Table 1. Characteristics of Ph(−) ALL patients undergoing myeloablative allo-SCT, according to the disease status

	CR1							Subsequent CR		non-CR	
	Related		Unrelated BM		Cord blood		*P*				
No. of patients	388	(%)	434	(%)	95	(%)		300	(%)	509	(%)
Median white blood cell (WBC) count at diagnosis /μl (range)	11 400 (400–801 000)		13 235 (500–892 000)		10 400 (400–771 300)		0.48	9000 (500–706 000)		19 400 (500–840 200)	
Median patient age at allo-SCT, year (range)	31 (16–66)		34 (16–59)		37 (16–70)		0.01	25 (16–65)		30 (16–65)	
Patient sex, *n* (%)							0.47				
Male	207	53	240	55	46	48		166	55	295	58
Female	181	47	194	45	49	52		134	45	214	42
Lineage							<0.0001				
T	66	17	74	17	17	18		53	18	118	23
B	276	71	325	75	57	60		226	75	346	68
Other	28	7	17	4	17	18		12	4	35	7
Unknown	18	5	18	4	4	4		9	3	10	2
Cytogenetics							0.65				
Normal	226	58	255	59	53	56		195	65	243	48
t(4;11)	9	2	12	3	4	4		2	1	20	4
t(8;14)	4	1	3	1	0	0					
Hypodiploid	3	1	3	1	2	2		3	1	6	1
Hyperdiploid	11	3	15	3	0	0		15	5	26	5
Other [no *t*(9;22)]	135	35	146	34	36	38		85	28	214	42
JALSG risk stratification							0.09				
Low	112	29	96	22	22	23		121	40	149	29
Intermediate	213	55	253	58	57	60		151	50	227	45
High	54	14	84	19	16	17		23	8	116	23
Unknown	9	2	1	0	0	0		5	2	17	3
Source											
Related								89	30	207	41
Unrelated BM								158	53	217	43
Cord blood								53	18	85	17
HLA matching							<0.0001				
Well matched	351	90	278	64	4	4		153	51	246	48
Partially matched	27	7	102	24	71	75		93	31	168	33
Mismatched	10	3	54	12	20	21		54	18	95	19
Time from diagnosis to transplantation, month (range)	6 (2–64)		9 (3–48)		6 (2–16)		<0.0001	23 (3–272)		10 (1–261)	
<6	189	49	35	8	40	42		13	4	88	17
6 ≤, <10	150	39	222	51	44	46		40	13	159	31
10≤	49	13	177	41	11	12		247	82	262	51
Preparative regimen							<0.0001				
CY + TBI (8–13.2 Gy)	216	56	228	53	36	38		114	38	145	28
CA + CY + TBI	58	15	88	20	27	28		58	19	125	25
Other TBI (8–13.2 Gy) regimens	89	23	105	24	28	29		112	37	203	40
BU + CY	17	4	13	3	3	3		9	3	15	3
Other non-TBI regimens	8	2	0	0	1	1		7	2	21	4
GVHD prophylaxis							<0.0001				
Cyclosporine A ± other	338	87	172	40	59	62		157	52	250	49
Tacrolimus ± other	40	10	253	58	36	38		135	45	245	48
Other	10	3	9	2	0	0		8	3	14	3
Year of allo-SCT							<0.0001				
1998–2004	203	52	229	53	27	28		181	60	259	51
2005–2009	185	48	205	47	68	72		119	40	250	49

CR, complete remission; PIF, primary induction failure; BM, bone marrow; allo-SCT, allogeneic stem cell transplantation; CY, cyclophosphamide; TBI, total body irradiation; CA, cytarabine; BU, busulfan; GVHD, graft-versus-host disease.

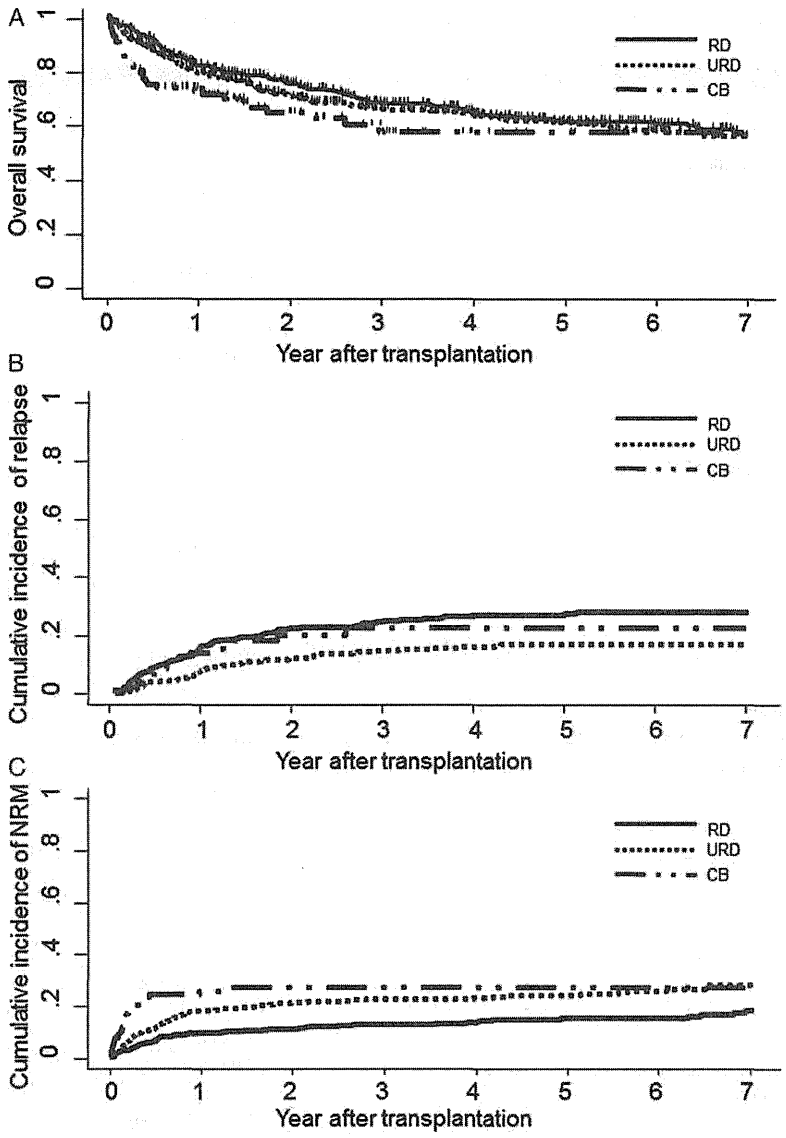

Figure 1. Transplant outcomes by a donor source for 917 Ph(−) ALL patients who underwent myeloablative allo-SCT in CR1. (A) Overall survival; (B) cumulative incidence of relapse; (C) cumulative incidence of non-relapse mortality (NRM). RD, related donor; URD, unrelated donor; CB, cord blood.

0.83–1.44), $P = 0.53$; CB: HR 1.20 (95% CI 0.76–1.89), $P = 0.43$ (versus RD)]. Among CB recipients in CR1, only age at allo-SCT (≥45 years) was a significant prognostic factor in multivariate analysis [HR 2.89 (95% CI 1.43–5.81), $P = 0.003$]. CD34-positive cell dose was not a significant prognostic factor. Subgroup analyses of CR1 patients younger than 45 years showed that OS after CB allo-SCT ($N = 65$) was significantly better than that after mismatched URD allo-SCT ($N = 47$) in CR1 (68% versus 49% at 4 years, $P = 0.04$).

relapse and NRM among patients transplanted in CR1

The cumulative incidence of relapse was significantly lower in patients who underwent URD allo-SCT than in those who underwent RD or CB allo-SCT (25% in RD, 17% in URD, and 22% in CB at 3 years; $P = 0.02$) (Figure 1B). The results of multivariate analysis showed that abnormal cytogenetics, non-TBI preparative regimens, and RD were significant risk factors for relapse (Table 2).

The cumulative incidence of NRM was significantly lower in patients who underwent RD allo-SCT than in those who underwent URD or CB allo-SCT (13% in RD, 23% in URD, and 27% in CB at 3 years; $P = 0.0001$) (Figure 1C). The results of multivariate analysis showed that ≥45 years of age at allo-SCT, JALSG intermediate or high risk, HLA partially matched or mismatched, non-TBI preparative regimens, transplantation between 1998 and 2004, <6 or ≥10 months from diagnosis to allo-SCT, and URD were significant risk factors for NRM (Table 2).

Table 2. Outcomes for adult Ph(−) ALL patients undergoing myeloablative allo-SCT in CR1: multivariate analyses ($N = 917$)

Covariates	Relative risk (95% CI)	P
Overall survival		
Favorable		
Year of allo-SCT (versus 1998–2004)		
2005–2009	0.75 (0.59–0.95)	0.02
Unfavorable		
Age at allo-SCT, year (versus 16≤, <45)		
45	1.73 (1.33–2.25)	<0.0001
JALSG risk (versus low)		
Intermediate	1.36 (1.01–1.82)	0.04
High	1.54 (1.06–2.22)	0.02
HLA (versus well matched)		
Partially matched	1.55 (1.87–2.02)	0.001
Mismatched	1.60 (1.11–2.31)	0.01
Conditioning (versus TBI regimens)		
Non-TBI regimens	2.02 (1.35–3.03)	0.001
Interval from diagnosis to allo-SCT, month (versus 6≤, <10)		
<6	1.42 (1.09–1.85)	0.009
Relapse		
Favorable		
Source (versus related)		
Unrelated BM	0.61 (0.44–0.84)	0.002
Unfavorable		
Cytogenetics (versus normal)		
Others [no hypodiploid, $t(4;11)$, $t(8;14)$, nor $t(9;22)$]	1.42 (1.05–1.93)	0.02
Conditioning (versus TBI regimens)		
Non-TBI regimens	1.90 (1.05–3.42)	0.03
Non-relapse mortality		
Favorable		
Year of allo-SCT (versus 1998–2004)		
2005–2009	0.60 (0.43–0.83)	0.002
Unfavorable		
Age at allo-SCT, year (versus 16≤, <45)		
≤45	2.46 (1.78–3.39)	<0.0001
JALSG risk (versus low)		
Intermediate	1.82 (1.16–2.86)	0.009
High	2.00 (1.36–3.40)	0.01
HLA (versus Well matched)		
Partially matched	1.81 (1.24–2.66)	0.002
Mismatched	2.13 (1.36–3.34)	0.001
Conditioning (versus TBI regimens)		
Non-TBI regimens	2.10 (1.21–3.64)	0.009
Interval from diagnosis to allo-SCT, month (versus 6≤, <10)		
<6	1.76 (1.19–2.61)	0.005
10≤	1.43 (1.00–2.06)	0.048
Source (versus related)		
Unrelated BM	1.53 (1.04–2.24)	0.03
Cord blood	1.64 (0.93–2.87)	0.09

Ph(−), ALL indicates Philadelphia chromosome-negative acute lymphoblastic leukemia; allo-SCT, allogeneic stem cell transplantation; CR, complete remission; TBI, total body irradiation; BM, bone marrow.

causes of death among patients transplanted in CR1

The frequency of relapse was significantly higher in patients who underwent RD allo-SCT ($P = 0.001$). Infection, organ failure, GVHD, and interstitial pneumonia were the major causes of NRM, and the incidence of hemorrhage was significantly higher in patients who underwent CB allo-SCT ($P = 0.009$) (Table 3).

other outcomes of allo-SCT among patients transplanted in CR1

The cumulative incidence of neutrophil recovery or platelet recovery was significantly lower in patients who underwent CB allo-SCT (neutrophil recovery: 98% in RD, 98% in URD, and 72% in CB at day 100, $P < 0.0001$; platelet recovery: 95% in RD, 91% in URD, and 81% in CB at 1 year, $P < 0.0001$). Among CB

Table 3. Causes of death for adult Ph(−) ALL patients undergoing myeloablative allo-SCT in CR1 ($N = 917$)

	Related		Unrelated BM		Cord blood		
No. of patients	130	(%)	148	(%)	33	(%)	P
Relapse	59	45	37	25	8	24	0.001
Infection	17	13	26	18	8	24	0.26
Organ failure	17	13	23	16	1	3	0.15
GVHD	11	8	19	13	2	6	0.34
Interstitial pneumonia	8	6	15	10	4	12	0.38
Hemorrhage	3	2	7	5	5	15	0.009
TMA	7	5	3	2	2	6	0.27
ARDS	1	1	5	3	0	0	0.20
Graft failure	2	2	2	1	2	6	0.18
SOS	2	2	2	1	0	0	0.78
Secondary malignancy	1	1	0	0	0	0	0.50
Other	2	2	9	6	1	3	0.14

Ph(−), ALL indicates Philadelphia chromosome-negative acute lymphoblastic leukemia; allo-SCT, allogeneic stem cell transplantation; CR, complete remission; BM, bone marrow; GVHD, graft-versus-host disease; TMA, thrombotic microangiopathy; ARDS, acute respiratory distress syndrome; SOS, sinusoidal obstruction syndrome.

recipients, time to neutrophil engraftment was associated with a CD34-positive cell number ($<1 \times 10^5$/kg: day 22 versus $\geq 1 \times 10^5$/kg: day 19, $P = 0.02$).

The cumulative incidence of grade III–IV acute GVHD was significantly higher in patients who underwent URD allo-SCT (8% in RD, 18% in URD, and 11% in CB at day 100; $P = 0.008$).

Among assessable patients who survived at least 100 days after allo-SCT, no significant difference was observed between RD, URD, and CB allo-SCTs in the incidence of chronic GVHD (34% in RD, 38% in URD, and 31% in CB at 3 years; $P = 0.52$)

allo-SCT in subsequent CR

Although it was concluded from the results of a study by MRC/ECOG that RD allo-SCT in CR1 could achieve the best result [10], there is still plenty of room to discuss allo-SCT beyond CR1 for patients who could not find a suitable donor or maintain CR1. Among 300 patients transplanted in subsequent CR, there were no significant differences in OS between RD, URD, and CB allo-SCTs (47% in RD, 39% in URD, and 48% in CB at 4 years; $P = 0.33$). The results of multivariate analysis showed that JALSG intermediate- or high-risk and cytogenetic abnormalities [hypodiploid, $t(4;11)$ or $t(8;14)$] were significant risk factors for OS (Table 4). The donor source was not a significant risk factor [URD: HR 1.28 (95% CI 0.90–1.82), $P = 0.17$; CB: HR 1.01 (95% CI 0.61–1.67), $P = 0.97$ (versus RD)].

The cumulative incidence of relapse was not statistically different among patients who underwent RD, URD, and CB allo-SCTs (31% in RD, 26% in URD, and 29% in CB at 3 years; $P = 0.48$). The results of multivariate analysis showed

Table 4. Outcomes for adult Ph(−) ALL patients undergoing myeloablative allo-SCT in subsequent CR: multivariate analyses ($N = 300$)

Covariates	Relative risk (95%CI)	P
Overall survival		
JALSG risk		
Low	1.00	
Intermediate	1.45 (1.05–2.02)	0.03
High	1.92 (1.08–3.41)	0.03
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	2.48 (1.11–5.52)	0.03
Others [no $t(9;22)$]	1.24 (0.89–1.71)	0.20
Relapse		
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	4.13 (1.54–11.1)	0.005
Others [no t(9;22)]	1.29 (0.82–2.02)	0.27
Non-relapse mortality		
Age at allo-SCT, year		
16≤, <45	1.00	
≥45	1.82 (1.08–3.07)	0.02
JALSG risk		
Low	1.00	
Intermediate	1.52 (0.94–2.47)	0.09
High	2.39 (1.12–5.10)	0.02

Ph(−) ALL indicates Philadelphia chromosome-negative acute lymphoblastic leukemia; allo-SCT, allogeneic stem cell transplantation; CR, complete remission.

that cytogenetic abnormalities [hypodiploid, $t(4;11)$, or $t(8;14)$] were significant risk factors for relapse (Table 4).

Similarly, the cumulative incidence of NRM was not statistically different among patients who underwent RD, URD, and CB allo-SCTs (21% in RD, 36% in URD, and 27% in CB at 3 years; $P = 0.46$). The results of multivariate analysis showed that ≥45 years of age at allo-SCT and JALSG intermediate or high risk were significant risk factors for NRM (Table 4).

allo-SCT in non-CR

Among 509 patients transplanted in non-CR, there were no significant differences in OS among patients who underwent RD, URD, and CB allo-SCTs (15% in RD, 21% in URD, and 18% in CB at 4 years; $P = 0.20$). The results of multivariate analysis showed that ≥45 years of age at allo-SCT, cytogenetic abnormalities, HLA partially matched or mismatched, and non-TBI preparative regimens were significant risk factors for OS (Table 5). The donor source was not a significant risk factor [URD: HR 0.99 (95% CI 0.79–1.24), $P = 0.96$; CB: HR 1.09 (95% CI 0.78–1.53), $P = 0.61$ (versus RD)].

The cumulative incidence of relapse was not statistically different among patients who underwent RD, URD, and CB allo-SCTs (59% in RD, 42% in URD, and 58% in CB at 3 years; $P = 0.35$). However, the results of multivariate analysis showed that the donor source as well as cytogenetic abnormalities [hypodiploid, $t(4;11)$, or $t(8;14)$] and non-TBI

Table 5. Outcomes for adult Ph(−) ALL patients undergoing myeloablative allo-SCT in non-CR: multivariate analyses ($N = 509$)

Covariates	Relative risk (95% CI)	P
Overall survival		
Age at allo-SCT, year		
16≤, <45	1.00	
≥45	1.65 (1.30–2.11)	<0.0001
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	2.04 (1.38–3.03)	<0.0001
Others [no $t(9;22)$]	1.26 (1.02–1.55)	0.03
HLA		
Well matched	1.00	
Partially matched	1.44 (1.15–1.81)	0.002
Mismatched	1.37 (1.04–1.81)	0.02
Conditioning		
TBI regimens	1.00	
Non-TBI regimens	1.83 (1.28–2.62)	0.001
Relapse		
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	2.45 (1.52–3.97)	<0.0001
Others [no $t(9;22)$]	1.26 (0.97–1.62)	0.08
Conditioning		
TBI regimens	1.00	
Non-TBI regimens	1.84 (1.19–2.84)	0.006
Source		
Related	1.00	
Unrelated BM	0.74 (0.56–0.97)	0.03
Cord blood (CB)	1.54 (1.09–2.17)	0.02
Non-relapse mortality		
Age at allo-SCT, year		
16≤, <45	1.00	
≥45	2.00 (1.47–2.73)	<0.0001
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	1.98 (1.18–3.34)	0.01
Others [no $t(9;22)$]	1.14 (0.86–1.51)	0.37
HLA		
Well matched	1.00	
Partially matched	1.59 (1.16–2.17)	0.004
Mismatched	1.67 (1.16–2.41)	0.006
Conditioning		
TBI regimens	1.00	
Non-TBI regimens	1.99 (1.26–3.14)	0.003

Ph(−), ALL indicates Philadelphia chromosome-negative acute lymphoblastic leukemia; allo-SCT, allogeneic stem cell transplantation; TBI, total body irradiation.

preparative regimens were significant risk factors for relapse (Table 5).

The cumulative incidence of NRM was not statistically different among patients who underwent RD, URD, and CB allo-SCTs (39% in RD, 42% in URD, and 45% in CB at 3 years; $P = 0.17$). The results of multivariate analysis showed that ≥45 years of age at allo-SCT, cytogenetic abnormalities, HLA partially matched or mismatched, and non-TBI preparative regimens were significant risk factors for NRM (Table 5). Post-transplant lymphoproliferative disorder was observed in one patient, and there was no association with the use of ATG.

discussion

This report presents the results for the largest series of adult Ph(−) ALL patients who underwent allo-SCT. There were no significant differences between RD, URD, and CB allo-SCTs in any disease stage, suggesting that CB allo-SCT could be a treatment of choice for all disease stages of patients without a suitable RD or URD. There were no significant survival differences between BM and PBSC recipients in any disease stage (data not shown), which was consistent with other studies [27, 28]. Interestingly, OS after CB allo-SCT was significantly better than that after HLA-mismatched URD allo-SCT for CR1 patients younger than 45 years of age. These results might indicate advantages of CB allo-SCT when carried out for patients without an HLA-matched donor at an appropriate timing.

The major finding in this study is that OS was compatible between RD, URD, and CB allo-SCTs for Ph(−) ALL in CR1, even though NRM rates were higher in URD and CB allo-SCTs than RD allo-SCT. This is because of higher relapse rates in RD allo-SCT compared with URD and CB allo-SCTs. The low NRM rates due to the lower incidence of acute GVHD in our population might result from the differences in ethnic background [29, 30]. Although the NRM rates after allo-SCT in CR1 were not significantly different from those of URD allo-SCT in CR1, the causes of NRM would be different between URD and CB allo-SCTs. Hemorrhage due to insufficient platelet recovery and infection due to graft failure or delayed neutrophil recovery would be the major causes of NRM after CB allo-SCT in CR1. Since delayed engraftment is one of the most common limitations of CB allo-SCT [31–33], several attempts such as double cord units [34–36], intra-BM injection [37–39], and *ex vivo* expansion [40, 41] have been made to ensure engraftment. Although CD34-positive cell dose was not a significant risk factor for OS in this study, engraftment was delayed among patients who received fewer CD34-positive cells as previously reported [42]. Although all patients who underwent CB allo-SCT administered single CB intravenously, the technical progression of CB all-SCT could also improve the outcome of Ph(−) ALL as well as other hematological malignancies [21, 43–49] by reducing NRM.

Our results also indicated that CB allo-SCT beyond CR1 could achieve OS similar to that of RD or URD allo-SCT. It is noteworthy that some, but not all, patients with refractory disease could be rescued by CB allo-SCT as well as RD or URD allo-SCT [12]. Among patients transplanted in non-CR, survival of patients transplanted at ≥10 months from diagnosis was significantly superior to that of those transplanted <10 months from diagnosis (data not shown), suggesting that patients who could await a suitable donor or those with late relapse could obtain the advantages of allo-SCT. These patients could not have survived long with chemotherapy alone, and therefore, CB could be a hope of survival for patients with refractory disease who do not have a suitable RD or URD.

To our knowledge, this is the first and largest analysis of CB allo-SCT for Ph(−) ALL alone ($N = 233$). Recently, the results of a large retrospective analysis of a donor source that included data of 1525 patients (including 165 patients who underwent CB allo-SCT) were reported by the Center for International Blood and Marrow Transplant Research, the National Cord Blood Program, the European Group for Blood and Marrow Transplantation, and the Eurocord-Netcord registry [50]. The number of ALL patients who underwent CB allo-SCT was limited to 89 including both Ph(+) and Ph(−) ALL patients. The results of disease-specific analyses were also reported from Japan [CB; Ph(+): $N = 43$, Ph(−): $N = 71$] [51] and Minnesota (CB: $N = 69$) [52], with data of Ph(+) and Ph(−) ALL patients being analyzed together. Statistical techniques to adjust heterogeneities of the study population were used in those studies. Although we agree with the methodology of the above-described studies and the conclusions that support the use of CB for ALL patients without a suitable RD or URD, Ph(+) and Ph(−) ALL should be analyzed separately in an era of TKIs to obtain data which would be useful in clinical situations [9]. Our study clearly confirmed the usefulness of CB for Ph(−) ALL in any disease status.

In this type of retrospective study, selection biases from different backgrounds of patients who underwent RD, URD, and CB allo-SCTs could not be eliminated [12]. Considering that CB allo-SCT has not yet been recognized as a standard treatment of Ph(−) ALL, the background of CB recipients might be worse than that of other sources, that is, CB allo-SCT might be carried out for patients whose prognosis is considered to be poor without allo-SCT. In addition, since the median interval from diagnosis to allo-SCT in CR1 was similar between RD and CB allo-SCTs, the time-censoring effect, a major bias described elsewhere [12, 53, 54], did not affect our results. Although we could not make a comparison between chemotherapy and allo-SCT, our study could suggest promising data to broaden the choices of donor source.

In conclusion, the outcomes were comparable between RD, URD, and CB allo-SCTs in any disease status, and these may be considered equivalent options for patients with Ph(−) ALL. In the absence of a suitable RD or URD, CB allo-SCT should be planned promptly for Ph(−) ALL patients so as not to miss the appropriate timing.

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disclosure

The authors have declared no conflicts of interest.

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ORIGINAL ARTICLE: CLINICAL

Clinical evaluation of WT1 mRNA expression levels in peripheral blood and bone marrow in patients with myelodysplastic syndromes

Yasunori Ueda[1], Chisato Mizutani[1], Yasuhito Nannya[2], Mineo Kurokawa[2], Sumiko Kobayashi[3], Jin Takeuchi[3], Hideto Tamura[4], Kiyoyuki Ogata[4], Kazuo Dan[4], Hirohiko Shibayama[5], Yuzuru Kanakura[5], Keiko Niimi[6], Ko Sasaki[7], Masato Watanabe[8], Nobuhiko Emi[8], Masanao Teramura[9], Toshiko Motoji[9], Michiko Kida[10], Kensuke Usuki[10], Satoru Takada[11], Toru Sakura[11], Yoshikazu Ito[12], Kazuma Ohyashiki[12], Hiroyasu Ogawa[13], Takahiro Suzuki[14], Keiya Ozawa[14], Kiyotoshi Imai[15], Masaharu Kasai[15], Tomoko Hata[16], Yasushi Miyazaki[16], Yasuyoshi Morita[17], Akihisa Kanamaru[17], Akira Matsuda[18], Kaoru Tohyama[19], Daisuke Koga[20], Hiroya Tamaki[13], Kinuko Mitani[7], Tomoki Naoe[6], Haruo Sugiyama[21] & Fumimaro Takaku[22]

[1]*Department of Hematology/Oncology, Transfusion and Hemapheresis Center, Kurashiki Central Hospital, Okayama, Japan,* [2]*Department of Hematology and Oncology, University of Tokyo Graduate School of Medicine, Tokyo, Japan,* [3]*Department of Hematology and Rheumatology, Nihon University School of Medicine, Tokyo, Japan,* [4]*Division of Hematology, Department of Medicine, Nippon Medical School, Tokyo, Japan,* [5]*Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Osaka, Japan,* [6]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan,* [7]*Department of Hematology and Oncology, Dokkyo Medical University Hospital, Tochigi, Japan,* [8]*Department of Hematology, Fujita Health University School of Medicine, Aichi, Japan,* [9]*Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan,* [10]*Division of Hematology, NTT Kanto Medical Center, Tokyo, Japan,* [11]*Department of Hematology, Saiseikai Maebashi Hospital, Gunma, Japan,* [12]*Division of Hematology, Tokyo Medical University Hospital, Tokyo, Japan,* [13]*Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan,* [14]*Division of Hematology, Department of Medicine, Jichi Medical University, Tochigi, Japan,* [15]*Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan,* [16]*Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan,* [17]*Division of Hematology, Kinki University School of Medicine, Osaka, Japan,* [18]*Department of Hemato-Oncology, Saitama International Medical Center, Saitama Medical University, Saitama, Japan,* [19]*Department of Laboratory Medicine, Kawasaki Medical School, Okayama, Japan,* [20]*Diagnostic Division, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan,* [21]*Department of Functional Diagnostic Science, Osaka University Graduate School of Medicine, Osaka, Japan, and* [22]*Jichi Medical University, Tochigi, Japan*

Abstract

A study to evaluate WT1 mRNA expression levels in peripheral blood (PB) and bone marrow aspirate (BM) was conducted in 172 patients, including 115 with myelodysplastic syndromes (MDS), in Japan. The level of WT1 mRNA expression was evaluated according to the French–American–British (FAB) and World Health Organization (WHO) classifications (2001, 2008) and using the International Prognostic Scoring System and the WHO Prognostic Scoring System scales. WT1 mRNA expression levels in PB and BM were well correlated ($r = 0.85$), and they tended to increase with disease stage progression and in those at higher risk of leukemic transformation. WT1 mRNA expression can be a useful marker for the diagnosis and risk evaluation of MDS.

Keywords: Myelodysplastic syndromes, WT1 mRNA expression, classification system, peripheral blood, bone marrow

Introduction

Myelodysplastic syndrome (MDS), a clonal disorder of pluripotent hematopoietic stem cells, is a blood disease characterized by dysplasia and ineffective hemopoiesis. Approximately 20–30% of cases of MDS undergo transformation to acute myeloid leukemia (AML) [1].

The expression of Wilms' tumor gene (WT1) has been found to be a new prognostic factor and marker for the detection of minimal residual disease (MRD) in acute leukemia, including AML and acute lymphocytic leukemia (ALL) [2]. A recent study has revealed the clinical relevance of measuring WT1 mRNA for monitoring MRD in AML, primarily due to its high rate of expression (93.9%) in the peripheral blood (PB) of incipient untreated patients with AML, secondarily due to its ability to predict relapse after complete remission (CR), and finally because its levels after consolidation therapy

Correspondence: Yasunori Ueda, MD, PhD, Department of Hematology/Oncology, Transfusion and Hemapheresis Center, Kurashiki Central Hospital, 1-1-1 Miwa, Kurashiki, Okayama 710-8602, Japan. Tel: +81-86-422-0210. Fax: +81-86-425-9879. E-mail: ueda-y@kchnet.or.jp

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show a significant correlation between disease-free survival, overall survival and early relapse [3]. WT1 mRNA expression occurs not only in AML but also in the PB and bone marrow (BM) of patients with MDS [4–9].

Tamaki *et al.* [4] examined the level of WT1 mRNA expression in PB and BM from 57 patients with MDS grouped by the French-American-British (FAB) classification, and 12 patients experienced AML-MDS progression. The results revealed that WT1 mRNA expression in both PB and BM progressively increased with disease stage progression, from refractory anemia (RA), refractory anemia with excess of blasts (RAEB), refractory anemia with excess of blasts in transformation (RAEB-t), and to AML, suggesting the possibility that the WT1 mRNA expression level reflects the disease stage progression of MDS. Particularly, the patient group who developed leukemia from RAEB or RAEB-t within 6 months showed significantly higher WT1 mRNA expression in PB compared with the group who did not [4].

In accordance with that study, Cilloni *et al.* [6] measured WT1 mRNA expression levels in PB and BM from 131 patients with MDS, and found that: (1) WT1 mRNA expression in PB and BM was confirmed in 78% and 65% of patients with RA, respectively; (2) WT1 mRNA expression in PB and BM was confirmed in all patients with RAEB and secondary AML; (3) the level of WT1 mRNA expression increased with disease stage progression; and (4) the WT1 mRNA expression level was well correlated with the International Prognostic Scoring System (IPSS) scores established by Greenberg *et al.* [10].

In addition to the IPSS, the World Health Organization (WHO) Classification-Based Prognostic Scoring System (WPSS) has been proposed as a prognostic scoring system for MDS [11]. The WPSS consists of three characteristics: WHO subtype classification, considered to be important as a prognostic factor; IPSS-based karyotype abnormalities; and transfusion dependency.

Both the IPSS and WPSS require a chromosomal test as a primary parameter. However, because there are cases in which chromosomal abnormalities cannot be determined [12–14], it is necessary to establish molecular- and genetic-based methods to diagnose and determine the prognosis of MDS. The relatively rapid quantitation of WT1 mRNA is considered to be a useful test to determine the prognosis of MDS and has potential for clinical application, to become a novel marker to complement the current IPSS and WPSS criteria. We performed a clinical study in patients with MDS to demonstrate the usefulness of measuring the WT1 mRNA expression level in PB and BM in the diagnosis and treatment of MDS.

Patients and methods

This study was conducted in accordance with the Declaration of Helsinki, and preliminary approval was obtained from the Institutional Review Board or equivalent organization of each participating institution. Explanations of the study protocol were provided to all patients, and written informed consent was obtained from them before study enrollment.

Patients

From December 2008 to September 2009, 175 patients with MDS, suspected MDS and AML-MDS examined at 17 Japanese medical institutions were enrolled in the study. The subjects were 20 years of age or older and entered in the study regardless of gender, inpatient/outpatient status, or presence or absence of treatment. The 175 patients comprised 106 men (age range 27–88 years, average 65.5 years) and 69 women (age range 22–85 years, average 64.5 years). PB and BM samples from each patient were collected on the same day and used for WT1 mRNA measurement. Three of the 175 enrolled patients were excluded because BM could not be collected due to a dry tap or because the subtype could not be diagnosed. A total of 172 patients were therefore included in the final analysis set.

Diagnosis

Diagnosis of MDS was carried out using a central review format based on the FAB classification [15], the 2001 WHO classification [16] and the 2008 WHO classification [17]. Central review of the bone marrow smear-stained specimens, blood smear-stained specimens, iron-stained specimens, and clot hematoxylin and eosin-stained specimens was carried out by two individuals, one each in the Department of Hemato-Oncology, Saitama International Medical Center, Saitama Medical University, and the Department of Laboratory Medicine, Kawasaki Medical School.

WT1 mRNA measurement method

mRNA was extracted from PB leukocytes and BM nucleated cells at SRL, Inc., Tokyo, Japan using the RNeasy Mini-Kit (Qiagen, Valencia, CA), and the amount containing WT1 mRNA was measured at the Research Laboratory, Diagnostic Division, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan using a WT1 mRNA Assay Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). cDNA was synthesized from 1 μg of extracted RNA in a reverse-transcription reaction using random hexamer primers. The amounts of WT1 and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) mRNA were quantitated using real-time polymerase chain reaction (PCR) with a COBAS TaqMan48 analyzer (Roche Diagnostics, Pleasanton, CA), and the respective amounts of WT1 and GAPDH RNA in the sample were calculated by simultaneous reaction with standards of known concentrations.

Method for calculating WT1 mRNA expression

mRNA of the universally expressed housekeeping gene GAPDH was used for correction of variations in the efficiencies of RNA extraction and reverse transcription. As shown in the following formula, the level of WT1 mRNA expression was calculated by dividing the measured amount of WT1 mRNA by the measured amount of GAPDH mRNA and multiplying that value by the average number of copies of GAPDH mRNA found in 1 μg of RNA from PB leukocytes of healthy adults (GAPDH mRNA expression). The average GAPDH mRNA expression in PB leukocytes of healthy adults was reported to be 2.7×10^7 copies/μg RNA based on independent tests in healthy adults [3].

WT1 mRNA expression (copies/μg RNA) = (measured WT1 mRNA [copies/mL]/measured GAPDH mRNA [copies/mL]) × 2.7 × 10^7 (copies/μg RNA)

PB cut-off value

The lower limit of the WT1 mRNA measurement range in the WT1 assay kit is 2500 copies/mL, or 50 copies/μg RNA when converted to copies per microgram of RNA. In this study, a value of 50 copies/μg RNA was set as the cut-off value for WT1 mRNA expression, and a value of 50 or more copies/μg RNA was judged as positive according to the instruction manual of the WT1 mRNA assay kit.

Statistical analysis

The mean ± SD for the log-transformed values of WT1 mRNA expression (copies/μg RNA) was calculated, and then converted back to base 10 and used as the geometric mean. All data below the detection limit were shown as 49 copies/μg RNA. For intergroup comparison of WT1 mRNA expression, a Tukey-Kramer honestly significant difference (HSD) test was performed at the level of significance of $p < 0.05$ using log-transformed values of WT1 mRNA expression (copies/μg RNA). For comparison of WT1 mRNA expression between the aplastic anemia (AA) and RA groups, a Wilcoxon rank-sum test and Steel test were performed at the level of significance of $p < 0.05$ using log-transformed values of WT1 mRNA expression (copies/μg RNA). The Pearson correlation coefficient was used for analysis of each correlation.

Results

As a result of the central review conducted on all 172 patients, 115 were classified as patients with MDS in the FAB classification, excluding chronic myelomonocytic leukemia (CMML). Similarly, 98 patients in the 2001 WHO classification and 97 in the 2008 WHO classification were classified as patients with MDS (Figure 1).

Analytical results based on FAB classification

WT1 mRNA expression in PB and BM

The 172 patients eligible for analysis were categorized by disease type, and their WT1 mRNA expression levels in PB and BM are shown in Table I. The mean WT1 mRNA expression level in the 115 patients with MDS (excluding CMML) was 360 copies/μg RNA in PB and 2240 copies/μg RNA in BM, and these values were the second highest after the values obtained in patients with AML-MDS (PB: 12 600 copies/μg RNA; BM: 33 100 copies/μg RNA). On the other hand, the WT1 mRNA expression level was less than 50 copies/μg RNA in PB and 90–630 copies/μg RNA in BM in patients with AA, idiopathic cytopenia of unknown significance (ICUS), idiopathic thrombocytopenic purpura (ITP), paroxysmal nocturnal hemoglobinuria (PNH), pure red-cell aplasia (PRCA) and erythroid hypoplasia, which were all lower compared with the level in MDS.

The relationship between WT1 mRNA expression in PB and BM was evaluated in all patients. The regression line formula $y = 0.7329x + 1.4407$ was obtained, indicating a strong correlation ($r = 0.85$) (Figure 2).

WT1 mRNA expression in PB and BM for each MDS disease stage

When the WT1 mRNA expression levels in PB and BM were compared for each MDS subtype based on the FAB classification [Figure 3(a)], the level in both increased proportionally with each MDS classification as the disease

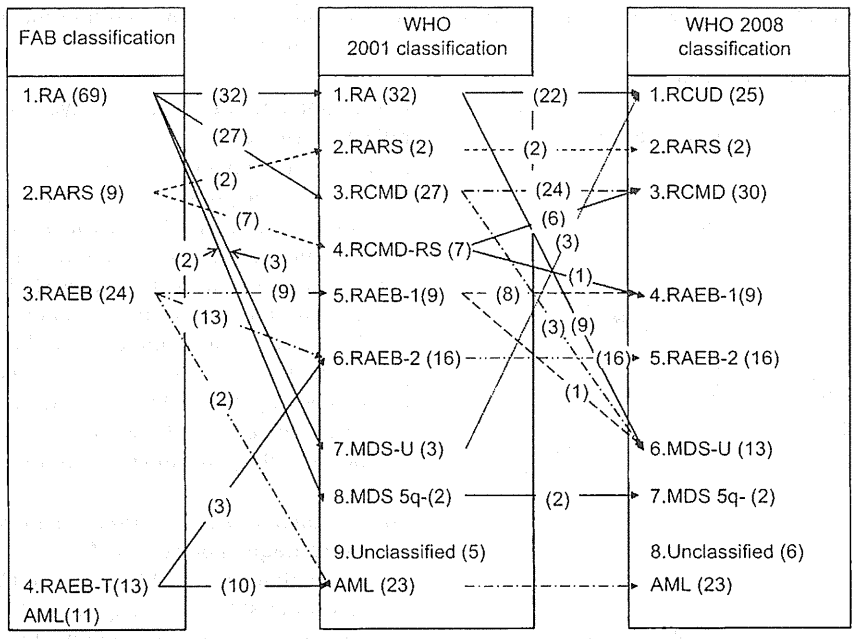

Figure 1. FAB and WHO classification of myelodysplastic syndromes in this study. FAB classification-based MDS subtypes (four subtypes: RA, RARS, RAEB and RAEB-t), 2001 WHO-based MDS subtypes (eight subtypes: RA, RARS, RCMD, RCMD-RS, RAEB-1, RAEB-2, MDS-U and MDS 5q−), 2008 WHO-based MDS subtypes (seven subtypes: RCUD, RARS, RCMD, RAEB-1, RAEB-2, MDS-U and MDS 5q−). Numbers in parentheses represent numbers of patients.

Table I. WT1 mRNA expression levels in PB and BM from patients with different MDS subtypes and AML-MDS according to FAB classification.

		WT1 mRNA expression level			
		Peripheral blood		Bone marrow	
Disease	No. of patients	Log (mean ± SD)	Geometric mean (copies/μg RNA)	Log (mean ± SD)	Geometric mean (copies/μg RNA)
MDS	115	2.56 ± 1.05	360	3.35 ± 0.87	2240
AML-MDS	11	4.10 ± 0.96	12 600	4.52 ± 0.77	33 100
AML-MDS (CR)	2	1.89 ± 0.20	80	2.98 ± 0.39	1000
CMML	3	2.17 ± 0.54	150	3.04 ± 0.54	1100
CLL	1	1.92	80	3.33	2140
Atypical CML	1	—	<50	1.95	90
AA	8	—	<50	2.64 ± 0.37	440
ICUS	3	—	<50	2.16 ± 0.36	140
ITP	1	—	<50	2.13	130
PNH	1	—	<50	2.8	630
PRCA	2	—	<50	2.17 ± 0.12	150
Erythroid hypoplasia	1	—	<50	1.94	90
Unclassified	23	2.14 ± 0.56	140	2.96 ± 0.61	910
Total	172	2.50 ± 1.05	320	3.27 ± 0.90	1860

PB, peripheral blood; BM, bone marrow; MDS, myelodysplastic syndromes; AML-MDS, acute myeloid leukemia-evolved MDS; FAB, French-American-British; CR, complete remission; CMML, chronic myelomonocytic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; AA, aplastic anemia; ICUS, idiopathic cytopenia of unknown significance; ITP, idiopathic thrombocytopenic purpura; PNH, paroxysmal nocturnal hemoglobinuria; PRCA, pure red-cell aplasia.

stage progressed. Significant differences in both PB and BM expression were seen between RA and RAEB, RA and RAEB-t, refractory anemia with ringed sideroblasts (RARS) and RAEB, and RARS and RAEB-t ($p < 0.05$).

WT1 mRNA expression in PB and BM for each IPSS risk group

WT1 mRNA expression levels in PB and BM for each IPSS risk group were compared in the 115 patients with MDS. A tendency for WT1 mRNA expression to increase in both PB and BM was observed in each IPSS risk group as the risk of transformation to AML increased from low to high. Significant differences ($p < 0.05$) in WT1 mRNA expression were observed in risk groups between low and intermediate-2, low and high, intermediate-1 and intermediate-2, and intermediate-1 and high in PB samples; and between low and intermediate-1, low and intermediate-2, low and high, intermediate-1 and intermediate-2, and intermediate-1 and higher in BM samples [Figure 3(b)]. The correlation between IPSS score and WT1 mRNA expression was evaluated, and a correlation of $r = 0.57$ was found for both PB and BM samples.

Next, the WT1 mRNA expression levels in PB and BM between IPSS risk groups were compared in the 69 patients with RA [Figure 3(c)]. As the risk increased from low to intermediate-2, the level of WT1 mRNA expression in both PB and BM increased. Moreover, when the distribution of WT1 mRNA expression between each risk group was evaluated, a significant difference ($p < 0.05$) was found in PB between low and intermediate-2; in BM, significant differences were found between low and intermediate-1, and low and intermediate-2.

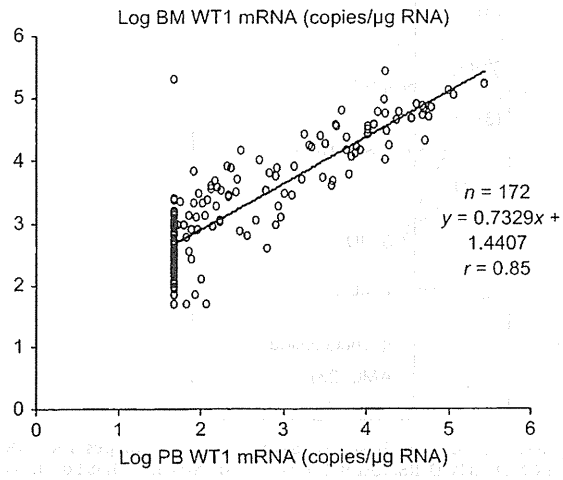

Figure 2. Correlation of WT1 mRNA expression in PB and WT1 mRNA expression in BM.

Correlation between IPSS karyotype and WT1 mRNA expression

A total of 114 patients with MDS were categorized into the three prognostic groups of good, intermediate and poor in accordance with their IPSS karyotype, and the levels of WT1 mRNA expression in their PB and BM samples were compared. One patient with MDS was excluded from this analysis because chromosome testing was not performed. The WT1 mRNA expression level increased in both PB and BM samples as the karyotype indicated a poorer prognosis. Among karyotypes, significant differences ($p < 0.05$) in WT1 mRNA expression were found between the good and intermediate and between the good and poor groups [Figure 3(d)].

Correlation between WT1 mRNA expression and percentage of blasts in BM

The correlation between blast ratio and WT1 mRNA expression in PB and BM was investigated in 114 patients with MDS (excluding one patient in whom the blast ratio could not be measured). The correlation between blast ratio and PB WT1 mRNA expression was $r = 0.51$, and the correlation between blast ratio and BM WT1 mRNA expression was $r = 0.48$.

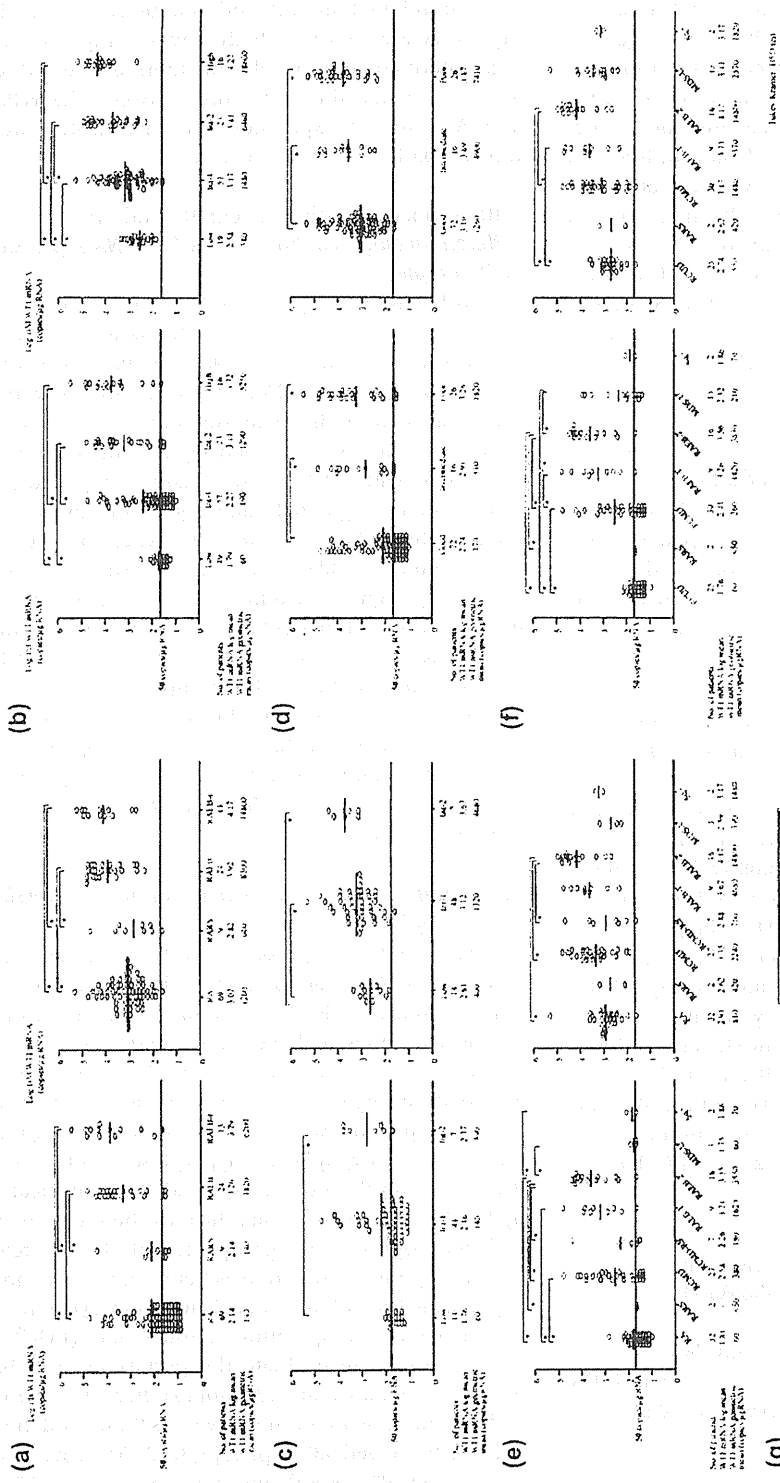

Figure 3. WT1 mRNA expression levels in PB and BM from patients with MDS (a) according to FAB classification, (b) according to IPSS category, (c) patients with RA according to IPSS category, (d) according to chromosomal karyotype, (e) according to WHO 2001 classification, (f) according to WHO 2008 classification, (g) according to WPSS category. In intergroup comparison of WT1 mRNA expression, the Tukey-Kramer HSD test was performed using log-transformed values of WT1 mRNA expression with a level of significance of $p < 0.05$. Bold lines represent mean WT1 mRNA expression after log transformation. Fine lines represent lower limit of detection of WT1 mRNA (50 copies/μg RNA).

Analytical results based on 2001 WHO classification

WT1 mRNA expression in PB and BM for each MDS disease stage based on 2001 WHO classification

Figure 3(e) shows the assay results for WT1 mRNA expression in PB and BM in 98 patients in various MDS disease stages categorized on the basis of the 2001 WHO classification. The WT1 mRNA expression levels in both PB and BM tended to increase with the progression to each MDS subtype. When the levels of WT1 mRNA expression in each disease stage were investigated, significant differences ($p < 0.05$) were found in PB between RA and refractory cytopenia with multilineage dysplasia (RCMD), RA and RAEB-1, RA and RAEB-2, RARS and RAEB-2, RCMD and RAEB-2, RCMD with ringed sideroblasts (RCMD-RS) and RAEB-2, RAEB-2 and unclassified MDS (MDS-U), and RAEB-2 and 5q− syndrome; in BM, significant differences were found between RA and RAEB-2, RCMD and RAEB-2, and RCMD-RS and RAEB-2.

Correlation between WT1 mRNA expression and percentage of blasts in BM based on 2001 WHO Classification

The correlation between the blast ratio and WT1 mRNA expression in PB and BM was investigated in 97 patients with MDS (excluding one patient in whom the blast ratio could not be measured). The correlations between the blast ratio and WT1 mRNA expression were $r = 0.50$ in PB and $r = 0.46$ in BM.

Analytical results based on 2008 WHO classification

WT1 mRNA expression in PB and BM for each MDS disease stage based on 2008 WHO classification

Figure 3(f) shows the assay results for WT1 mRNA expression in PB and BM in a total of 97 patients in various MDS disease stages categorized on the basis of the 2008 WHO classification. WT1 mRNA expression in both PB and BM tended to increase with the progression to each MDS subtype.

When the distribution of WT1 mRNA expression for each disease stage was examined, significant differences ($p < 0.05$) were found in PB between refractory cytopenia with unilineage dysplasia (RCUD) and RCMD, RCUD and RAEB-1, RCUD and RAEB-2, RARS and RAEB-2, RCMD and RAEB-1, RCMD and RAEB-2, RAEB-1 and MDS-U, and RAEB-2 and MDS-U; in BM, significant differences were found between RCUD and RAEB-1, RCUD and RAEB-2, and RCMD and RAEB-2.

Correlation between WT1 mRNA expression and percentage of blasts in BM based on 2008 WHO classification

The correlations between blast ratio and WT1 mRNA expression in 96 patients (excluding one patient with MDS whose blast ratio could not be measured) were $r = 0.50$ in PB and $r = 0.46$ in BM.

WT1 mRNA expression in PB and BM for each WPSS risk group

WT1 mRNA expression in PB and BM was compared in 98 patients with MDS classified according to WPSS risk group [Figure 3(g)]. As the risk increased from very low to very high, WT1 mRNA expression in both PB and BM also tended to rise. When the distribution of WT1 mRNA for each risk group was evaluated, significant differences ($p < 0.05$) were found in both PB and BM between very low and high, very low and very high, low and high, low and very high, intermediate and high, and intermediate and very high. Moreover, when the correlation between the WPSS score and WT1 mRNA expression was investigated, the values were $r = 0.61$ in PB and $r = 0.55$ in BM.

Differential diagnosis between RA and AA

Differential diagnosis based on WT1 mRNA expression in PB samples

The WT1 mRNA expression level in PB was less than 50 copies/μg RNA in all eight patients with AA, whereas it was less than 50 copies/μg RNA in 34 patients with RA and 50–52 100 copies/μg RNA in 35 of 69 patients with RA. The statistical analysis by Wilcoxon rank-sum test revealed a statistical difference between eight patients with AA and 65 patients with RA ($p = 0.01$). Sixty-nine patients with RA were further categorized into three groups by bone marrow findings: hypoplastic RA ($n = 20$), hyperplastic RA ($n = 15$) and normoplastic RA ($n = 30$), excluding the non-categorized RA ($n = 4$). Significant differences were observed between AA and each of hypoplastic ($p = 0.04$) or normoplastic RA ($p = 0.02$), whereas no difference was shown between the AA and hyperplastic RA group ($p = 0.10$) by Steel test (Figure 4). From these findings, a differential diagnostic cut-off value between RA and AA of 50 copies/μg RNA for WT1 mRNA expression in PB is considered appropriate, for which the sensitivity was 50.7% (35/69) and the specificity was 100% (8/8).

Differential diagnosis based on WT1 mRNA expression in BM samples

The WT1 mRNA expression level in BM was 251–2600 copies/μg RNA in eight patients with AA, whereas it was less than 50 copies/μg RNA in one of 69 patients with RA and 69–196 000 copies/μg RNA in the others. The statistical analysis by Wilcoxon rank-sum test revealed no statistical difference between eight patients with AA and 65 patients with RA. Sixty-nine patients with RA were similarly categorized into three groups: hypoplastic, hyperplastic and normoplastic RA, excluding the non-categorized RA. Statistical analysis by Steel test revealed a significant difference between AA and normoplastic RA groups ($p = 0.04$), whereas there were no significant differences between the AA and each of hypoplastic RA and hyperplastic RA groups (Figure 4).

When receiver operating characteristic (ROC) analysis was performed to evaluate the performance of BM WT1 mRNA expression as an indicator to differentiate between RA and AA, the area under the curve was 0.713, and the Youden index [18] showed 432 copies/μg RNA. Moreover, the sensitivity was 69.6% (48/69), and the specificity was 75.0% (6/8) (Supplementary Figure to be found online at http://informahealthcare.com/doi/abs/10.3109/10428194.2012.745074).

When the PB cut-off value of 50 copies/μg RNA was inserted into the regression line formula obtained

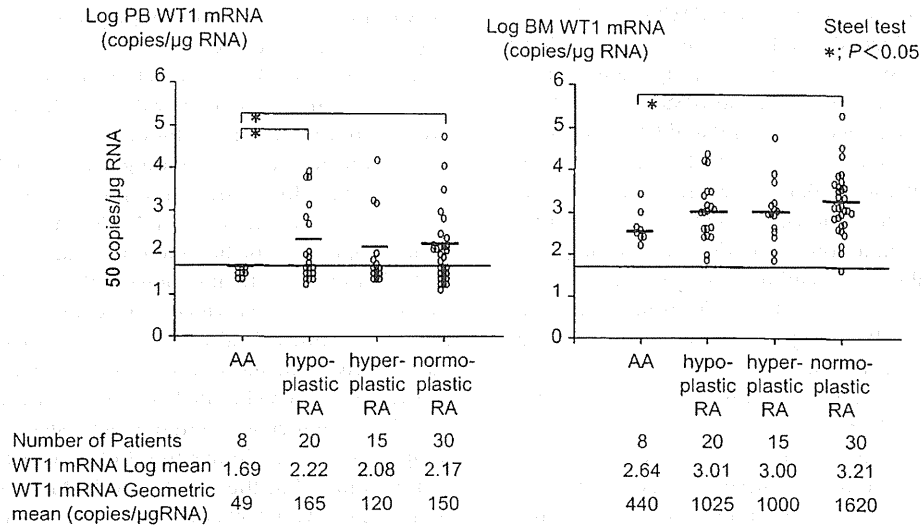

Figure 4. Comparison of WT1 mRNA expression between AA and RA groups (hypoplastic, hyperplastic and normoplastic RA). In intergroup comparison of WT1 mRNA expression, Steel test was performed using log-transformed values of WT1 mRNA expression with a level of significance of $p < 0.05$. Bold lines represent mean WT1 mRNA expression after log transformation. Fine lines represent lower limit of detection of WT1 mRNA (50 copies/μg RNA).

from the correlation between WT1 mRNA expression in PB and BM (Figure 2), BM WT1 mRNA expression became 480 copies/μg RNA. When 500 copies/μg was evaluated as the cut-off value for BM WT1 mRNA expression, the sensitivity was 68.1% (47/69) and the specificity was 75.0% (6/8). Based on these results, 500 copies/μg RNA was considered to be an appropriate cut-off value for the differential diagnosis between RA and AA using WT1 mRNA expression in BM.

Comprehensive analysis using cut-off values

The PB and BM samples in each disease and MDS subtype were further evaluated for their WT1-positive rates, using the WT1 mRNA expression cut-off values determined above (PB: 50 copies/μg RNA; BM: 500 copies/μg RNA) (Table II). For AML-MDS (11 patients), the WT1 mRNA-positive rates were a high 100% (11/11) for PB and 90.9% (10/11) for BM, and in MDS (115 patients), the WT1 mRNA-positive rates were 61.7% (71/115) for PB and 73.0% (84/115) for BM, which were the second highest after AML-MDS. In contrast, all patients with AA, ICUS, ITP, PNH, PRCA and erythroid hypoplasia had low positive rates of 0% for PB and 18.8% (3/16) for BM. The WT1 mRNA-positive rates for PB and BM increased with MDS disease stage progression (Table II).

Table II. WT1 mRNA-positive rate in PB and BM from patients with different MDS subtypes and AML-MDS according to FAB classification.

Subtype	No. of patients	WT1 mRNA-positive rate (%)	
		Peripheral blood	Bone marrow
RA	69	50.7 (35/69)	68.1 (47/69)
RARS	9	44.4 (4/9)	44.4 (4/9)
RAEB	24	83.3 (20/24)	87.5 (21/24)
RAEB-t	13	92.3 (12/13)	92.3 (12/13)
AML-MDS	11	100.0 (11/11)	90.9 (10/11)
Total	126	65.1 (82/126)	74.6 (94/126)

PB, peripheral blood; BM, bone marrow; MDS, myelodysplastic syndromes; AML-MDS, acute myeloid leukemia-evolved MDS; FAB, French-American-British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation.

Discussion

In this study, the clinical usefulness of the measurement of WT1 mRNA expression in risk assessment of MDS was evaluated using a WT1 assay kit. Recently, a steady stream of reports has indicated the usefulness of WT1 mRNA measurement. The group of Cilloni [6] confirmed that WT1 mRNA expression potentially fulfills all the requirements for an additional marker for risk assessment in MDS, compared with the conventional methods. The measurement of WT1 can be effective, particularly in cases in which BM aspiration and/or cytogenetic analysis fail or are not informative [6].

Furthermore, in their findings in a long-term prospective study, Tamura *et al.* [19] reported that a significant correlation ($p = 0.0186$) was seen between WT1 mRNA expression and survival time when WT1 mRNA expression in PB was categorized into three groups of less than 10^2, 10^2–10^4, and greater than 10^4 copies/μg RNA, that the median survival time for each group was 62.7 months, 29.9 months and 11.6 months, respectively; and that the time until transformation to leukemia was the shortest in the group with the highest WT1 mRNA expression. In addition, they reported that in univariate analysis, WT1 mRNA expression was a predictive parameter for transformation to leukemia, and in multivariate analysis, it was a significant predictive parameter along with the IPSS score [19]. As described above, Tamaki *et al.* reported similar findings [4].

This study was conducted using not only the FAB classification system but also the 2001 and 2008 WHO classification systems. It was confirmed that in all three classification systems, WT1 mRNA expression in both PB and BM increases significantly in MDS subtypes with disease stage

progression. In addition, both PB and BM WT1 mRNA expression increased significantly as the risk of transformation to AML rose in the IPSS and WPSS risk groups. Furthermore, a correlation of $r = 0.57$ between the IPSS score and WT1 mRNA expression was seen in both PB and BM. The correlations between the WPSS score and WT1 mRNA expression were $r = 0.61$ in PB and $r = 0.55$ in BM. In comparison with the IPSS, the WPSS allows the assessment of survival time and progression of leukemic transformation at all time periods during the clinical course, leading to continued prognostic evaluation while reviewing the risk. WT1 mRNA expression correlates with the WPSS prognosis, and despite the single-point quantitation, the results in this study indicate that WT1 mRNA is useful as a time-course prognostic marker in the same manner as the WPSS.

At present, allogeneic hematopoietic stem cell transplant is the only curative treatment for MDS. However, determination of the timing of allogeneic transplant is very difficult because many patients are older, treatment-related deaths frequently occur, and there are large individual differences in the rate of disease progression. Allogeneic transplant is selected as the therapeutic regimen for MDS when no increase in blast cells is confirmed, taking into consideration the development of transfusion dependency and frequency of infections [20]. In addition, allogeneic transplant is selected when a future increase in blast cells is predicted by karyotypic analysis even though no increase is currently observed. It is recommended that transplant be performed before the progression to cytopenia caused by an increase in blast cell clones and before the progression to acute leukemia, although induction chemotherapy may be required when an increase in blast cells is observed [21]. On the other hand, another study suggested that delaying transplant until the advanced stage of disease results in a longer survival time for low and intermediate-1 IPSS risk groups, while early transplant was recommended for the intermediate-2 and high groups [22]. The period after CR is achieved is considered to be the standard timing to perform transplant for acute leukemia, but determining CR is extremely challenging. Our results revealed that periodic monitoring of WT1 mRNA expression in patients with MDS provided useful information for predicting the timing of transplant.

RA, a subtype in the early MDS disease stage, is often difficult to differentiate from AA [23]. In a previous study by Iwasaki *et al.*, no difference in WT1 mRNA expression was observed between RA and AA [9]. However, our data revealed the possibility of WT1 expression level to differentiate AA and RA groups using both peripheral blood and bone marrow samples (Figure 4). In the present statistical analysis, significant differences were observed between AA and hypoplastic RA ($p = 0.04$) in PB. The number of subjects was limited, and further trial is required for more detailed analysis. Moreover, tentative cut-off values for WT1 mRNA expression were set at 50 copies/μg RNA in PB and 500 copies/μg RNA in BM. Although the number of patients was small, the results showed that the level of WT1 mRNA expression could differentiate between RA and AA, with specificity in PB and BM of 100% (8/8) and 75.0% (6/8), respectively. This provides evidence that the measurement of WT1 mRNA expression can play a role in the differential diagnosis of RA and AA.

The WT1 assay kit is used clinically in Japan as a marker to monitor MRD in patients wth AML. In MDS, a clonal disorder of pluripotent hematopoietic stem cells, WT1 mRNA expression increases depending on the MDS subtype and disease stage. In contrast, the mechanism by which WT1 mRNA expression increases in MDS is not considered to correlate simply with the fluctuation in leukemic clones, as seen in AML. In normal hematopoiesis, WT1 mRNA is expressed mainly in CD34-positive cells. In contrast, in patients with MDS, WT1 mRNA is also expressed in CD34-negative cells, particularly in lineages exhibiting abnormalities [24]. In our study, the level of WT1 mRNA expression within the RA group was shown to increase with the increase in IPSS risk [Figure 3(c)]. Moreover, a similar trend of increasing WT1 expression was found in the RCUD and RCMD groups according to the 2008 WHO classification, although no significant increase in blast cells in BM was observed in these groups. Taken together, these findings indicate that the increase in WT1 mRNA expression in patients with MDS may reflect the divergence of MDS clones from normal clones and preleukemic changes.

In patients with MDS, evaluating the changes in WT1 mRNA levels simultaneously in PB and BM samples provides useful information on disease stage progression or risk assessment in individual patients. In addition, the WT1 mRNA-positive rate in each subtype of MDS was high (50–90%) in both PB and BM in this study, suggesting that a single measurement of WT1 mRNA is sufficient for MDS diagnosis, particularly for differentiating RA from AA.

Overall, this study provides evidence that the measurement of the level of WT1 mRNA expression in PB and BM serves as a supplemental marker for MDS diagnosis and prognostic assessment. This assay has great potential to contribute to more appropriate diagnoses and therapeutic decisions in patients with MDS and to evaluate the timing of allogeneic transplant.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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Supplementary material available online

Supplementary figure showing ROC analysis of WT1 mRNA expression in BM in RA and AA groups

話　題

非血縁者間同種末梢血幹細胞移植：日本と米国の現状の比較*

上　田　恭　典**,***

Key Words : unrelated peripheral blood stem cell transplantation, unrelated bone marrow transplantation, stem cell donor, National Marrow Donor Program, Japan Marrow Donor Program

はじめに

非血縁者間末梢血幹細胞移植(unrelated peripheral blood stem cell transplantation；以下URPBSCT)がわが国で施行可能となったのは2010年10月であった．1例目は，東北大震災のあった2011年3月に施行され，その後2013年5月31日までにわずか24例が施行されたのみである．一方欧米においては，非血縁者間の移植においても骨髄移植(bone marrow transplantation；以下BMT)に比べてPBSCTの頻度は高く，むしろ標準的な方法となっている[1](図1)．著者は昨年，厚生労働科学研究 難治性疾患等克服研究事業(免疫アレルギー疾患等予防・治療研究事業)「非血縁者間同種末梢血幹細胞移植開始におけるドナーおよびレシピエントの安全性と移植成績向上に関する研究班(班長 宮村耕一)より派遣されて，米国のURPBSCTの状況を視察する機会を得た．本稿では，米国におけるURPBSCTの実施体制の現状とわが国の現状を紹介しつつ今後の方向を探りたい．

末梢血幹細胞移植の歴史

造血幹細胞移植の歴史の中で，末梢血中に造血幹細胞(以下PBSC)が骨髄に比べて低濃度ではあるが存在することは以前より知られていた[2]．その後，化学療法ののちに一時的に末梢血中にPBSCが動員されることが確認され[3]，アフェレシスによって確保したPBSCを用いた自家(以下auto)PBSCTが臨床の場に登場した．さらに，造血回復期にG-CSFを併用することで動員されるPBSCの数が著増することが判明し[4]，簡便な凍結保存法の開発[5]とあいまって，autoPBSCTは急速に広まっていった．移植に必要十分な造血幹細胞の指標として，当初は幹細胞コロニーアッセイの代替とし

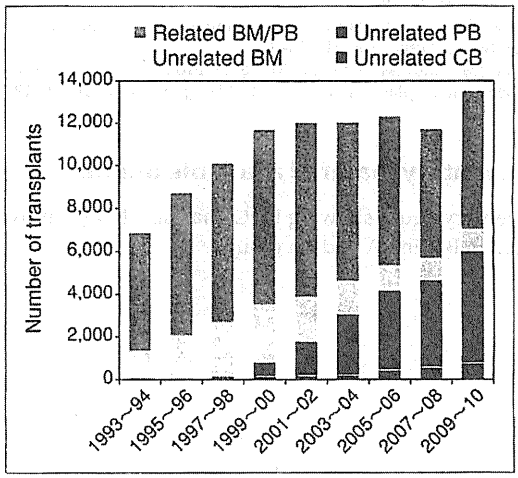

図1　CIBMTRに登録された21歳以上の同種移植症例数(1993～2010)
CIBMTR：Center for International Blood & Marrow Research　(文献[1]より引用改変)

* Current status of unrelated peripheral blood stem cell transplantation in Japan and the United States.

** Yasunori UEDA, M.D., Ph.D.: 公益財団法人大原記念倉敷中央医療機構倉敷中央病院血液内科〔〒710-8602 岡山県倉敷市美和1-1-1〕; Department of Haematology/Oncology, Kurashiki Central Hospital, Kurashiki, Okayama 710-8602, JAPAN

*** 血液治療センター/外来化学療法センター

て，レシピエント体重あたりのCFU-GM(顆粒球単球コロニー数)が用いられていたが，造血前駆細胞に表現される表面マーカーであるCD34陽性細胞数で代替可能なことが明らかとなったことも，PBSCTの普及を容易にした．その後，顆粒球コロニー刺激因子(granulocyte-colony stimulating factor；以下G-CSF)を通常の治療量の数倍投与することで，抗腫瘍剤の併用なしにPBSCを増加させうることが示され[6]，患者のautoPBSCTのみならず，同種，同系の健常人ドナーからのPBSCTの道が開けた．その際，問題となったのは，ドナーについては，G-CSF大量投与に関連する短期，中長期の安全性，患者については，PBSC浮遊液を輸注する際に，骨髄細胞を輸注する場合に比べて，多量のT細胞が輸注されること[7]，造血微小環境を創る細胞が含まれない可能性があること，長期間にわたっての造血を保証するレベルの幹細胞が含まれているか不明であることなどであった．これらの点を少しずつ解決しつつ，血縁者間(related；以下R)の同種末梢血幹細胞移植(以下alloPBSCT)が行われるようになった[8]．わが国では2000年に，RPBSCTが保険収載となった．その後，アフェレシス中のドナーに心停止が生じ，またドナーフォローアップ中に白血病を発症したドナーが報告され，RPBSCTは継続されたものの，非血縁ボランティアドナーからのURPBSCTは，長期の安全性に関する調査が終了するまで留保される状態となった．日本造血細胞移植学会(JSHCT)による10年間にわたるRPBSCドナーの長期フォローアップの成績では骨髄(BM)ドナーと末梢血(PB)ドナーの間に造血器腫瘍発症率の有意差はなく[9]，Koderaの提案によって行われた，ヨーロッパ骨髄移植学会(EBMT)を中心に行われた，BMドナー27,770人とPBドナー23,254人での後方視的調査では，一般集団の造血器悪性腫瘍の発症率よりむしろ低率であり[10]，米国骨髄バンク(National Marrow Donor Program；以下NMDP)の2,408人の非血縁(UR)ドナーの前向き調査でも，造血器腫瘍発症は慢性リンパ性白血病の1人であり，一般人での発症率を上まわることはなかった[11]．これらの成績を踏まえて，2010年10月より，日本骨髄バンク(Japan Marrow Donor Program；以下JMDP)による，URPBSCTが開始された．

非血縁者間骨髄移植と末梢血幹細胞移植

遺伝子的背景の異なる造血幹細胞を用いる同種移植の目的として，造血・免疫障害や先天性代謝障害における正常な幹細胞を用いた造血能免疫能の回復や酵素活性の回復と，腫瘍性疾患に対する大量放射線化学療法後の造血能の再構築があげられる．一方で，最も問題となる合併症として，ドナーリンパ球がレシピエント(患者)を傷害する，移植片対宿主病(graft versus host disease；以下GVHD)がある[12]．HLAのDNAタイピングを用いた適合ドナーの検索と，その予防方法，治療方法の進歩が移植成績の改善をもたらしたが，ドナーリンパ球は患者由来の腫瘍細胞も同時に傷害することが判明し[13]，現在では，腫瘍性疾患に対する同種移植は大量放射線化学療法であると同時に，むしろドナーリンパ球が患者由来の腫瘍を攻撃する移植片対腫瘍効果(graft versus tumor effect；以下GVT)を期待した免疫療法として重要であるととらえられている[14]．PBSCをドナーから採取する場合には，先に述べたようにG-CSFを通常の5倍量程度連日皮下注し，4日目注射後2～4時間以降もしくは，5日目6日目に，血小板採取時とほぼ同様に，遠心式血液成分分離装置で採取する[15]．PBSCは，高濃度の単核球，血小板浮遊液として採取される．採取細胞の中には，早期の血球回復をもたらす造血前駆細胞と，Tリンパ球とが多く含まれている．PBSCTでは，通常のBMTに比べて約10倍程度のGVHD，GVTに関連するとされるTリンパ球が輸注されると考えられるため[7]，従来のBMTとは異なった治療成績となる可能性が推測される．血縁者間においては，米国からの前向きのメタアナリシスでは，進行期急性骨髄性白血病(以下AML)が対象の場合，全生存率と無病生存率は，PBSCT群で優れているという結果がでており，細胞組成の差異の影響を推測させるものであった[16]．一方長藤らが行った，JSHCTに2000年から2005年までに報告されたわが国における18歳以上の白血病患者707例に対する骨髄破壊的移植を対象とした後ろ向き研究では，全生存率については，標準リスク群で有意にPBSCTの予

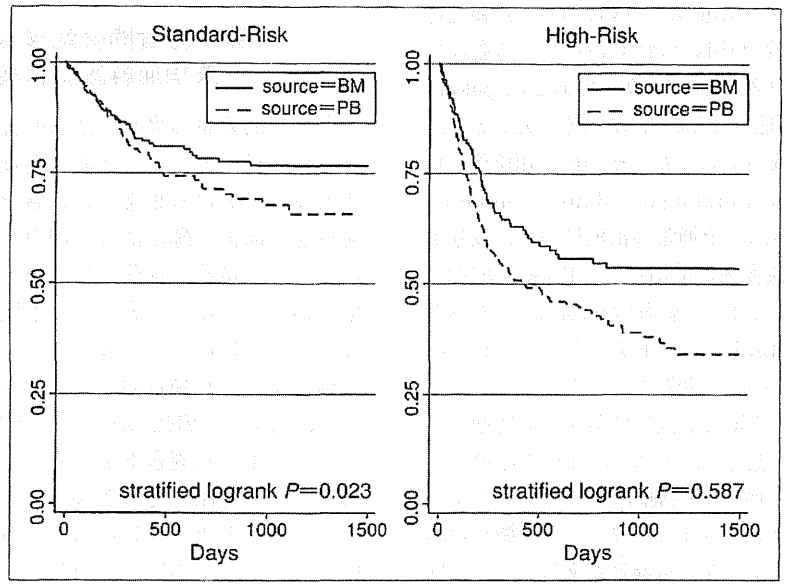

図2　わが国での血縁者間BMTとPBSCTの後ろ向き検討(2000～2005)
(文献[17]より引用改変)

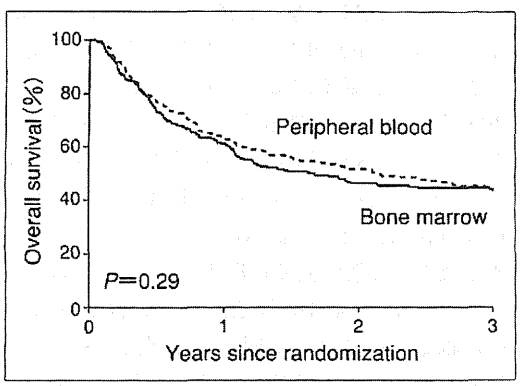

図3　米国での非血縁者間BMTとPBSCTの前向き比較
(全生存率)　(文献[19]より引用改変)

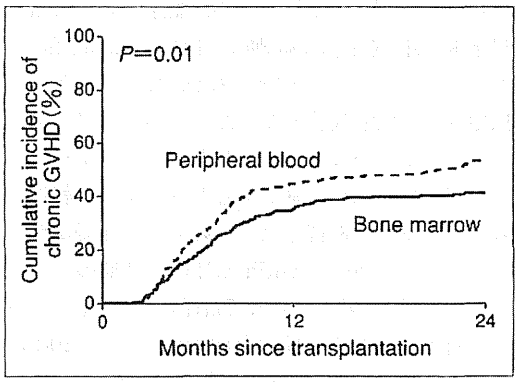

図4　米国での非血縁者間BMTとPBSCTの前向き比較
(慢性GVHD)　(文献[19]より引用改変)

後が低下しており，高リスク群では，有意差なし，造血回復はPBSCTのほうが有意に速く，重度の急性GVHDやextensive typeの慢性GVHDはPBSCTで多い，移植後再発に差はないとの結果であった[17](図2)．また，2012年の日本血液学会において，2000年から2010年までに対象期間を延ばした1,835症例に対する検討が報告された[18]が，ほぼ2005年までと同等の結果であった．わが国において，後ろ向き研究とはいえ，欧米の成績からPBSCTで予想された結果が認められなかった点については，前向き研究を含め，今後の検討が必要である．URPBSCTについては，NMDPを介しての，移植前処置とGVHD予防が，割り付け前に決定された形でのランダム化前向き試験の2004年から2009年に移植が行われた551症例での結果が報告され，全生存率には有意差がなく，PB群で造血回復は早く，生着不全は少ないが，急性GVHDの発症は同等で，慢性GVHDは有意にPBSCT群に多いという報告がなされている[19](図3, 4)．

末梢血幹細胞採取

ドナーへの侵襲に関しては，PBSCTのドナーについては，全身麻酔が行われず，多数の骨髄

表1　末梢血幹細胞提供ドナーにおける死亡症例(すべて海外)

	年齢	発生時期	死因	並存症
血縁	61歳	採取4日後	心不全	高血圧，冠動脈疾患
血縁	57歳	帰宅24時間以内	脳卒中	
血縁	64歳	動員終了後	心筋梗塞	冠動脈疾患
血縁	73歳	採取数日後	脳血管障害	高血圧，狭心症
血縁	67歳	採取2日後	硬膜下血腫	心筋梗塞，大動脈瘤手術
血縁	47歳	G-CSF投与4日目	鎌状赤血球症発作	鎌状赤血球症
血縁	未報告	未報告	脳血管障害	
血縁	50歳	カテーテル抜去後	空気塞栓(技術ミス)	
血縁	43歳	不明(採取15日死亡)	心停止	高血圧
血縁	52歳	不明(採取17日後死亡)	心停止	喫煙
血縁	27歳	採取時	心停止(技術ミス)	
非血縁	21歳	カテーテル挿入時	出血(技術ミス)	

(文献[20]より引用)

穿刺が行われない点で非侵襲的と思われがちであるが，報告されたドナーの死亡例は，骨髄採取(以下BMH)の4例に比べて，末梢血幹細胞採取(以下PBSCH)では12例と多い[20](表1)．最近URドナーでの死亡例の報告が1例なされた．幸いわが国では，PBSCHでのドナーの死亡例は報告されていない．PBSCHについては，体外循環時に必要なバスキュラーアクセスの問題と，G-CSF使用による白血球増加，血小板減少，機能亢進，サイトカイン産生などによる，骨痛，発熱，全身倦怠等の身体症状の発生とともに，臓器障害を伴う重篤な合併症のリスクがある[21]．このため，ドナー選択には，BMHと異なった，むしろさらに慎重な全身検索が必要である．白血病発症のリスクについては，完全には解決できておらず[22]，さらに長期間の慎重な経過観察が必要である．BMHの際には，対象が成人であれば，全身麻酔下に合わせて数十か所の穿刺を行うことになるが，PBSCHの場合には，基本的には赤十字血液センターでのドナーからの血小板採取に準じて，血液処理量を増したアフェレシスが行われるのみであるため，採取以降のドナーへの負担は明らかに少ない．

日本の非血縁者間末梢血幹細胞採取の現状

1．ドナーコーディネート

すでに述べたような経緯で，2010年10月に，非血縁者間末梢血幹細胞移植が，JMDPを介して開始された．特に，慎重な立ち上げに配慮し，ドナー，PBSC採取施設については，特別な要件が定められた[23]．また，適格ドナーはHLAのA, B, C, DRについて，アリルで8/8適合者のみで開始することとなった．ドナーについては，骨髄採取の場合と同様，ドナー候補に挙がると，骨髄バンクコーディネーターと調整医師による具体的なBM, PB両方の採取の説明とそれに同意した場合に，全身状態のチェック，不足しているHLA情報のチェックが行われる(確認検査)．この際に採取方法についての希望が調査され，その後は，希望されなかった方法を除いて，コーディネートが進行し，採取方法も含めた移植施設のドナー選定が行われると，ドナー候補は，家族，調整医師，バンクコーディネーター，立会人との5者で最終同意面談を行い，自由意思での採取の同意を行う．その後採取施設での健康診断に臨み，問題がないことが確認されれば，最終的に採取が決定することになる．ドナーの適格性判定はJMDPのドナー適格性基準[24]に従う．年齢は20～55歳とBMHと同様であるが，全身麻酔，多数の腸骨穿刺に伴う合併症のリスク回避に関する要件がとれた反面，幹細胞動員，幹細胞採取に関連した血管イベントのリスクを回避するため，総コレステロール値が240 mg/dlを超えれば不適格とされた．また，原則として，末梢の皮静脈の穿刺となるため，特に脱血路については，確認検査時に採取可能な末梢静脈の存在が適格性に含まれる．