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研究分担報告書

同種造血幹細胞移植後合併症におけるHMGB1-RAGE系の関与の検討

研究分担者　前田嘉信

研究要旨　同種造血幹細胞移植後の合併症の克服は、移植成績を向上させる上で重要である。移植後早期に起こる生体内の変化“danger signal”は、その後の移植合併症を誘発させる可能性がある。“danger signal”には、外的要因（微生物）としてのPAMP(Pathogen-associated molecular pattern；病原体関連分子パターン)に対し、内的要因(細胞障害など)の分子群はDAMP (Damage associated molecular pattern；ダメージ関連分子パターン) として大別される。High-mobility group box 1 protein (HMGB-1)は、核内において転写調節に重要な核内蛋白質として知られていたが、免疫担当細胞から能動的に細胞外に分泌、または細胞死に伴って受動的に細胞外へ放出される。受容体としては、receptor for advanced glycation endproducts (RAGE)やTRLが知られている。“danger signal”として、炎症性サイトカインと微生物特有の分子群PAMPの役割が明らかにされているが、今回、我々はマウスモデルを使ってDAMPであるHMGB1-RAGE系と同種造血幹細胞移植後合併症の関連について検討した。その結果、同種移植後にはHMGB1濃度が上昇し、RAGEを欠損したホストではGVHDの上昇度が異なることが明らかとなり、HMGB1-RAGE系がGVHDに関与していることが示唆された。

A. 研究目的

同種造血幹細胞移植は、白血病などの悪性疾患に対する根治的治療として確立しているが、致死的合併症である移植片対宿主病（GVHD）は今日なお克服すべき課題である。移植後早期に起こる生体内の変化“danger signal”は、その後の移植合併症を誘発させる可能性がある。今回、我々は、内的要因(細胞障害など)の分子群DAMP (Damage associated molecular pattern；ダメージ関連分子パターン)であるHigh-mobility group box 1 protein (HMGB-1)に注目し、その受容体 receptor for advanced glycation endproducts (RAGE)とHMGB-1の同種造血幹細胞移植後合併症における役割を明らかにすることを目的とした。

B. 研究方法

ドナーにB6、ホストにBALB/cを使ったマウスGVHDモデルを作成した。また、HMGB-1の受容体であるRAGEを欠損したRAGE KOマウスをレシピエントに使用した。骨髄幹細胞（T細胞除去したBM $5x10^6$）と　脾臓から採取した細胞（$5x10^6$）を13Gy照射したマウスに移植。急性GVHDは臨床的GVHDスコアに加え病理スコアにて評価した。血清中のHMGB-1は

ELISA にて、また、Th 細胞への影響、細胞内サイトカインを FACS にて解析した。

本実験計画は岡山大学実験動物実験委員会に承認済みである。すべての実験動物は動物愛護の観点から、計画的にできるだけ少ない個体数での実験とした。

C. 研究結果

まず、血清中の HMGB-1 が移植後の障害により血中に放出されるかを 移植後 6 時間、24 時間、7 日目、14 日目、21 日目と経時的に測定した。同種移植後 7 日目には他の群に比べ、有意差をもって HMGB-1 が上昇しており、免疫反応による組織障害を反映したと考えられた（図 1）。

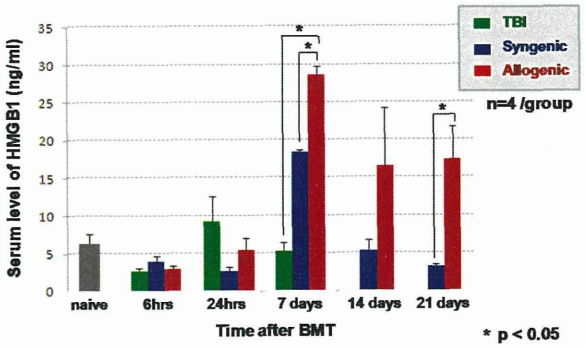
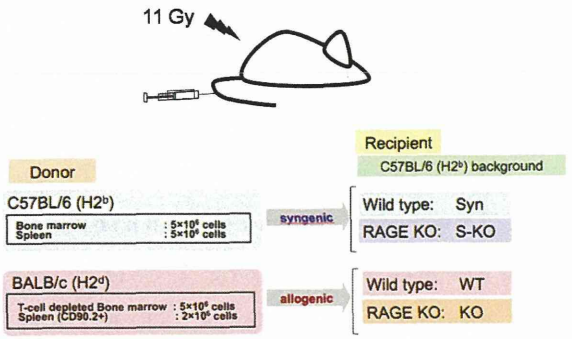


図 1 血清 HMGB-1 濃度

次に、HMGB-1 の受容体である RAGE を欠損した RAGE KO マウスをレシピエントに使用し急性 GVHD の発症を野生型 WT と比較した。



RAGE KO マウス群では、移植後 7-10 日の 1 週間前後で多くが早期死亡を認め WT と比較しても有意に生存率は低い傾向を示した。GVHD スコア、体重減少と合わせて KO 群で WT より強い同種免疫反応が生じている可能性があると考えられた。

D. 考察

同種移植後 7 日目には有意差をもって HMGB-1 が上昇した。また、受容体を欠損した RAGE KO マウスをレシピエントに使用した場合に急性 GVHD の重症度に差を認め、以上から HMGB-1 ・RAGE の系が GVHD に関与している可能性が示唆された。

E. 結論

HMGB-1 ・RAGE の系が GVHD に関与している可能性が示唆され、今後詳細なメカニズムと治療標的となり得るかと検討していく必要がある。

F. 健康危険情報

特になし。

G.研究発表

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H.知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

特になし。

2. 実用新案登録

特になし。

3. その他

特になし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Murata M, Nishida T, Taniguchi S, Ohashi K, Ogawa H, Fukuda T, Mori T, Kobayashi H, Nakaseko C, Yamagata N, Morishima Y, Nagamura-Inoue T, Sakamaki H,Atsuta Y, Suzuki R, Naoe T.	Allogeneic transplantation for primary myelofibrosis with BM, peripheral blood or umbilical cord blood: an analysis of the JSHCT.	*Bone Marrow Transplant.*		Epub ahead of print	2013
Muroi K, Miyamura K, Ohashi K, Murata M, Eto T, Kobayashi N, Taniguchi S, Imamura M, Ando K, Kato S, Mori T, Teshima T, Mori M, Ozawa K.	Unrelated allogeneic bone marrow-derived mesenchymal stem cells for steroid-refractory acute graft-versus-host disease: a phase I/II study.	*Int J Hematol.*	98(2)	206-13	2013
Fujioka T, Tamaki H, Ikegame K, Yoshihara S, Taniguchi K, Kaida K, Kato R, Inoue T, Nakata J, Ishii S, Soma T, Okada M, Ogawa H.	Frequency of CD4(+)FOXP3(+) regulatory T-cells at early stages after HLA-mismatched allogeneic hematopoietic SCT predicts the incidence of acute GVHD.	*Bone Marrow Transplant.*	48(6)	859-64	2013
Koyama M, Hashimoto D, Nagafuji K, Eto T, Ohno Y, Aoyama K, Iwasaki H, Miyamoto T, Hill GR, Akashi K, Teshima T.	Expansion of donor-reactive host T cells in primary graft failure after allogeneic hematopoietic SCT following reduced-intensity conditioning.	*Bone Marrow Transplant.*	49(1)	110-5	2014
Sugiyama H, Maeda Y, Nishimori H, Yamasuji Y, Matsuoka K, Fujii N, Kondo E, Shinagawa K, Tanaka T, Takeuchi K, Teshima T, Tanimoto M.	mTOR inhibitors permit regulatory T cell reconstitution and inhibit chronic GVHD.	*Biol Blood Marrow Transplant.*	20(2)	183-91	2014

Ⅳ. 研究成果の刊行物・別刷

OPEN

Bone Marrow Transplantation (2013), 1–6

www.nature.com/bmt

ORIGINAL ARTICLE

Allogeneic transplantation for primary myelofibrosis with BM, peripheral blood or umbilical cord blood: an analysis of the JSHCT

M Murata[1], T Nishida[1], S Taniguchi[2], K Ohashi[3], H Ogawa[4], T Fukuda[5], T Mori[6], H Kobayashi[7], C Nakaseko[8], N Yamagata[9], Y Morishima[10], T Nagamura-Inoue[11], H Sakamaki[3], Y Atsuta[12], R Suzuki[12] and T Naoe[1]

To determine whether a difference in donor source affects the outcome of transplantation for patients with primary myelofibrosis (PMF), a retrospective study was conducted using the national registry data on patients who received first allogeneic hematopoietic cell transplantation (HCT) with related BM ($n=19$), related PBSCs ($n=25$), unrelated BM ($n=28$) or unrelated umbilical cord blood (UCB; $n=11$). The 5-year OS rates after related BM, related PBSC and unrelated BM transplantation were 63%, 43% and 41%, respectively, and the 2-year OS rate after UCB transplantation was 36%. On multivariate analysis, the donor source was not a significant factor for predicting the OS rate. Instead, performance status (PS) $\geqslant 2$ (vs PS 0–1) predicted a lower OS ($P=0.044$), and RBC transfusion $\geqslant 20$ times before transplantation (vs transfusion $\leqslant 9$ times) showed a trend toward a lower OS ($P=0.053$). No advantage of nonmyeloablative preconditioning regimens in terms of decreasing nonrelapse mortality or increasing OS was found. Allogeneic HCT, and even unrelated BM and UCB transplantation, provides a curative treatment for PMF patients.

Bone Marrow Transplantation advance online publication, 25 November 2013; doi:10.1038/bmt.2013.180

Keywords: idiopathic myelofibrosis; hematopoietic SCT; donor source; engraftment; survival

INTRODUCTION

Primary myelofibrosis (PMF) is a clonal stem cell disorder characterized by anemia, BM fibrosis, progressive splenomegaly, constitutional symptoms and a significant risk of evolution into acute leukemia.[1,2] The median age at diagnosis is ~65 years, with a median survival of ~5 years after diagnosis, depending on the presence or absence of clinically defined prognostic factors, such as those defined by the International Prognostic Scoring System (IPSS), Dynamic IPSS and Dynamic IPSS plus.[3–5] No available conventional drug therapies for PMF have been shown to prolong survival. Palliative therapeutic options include agents such as hydroxyurea, prednisone, EPO, androgens, thalidomide and lenalidomide, and nonpharmacological approaches such as blood transfusion, splenic irradiation and splenectomy.[6,7] The impact of new agents, such as Janus kinase 2 (JAK2) inhibitors, pomalidomide and histone deacetylase inhibitors, on the long-term management of PMF is under investigation.[7,8] The only known curative therapy for PMF is allogeneic hematopoietic cell transplantation (HCT).[9]

The largest retrospective study of PMF patients undergoing allogeneic BM or PBSC transplantation reported OS of 30–40% at 5 years after transplantation with nonrelapse mortality (NRM) of 24–43% at 1 year after transplantation.[10] The prospective study in patients with PMF or secondary myelofibrosis to evaluate a nonmyeloablative preconditioning regimen followed by mainly PBSC transplantation achieved an OS of 51% at 5 years after transplantation with NRM of 16% at 1 year after transplantation.[11] The issues of the choice of stem cell source, the choice of conditioning regimen and the timing of transplantation are currently under debate.[6–9,12,13]

To determine whether a difference in stem cell source affects the outcome of HCT for PMF patients, a retrospective study was conducted using the national registry data on patients who received first allogeneic HCT in Japan with BM, PBSCs or umbilical cord blood (UCB).

PATIENTS AND METHODS

Patients

Clinical data for patients with PMF who received first allogeneic HCT in Japan were extracted from the Transplant Registry Unified Management Program (TRUMP) system, which is a registry of the outcomes of Japanese transplant patients.[14] Patients who had progressed to myelofibrosis from polycythemia vera, essential thrombocythemia, leukemia or other disease were excluded. This study was approved by the Data Management Committee of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and by the ethics committee of the Nagoya University School of Medicine (no. 2012–0270).

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Definitions

Hematopoietic recovery was defined as time to ANC $\geqslant 0.5 \times 10^9$/L, time to reticulocytes $\geqslant 10‰$ and time to platelets $\geqslant 50 \times 10^9$/L for 3 consecutive days. Engraftment failure was defined as no neutrophil recovery by day 60. Acute and chronic GVHD were diagnosed and graded according to established criteria.[15,16] Based on the report by the Center for International Blood and Marrow Transplant Research (CIBMTR),[17] the conditioning regimens were classified as myeloablative if TBI >8 Gy, oral BU $\geqslant 9$ mg/kg, i.v. BU $\geqslant 7.2$ mg/kg or melphalan >140 mg/m^2 was included in the conditioning regimen, whereas other conditioning regimens were classified as nonmyeloablative.

End points

The primary end point was OS. The secondary end points were engraftment, GVHD, relapse and NRM.

Statistical analysis

The probabilities of hematopoietic recovery, acute and chronic GVHD, relapse and NRM were estimated on the basis of cumulative incidence curves.[18] The probability of OS was estimated according to the Kaplan–Meier method.[19] The groups were compared using the log-rank test. Competing risk regression analysis was used to identify factors associated with NRM. The adjusted probability of OS was estimated using Cox's proportional hazards model, with consideration of other significant clinical variables in the final multivariate models.[20] All variables significant at $P<0.10$ on univariate analysis were included in multivariate stepwise analyses. All tests were two sided, and $P<0.05$ was considered significant. The data were analyzed by STATA version 12 statistical software (StataCorp, College Station, TX, USA).

RESULTS

Patient and transplantation characteristics

A total of 83 patients met the inclusion criteria. Patient and transplantation characteristics are summarized in Table 1. The median age at transplantation was 53 years, and most patients (66%) were male. Transplants were performed between 1993 and 2009, but the majority (90%) of them were performed after 2000. This population consisted of 47 BM transplants, 25 PBSC transplants and 11 UCB transplants. Of the 44 related donor transplants, 40 (91%) were performed from serological HLA-A, B and DR 6/6 matched donor; 28 unrelated BM transplants included 16 (57%) HLA-A, B and DRB1 alleles 6/6 matched donors and 11 (39%) HLA-A, B and DRB1 alleles 5/6 matched donors; all (100%) unrelated UCB transplants were performed from serological HLA-A, B and DR 5/6 or 4/6 matched donors. Most patients (76%) received a nonmyeloablative regimen. The median follow-up for living patients was 40 (range, 0.4–150) months.

Engraftment

Seven patients (8%) died without engraftment within 60 days after transplantation, including heart failure on day 5 after UCB transplant ($n=1$), primary disease on day 7 after related PBSC transplant ($n=1$), infection on day 11 after unrelated BM transplant ($n=1$), multiple organ failure on day 12 after unrelated BM transplant ($n=1$), heart failure on day 18 after unrelated BM transplant ($n=1$), infection on day 30 after unrelated BM transplant ($n=1$) and thrombotic microangiopathy on day 56 after UCB transplant ($n=1$). Another patient (1%) received a second transplant on day 28 because of lack of engraftment signs at that time.

Neutrophil recovery on day 60 occurred in 92% (95% confidence interval (CI), 57–99%) of related BM, 92% (71–98%) of related PBSCs, 79% (58–90%) of unrelated BM and 82% (45–95%) of unrelated UCB (Figure 1a). Unrelated BM and unrelated UCB (vs related BM) transplantations were significantly associated with a lower probability of neutrophil recovery ($P=0.015$ and $P=0.016$, respectively), whereas related PBSC transplantation was

Table 1. Patient and transplantation characteristics ($n=83$)

	N *(%)*
Age at transplant, evaluable n	83
21–39 Years	9 (11)
40–49 Years	22 (27)
50–59 Years	37 (44)
60–79 Years	15 (18)
Median age (range), years	53 (21–79)
Sex, evaluable n	83
Female	28 (34)
Male	55 (66)
Transplant year, evaluable n	83
1993–1999	8 (10)
2000–2004	22 (27)
2005–2009	53 (63)
Performance status at transplant, evaluable n	70
0–1	54 (77)
$\geqslant 2$	16 (23)
Time from diagnosis to transplant, evaluable n	80
<1 Years	33 (41)
1–2 Years	16 (20)
$\geqslant 2$ Years	31 (39)
Median (range), years	1.5 (0.1–21.0)
Frequency of RBC transfusion before transplant, evaluable n	51
$\leqslant 9$	26 (51)
10–19	8 (16)
$\geqslant 20$	17 (33)
Frequency of PLT transfusion before transplant, evaluable n	51
$\leqslant 9$	38 (74)
10–19	4 (8)
$\geqslant 20$	9 (18)
Use of JAK2 inhibitor before transplant, evaluable n	77
Yes	0 (0)
No	77 (100)
Splenectomy before transplant, evaluable n	78
Yes	2 (3)
No	76 (97)
DIPSS at transplant	78
Low	8 (10)
Intermediate-1	17 (22)
Intermediate-2	50 (64)
High	3 (4)
Splenomegaly at transplant	78
Yes	59 (76)
No	19 (24)
CMV serostatus, evaluable n	58
Negative	5 (9)
Positive	53 (91)
Donor source, evaluable n	83
Related BM	19 (23)
Related PBSCs	25 (30)
Unrelated BM	28 (34)
Unrelated umbilical cord blood	11 (13)
Sex matching between patient and donor, evaluable n	71
Match	35 (49)
Female patient and male donor	15 (21)
Male patient and female donor	21 (30)
ABO matching between patient and donor, evaluable n	65
Match	34 (52)
Mismatch	31 (48)
Preconditioning regimen, evaluable n	71
Myeloablative	17 (24)
Nonmyeloablative	54 (76)
Prophylaxis for GVHD, evaluable n	81
CsA based	37 (46)
Tacrolimus based	42 (52)
Others	2 (2)
Use of JAK2 inhibitor after transplant, evaluable n	78
Yes	0 (0)
No	78 (100)

Abbreviations: DIPSS = Dynamic International Prognostic Scoring System; JAK2 = Janus kinase 2.

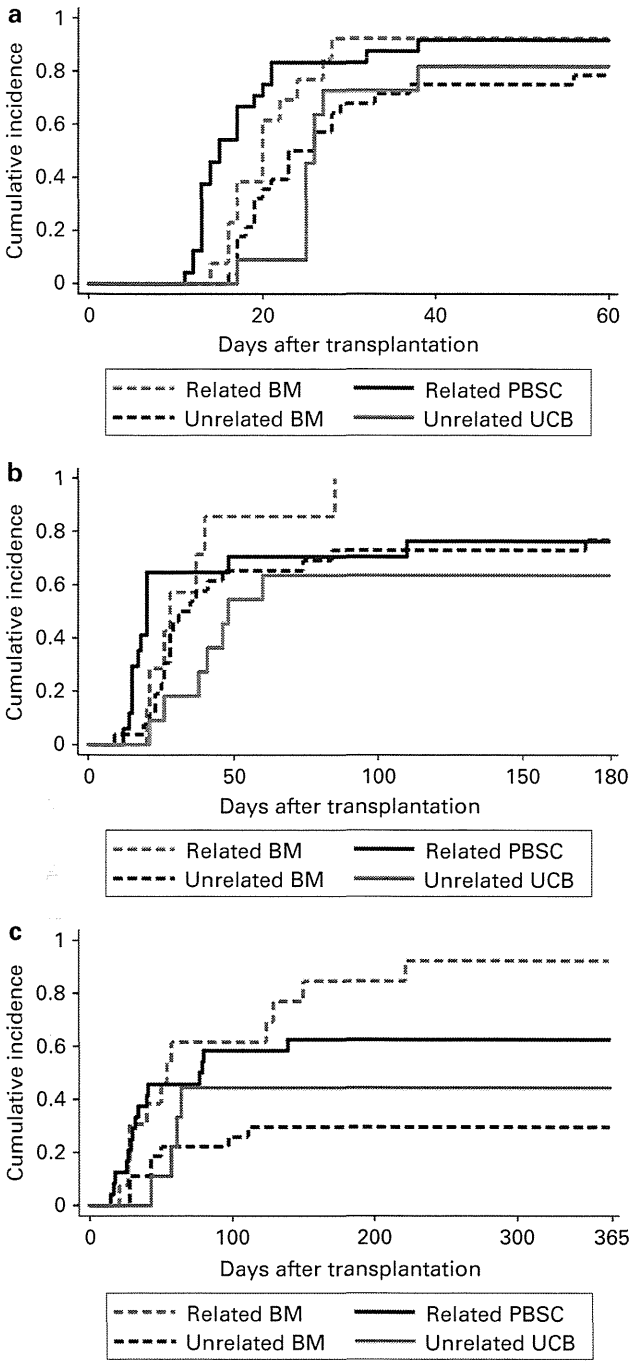

Figure 1. Hematopoietic recoveries after transplantation in PMF patients. (**a**) Cumulative incidences of neutrophil recovery after related BM (gray and dash line), related PBSC (black and solid line), unrelated BM (black and dash line) and unrelated UCB (gray and solid line) transplantations are shown. (**b**) Cumulative incidences of reticulocyte recovery after related BM (gray and dash line), related PBSC (black and solid line), unrelated BM (black and dash line) and unrelated UCB (gray and solid line) transplantations are shown. (**c**) Cumulative incidences of platelet recovery after related BM (gray and dash line), related PBSC (black and solid line), unrelated BM (black and dash line) and unrelated UCB (gray and solid line) transplantations are shown.

not significantly different from related BM transplantation ($P=0.46$). The median days for neutrophil recovery in patients receiving related BM, related PBSCs, unrelated BM and unrelated UCB were 20, 14, 21 and 25, respectively.

Reticulocyte recovery on day 180 occurred in 100% of related BM, 75% (46–90%) of related PBSC, 77% (56–89%) of unrelated BM and 64% (30–85%) of unrelated UCB transplantations (Figure 1b). Unrelated UCB (vs related BM) transplantation was significantly associated with a lower probability of reticulocyte recovery ($P=0.012$), whereas related PBSC and unrelated BM transplantations were not significantly different from related BM transplantation ($P=0.57$ and $P=0.076$, respectively). The median days for reticulocyte recovery in patients receiving related BM, related PBSCs, unrelated BM and unrelated UCB were 28, 17, 28 and 41, respectively.

Platelet recovery on day 365 occurred in 92% (57–99%) of related BM, 63% (40–78%) of related PBSC, 30% (14–47%) of unrelated BM and 44% (14–72%) of unrelated UCB transplantations (Figure 1c). Unrelated BM and unrelated UCB transplantations (vs related BM) were significantly associated with a lower probability of platelet recovery ($P<0.001$ and $P=0.027$, respectively), whereas related PBSC transplantation was not significantly different from related BM transplantation ($P=0.20$). The median days for platelet engraftment in patients receiving related BM, related PBSCs, unrelated BM and unrelated UCB were 50, 32, 43 and 57, respectively.

GVHD

The incidences of grade II–IV and III–IV acute GVHD on day 100 were 17% (95% CI, 4–37%) and 6% (0–22%) in related BM, 32% (15–50%) and 16% (5–33%) in related PBSC, 29% (14–46%) and 14% (4–30%) in unrelated BM and 10% (1–36%) and 0% in unrelated UCB transplantations, respectively. There was no significant difference in the incidence of grade II–IV acute GVHD among stem cell sources, whereas the incidence of grade III–IV acute GVHD was significantly lower after unrelated UCB transplantation than after related BM transplantation ($P<0.001$).

The incidences of chronic GVHD at 2 years after transplantation were 35% (95% CI, 14–57%) in related BM, 52% (31–69%) in related PBSC, 25% (11–42%) in unrelated BM and 18% (3–44%) in unrelated UCB transplantations. There was no significant difference in the incidence of chronic GVHD among stem cell sources.

Relapse

Relapse rates at 2 and 5 years after transplantation were 5% (95% CI, 0–21%) and 12% (2–33%) in related BM, 8% (1–22%) and 12% (3–28%) in related PBSC and 4% (0–18%) and 4% (0–18%) in unrelated BM transplantations, respectively. No patient relapsed after UCB transplantation, in which the longest follow-up was 48 months.

NRM

NRM rates at 2 and 5 years after transplantation were 33% (95% CI, 13–54%) and 33% (13–54%) in related BM, 45% (24–63%) and 50% (28–69%) in related PBSC and 61% (38–77%) and 61% (38–77%) in unrelated BM transplantations, respectively (Figure 2). NRM at 2 years after unrelated UCB transplantation was 64% (30–85%), and NRM at 5 years after UCB transplantation was not evaluable because of lack of patients alive beyond 5 years after transplantation. NRM rates after related PBSC and unrelated BM transplantation were not significantly different from that after related BM transplantation ($P=0.28$ and $P=0.068$, respectively), whereas unrelated UCB transplantation (vs related BM) was significantly associated with a significantly higher NRM ($P=0.021$).

To identify predictive factors for higher NRM, multivariate analysis for all clinical features listed in Table 1 was performed, and the final multivariate model is shown in Table 2. PS $\geqslant 2$ and unrelated BM were predictive factors for higher NRM. For patients with performance status (PS) 0–1 ($n=54$), NRM rates at 2 and 5 years after transplantation were 37% (23–50%) and 40% (26–54%),

respectively. For patients with PS $\geqslant 2$ ($n = 16$), NRM at 2 years was 77% (45–92%), and NRM at 5 years was not evaluable because of lack of patients alive beyond 5 years after transplantation.

OS

OS rates at 2 and 5 years after transplantation were 63% (95% CI, 38–80%) and 63% (38–80%) in related BM, 48% (28–66%) and 43% (23–61%) in related PBSC and 41% (21–59%) and 41% (21–59%) in unrelated BM transplantations, respectively (Figure 3). The OS rate at 2 years after unrelated UCB transplantation was 36% (11–63%), and the OS rate at 5 years after UCB transplantation was not evaluable because of a lack of patients alive beyond 5 years after transplantation (longest follow-up, 48 months). There was no significant difference among stem cell donor sources ($P = 0.15$).

Cox's proportional hazards model was used with all clinical features listed in Table 1, and the final multivariate model is shown in Table 2. After adjustment by PS and frequency of RBC transfusion, which were significant on univariate analysis, donor source was not a significant factor for predicting OS. Instead, PS $\geqslant 2$ predicted a lower OS rate, and RBC transfusion $\geqslant 20$ times before transplantation showed a trend toward a lower OS. We confirmed that there was no significant difference in the frequencies of PS $\geqslant 2$ between patients receiving different stem cell sources (2 of 13 related BM, 6 of 24 related PBSC, 5 of 27 unrelated BM and 3 of 6 unrelated UCB transplantations; $P = 0.30$). Similarly, we confirmed that there was no significant difference in the frequencies of RBC transfusion $\geqslant 20$ times between patients receiving different stem cell sources (2 of 8 related BM, 5 of 18 related PBSC, 8 of 20 unrelated BM and 2 of 5 unrelated UCB transplantations; $P = 0.80$).

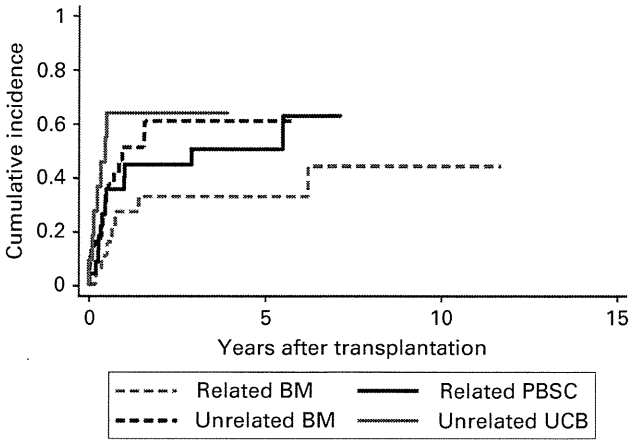

Figure 2. NRM after transplantation in PMF patients. Cumulative incidences of NRM after related BM (gray and dash line), related PBSC (black and solid line), unrelated BM (black and dash line) and unrelated UCB (gray and solid line) transplantations are shown.

Causes of death

The causes of death after transplantation are summarized in Table 3. For patients after related donor transplantation ($n = 23$), the most common cause of death was primary disease ($n = 9$, 39%), followed by infection ($n = 4$, 17%) and organ failure ($n = 3$, 13%). For patients after unrelated donor transplantation ($n = 22$), the most common causes of death were infection ($n = 7$, 32%) and organ failure ($n = 7$, 32%), followed by GVHD ($n = 3$, 14%), and only 1 patient (5%) died of primary disease.

DISCUSSION

The present study confirmed 5-year OS of 63%, 43% and 41% after related BM, related PBSC and unrelated BM transplantations, respectively. These results are comparable to previous reports in which long-term survival rates in patients with PMF or secondary myelofibrosis were 30–67% after transplantation.[10,11,21–26] This is the first report of UCB transplantation for more than 10 patients with PMF, and a 2-year OS of 36% was confirmed.

Several investigators have examined factors to predict outcomes after allogeneic HCT for PMF patients. The largest retrospective study of PMF patients from the CIBMTR demonstrated that Karnofsky score of $<90\%$ and the presence of blasts in peripheral blood, but not donor source, predicted lower disease-free survival of patients who had received BM or PBSC transplantation from related or unrelated donors.[10] Other retrospective studies including both PMF and secondary myelofibrosis demonstrated negative predictors for OS of higher patient age, nonchronic phase disease, RBC transfusion >20 times, increased comorbidity score, intermediate-2 and high scores of the Dynamic IPSS and non-HLA-matched sibling donor.[11,21,24,26,27] In the present study, multivariate analysis demonstrated that PS $\geqslant 2$ predicted a lower OS and that RBC transfusion $\geqslant 20$ times before transplantation showed a trend toward a lower OS (Table 2). Unexpectedly, the stem cell source was not a significant factor for OS. One possibility is that a significant association between stem cell source and OS was not detected because of a lack of statistical power, namely, the small

Table 2. Significant factors in multivariate analyses for nonrelapse mortality and OS after transplantation

	Nonrelapse mortality HR (95% CI)	P-*value*	*Overall survival* HR (95% CI)	P-*value*
Performance status at transplant				
0–1	1.0		1.0	
$\geqslant 2$	3.36 (1.42–7.95)	0.006	2.67 (1.03–6.95)	0.044
Frequency of RBC transfusion[a]				
$\leqslant 9$	NA		1.0	
10–19	NA		0.48 (0.97–2.36)	0.37
$\geqslant 20$	NA		2.42 (0.99–5.93)	0.053
Donor source				
Related BM	1.0		1.0	
Related PBSCs	2.43 (0.73–8.07)	0.15	3.86 (0.81–18.44)	0.091
Unrelated BM	3.58 (1.07–12.01)	0.039	3.13 (0.66–14.79)	0.15
Unrelated umbilical cord blood	2.71 (0.49–14.86)	0.25	3.79 (0.60–23.91)	0.16

Abbreviations: CI = confidence interval; HR = hazard ratio; NA = not applicable.
[a]Frequency of RBC transfusion before transplantation.

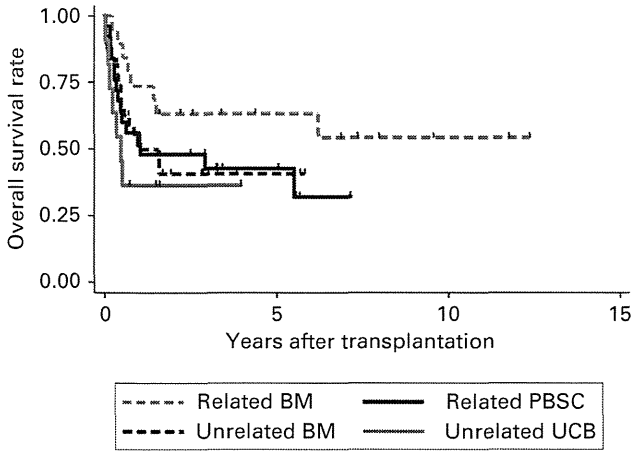

Figure 3. OS rates after transplantation in PMF patients. OS rates after related BM (gray and dash line), related PBSC (black and solid line), unrelated BM (black and dash line) and unrelated UCB (gray and solid line) transplantations are shown.

Table 3. Causes of death

	Related BM, n (%)	*Related PBSC, n (%)*	*Unrelated BM, n (%)*	*Unrelated UCB, n (%)*
Primary disease	2 (25)	7 (46)	1 (7)	0
Infection	1 (13)	3 (20)	6 (40)	1 (14)
Interstitial pneumonitis	2 (25)	0	1 (7)	0
ARDS	0	0	1 (7)	0
GVHD	1 (13)	1 (7)	2 (13)	1 (14)
Organ failure	1 (13)	2 (13)	3 (20)	4 (58)
Graft failure	1 (13)	0	0	0
Bleeding	0	1 (7)	1 (7)	0
Other	0	1 (7)	0	1 (14)
Total	8 (100)	15 (100)	15 (100)	7 (100)

Abbreviations: ARDS = acute respiratory distress syndrome; UCB = umbilical cord blood.

number of patients in each group, and the short-term follow-up. In particular, the number of patients with UCB transplantation was very small, and therefore, careful interpretation of these data is required. Further analysis with data including more patients undergoing UCB transplantation is required in order to determine the effect of UCB transplantation on outcomes of PMF patients. Another possibility is that the HCT outcome for PMF patients is more adversely affected by the deterioration in a patient's systemic condition as a consequence of multiple transfusions of blood and so on, rather than by the difference in stem cell sources.

In practice, UCB transplantation may be avoided in the treatment of PMF patients because of delayed engraftment and a higher probability of graft failure.[9] The present study demonstrated that UCB transplantation was significantly associated with a lower probability of hematopoietic recovery in comparison with related BM transplantation (Figure 1). The incidences of neutrophil recovery at 60 days and platelet recovery at 1 year were 82% and 44% for UCB transplantation, respectively. In a recent report of nonmyeloablative UCB transplantation for 14 patients with myelofibrosis, including 1 patient with PMF and 13 patients with secondary myelofibrosis, the incidences of neutrophil recovery at 60 days and platelet recovery at 100 days were 93% and 43%, respectively.[28] Thus, careful management is required for PMF patients, especially in the early period after unrelated UCB transplantation.

NRM was 30–60% (Figure 2), which is higher than in previous studies from large, well-known transplant center(s).[22–24,26,27,29–32] This may be explained by the large number of the participating centers, the heterogeneity of patients' clinical features and the fact that 18% of patients were ⩾60 years in the present study.

Nonmyeloablative preconditioning regimens have advantages of less NRM and a broader applicability in elderly patients and may, therefore, be appropriate for PMF patients. After small studies demonstrated the feasibility of allogeneic HCT with nonmyeloablative preconditioning for myelofibrosis,[33–35] Kröger *et al.*[11] prospectively treated 103 patients with PMF or post essential thrombocythemia and post polycythemia vera myelofibrosis with BU and fludarabine-based nonmyeloablative preconditioning. They reported encouraging 1-year NRM of 16% and 5-year OS of 67%. The Swedish group compared results from 10 patients undergoing nonmyeloablative transplant with 17 patients undergoing myeloablative transplant for secondary myelofibrosis. NRM was lower in the nonmyeloablative group than in the myeloablative group (10% vs 30%). With a median follow-up of 55 months, 9 (90%) of 10 patients undergoing nonmyeloablative transplant and 9 (55%) of 16 patients undergoing myeloablative transplant survived.[36] In contrast, the present study could not find any advantage of nonmyeloablative preconditioning in terms of decreasing NRM or increasing OS (Table 2). Other retrospective studies, including a large study ($n = 289$), also did not find any favorable affect with nonmyeloablative preconditioning.[10,22,24] In retrospective studies, drugs and doses of preconditioning regimens were heterogeneous, which could partly explain the failure to detect an advantage of nonmyeloablative preconditioning. There has been no randomized study to compare the efficacy of nonmyeloablative and myeloablative preconditioning for patients with PMF. The advantage of nonmyeloablative preconditioning for patients with PMF remains in question.

The molecular assessment of the *JAK2* mutation was performed in a very limited number of patients (six cases for pretransplant mutation and four cases for post transplant mutation). Therefore, we were unable to analyze association between the presence of pretransplant *JAK2* mutation and transplant outcomes or between the minimum residual disease and relapse after transplant. However, the present study clearly demonstrated that allogeneic BM and PBSC transplantations provide long-term survival for PMF patients and suggested the feasibility of UCB transplantation for PMF patients. Given the constant improvement in supportive care for transplant patients and the beginning of the use of molecular targeted therapy for myelofibrosis, the NRM and relapse rates may be further decreased. Allogeneic HCT should be considered in the treatment plan for PMF patients. The indications for allogeneic HCT in PMF patients have to be defined in a future study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX

Institutes participating in this study: Japanese Red Cross Asahikawa Hospital; Hokkaido University Hospital; Sapporo Medical University Hospital; Sapporo Hokuyu Hospital; Akita University Hospital; Iwate Medical University; Tohoku University Hospital; Fukushima Medical University Hospital; Nagaoka Red Cross Hospital; Gunmaken Saiseikai Maebashi Hospital; Tsukuba Memorial Hospital; Chiba University Hospital; Kameda Medical Center; National Defense Medical College Hospital; Saitama Medical Center, Jichi Medical University; Keio University Hospital; Tokyo Metropolitan Cancer and Infectious diseases Center, Komagome Hospital; Toranomon Hospital; National Cancer Center Hospital; Tokyo Women's Medical University Hospital; Institute of Medical Science, University of Tokyo; Nippon Medical School Hospital; Kanagawa Cancer Center; Yokohama City University Medical Center; Nagano Red Cross Hospital; Shinshu University Hospital; Toyama Prefectural Central Hospital; Kurobe City Hospital; Kanazawa University Hospital; Shizuoka General Hospital; Japanese Red Cross Shizuoka Hospital; Hamamatsu University Hospital; Hamamatsu Medical Center; Anjo Kosei Hospital; Fujita Health University Hospital; Japanese Red Cross Nagoya Daiichi Hospital; Japanese Red Cross Nagoya Daini Hospital; Meitetsu Hospital; Nagoya University Hospital; Nara Medical University Hospital; Tenri Hospital; Takanohara Central Hospital; Kyoto University Hospital; Kyoto-Katsura Hospital; Osaka Red Cross Hospital; Osaka Medical Center for Cancer and Cardiovascular Diseases; Takatsuki Red Cross Hospital; Seichokai Fuchu Hospital; Kinki University Hospital; Wakayama Medical University Hospital; Hyogo College of Medicine; Institute of Biomedical Research and Innovation; Kurashiki Central Hospital; Okayama Medical Center; Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital; Shimane Prefectural Central Hospital; Yamaguchi University Hospital; Ehime University Hospital; Ehime Prefectural Central Hospital; Kochi Medical School Hospital; Kitakyushu Municipal Medical Center; University of Occupational and Environmental Health; Kyushu Cancer Center; Kyushu Medical Center; Kyushu University Hospital; Kurume University Hospital; Ryukyu University Hospital.

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ORIGINAL ARTICLE

Unrelated allogeneic bone marrow-derived mesenchymal stem cells for steroid-refractory acute graft-versus-host disease: a phase I/II study

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Abstract We conducted a multicenter phase I/II study using mesenchymal stem cells (MSCs) manufactured from the bone marrow of healthy unrelated volunteers to treat steroid-refractory acute graft-versus-host disease (aGVHD). Fourteen patients with hematological malignancies who suffered from grade II (9 patients) or III aGVHD (5) were treated. Affected organs were gut (10 patients), skin (9 patients), and liver (3 patients). Seven patients had two involved organs. The median age was 52. No other second-line agents were given. MSCs were given at a dose of 2×10^6 cells/kg for each infusion twice a week for 4 weeks. If needed, patients were continuously given MSCs weekly for an additional 4 weeks. By week 4, 13 of 14 patients (92.9 %) had responded to MSC therapy with a complete response (CR; $n = 8$) or partial response (PR; $n = 5$). At 24 weeks, 11 patients (10 with CR and 1 with PR) were alive. At 96 weeks, 8 patients were alive in CR. A total of 6 patients died, attributable to the following: underlying disease relapse (2 patients), breast cancer relapse (1), veno-occlusive disease (1), ischemic cholangiopathy (1), and

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pneumonia (1). No clear adverse effects associated with MSC infusion were observed. Third party-derived bone marrow MSCs may be safe and effective for patients with steroid-refractory aGVHD.

Keywords Mesenchymal stem cells · GVHD · Steroid

Introduction

Allogeneic hematopoietic stem cell transplantation (AlloHSCT) is a curative therapy for hematological malignancies and hemopoietic stem cell disorders. Acute graft-versus-host disease (aGVHD), the most important complication associated with AlloHSCT, develops in a significant number of patients who receive AlloHSCT despite GVHD prophylaxis [1]. Levine et al. [2] showed using Cox regression analysis that GVHD grade had a significant impact on non-relapse mortality and overall survival (OS) in a phase II GVHD treatment trial. The relative risk of non-relapse mortality was 1.72 for patients with grade III–IV GVHD, compared to patients with grade 0–II GVHD. Significant factors on OS were aGVHD grade (0–II versus III–IV), donor type (related versus unrelated), and stem cell source (peripheral blood versus bone marrow versus cord blood).

In general, a steroid is first given to patients with aGVHD; however, about half of the patients do not respond to the therapy [3]. Unfortunately, second-line agents have not clearly shown effectiveness against steroid-refractory aGVHD, because they act as a non-specific immunosuppressant and reduce host immunity, leading frequently to infections caused by bacteria, fungi and viruses [4, 5]. Indeed, most patients with steroid-refractory aGVHD die of aGVHD itself, organ damage, and infections even if such second-line therapy is conducted. Recently, the American Society of Blood and Marrow Transplantation evaluated 29 studies in which agents were administered as secondary therapy in aGVHD [6]. Evaluated agents included mycophenolate mofetil, daclizumab, alemtuzumab, infliximab, etanercept, horse antithymocyte globulin, and so on, but excluded mesenchymal stem cells (MSCs). Importantly, the evaluation of complete response (CR) rates, overall response (OR) rates, and 6-month survival estimates did not support the choice of any specific agents for second-line therapy in aGVHD. Therefore, a new agent for steroid-refractory aGVHD is desirable.

MSCs have unique characteristics: specific immunosuppressive properties, no immunogenicity on its own, supportive activity for hemopoiesis, and differentiation abilities into fat cells, chondrocytes, and osteoblasts. Since the first dramatic report by LeBlanc et al., there have been several reports on the effectiveness of MSCs against steroid-refractory aGVHD [7–20]. However, there are several problems when evaluating MSCs against steroid-refractory aGVHD in these studies: before MSC administration, patients have already received one or more immunosuppressants other than for GVHD prophylaxis to steroid-refractory aGVHD and the follow-up time of the patients who received MSCs was relatively short. The source of MSCs used for steroid-refractory aGVHD in these studies was heterogeneous: HLA-identical siblings, HLA-haploidentical related donors, and HLA-mismatched unrelated (third-party) donors. The production of MSCs in different institutes leads to concern about purity and cell function. We report a phase I/II trial on steroid-refractory aGVHD using third party-derived bone marrow MSCs. Before MSC administration, patients only received steroids for aGVHD as a first-line therapy.

Materials and methods

Patients

During the period from January 2009 and November 2010, 14 patients were enrolled in a phase I/II trial using third party-derived bone marrow MSCs for steroid-refractory aGVHD across major transplant centers in Japan. This trial, sponsored by JCR Pharmaceuticals Co., Ltd (Ashiya, Japan) and designated JR-031-201, was approved by the ethics committee in each participating facility. Informed consent was obtained from all the patients.

The eligibility requirements included patients with steroid-refractory grade II to IV aGVHD and age over 6 months. Steroid-refractory aGVHD was defined as progression of aGVHD for 3 days with standard-dose steroid administration or no change in aGVHD for 5 days with the therapy. The standard steroid dose (prednisolone or methylprednisolone) was 1–2 mg/kg. Exclusion criteria were as follows: chemorefractory disease, severe infection, positive results of viral infections including human immunodeficiency virus, human T-lymphotropic virus type I, hepatitis B virus, and hepatitis C virus, severe organ damage including heart, lung, kidney, and liver except liver GVHD, uncontrolled hypertension, oxygen saturation at a steady state less than 94 %, and new immunosuppressive agents added other than steroids for aGVHD. In cases where attending physicians did not predict early relapse after AlloHSCT, no remission in acute leukemia, myelodysplastic syndrome, or hematological malignancies was included. All patients received prophylaxis against GVHD with a calcineurin inhibitor (tacrolimus or cyclosporine) alone or a combination of a calcineurin inhibitor and methotrexate or mycophenolate mofetil. The source of hemopoietic stem cell transplants was bone marrow, peripheral blood stem cells, or cord blood. Conditioning

was either myeloablative conditioning such as total body irradiation-based and intravenous busulfan-based regimens or non-myeloablative conditioning such as fludarabine-based regimens. aGVHD was defined according to the 1994 Consensus Conference on Acute GVHD Grading [21].

MSCs

MSCs were manufactured by JCR based on a license from Osiris Therapeutics Inc (Columbia, Merryland, USA) and named JR-031. JR-031 is almost the same as Prochymal produced by Osiris [12, 17]. Briefly, an aliquot of bone marrow obtained from healthy volunteers was cultured in a medium supplemented with 10 % fetal bovine serum from New Zealand (Life Technologies, New York, USA). The fetal bovine serum products were free of bacteria, viruses, mycoplasma, and endotoxins in the checking tests. The products met standards for Code of Federal Regulations 9CFR113.53 and the United States Department of Agriculture. Adherent cells were expanded by culture and used as MSCs. Before freezing, cells were examined in the terms of MSC characteristics [22]. Isolated cells showed positivity for CD73, CD90, CD105, and CD166 and negativity for CD34, CD45, and HLA-DR. The cells inhibited the mixed-lymphocyte reaction and differentiated to fat cells, chondrocytes, and osteoblasts. The cells had the ability to produce prostaglandin E2. Multicolor-fluorescence in situ hybridization showed that the cells had no chromosomal abnormalities. No infectious agents such as bacteria, mycoplasma, or viruses were detected in the supernatants of the cells or the cells themselves. No endotoxin was detected in the supernatant.

Treatment schedule and evaluation

Initially, patients received a dose of 2×10^6 MSCs/kg twice a week for 4 weeks. The first infusion of MSCs was given within 48 h of the diagnosis of steroid-refractory aGVHD. The interval between each MSC infusion was 3 or 4 days. The volume of one bag of JR-031 was 15 ml containing 100×10^6 MSC, 1.5 g DMSO, 750 mg of human albumin, and other electroride elements. A solution of 25 ml saline was added to thawed MSCs and they were infused at a speed of around 4 ml/min. Before infusion, either 100–200 mg of hydrocortisone or 5–10 mg of chlorpheniramine or both were given to prevent an infusion reaction. During the total course of MSC infusions, no increase in the dose of immunosuppressants given for GVHD prevention was allowed. As for the steroid dose for GVHD treatment, no increase of more than the initial dose of steroid was allowed. Steroid dose reduction including the start of tapering timing and the reduction dose was left to the physicians who took care of the patient.

Response to aGHVD was evaluated for each involved organ. CR was defined as the complete resolution of aGVHD; partial response (PR), as a decrease in organ stages of aGVHD; no response (NR), as no change in aGVHD; progression (PG), as progressive worsening of aGVHD; mixed response (MR), as a mixture of a decrease and increase in organ stages of aGVHD. Patients were dropped out of this JR-031-201 trial if there was PG after the infusion of 3 doses of MSCs or NR after the infusion of 5 doses of MSCs. After completing MSC infusion for 4 weeks, i.e., 8 doses of MSCs, the response was evaluated. When patients showed PR or MR, 2×10^6 MSCs/kg were further given weekly for 4 weeks. The response of MSC therapy to aGVHD was evaluated as follows: CR or PR by the end of 4, 12, and 24 weeks from the first MSC infusion, as wells as continuous CR for more than 28 days. Other evaluable factors associated with MSC therapy were survival, disease relapse, infection, chronic GVHD, and so on.

Monitoring of adverse effects

To monitor adverse effects associated with MSC therapy, laboratory studies, electrocardiogram (ECG), chest X-ray, and computed tomography (CT) of the chest and abdomen were done according to the schedule; ECG was conducted before the first MSC infusion, at 4, 12, and 24 weeks (the cessation of the study) from the first MSC infusion. Chest X-ray was performed before the first MSC infusion, at 4 and 24 weeks. CT was conducted before the first MSC infusion and at 24 weeks. Vital signs, including percutaneous oxygen saturation concentration, were measured before and after each MSC infusion.

Long-term follow-up

After completing JR-031-201, a long-term follow-up study, JR-031-202, was conducted. The observation period was from the week following the end of JR-031-201, i.e., 25 weeks after the first MSC infusion, to 96 weeks (2 years). Informed consent was obtained from each patient. Evaluated valuables included adverse effects associated with MSC therapy such as the status of aGVHD, development of chronic GVHD (cGVHD), disease relapse, ectopic tissue formation, and so on.

Statistical analysis

Survival was described as time from the first MSC infusion and calculated by the Kaplan–Meier method.

Results

Table 1 shows the characteristics of the patients who received MSCs. The median age was 52 years (range 4–62 years). Thirteen patients were adults, while only one was a child. All patients had hematological malignancies as follows: acute myeloid leukemia, 4 patients; acute lymphoblastic leukemia, 3; myelodysplastic syndrome, 3; chronic lymphocytic leukemia, 1; follicular lymphoma, 1; multiple myeloma, 1; and juvenile myelomonocytic leukemia, 1. Of these, 2 patients (no. 4 and 10) had MLL-related leukemia due to chemotherapy for breast cancer. Breast cancer in both patients was in complete remission before AlloHSCT. No patients with refractory disease to chemotherapy were included. Most patients received a transplant from HLA-mismatched unrelated donors after myeloablative or non-myeloablative conditioning. The source of hematopoietic stem cells for transplantation was bone marrow (9 patients), peripheral blood stem cells (1), and cord blood (4). HLA disparity was shown in the eight pairs. All except 3 patients received a combination of a calcineurin inhibitor and methotrexate as GVHD prophylaxis. All patients were first given either prednisolone or methylprednisolone to treat acute GVHD.

Table 2 shows the aGVHD severity and organ involvement before the first MSC infusion and response to aGVHD. The grade of aGVHD was grade II (9 patients) and III (5 patients). Grade IV aGVHD was not enrolled. The most affected organs were the skin (9 patients) and gut (10 patients). Seven patients had two involved organs. MSCs were first infused on the median 47 days after AlloHSCT. The median number of MSC infusions was eight. By 4 weeks after the first MSC infusion, 8 and 5 patients had achieved CR and PR, respectively. The OR rate was 92.9 % (13 of 14 patients). By 24 weeks, 4 of 5 patients with PR achieved CR. At 96 weeks, 8 patients were alive and in CR. As shown in Fig. 1, the estimated time to reach 50 % CR after the first MSC infusion was 3 weeks (MSC infusion six times). There was no difference in the time to reach CR between grade II and grade III aGVHD (data not shown). Relapse of aGVHD after MSC therapy occurred in one patient (no. 12). At 78 days after the first MSC infusion, he was admitted again because of bloody diarrhea. Endoscopic biopsy showed aGVHD in the cecum and colon. The patient was put on parenteral hyperalimentation with tacrolimus administration. His aGVHD gradually disappeared.

By the end of the follow-up (2 years), 6 patients had died (Table 2). Five of the 6 patients (no. 2, 3, 4, 9, and 10) died due to factors not directly related to aGVHD as follows: no. 9 patient, veno-occlusive disease on day 25; no. 7, hepatic failure on day 36; no. 2, pneumonia on day 82; no. 3 and 10, disease relapse on days 191 and 696, respectively; and no. 4, metastatic breast cancer on day

Table 1 Patient characteristics

Case no.	Age	Sex	Disease	HSCT				GVHD prophylaxis	First line therapy for acute GVHD
				Source	Donor	HLA disparity	Conditioning		
1	56	F	MDS/RCMD	BM	Unrelated	7/8	Myeloab	CyA + sMTX	mPSL
2	59	F	AML/2nd CR	BM	Unrelated	8/8	Myeloab	CyA	mPSL
3	44	M	AML/1st CR	BM	Unrelated	7/8	Myeloab	FK506	mPSL
4	36	F	ALL/2nd Rel	BM	Unrelated	6/8	Myeloab	FK506	mPSL
5	57	F	FL	PB	Sibling	8/8	Myeloab	CyA + sMTX	PSL
6	42	F	ALL/1st CR	BM	Unrelated	8/8	Myeloabl	FK506 + sMTX	PSL
7	29	M	MDS/RAEB	CB	Unrelated	4/8	Myeloab	FK506 + sMTX	mPSL
8	62	F	CLL/PR	CB	Unrelated	5/8	Non-myeloab	FK506 + sMTX	mPSL
9	55	M	ALL/1st CR	BM	Unrelated	8/8	Myeloab	CyA + sMTX	mPSL
10	49	F	AML/1st CR	BM	Unrelated	6/8	Myeloab	FK506 + sMTX	mPSL
11	4	M	JMML/1st CP	CB	Unrelated	5/6	Myeloab	CyA + sMTX	PSL
12	61	M	AML-MRC[a]	CB	Unrelated	4/6	Myeloab	FK506 + MMF	PSL
13	35	F	MM/1st CR	BM	Unrelated	8/8	Non-myeloab	FK506 + sMTX	PSL
14	61	F	MDS/RAEB	BM	Sibling	6/6	Non-myeloab	CyA + sMTX	PSL

HSCT hemopoietic stem cell transplantation, *GVHD* graft-versus-host disease, *F* female, *M* male, *MDS* myelodysplastic syndrome, *RCMD* refractory cytopenia with multilineage dysplasia, *AML* acute myeloid leukemia, *AML-MRC* acute myeloid leukemia with myelodysplasia-related changes, *ALL* acute lymphoblastic leukemia, *FL* follicular lymphoma, *RAEB* refractory anemia with excess of blasts, *CLL* chronic lymphocytic leukemia, *MM* multiple myeloma, *CR* complete remission, *PR* partial remission, *Rel* relapse, *BM* bone marrow, *PB* peripheral blood stem cell, *CB* cord blood, *Myeloab* myeloablative, *Non-myeloab* non-myeloablative, *CyA* cyclosporine, *FK506* tacrolimus, *sMTX* short-term methotrexate, *PSL* prednisolone, *mPSL* methylprednisolone

[a] No chemotherapy before HSCT

Table 2 GVHD and outcome

Case	GVHD				1st MSC infusion	No. of MSC infusions	Response		Survival/ death	Cause of death (days)
	Grade	Skin	Liver	Gut	Days after HSCT		By 4 weeks	By 24 weeks	At 96 weeks	
1	II	3	0	0	38	8	PR	PR	Alive in CR	N/A
2	II	1	0	1	50	10	PR	CR	Dead	Pneumonia (82)
3	II	3	0	0	43	12	PR	CR	Dead	Relapse (696)
4	III	1	0	2	51	12	CR	CR	Dead	Breast cancer (244)
5	II	3	0	1	46	8	CR	CR	Alive in CR	N/A
6	II	3	0	0	48	8	CR	CR	Alive in CR	N/A
7	III	0	1	2	33	5	PG	–	Dead	IC (36)
8	III	3	0	3	57	12	PR	CR	Alive in CR	N/A
9	II	1	0	1	38	3	CR	CR	Dead	VOD (25)
10	II	0	0	1	45	8	CR	CR	Dead	Relapse (191)
11	III	0	2	4	78	12	PR	CR	Alive in CR	N/A
12	II	0	1	0	52	8	CR	CR	Alive in CR	N/A
13	III	3	0	4	44	8	CR	CR	Alive in CR	N/A
14	II	0	0	1	108	7	CR	CR	Alive in CR	N/A

GVHD graft-versus-host disease, *MSC* mesenchymal stem cell, *HSCT* hemopoietic stem cell transplantation, *CR* complete response, *PR* partial response, *PG* progression, *VOD* veno-occlusive disease, *IC* ischemic cholangiopathy, *N/A* not applicable

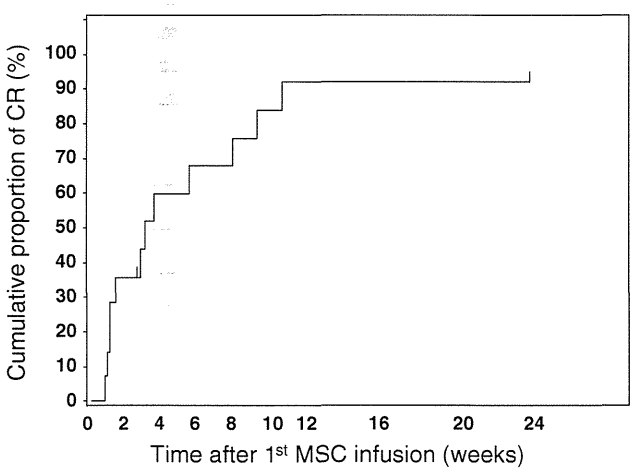

Fig. 1 Time to achieve complete response

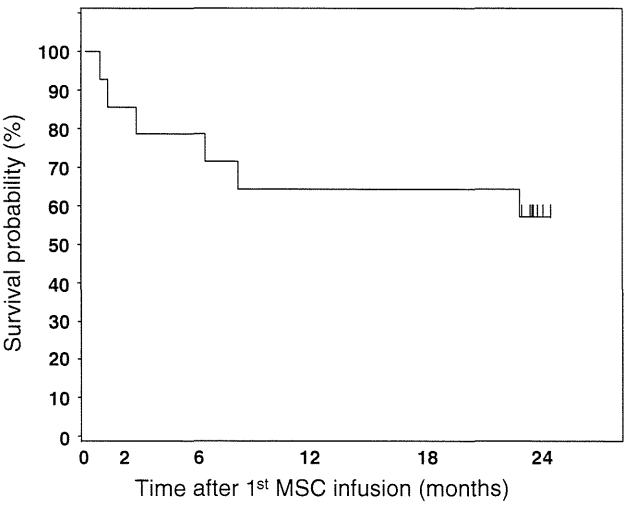

Fig. 2 Overall survival

244. The no. 2 patient showed pancytopenia caused by ganciclovir treatment for CMV antigenemia and died of pneumonia with massive pleural effusion. Autopsy findings showed pulmonary aspergillosis. Two patients with acute myeloid leukemia (no. 3 and 10) relapsed; the former received a second bone marrow transplant from another donor, but he died of sepsis. The latter died of septic shock after chemotherapy for disease relapse. Patient no. 4 maintained CR after MSC therapy, but a scheduled CT scan incidentally showed multiple low-density areas in both liver lobes on day 145. A liver biopsy demonstrated adenocarcinoma, leading to the suspicion of liver metastasis of breast cancer. Bone scintigraphy showed multiple isotope uptake regions in vertebrae and pelvic bone. The patient was diagnosed with recurrent breast cancer in the liver and bone and died of breast cancer. Patient no. 7 was evaluated as having PG of aGVHD. Serum liver enzyme levels and bilirubin values progressively worsened, leading to death. Necropsy of the liver showed ischemic cholangiopathy characterized by massive hepatocyte necrosis, marked congestion in the bile ducts, hyaline degeneration in the arterioles, disappearance of endothelial cells in the arterioles, and slight infiltration of lymphocytes. OS is shown in Fig. 2. There was no

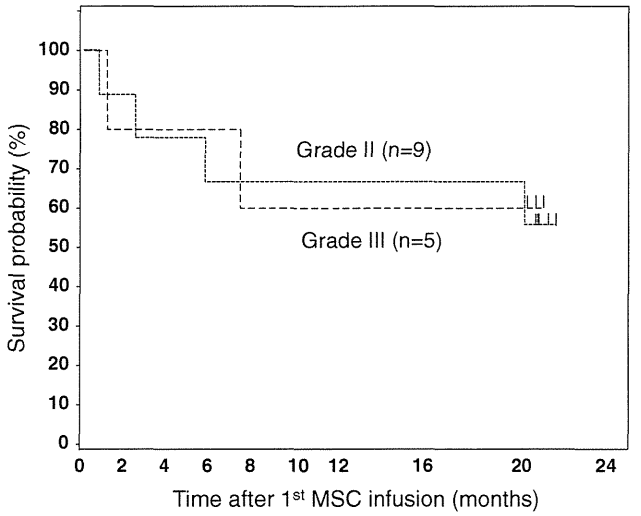

Fig. 3 Overall survival in the category of grade II and III acute GVHD. There was no difference between the two groups

difference in survival between groups of patients with grade II and grade III aGVHD (Fig. 3).

Adverse effects associated with MSC therapy were monitored. No infusion toxicity such as fever or decrease in oxygen saturation was observed. In the JCR-031-201 study, 27 events of infection episodes in 13 patients were collected as follows: bacteremia (3 events, 3 patients), pneumonia (4, 3), herpes zoster (3, 2), oral candidiasis (2, 2), infectious enterocolitis (2, 2), CMV antigenemia (2, 2), sepsis (1, 1), CMV colitis (1, 1), hemorrhagic cystitis (1, 1), and others (7, 7). In the JCR-031-202 study, 28 events in 9 patients were collected as follows: pneumonia (3 events, 3 patients), septic shock (3, 2), sinusitis (2, 2), upper respiratory tract infection (2, 2), oral herpes (4, 2), herpes zoster (1, 1), varicella (1, 1), infectious enterocolitis (1, 1), CMV antigenemia (1, 1), and others (8, 8). Ectopic tissue formation was not detected by scheduled CT scans. cGVHD developed in 7 patients; 4 patients (no. 8 at 24 weeks, 11 at 24 weeks, 12 at 48 weeks, and 14 at 36 weeks) with a limited form, and 3 patients (no. 1 at 24 weeks, 3 at 24 weeks, and 6 at 24 weeks) with an extensive form, of cGVHD.

Discussion

Reports of bone marrow MSCs used for steroid-refractory aGVHD are divided into two approaches; one approach used MSCs produced in the institution where patients were scheduled to receive the cells, while the another used MSCs manufactured in a company. In the former, the largest study was a phase II study conducted by the European Group for Blood and Bone Marrow [11]. Fifty-five patients with a median age of 22 years received MSCs for steroid-refractory aGVHD. Most patients had grade III or IV aGVHD. MSC donors were either HLA-identical, haploidentical, or HLA-mismatched unrelated donors. The median time from aGVHD onset to the first MSC infusion was 25 days. Of note, 33 patients had already received second-line therapy for aGVHD before MSC administration. Most patients received MSCs at a median dose of 1.4×10^6 MSCs/kg once or twice. The overall response rate was 71 % (CR, 30; PR, 9 patients). Twenty-four of these responders received MSCs from third-party donors. The overall estimated 2-year survival in this trial was 35 % and was significantly better in complete responders (53 %) versus non-complete responders (16 %). There was a better trend for 2-year estimated survival in the pediatric population compared to adults. No severe–adverse effects associated with MSC infusions were reported. Except for the report from the European Group for Blood and Bone Marrow, other reports were small-sized clinical studies including a phase I or a phase I/II study to treat steroid-refractory aGVHD with MSCs [7–10, 13–16, 18–20]. Importantly, in all of these studies, any second- or third-line immunosuppressive agent in combination with MSCs was allowed. Therefore, it is difficult to exactly evaluate the effects of MSCs on steroid-refractory aGVHD.

MSCs manufactured by Osiris, Prochymal, were given to steroid-refractory GVHD patients. Kebriaei et al. compared a dose of 2×10^8 Prochymal cells/kg with 8×10^8 Prochymal cells/kg in combination with steroids to treat patients with de novo aGVHD. Thirty-one patients were evaluated: there was no difference between the two groups in terms of safety and efficacy [12]. Prasad et al. [17] showed the efficacy of Prochymal for pediatric patients with severe refractory aGVHD. Most patients received Prochymal at a dose of 2×10^8 cells/kg. Following positive results in these two studies, Osiris conducted a phase III trial investigating Prochymal for steroid-refractory aGVHD across transplant centers in the United States, Canada, and Australia [23]. This was a double-blind placebo-controlled study. Patients were randomized at a 2:1 ratio for either Prochymal or the placebo. The dose of Prochymal was 2×10^8 cells/kg. Of note, most patients had already received a second-line therapy before MSC therapy. This trial enrolled 260 patients. The primary endpoint was durable CR for 28 days. The preliminary analysis did not show a statistical difference between Prochymal and the placebo for the primary endpoint (Prochymal 35 % versus placebo 30 %). However, subpopulation analysis showed that Prochymal significantly improved the response in liver aGVHD (76 versus 47 %) and gastrointestinal aGVHD (82 versus 68 %). Infection rates were not different between the two groups. Rates of severe–adverse effects associated with MSC administration were not different in the two arms. Now, Prochymal is approved for use