from other sources, have distinct capacity for self-renewal while maintaining their multipotency, *i.e.*, the ability to differentiate into adipocytes, osteocytes, chondrocytes, neurons and hepatocytes, although some differentiation abilities are known to be partial[11-13]. Moreover, UC-MSCs have also attracted great interest because of their immunomodulatory properties. Nowadays, UC-MSCs are proposed as a possible versatile tool for regenerative medicine and immunotherapy.

HISTORY OF UC-MSCs

During pregnancy, the fetus and mother are connected by UC. UC prevents umbilical vessels from compression, torsion and bending, while providing good blood circulation. McElreavey *et al*[4] for the first time reported isolation of fibroblast-like cells from WJ of human UC in 1991. The UC-derived cells have the similar surface phenotype, plastic adherence and multipotency as those of MSCs derived from other sources. It was 3 years earlier that the first cord blood (CB) transplantation was performed in France in 1988[14]. After that, together with the development of CB banking, CB transplantation has become the alternative source of hematopoietic stem cells. Although CB-derived MSCs cannot be consistently isolated[15-18], MSCs were considered to circulate in the blood of preterm fetuses and able to be successfully isolated and expanded[3]. Where these cells home at the end of gestation is not clear[13]. Thus, UC has inevitably become a focus of attention as a source of MSCs because it contains CB[18]. One key study appeared concerning CB-derived MSCs appeared around 2003[19]. Mitchell *et al*[20] successfully isolated matrix cells from UC WJ using explant culture and Romanov *et al*[19] isolated MSCs-like cells from the subendothelial layer of UCV.

ADVANTAGES OF UC-MSCs

Stem cell populations can be isolated from embryonic, fetal and adult tissues. Embryonic stem cells (ESCs) are a leading candidate for tissue engineering because of their high self-renewal capacity and pluripotency (ability to differentiate into all germ layers) *in vitro* and *in vivo*. Nonetheless, in addition to ethical restrictions, the clinical applications of ESCs are severely limited by technical difficulties with the depletion of immature cells that may result in the formation of a teratoma.

In contrast, adult stem cells, such as those in the skin, bone marrow (BM) and adipose tissue, may have wider clinical applications. BM-MSCs have been used for autologous and allogeneic purposes. Recently, successful clinical application of autologous BM-MSCs was reported for conditions such as cardiac infarction[21], graft-versus-host disease (GVHD)[22,23], Crohn's disease[24] and bone tissue engineering[25]. On the other hand, the autologous use was sometimes limited by cell numbers and age-related changes such as decreased growth and differentiation capacity[26,27].

Compared with BM-MSCs and ES cells, UC-MSCs show a gene expression profile more similar to that of ESCs and faster self-renewal rather than BM-MSCs[11,12].

It is easy to obtain a substantial number of UC-MSCs after several passages and extensive *ex vivo* expansion[28]. The most appreciable advantage is that the collection procedure is noninvasive and ethically acceptable.

Similar to BM-MSCs, UC-MSCs can be considered for autologous and allogeneic use. Autologous UC-MSCs might be used as gene therapy for genetic diseases and as regenerative or anti-inflammatory therapy for neonatal injury, such as cerebral palsy or hypoxic brain damage. On the other hand, allogeneic UC-MSCs can be expanded and cryopreserved in a cell bank for patients in need. The only disadvantage is that physicians need to confirm the baby's health as a donor because it cannot be ascertained in advance whether the donor will grow normally without health problems; thus, genomic or chromosomal tests need to be performed. In contrast, in the case of a BM donor, the physician can directly see and examine the donor and then decide to collect BM. In the case of CB banking, many CB banks monitor the baby's health after birth. Thus, it is important to know the advantages and disadvantages of UC-MSCs for each clinical application.

DIFFERENT METHODS FOR ISOLATION OF MSCs FROM DIFFERENT COMPARTMENTS OF UC

There are two methods to obtain MSCs from various UC compartments or from the whole UC: the explant method and the enzymatic digestion method.

The explant method

UC, or its compartments, is manually minced into small fragments 1-2 mm^3. These fragments are aligned and seeded regularly on the tissue culture-treated dishes. After the tissue fragments are attached to the bottom of the dish, the culture medium is poured slowly and gently, so as not to detach the fragments, and the culture is started[29-31]. The culture medium is replaced every 3-7 d for 2-4 wk until fibroblast-like adherent cells reach 80%-90% confluence. The adherent cells and tissue fragments are rinsed once with PBS and detached using a trypsin solution, followed by washing with the medium. The cells and tissue fragments are filtered to remove the tissue fragments.

The disadvantage of this method is that the fragments often float in the medium, resulting in poor cell recovery. No MSCs can be obtained from the floating fragments. To collect a consistent number of MSCs each time, it is important to prevent the exfoliation of the tissue fragments from the bottom of plastic dishes.

The enzymatic digestion method

WJ is either directly exposed to enzymatic solutions to

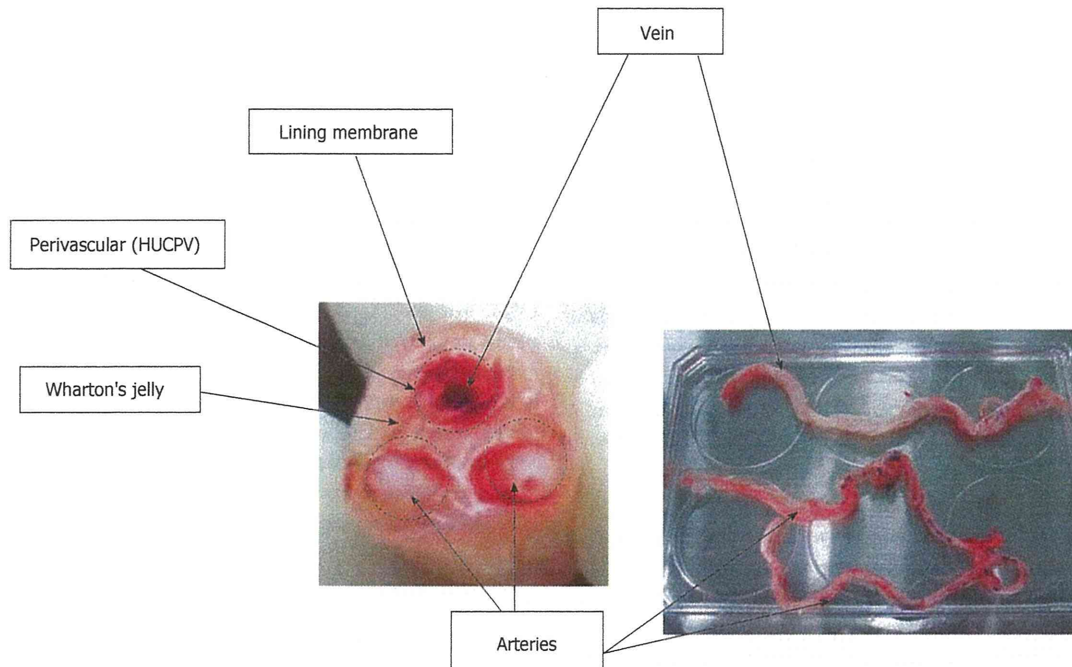

Figure 1 Various compartments of umbilical cord from which mesenchymal stem cells can be isolated. HUCPV: Human umbilical cord perivascular.

release the cells or it is cut into small pieces followed by enzymatic digestion. The enzymes used for digestion vary from simple collagenase[31,32] to a combination of either collagenase and hyaluronidase with or without trypsin[33,34] or collagenase, dispase II and hyaluronidase[33]. The digestion time and concentrations varied by researchers.

There are four compartments of UC as a source of MSCs: (1) Whole UC-MSCs: the whole UC is cut into smaller pieces followed by either an explant procedure or enzyme digestion[30,35,36]; (2) UCWJ-, UCA- and UCV-MSCs: UCWJ-MSCs are obtained after removing umbilical vessels. Umbilical vessels [two arteries (UCAs) and one vein (UCV)] can also be minced into 1-2 mm^3 fragments. The fragments are aligned regularly on the plate and cultured until MSCs start growing; (3) UC lining and subamnion-derived MSCs: the subamnion region of UC lining membrane is removed with a razor blade and then cut into small pieces. These fragments are plated in plastic culture dishes until MSCs start growing (explant method). With this method, however, it might be difficult to remove the adjacent region underneath the amniotic epithelium completely[37,38]; and (4) Human UC perivascular stem cells (HUCPVC): the vessels are extracted from UC and tied at both ends into loops. The loops are then placed into an enzymatic solution for a defined period of time to allow the cells to separate from the perivascular region. The detached cells are cultured and collected as HUCPVCs[26,28,39].

It is still controversial whether the isolation of the cells from the whole or some compartment is superior to others with respect to their proliferation ability, differentiation ability and immunosuppressive capacity.

Proliferation assays

The frequency of colony-forming unit fibroblasts (CFU-F) is significantly higher in whole UC-derived MSCs than in BM-MSCs with limiting dilution[26,30,40]. The authors first compared UCWJ-MSCs, UCA-MSCs and UCV-MSCs. UCV-MSCs exhibited a significantly higher frequency of CFU-F than UCWJ-MSCs and UCA-MSCs, but the doubling time was not different among these cell types[5]. The Mennan group also reported that there are no significant differences among the various compartments of UC, although the cells derived from any UC compartment proliferate significantly faster than BM-MSCs, with mean doubling times of 2-3 d at P0 through P3[41]. Depending on the purpose, researchers need to select either a compartment or the whole UC.

Biomarkers of UC-MSCs

The immunoprofile of UC-MSCs is analyzed using flow cytometry, according to the standard definitions for MSCs described by the position paper of the International Society for Cellular Therapy[42]. There are no single specific markers that can be used to identify multipotent MSCs. MSCs are positive for adhesion markers such as CD29 and CD44; mesenchymal markers such as CD90, CD73 and CD105; and human leukocyte antigen class I (HLA-ABC). MSCs are negative for endothelial cell marker CD31; hematopoietic cell markers such as CD34, CD45 and CD117; and human leukocyte differentiation antigen class II (HLA-DR)[43]. Among the different UC compartments, UCWJ-, UCV- and UCA-derived MSCs show a similar fibroblast-like spindle shape and the MSCs from these three types of tissues demonstrate no significant differences in the immunoprofile. These cells are positive for CD13, CD29 (integrin β1), CD73 (SH3), CD90 (Thy-1), CD105 (SH2; endogrin) and HLA-ABC at the cellular frequency greater than 90% and are negative for CD34,

CD45, CD133 and HLA-DR, with the cellular frequency less than 1%[5]. Mennan *et al*[41] also confirmed that MSC immunophenotypes showed no significant differences among different sources: BM, umbilical cord arteries, vein, WJ and UC lining membrane. Even although the authors could not find any major differences in their immunophenotypes, the cell populations derived from the different compartments may consist of different proportions of multipotent stem cells. Karahuseyinoglu *et al*[44] demonstrated that CD73 is expressed throughout the vessels and endothelium and is absent in the perivascular region, but the strongest expression is observed in the epithelial and subepithelial regions of WJ. CD90 is positive in most compartments but negative in the endothelial lumen lining. A high expression of vimentin, CKs (1, 4, 5, 6, 8, 10, 13, 18 and 19), desmin and SMA has been detected in the subamniotic layer and the perivascular region. Schugar *et al*[45] reported that CD146 (endothelial progenitor marker) is expressed in the vessel walls (100%) and the perivascular region of UC (62%) but is no longer expressed in UCWJ-MSCs[26,46]. These markers might aid in determining the multipotency of the isolated cell population. Phenotypic characterization of UC-MSCs might be influenced by the culture passage number, culture medium and culture method.

Furthermore, ESC markers such as Oct4, Nanog, Sox-2 and KLF4 are expressed only at low levels in UC-MSCs[47]. This suggests that MSCs are primitive stem cells, somewhere between ESCs and mature adult stem cells. Nonetheless, a precise isolation of pluripotent MSCs using specific markers remains a challenge.

The role of SSEA3 and SSEA4 in MSCs remains controversial. Gang *et al*[48] reported that SSEA4$^+$ cells proliferate predominantly when the culture is initiated from primary BM cells in the medium supplemented with special cocktails of cytokines. In contrast, the authors failed to reproduce the same phenomena in UCWJ-MSCs in the medium consisting of α-MEM and 10% FBS. Furthermore, SSEA4 expression in UCWJ-MSCs significantly correlates with the FBS concentration in the culture medium, whereas SSEA3 expression was inversely correlated. We concluded that SSEA4 in UCWJ-MSCs is not a marker of either proliferation capacity or multipotency[31]. Schrobback *et al*[49] assessed SSEA4 expression in human articular chondrocytes, osteoblasts and BM-derived MSCs and characterized their differentiation potential. Their results showed that SSEA4 levels in these cells are not related to the capacity for chondrogenic and osteogenic differentiation and the proliferation potential *in vitro*[49].

THE ABILITY OF UC-MSCs TO DIFFERENTIATE INTO ADIPOGENIC, CHONDROGENIC AND OSTEOGENIC LINEAGES

UC-MSCs originating from the extraembryonic mesoderm and their capacity for differentiation into adipogenic, chondrogenic and osteogenic lineages have been extensively studied[50]. Regarding the osteogenic differentiation ability, Hsieh *et al*[11] demonstrated that the gene profiles of UC-MSCs are close to ESCs; UC-MSCs show delayed and insufficient differentiation into osteocytes. On the other hand, BM-MSCs have already expressed an osteogenic gene profile and can easily differentiate into osteocytes. Among the three compartments, UCWJ, UCV and UCAs, UCWJ-MSCs demonstrate an obviously defective ability to differentiate into osteocytes, even although the expression of osteocyte-related genes is detected by reverse-transcriptase PCR, at levels similar to those in the other two tissues/compartments[5]. Mennan *et al*[41] compared the osteogenic differentiation among cord regions in six samples and found that the best differentiation is seen with UCWJ-MSCs and whole UC-derived MSCs, rather than with UCA-, UCV- and UC lining MSCs.

As for adipocytic differentiation, Mennan *et al*[41] reported that UC-MSCs produce small lipid vacuoles, whereas BM-MSCs produce more mature adipocytes (unilocular lipid vacuoles). UC-MSCs might maintain their multipotency for longer periods than BM-MSCs can[51], although there were no obvious differences among MSCs derived from UC compartments in our research[5].

With respect to chondrogenic differentiation, UC-MSCs show no apparent differences among the different cord regions (sources)[41]. Moreover, the comparison of the chondrogenic potential between BM-MSCs and UC-MSCs revealed that UC-MSCs produce thrice as much collagen as BM-MSCs; this finding indicates that the former may be a better option for fibrocartilage tissue engineering[52].

In relation to other differentiation abilities, UCWJ-MSCs are the most studied cell type among various UC compartments and many papers have been published[53,54]. In addition to differentiating into osteocytes, chondrocytes and adipocytes, UCWJ-MSCs can differentiate into cardiomyocytes (with the gene expression of N-cadherin and cardiac troponin I [55]), neurons and glia[20], oligodendrocytes[56] and hepatocytes[57]. Recently, clinical trials have been conducted using UC-MSCs for neurogenic disorders (spinocerebellar ataxia and multiple system atrophy of the cerebellar type)[58] and liver disorders[59,60].

IMMUNOSUPPRESSIVE PROPERTIES OF UC-MSCs

Immunosuppressive effects have now become the most popular property of MSCs for potential clinical use. First, MSCs themselves are weakly immunogenic owing to the lack of HLA-DR and low expression of MHC class I molecules. MSCs have been shown to have immunomodulatory properties *in vitro*[61]. Furthermore, MSCs lack both CD80 and CD86 proteins[36,62], which

are costimulatory molecules inducing T cell activation and survival. The lack of HLA-DR, CD80 and CD86 suggests that MSCs do not elicit acute rejection and are suitable for allogeneic cell-based therapy.

Second, UC-MSCs have immunosuppressive properties *in vitro* and *in vivo*. Many studies have been published about the immunosuppressive effect of UCWJ-MSCs[63], UC lining-MSCs[37,64], HUCPV[65] and whole UC-derived MSCs[66]. The immunosuppressive effect of UC-MSCs is mediated by soluble factors and cell-to-cell contacts. PGE2, galectin-1 and HLA-G5 are released from MSCs and serve as effective factors of immunosuppression[67]. Among these factors, indoleamine 2,3-dioxygenase (IDO) is one of the most relevant because it is inducible by IFN-γ and catalyzes conversion from tryptophan to kynurenine[62,68]. This depletion of tryptophan from the environment can suppress T cell proliferation. UCWJ-MSC-mediated immunosuppression may require preliminary activation by proinflammatory cytokines, such as IFN-γ, with or without TNF-α, IL-1α or IL-1β.

It was recently suggested that the inflammatory environment produced by the upregulation of cytokines such as IFN-γ and TNF-α might alter the biological activity of MSCs from immunosuppression to immunostimulation[68]. In this case, UC-MSCs maynot prevent GVHD *in vivo*. It is known that upon stimulation by activated immune cells or cytokines (priming), MSCs are primed and become functional immunosuppressors. The extent of immunosuppression is greater with UCWJ-MSCs than with BM-MSCs[62]. Polchert *et al*[68] demonstrated that MSCs primed with IFN-γ are effective in a mouse GVHD model despite upregulated MHC class II molecules. In order to ensure the effective and safe therapeutic use of UC-MSCs, more *in vivo* experiments need to be conducted because of the many discrepancies with *in vitro* data.

CONCLUSIONS

Compared with the counterparts of other origins, UC-MSCs have attractive advantages as MSCs and as UC-derived cells: (1) a noninvasive collection procedure for autologous or allogeneic use; (2) a lower risk of infection; (3) a low risk of teratoma; (4) multipotency; and (5) low immunogenicity with a good immunosuppressive ability. It is still unclear which compartment in UC is the best for clinical use; nonetheless, the era of the clinical use of UC-MSCs is approaching quickly.

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ORIGINAL ARTICLE

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

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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.

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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)
⩾2	16 (23)
Time from diagnosis to transplant, evaluable n	80
<1 Years	33 (41)
1–2 Years	16 (20)
⩾2 Years	31 (39)
Median (range), years	1.5 (0.1–21.0)
Frequency of RBC transfusion before transplant, evaluable n	51
⩽9	26 (51)
10–19	8 (16)
⩾20	17 (33)
Frequency of PLT transfusion before transplant, evaluable n	51
⩽9	38 (74)
10–19	4 (8)
⩾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.

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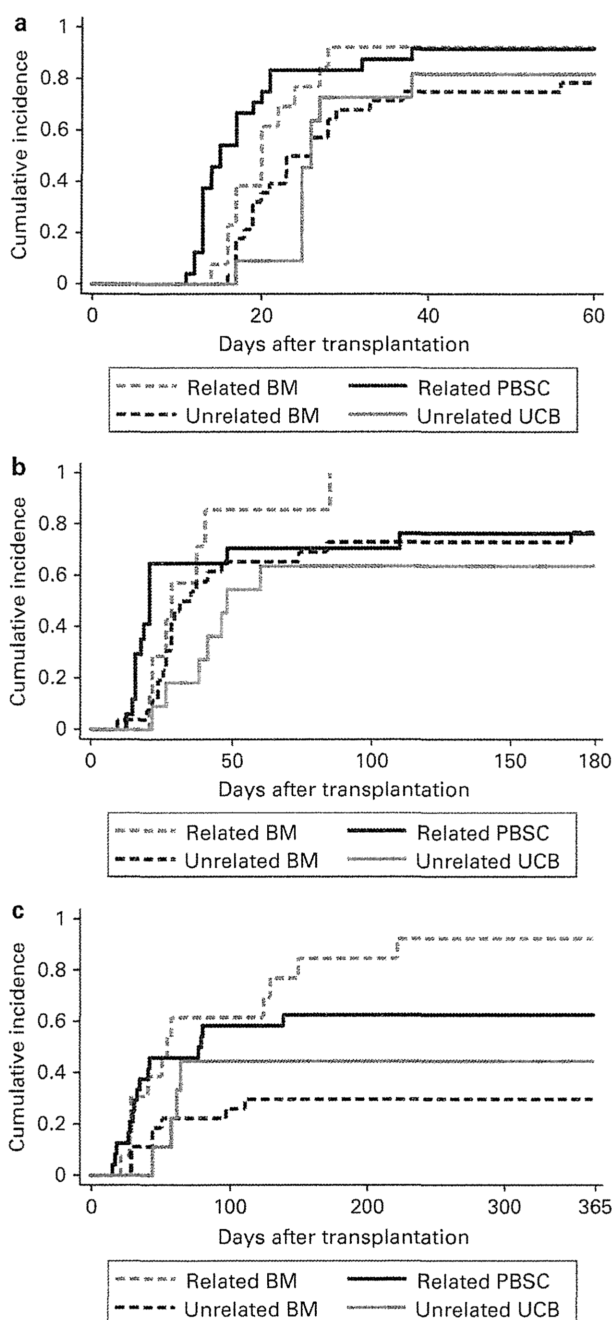

Figure 1. Hematopoietic recoveries after transplantation in PMF patients. (**a**) Cumulative incidences of neutrophil recovery after related BM (gray and dash line), related PBSC (black and solid line), unrelated BM (black and dash line) and unrelated UCB (gray and solid line) transplantations are shown.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

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