported that JMML patients with AML-type expression profile had a worse prognosis (Bresolin *et al*, 2010). In their report, monosomy 7 was observed in six of 20 patients with an AML-type profile, whereas it was observed in only one of 20 patients with a non-AML type profile. It is possible that children presenting blast crisis may have the AML-type expression profile. Taken together, the number of patients who need HSCT may decrease as more precise prognostic factors become available. Further studies including genotyping of JMML are necessary to identify those patients who may survive without HSCT and those who require HSCT. Those JMML patients identified as requiring HSCT, should receive HSCT as soon as possible to avoid blast crisis.

We recommend that for JMML cases, karyotype should be carefully monitored using fluorescence *in situ* hybridization to detect monosomy 7 or any other types of karyotypic abnormalities, especially if the patient does not undergo HSCT.

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## Authorship

Conception, design and writing the manuscript: YH, AM. Pathological diagnosis: MT, YZ, AMan, AY, SK, MI, AMas. Collection and assembly of data: YH, AK, AMan. Data analysis and interpretation: YH, TN, AMan. Final approval of manuscript: All authors.

## Disclosures

The authors declare no competing financial interests.

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# Criteria for evaluating response and outcome in clinical trials for children with juvenile myelomonocytic leukemia

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## ABSTRACT

Juvenile myelomonocytic leukemia is a rare myeloproliferative disease in young children. While hematopoietic stem cell transplantation remains the only curative therapeutic option for most patients, children with juvenile myelomonocytic leukemia increasingly receive novel agents in phase I-II clinical trials as pre-transplant therapy or therapy for relapse after transplantation. However, response criteria or definitions of outcome for standardized evaluation of treatment effect in patients with juvenile myelomonocytic leukemia are currently lacking. Here we propose criteria to evaluate the response to the non-transplant therapy and definitions of remission status after hematopoietic stem cell transplantation. For the evaluation of non-transplant therapy, we defined 6 clinical variables (white blood cell count, platelet count, hematopoietic precursors and blasts in peripheral blood, bone marrow blast percentage, spleen size and extramedullary disease) and 3 genetic variables (cytogenetic, molecular and chimerism response) which serve to describe the heterogeneous picture of response to therapy in each individual case. It is hoped that these criteria will facilitate the comparison of results between clinical trials in juvenile myelomonocytic leukemia.

## Introduction

Juvenile myelomonocytic leukemia (JMML) is a clonal disease in young children.[1,2] Patients with JMML present with leukocytosis, monocytosis and splenomegaly, features similar to those observed in the myeloproliferative subtype of chronic myelomonocytic leukemia (CMML) in adults.[3] Other clinical signs of JMML include thrombocytopenia, leukemic skin infiltration, elevation of fetal hemoglobin (HbF), and hypersensitivity of hematopoietic progenitors to granulocyte-macrophage colony-stimulating factor (GM-CSF).[4] Approximately 90% of patients with JMML harbor largely mutually exclusive mutations in *PTPN11, NF1, NRAS, KRAS,* or *CBL* in their leukemic cells resulting in hyperactivation of the RAS-MAPK pathway.[5-12]

Hematopoietic stem cell transplantation (HSCT) is still the only curative therapy for the vast majority of JMML patients.[13-15] However, with advances in understanding the underlying molecular mechanisms in JMML, the potential for the introduction of novel therapeutic agents has been recognized for some time. Several molecules, such as isotretinoin, zoledronic acid, and farnesyl transferase inhibitor R115777 have been evaluated in pre-HSCT windows or compassionately used.[16-18] Recently, azacitidine, a DNA-hypomethylating agent, was reported to induce hematologic and molecular remissions in some children with JMML,[19,20] and is currently being tested in clinical trials in Europe. Additional efforts are underway to employ therapeutic inhibition of the MEK/ERK and PI3K pathways.[21-28]

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In order to evaluate the efficacy of any conventional or novel interventions for JMML, standardized criteria to define responses and relapse are urgently required. With the goal of defining widely accepted criteria of response to therapy, international experts from the European Working Groups of Myelodysplastic Syndromes in Childhood (EWOG-MDS), the Children's Oncology Group (COG) from the US, and from the Japanese Society of Pediatric Hematology/Oncology met to find an agreement at the JMML International Symposium, New Orleans, USA, (6 December 2013). The agreed criteria are presented in this manuscript.

## Proposal of response criteria in clinical trials of therapy in JMML

### Previous efforts for standardized response criteria for non-HSCT therapy in JMML

In adults, standardized response criteria proposed by an International Working Group of MDS have been widely used in clinical trials for MDS as well as CMML.[24-28] These criteria are, however, not applicable to JMML and myeloproliferative CMML because they focus on reduction of blast percentage and improvement of blood counts, and are not designed to evaluate myeloproliferative features such as organomegaly.

Bergstraesser *et al.* defined response criteria in JMML considering white blood cell (WBC) count, platelet count, as well as liver and spleen size.[26] These authors retrospectively evaluated the efficacy of 129 treatment courses other than HSCT administered to 63 children with JMML and reported a significant correlation between WBC count or spleen size and the efficacy of non-HSCT therapy. This finding was later applied to response criteria proposed by Chan *et al.* describing complete response (WBC <20x10$^9$/L and normalization of spleen size) and partial response (<50% of initial WBC but total still greater than 20x10$^9$/L and 25% decrease in spleen size from initial size) based solely on these two criteria, WBC count and spleen size.[27] Due to the rarity of JMML there is no published prospective clinical trial that has actually applied these criteria, and there are evident limitations when only these two variables are used. The definitions are only applicable in patients with leukocytosis (WBC ≥20x10$^9$/L) and splenomegaly (≥2 cm below the costal margin). However, among 497 JMML patients currently registered in the EWOG-MDS studies, 30% had a WBC count less than 20x10$^9$/L, and 12% a spleen size of less than 2 cm below the costal margin at diagnosis (*EWOG-MDS, unpublished data, 2014*). In addition, the criteria are not applicable to patients relapsing post HSCT who have reappearance of cytogenetic or molecular abnormalities after HSCT but who do not yet show the full clinical picture of relapse. Response criteria thus need to be applicable in many different clinical situations for a broad range of patients. Therefore, clinical variables other than WBC count and spleen size are needed, and cytogenetic and molecular variables are also necessary to describe disease status.

### The concept behind the proposed response criteria

As outlined above, response measurements in JMML need to be applicable to the highly heterogeneous clinical features at presentation and to individual response patterns to different therapeutic agents. Therefore, 6 clinical variables and 3 genetic variables were selected (Table 1). For each of these variables (v), complete response (vCR), partial response (vPR) and progressive disease (vPD) are defined. This makes the evaluation of heterogeneous effects of each intervention possible. Because we recognize that each patient can present with different clinical, cytogenetic and molecular features, the number of evaluable variables at the start of therapy differs among patients. Based on the cumulative response of these 9 variables, the clinical and genetic remission status can be described (Tables 2).

### Variables to evaluate response

In addition to WBC count and spleen size, clinical variables include presence of myeloid/erythroid precursors and blasts in peripheral blood, platelet count, percentage of blasts in bone marrow, and presence of extramedullary disease (Table 1).[4] Two additional hallmarks of JMML, the level of hemoglobin F (HbF) corrected for age and the presence of monocytosis, have intentionally been excluded as response criteria for various reasons. High HbF levels at diagnosis are known to predict outcome but are dependent on karyotype.[4] Moreover, so far there has been no report on serial HbF levels during the natural course of JMML or following HSCT. Therefore, further studies to evaluate HbF during the clinical course of JMML patients are necessary. Monocytosis more than 1.0x10$^9$/L is one of the diagnostic criteria of JMML,[4] but the absolute monocyte count generally correlates with the WBC. Since the usefulness of the WBC count has been previously confirmed, we chose the WBC count, but not the monocyte count, as a variable to be evaluated.[26] Moreover, monocytosis can be non-specific since it is observed in various conditions, such as infections or an early sign of bone marrow recovery after HSCT, and leukocytosis in JMML is manifested not only by monocytosis but also by circulating immature myeloid cells in the peripheral blood.

Since the spleen size is one of the variables evaluating treatment efficacy in JMML,[26] reliable methods to measure the size of this variably shaped organ are required. Size determination by palpation with measurement of the length between the spleen tip and the left costal margin is clinically appropriate[26,29-30] but may not be sufficient for clinical trials since its results are not verifiable. Measurements by computed tomography are to be avoided in children with JMML because of radiation issues, and magnetic resonance imaging generally requires deep sedation or anesthesia. For these reasons, we currently recommend evaluation of spleen size by ultrasound with the linear measurement of the splenic length, defined as the maximum distance between the dome and the tip of spleen in the right lateral decubitas position.[31]

The three genetic variables selected for evaluating response were cytogenetics, molecular alterations, and chimerism. Cytogenetic and molecular data must have been collected at diagnosis while chimerism is only applicable after HSCT. Abnormal karyotypes are observed in approximately 35% of JMML patients, with monosomy 7 being the most common aberration (25%).[4] Oncogenic molecular alterations in *PTPN11, NF-1, NRAS, KRAS* or *CBL*, noted in approximately 90% of patients are increasingly important tools for diagnosis and follow up of JMML.[5-12] We anticipate that additional somatic mutations will be discovered in JMML. Indeed, recently *SETBP1* and *JAK3* mutations were reported as a result of an exome sequencing project.[32] However, until these markers are fur-

ther validated, we would suggest that these mutations, which might indicate sub-clones, fall under the category of "acquired molecular abnormalities". Analysis of donor chimerism has been a standard measurement to follow JMML patients given allogeneic HSCT. While discussion of the various methods used to determine donor chimerism is beyond the scope of this consensus report, there was broad agreement that unsorted cell donor chimerism should be a common measurement in clinical trials. Most JMML patients with persistent mixed chimerism experience clinical relapse of JMML,[35] and are thus candidates for early intervention with innovative therapies prior to development of a full clinical relapse.

### Definitions of response to therapy other than HSCT in JMML

Based on the response of each applicable variable listed in Table 1, the clinical and genetic remission status can be

**Table 1. Variables for evaluation of response to therapy in JMML.**

| Variables for response | | Definition of response | | Definition of disease progression or relapse (applicable to all patients) |
|---|---|---|---|---|
| | Assessment of CR and PR is feasible if the following are present before therapy | Requirement for CR for each variable (vCR) | Requirement for PR for each variable (vPR) | Requirement for PD for each variable (vPD) |
| Clinical variables | | | | |
| 1) WBC count | >20×10⁹/L | 3.0-15.0×10⁹/L | Decreased by ≥50% over pretreatment but still >15×10⁹/L | Increase by ≥50% and ≥20×10⁹/L |
| 2) Myeloid and erythroid precursors and blasts in PB* | ≥ 5% | 0-1% | Decreased by ≥50% over pre-treatment but still ≥ 2% | Increase from the baseline: < 5%: ≥ 50% increase and ≥ 5% ≥ 5%: ≥ 50% increase of total % of myeloid and erythroid precursors and blasts |
| 3) Platelet count | <100×10⁹/L | ≥100×10⁹/L | For patients starting with ≥ 20×10⁹/L platelets: absolute increase of ≥ 30×10⁹/L. For patients starting with < 20 ×10⁹/L platelets: increase by ≥ 100% and > 20×10⁹/L | Development of transfusion dependency or if patients have the baseline of the platelet count of ≥30×10⁹/L, decrease by ≥50% and <100×10⁹/L |
| 4) BM blasts | ≥5% | <5% | Decreased by ≥50% over pre-treatment but still ≥5% | Increase from baseline; < 5%: ≥ 50% increase and ≥ 5% ≥ 5%: ≥ 50% increase of BM blasts |
| 5) Spleen size a) Clinical evaluation or | ≥2 cm under the costal margin | No splenomegaly | 50% decrease by cm under the costal margin | Increase by ≥100% if baseline <4cm from under the costal margin ≥50% if baseline 5-10 cm >30% if baseline >10 cm |
| b) Sonography | Length of spleen ≥ 150% of upper limit of normal range | No splenomegaly | >25% decrease by length, but still splenomegaly | Increase by ≥25% of length |
| 6) Extramedullary disease# | Extramedullary leukemic infiltration | No evidence of extramedullary leukemic infiltration in any organ | – | Worsening or new lesions of extramedullary leukemic infiltration |
| 7) Cytogenetic response | Somatic cytogenetic abnormality detected | Normal karyotype | – | Reappearance or additional acquirement of cytogenetic abnormalities |
| 8) Molecular response | Somatic genetic anomalies detected ** | Absence of somatic genetic anomalies | – | Reappearance or additional acquirement of JMML-specific somatic gene abnormalities |
| 9) Chimerism response (only for patients after HSCT) | >15% autologous cells after allo-HSCT | Complete donor chimerism | – | 50% increase and >5% increase of autologous cells and >5% |

*CR: complete response; PR: partial response; PD: progressive disease; WBC: white blood cell; PB: peripheral blood; BM: bone marrow. *Myeloid precursors include promyelocytes, myelocytes and metamyelocytes. The myeloid and erythroid precursors and blasts in PB are given as percentage of the total nucleated cells in PB (WBC including erythroblasts). **In NF-1, PTPN11, NRAS, KRAS, or CBL, thus the mutations are thought to be initiating, in patients with germ-line NF-1, PTPN11 or CBL mutation, only acquired mutations can be evaluated for response and relapse after therapy. The germ-line mutation remains even if patients achieved complete molecular response. #Extramedullary disease includes infiltration of skin, lung, and, very rarely, cranial nerves or central nervous system.*

defined for each patient (Table 2). In patients who achieve genetic complete remission (gCR), JMML cells are considered to have been eradicated, irrespective of clinical remission status. Patients with gCR may have persistent splenomegaly or leukocytosis from causes other than JMML, such as infections. However, it is unlikely that such a patient has clinical signs of progressive disease of JMML in the presence of gCR. In such a patient, any new clonal abnormalities, other possible errors of genetic examinations, or other disorders which give rise to JMML-like clinical features, should be excluded.

## Response criteria for clinical trials of HSCT

There is a consensus that criteria for remission after HSCT are somewhat different from those stated above for remission after non-HSCT. The remission criteria in HSCT recipients include the results of chimerism analyses (Table 3). Appraisal of methodological consideration of chimerism studies will change over time and is beyond the scope of this consensus document. Patients undergoing HSCT who achieve neutrophil engraftment and complete donor chimerism with disappearance of acquired cytogenetic and molecular abnormalities are considered to have a complete remission of JMML. In these individuals, complete remission is defined irrespective of the spleen size or WBC counts, since post HSCT, patients often have persistent splenomegaly and leukocytosis without active JMML due to infections, graft-*versus*-host disease or other hepatic complications. For the very few cases with mixed chimerism after HSCT and no diagnostic cytogenetic or molecular marker, definition of complete remission requires the resolution of all clinical features indicative of JMML (Table 3).

Table 2. Definition of response following therapy other than HSCT in JMML.

| Clinical remission status: variable 1-6 in Table 1 | | Genetic remission status: variable 7-9 in Table 1 | |
|---|---|---|---|
| Clinical complete remission (cCR) | Patient fulfills the criteria of CR of all applicable clinical variables 1-6 of Table 1<br>The response variables must be maintained for at least 4 weeks. | Genetic complete remission (gCR) | Defined if the patient shows a normal karyotype and absence of acquired mutations in *PTPN11*, *NF-1*, *NRAS*, *KRAS*, or *CBL*. |
| Clinical partial remission (cPR) | Defined if the patient does not fulfill the criteria of cCR, but vPR was achieved in at least one of clinical variables (1-6) and none of clinical variables showed vPD. | – | – |
| Clinical stable disease (cSD) | Defined if the patient does not fulfill the criteria of cCR and cPR, but none of the variables showed vPD. | Genetic stable disease (gSD) | Defined if the patient does not fulfill the criteria of gCR, but none of the genetic variables (7-9) showed vPD. |
| Clinical progressive disease (cPD) | Defined if any of the variables 1-6 showed vPD. | Genetic progressive disease (gPD) | Defined if any of the variables 7-9 showed vPD. |
| Clinical relapse (cRel) | Defined if any of the variables 1-6 showed vPD after the achievement of cCR or cPR. | Genetic relapse (gRel) | Reappearance of an abnormal karyotype and/or mutation of genes related with JMML if previously undetected, and/or (only for patients after HSCT) increase in recipient chimerism with at least 10% of autologous cells and >50% increase above the baseline. |

**In patients with germ-line NF-1, PTPN11 or CBL mutation, the germ-line mutation remains even if patients achieved a genetic complete remission.*

Table 3. Definition of complete remission and relapse after hematopoietic stem cell transplantation in children with JMML.

**Complete remission** is defined in the presence of neutrophil engraftment and:
1. full donor chimerism of unsorted cells from PB or BM and
2. disappearance of acquired cytogenetic and molecular abnormalities in patients with such a previously identified abnormality

For patients without cytogenetic or acquired molecular abnormalities at diagnosis, who do not achieve full donor chimerism (as defined above), all of the following features of clinical remission must be achieved for definition of complete remission:
a. absence of splenomegaly on exam and imaging, if splenomegaly was present at diagnosis
b. absolute leukocyte count < $15\times10^9$/L
c. blasts in BM of <5%,
d. myeloid/erythroid precursors including blasts in PB ≤1%

**Relapse** is defined if one of the following criteria is fulfilled:
1. clinical JMML features and mixed chimerism >5%
2. blasts in BM ≥5%, total blasts and myeloid/erythroid precursors in PB ≥5%*
3. cytogenetic relapse: if applicable, reappearance of clonal cytogenetic abnormality
4. molecular relapse: if applicable, reappearance of acquired genetic anomalies

*PB: peripheral blood; BM: bone marrow. *Exclude other causes of appearance of blasts and myeloid/erythroid precursors in PB such as the regenerating phase after engraftment, severe infections, and effect of granulocyte-colony-stimulating factor.*

## Conclusion

In this paper we propose response and relapse criteria for patients who are diagnosed and treated for JMML, recognizing the complexities of disease presentation and therapeutic interventions. The usefulness and suitability of these criteria need to be proven in prospective clinical trials. It is likely that the proposed response criteria will require modifications in the future based on the accumulated experiences and advances of molecular biology in JMML. Because the goal of therapy of children with JMML is a cure, it is also important to be aware that responses need to be translated into an increase in long-term survival in well-controlled clinical trials.

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### *Authorship and Disclosures*

*Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.*

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ORIGINAL ARTICLE

# Transplantation for juvenile myelomonocytic leukemia: a retrospective study of 30 children treated with a regimen of busulfan, fludarabine, and melphalan

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**Abstract** We report the outcomes of 30 patients with juvenile myelomonocytic leukemia (JMML) who received unmanipulated hematopoietic stem cell transplantation (HSCT) with oral or intravenous busulfan, fludarabine, and melphalan between 2001 and 2011. Mutations in *PTPN11* were detected in 15 patients. Six patients received human leukocyte antigen (HLA)-matched HSCT from related donors, and 24 patients received HSCT from alternative donors, including 13 HLA-mismatched donors. Primary engraftment failed in five patients, all of whom had received allografts from HLA-mismatched donors. HLA-mismatched HSCT resulted in poorer event-free survival than HLA-matched HSCT (28.8 vs. 70.6 %). Three patients died of transplantation-related causes, and eight patients experienced hematological relapse (including five patients who died due to disease progression). Eight patients received a second HSCT, and four of these patients have survived. The 5-year estimated overall survival for all patients was 72.4: 88.9 % for the patients without a mutation in *PTPN11* ($n = 10$) and 58.3 % for the patients with a mutation in *PTPN11* ($n = 15$) ($P = 0.092$). The conditioning regimen reported in the present study achieved hematological and clinical remission in >50 % of patients with JMML who received HSCT from alternative donors, and may also be effective for JMML patients with *PTPN11* mutation.

**Keywords** Juvenile myelomonocytic leukemia · Hematopoietic stem cell transplantation · Fludarabine · Alternative donors · *PTPN11*

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## Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare clonal myelodysplastic/myeloproliferative disorder that occurs during infancy and early childhood [1], and is characterized by excessive proliferation of monocytes and hypersensitivity of myeloid progenitor cells to granulocyte-macrophage colony-stimulating factor (GM–CSF) in vitro. Most patients present with pallor, fever, marked splenomegaly, and skin rash, and typically experience an aggressive clinical course [2]. Interestingly, JMML usually involves somatic and/or germ-line mutations in the genes of the *RAS* pathway, such as *PTPN11*, *NRAS*, *KRAS*, *NF1*, and *CBL*, in the leukemic cells; *PTPN11* mutations are particularly associated with an unfavorable prognosis [3]. Moreover, exome sequencing has identified secondary mutations in *SETBP1* and *JAK3* among JMML patients, more commonly in patients with *PTPN11* mutations. Furthermore, individuals with secondary mutations have poorer survival than those without secondary mutations [4]. Although a few JMML patients with germ-line mutations in *CBL* and *NRAS* show spontaneous resolution [5], the median survival time without allogeneic hematopoietic stem cell transplantation (HSCT) is approximately 1 year [6]. Thus, HSCT is currently the only curative treatment for JMML, and we have previously reported our early experience with JMML patients who received HSCT with a conditioning regimen of busulfan (Bu), fludarabine (Flu), and melphalan (L-PAM) [7]. In this report, we present updated data on our early experience with 10 patients and retrospectively analyze the data of 20 additional patients who were administered intravenous or oral Bu combined with Flu and L-PAM. In addition, we studied mutations in the *RAS* pathway and their prognostic implications after HSCT.

## Materials and methods

### Patients

Patients were diagnosed with JMML according to previously published criteria [8]. The Myelodysplastic Syndrome (MDS) committee of the Japanese Society of Pediatric Hematology/Oncology and the Japanese Pediatric MDS Study Group supported the detection of oncogenic mutations (i.e., *PTPN11*, *RAS*, and *CBL*) and the experiments on spontaneous growth or GM–CSF hypersensitivity in colony assays. Thirty patients with JMML who underwent HSCT between July 2001 and April 2011 were followed in the observational study of the Japanese Pediatric MDS Study Group until October 2012. Patient data were retrospectively collected, including patient characteristics, transplantation procedure, and outcomes. The Japanese Pediatric MDS Study Group and the Tokai University Hospital Institutional Review Board approved this retrospective study. All patients or their guardians provided informed consent prior to HSCT, in accordance with the Declaration of Helsinki.

### Transplantation procedures

The characteristics of the 30 JMML patients who underwent are shown in Table 1.

Serological typing data for the human leukocyte antigen (HLA)-A and HLA-B and DNA typing data for HLA-DR were available for all the related donor–recipient pairs. DNA typing data were obtained from each participating facility for all the loci for the unrelated donor–recipient pairs and the mismatched related donor–recipient pairs.

Before HSCT, 25 patients received mild chemotherapy, with 6-mercaptopurine or low-dose cytarabine for example, and 3 patients received a short course of intensive chemotherapy for acute myeloid leukemia (AML) (2 of these patients had an abnormal karyotype: monosomy-7). One patient underwent splenectomy, and 2 patients received 6 Gy of splenic irradiation for marked splenomegaly. All patients were conditioned with oral Bu (140–160 mg/m$^2$ in divided doses daily for 4 days, total dose 560–645 mg/m$^2$) or intravenous iv Bu (3.2–6.0 mg/kg in divided doses daily for 4 days, total dose 12.8–24.0 mg/kg) on days −11, −10, −9, and −8. Conditioning with iv Flu (30 mg/m$^2$ once daily for 4 days, total dose 120 mg/m$^2$) was performed on days −7, −6, −5, and −4. Conditioning with iv L-PAM (90 mg/m$^2$ once daily for 2 days, total dose 180 mg/m$^2$) was performed on days −3 and −2. When a patient was scheduled to receive iv L-PAM on days −4, −3, and −2 (70 mg/m$^2$ once daily for 3 days, total dose 210 mg/m$^2$), their Bu conditioning was started on day −12. The regimen-related toxicity was scored according to the Bearman scale, and chimerism of the bone marrow (BM) or peripheral blood (PB) was evaluated using short tandem repeat analysis, XY chromosomal analysis, or fluorescence in situ hybridization analysis.

The patients' clinical status after HSCT was evaluated as follows. Engraftment was defined as an absolute neutrophil count $\geq 0.5 \times 10^9$/L for 3 consecutive days, with the day of engraftment considered the first day. Secondary graft failure was defined as loss of graft (absolute neutrophil count $<0.5 \times 10^9$/L for $\geq 3$ consecutive days after the initial engraftment) due to events that were not related to disease progression or relapse (e.g., infection and hemophagocytic syndrome). Relapse was defined as an increase in recipient chimerism in the BM or PB cells as well as hematological and clinical signs of JMML.

Overall survival (OS) was calculated using Kaplan–Meier analysis, and event-free survival (EFS) was defined as the probability of survival with remission; death, relapse, and engraftment failure were considered events.

## Results

The clinical responses of the 30 patients to HSCT are listed in Table 2. Twenty-five patients achieved engraftment (i.e., >0.5 × $10^9$ neutrophils/L) at a median of 21 days (range 11–55 days). Five patients experienced primary engraftment failure, all of whom had received an allograft from an HLA-mismatched donor, including the patient who underwent splenectomy and 1 patient who received splenic irradiation. Engraftment was successful in 3 patients who received a short course of intensive chemotherapy for AML without severe organ toxicity or relapse. Table 3 shows the details of the engraftment and patient outcomes according to HLA matching and the stem cell source. Three of 4 patients who received HLA-mismatched unrelated cord blood transplant (CBT) achieved primary engraftment, although 1 patient developed secondary graft failure. Two patients also successfully received HLA-matched PB stem cell transplants (PBSCT) from a related donor and an unrelated donor, respectively.

Chimerism analysis of the BM or PB was performed for 22 patients on days 27–113. Although all patients exhibited complete chimerism (i.e., >95 % donor cells) at least once, 5 patients experienced hematological relapse. Eight patients experienced hematological relapse 47–296 days after HSCT, and 6 of these patients had mutations in *PTPN11*.

The most common organ toxicities were stomatitis (grades I–II, 12 patients; grade III, 1 patient) and

**Table 1** Patient characteristics and transplantation procedures

| Variable | Number of patients or median (range) |
|---|---|
| Gender | |
| Male | 21 |
| Female | 9 |
| Age at diagnosis (years) | 1.3 (0.1–6.7) |
| Age at HSCT (years) | 2.2 (0.3–6.8) |
| Interval between diagnosis and HSCT (months) | 6.0 (1.2–41.0) |
| Status at diagnosis | |
| WBC (× $10^9$/L) | 27.7 (9.6–126.2) |
| Fetal hemoglobin (%) | 25.0 (0–63.3) |
| Karyotype | |
| Normal | 21 |
| −7/7q | 4 |
| Other | 5 |
| Mutation | |
| *PTPN11* | 15 |
| *RAS* | 3 |
| *NF-1* | 2 |
| Negative | 5 |
| Not tested | 5 |
| Donor | |
| Related donor | |
| HLA-matched | 6 |
| HLA-mismatched | 2 |
| Unrelated donor | |
| HLA-matched | 11 |
| HLA-mismatched | 11 |
| Stem cell source | |
| Bone marrow | 23 |
| Peripheral blood | 2 |
| Cord blood | 5 |
| GVHD prophylaxis | |
| Tacrolimus ± MTX | 22 |
| CsA + MTX | 6 |
| MTX | 2 |

*HSCT* hematopoietic stem cell transplantation, *WBC* white blood cell, *HLA* human leukocyte antigen, *MTX* methotrexate, *CsA* cyclosporine A, *GVHD* graft-versus-host disease

**Table 2** Transplantation outcomes

| Variable | Number of patients |
|---|---|
| Engraftment | |
| Yes | 25 |
| No | 5 |
| Secondary graft failure | 1 |
| Acute GVHD | |
| 0, I | 14 |
| II–IV | 11 |
| Chronic GVHD | |
| Yes | 10 |
| No | 12 |
| Outcome | |
| Second HSCT | 8 |
| Complications in the late post-transplant phase (>100 days) | |
| Interstitial pneumonia | 1 |
| Bronchiolitis obliterans | 2 |
| Membranous nephropathy | 1 |
| Hemorrhagic cystitis | 1 |
| Protein-losing gastroenteropathy | 1 |
| EB-PTLD | 1 |
| Alive | 22 |
| In remission | 20 |
| With persistent disease | 2 |
| Dead | 8 |
| Due to progressive disease | 5 |
| Transplantation related | 3 |

*GVHD* graft-versus-host disease, *HSCT* hematopoietic stem cell transplantation, *EB-PTLD* epstein–barr virus-related post-transplant lymphoproliferative disorder

**Table 3** Details of the engraftment and outcome according to HLA matching and stem cell source

| Donor | HLA matching | Stem cell source/ number of patients | Primary engraftment Yes/no (number of patients) | Outcome Alive/dead (number of patients) |
|---|---|---|---|---|
| Related donor | Matched | BM/5 | 5/0 | 3/2 |
| | | PB/1 | 1/0 | 1/0 |
| | Mismatched | | | |
| | 1-antigen-mismatched parent | BM/1 | 1/0 | 1/0 |
| | 2-antigen-mismatched parent | BM/1 | 0/1 | 0/1 |
| Unrelated donor | Matched | BM/9 | 9/0 | 8/1 |
| | | PB[a]/1 | 1/0 | 1/0 |
| | | CB/1 | 1/0 | 1/0 |
| | Mismatched | | | |
| | 1-antigen-mismatched | BM/4 | 3/1 | 2/2 |
| | 1-allele-mismatched | BM/3 | 1/2 | 3/0 |
| | 1-antigen-mismatched | CB/1 | 1/0 | 0/1 |
| | 1-antigen and 3-allele-mismatched | CB/1 | 0/1 | 0/1 |
| | 1-allele-mismatched | CB/2 | 2/0 | 2/0 |

*HLA* human leukocyte antigen, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood

[a] Peripheral blood graft was obtained from an overseas marrow and stem cell donor program

gastrointestinal mucositis (grades I–II, 7 patients). Pulmonary, cardiac, renal, and bladder toxicities were uncommon (2, 1, 1, and 2 patients, respectively), and none were serious (i.e., grades I–II). One patient with veno-occlusive disease (VOD) developed grade IV liver toxicity. Three patients died of transplantation-related causes: bronchiolitis obliterans 23 months after HSCT, interstitial pneumonia 9 months after HSCT, and hepatic VOD 1 month after HSCT. In addition, 5 patients died of disease progression after relapse.

Eight patients received a second HSCT. Three of them received a mismatched unrelated CBT 35–65 days after the first HSCT because of a lack of engraftment (including secondary graft failure); all 3 patients survived. Furthermore, 5 patients with relapse received a second HSCT [4 received unrelated BM transplant (BMT) and 1 received unrelated CBT] 110–285 days after the first HSCT; only 1 patient who received an unrelated BM graft survived.

The median follow-up durations for the entire cohort and surviving patients were 50.5 months (range 1.0–127.0 months) and 61.3 months (range 14.7–127.0 months), respectively, enabling the analysis of long-term outcomes. The 5-year OS and EFS rates for the entire cohort were 72.4 and 53.1 %, respectively (Fig. 1a). Patients who received HLA-mismatched HSCT had poorer EFS than those who received HLA-matched HSCT, because of the low incidence of engraftment (28.8 vs. 70.6 %, $P = 0.009$) (Fig. 1b). Although only a limited number of patients received HSCT from a related donor or cord blood, there was no significant difference in 5-year OS between the three groups (BMT or PBSCT from a related donor with 0 or 1 HLA disparity vs. BMT or PBSCT from an unrelated donor with 0 or 1 HLA disparity vs. cord blood) (Fig. 1c). The estimated 5-year OS rates for the patients without *PTPN11* mutation ($n = 10$) and the patients with *PTPN11* mutation ($n = 15$) were 88.9 and 58.3 %, respectively ($P = 0.092$) (Fig. 1d). No significant associations were observed between poorer outcomes and the Bu administration route [oral vs. iv 70.6 %, 95 % confidence interval (CI) 48.9–92.2 vs. 74.0 %, 95 % CI 48.1–99.9 %; $P = 0.790$], acute graft-versus-host disease grade (0–I vs. II–IV 71.4 %, 95 % CI 47.8–95.1 vs. 79.5 %, 95 % CI 54.0–100.0 %; $P = 0.547$), or chronic graft-versus-host disease (presence vs. absence 78.8 %, 95 % CI 52.5–100.0 vs. 83.3 %, 95 % CI 62.2–100.0 %; $P = 0.867$). In addition, age at diagnosis, sex, abnormal karyotype, and fetal hemoglobin were not risk factors for long-term survival (data not shown).

## Discussion

Relapse and engraftment failure are the major causes of HSCT failure in JMML. We began administering a Flu regimen without irradiation to improve the outcomes of HSCT in JMML patients, as JMML occurs in infancy and early childhood [7]. Allogeneic HSCT from an HLA-matched related or unrelated donor cured >50 % of JMML patients in the EWOG-MDS/EBMT group trial, which reported the outcomes of 100 children with JMML who received HSCT after homogenous conditioning with 3 alkylating agents: Bu, L-PAM, and cyclophosphamide [9]. In that

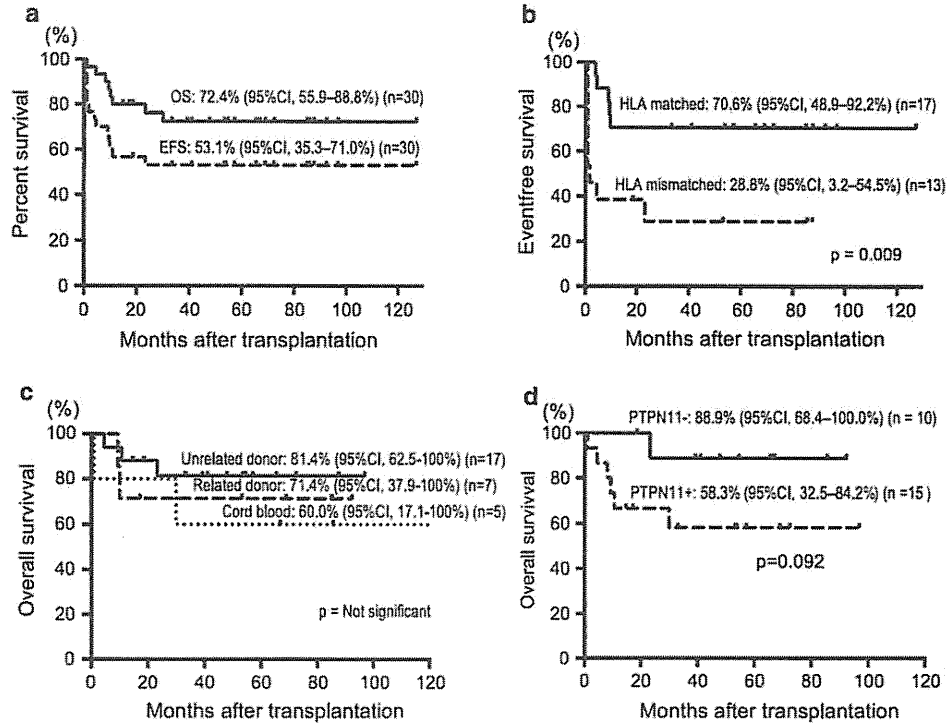


**Fig. 1** Outcomes of the busulfan/fludarabine/melphalan regimen. a Five-year overall survival (OS) and event-free survival (EFS). b Five-year EFS according to human leukocyte antigen (HLA) matching. c Five-year OS according to stem cell source (bone marrow transplant (BMT) or peripheral blood stem cell transplant (PBSCT) from a related donor with 0 or 1 HLA disparity, BMT or PBSCT from an unrelated donor with 0 or 1 HLA disparity, or cord blood). d Five-year OS of the patients with and without *PTPN11* mutation

trial, primary and secondary graft failure was observed in 3 patients and 2 patients, respectively. In contrast, all of our patients who received HSCT from an HLA-matched donor achieved engraftment. However, 5 of 13 patients who received HSCT from an HLA-mismatched donor experienced primary engraftment failure, and 1 patient experienced secondary graft failure. Therefore, overcoming graft failure in mismatched transplantation may require more intensive chemotherapy before the transplantation or the administration of more immunosuppressive agents for conditioning. In addition, a pharmacokinetics study is needed to determine the sufficient and adjusted dosages of Bu to maximize engraftment while avoiding fatal hepatic VOD [10].

Because of the lower possibility of engraftment and the weaker effect of graft-versus-leukemia, cord blood is not typically considered a stem cell source for HSCT in JMML patients. However, unrelated donor CBT has recently been reported as a suitable option for JMML patients [11]. In that study, 90 of 110 patients achieved neutrophil engraftment, with various conditioning regimens, and the 5-year disease-free survival and OS rates were 44 and 52 %, respectively. In the present series, 4 of 5 patients who received unrelated CBT subsequently achieved primary engraftment, and 3 of these patients have survived. The ultimate outcome for CBT patients appears to be comparable to that for BMT or PBSCT patients. Although the number of patients who received CBT using Bu/Flu/L-PAM conditioning was too small for statistical analysis, this conditioning may decrease the mortality rate and risk of graft failure with CBT in JMML patients who lack a compatible related or unrelated BMT or PBSCT donor.

Disease recurrence after HSCT is the main cause of treatment failure in JMML patients, and the relapse rate at 36 months after HSCT ranges from 25 to 40 % [9, 12]. Among JMML patients, relapse typically occurs early, often within the first year after HSCT. Although only a few relapsed JMML patients have been successfully treated using donor lymphocyte infusion [13], approximately 40 % of patients who receive a second transplant achieve remission [14]. In addition, Inagaki et al. [15] have reported that among 11 patients (4 with graft failure, 3 with mixed chimerism and clinical signs of JMML, and 4 with relapse) who received a second transplant, 5 survived with remission. Among the 8 patients in our series who received a second transplant, 4 patients (3 with engraftment failure and 1 with relapse) achieved remission. However, JMML patients with failed engraftment will progress to hematological

relapse; therefore, a prompt second transplant (e.g., CBT) may be helpful. The main advantages of CBT in JMML patients are its use as an allograft in urgent situations and obtaining an adequate quantity of stem cells, given the limited body weight of these patients. Moreover, Inagaki et al. and Yoshimi et al. [15, 16] have reported that several cases achieved complete chimerism after the withdrawal of immunosuppressive therapy. In addition, chimerism studies can identify patients with increased mixed chimerism who are at high risk of JMML relapse.

The EWOG-MDS/EBMT group has reported that the use of AML-like chemotherapy and splenectomy before transplantation did not appear to be associated with a lower risk of disease recurrence [9]. In our series, a short course of AML therapy in 3 patients (including 2 patients with monosomy-7) appeared to be effective and safe. However, we could not evaluate the engraftment and relapse rates for pre-HSCT treatment with AML chemotherapy and splenectomy/splenic irradiation because of the small sample size.

Yoshida et al. [3] have reported that OS in 19 patients with a mutation in *PTPN11* who received HSCT (25 %) was significantly poorer than OS in 14 patients without a mutation in *PTPN11* (64 %). In the present series, mutations in *PTPN11* were detected in 15 patients (50 %), and our results indicate a more favorable prognosis than the previous studies, with an estimated 5-year OS rate of 58.3 and 88.9 % for the patients with *PTPN11* mutation and the patients without *PTPN11* mutation, respectively ($P = 0.092$). The results of the survival analyses, according to mutation status, suggest that mutations in *PTPN11* have poor prognostic implications among Japanese JMML patients. Although Yoshida et al. did not specify the conditioning regimens that they used, the increased survival in our series may be attributable to the Bu/Flu/L-PAM regimen.

In conclusion, our results indicate that non-irradiation conditioning with Bu/Flu/L-PAM may achieve hematological and clinical remission in >50 % of JMML patients when alternative donors (including cord blood) are used for HSCT. In addition, this conditioning regimen appeared to be effective for JMML patients with *PTPN11* mutation. However, additional efforts are needed to overcome engraftment failure in cases of mismatched transplantation and disease recurrence after HSCT. Our group (the Committee of JMML-associated Japanese Pediatric Leukemia/Lymphoma Study Group) has initiated a prospective HSCT study to evaluate the Bu/Flu/L-PAM regimen with targeted iv Bu and serial monitoring with chimerism analyses.

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**Conflict of interest** The authors declare no conflicts of interest.

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