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Different Risk Factors Related to Adenovirus- or BK Virus-Associated Hemorrhagic Cystitis following Allogeneic Stem Cell Transplantation

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Virus-associated hemorrhagic cystitis (HC) is a major cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (HSCT). Although numerous studies have attempted to identify factors that predispose patients to viral HC, its causes remain controversial. We analyzed retrospectively the results of 266 allogeneic HSCTs to identify factors associated with HC. Of this group, 42 patients (15.8%) were diagnosed with viral HC, because of either adenovirus (ADV; n = 26; 9.8%) or BK virus (BKV; n = 16; 6.0%). ADV-HC was frequently associated with T cell purging, and was less common in patients with acute graft-versus-host-disease (GVHD). Conversely, BKV-HC was more frequently observed in patients with excessive immune reactions such as GVHD, preengraftment immune reaction, and hemophagocytic syndrome. These observations indicate that ADV- and BKV-HC may differ significantly in their risk factors and pathogenesis. Profound immune deficiency is more likely to be associated with ADV-HC, whereas immune hyperactivity might play a key role in BKV-HC.

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INTRODUCTION

Hemorrhagic cystitis (HC) is one of the most common complications following hematopoietic stem cell transplantation (HSCT), which remarkably decreases patients' quality of life, and potentially causes therapy-related mortality [1-3]. Clinical manifestations of HC vary from painless microscopic hematuria to gross hematuria, clot formation within the urinary tract, and obstructive renal failure [4]. Early-onset HC that occurs during or shortly after high-dose chemotherapy as part of the conditioning regimen is generally related to cyclophosphamide (CY) toxicity, whereas late-onset HC is mainly attributed to viral infection. BK virus (BKV) is most frequently associated with late-onset HC [5-10], although adenovirus (ADV)- and JC virus (JCV)-associated HC also occur: ADV type 11 is the prominent pathogen for HC, especially in Japan [11-17]. In general, primary ADV and BKV infections typically occur during childhood and remain latent in the genitourinary tract, but these viral infections are prevalent in allo-HSCT recipients and can cause viral-induced HC [1,2].

A number of retrospective studies have proposed a variety of risk factors for HC following allogeneic HSCT (allo-HSCT), including busulfan (BU)-containing myeloablative conditions, unrelated donors, and the occurrence of graft-versus-host disease (GVHD); however, these risk factors were not observed consistently. The analysis of risk factors is likely to be complicated by many variables, including the clinical definitions of HC, the HSCT protocols, or the number and age of patients analyzed. We performed a retrospective analysis of 42 Japanese adult allo-HSCT recipients with either ADV-HC (n = 26) or BKV-HC (n = 16), confirmed by polymerase chain reaction (PCR) examination, to identify risk factors for viral HC.

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Table 1. Pretransplantation Characteristics of the 266 Patients

Characteristics	Total	ADV-HC (n = 26)	BKV-HC (n = 16)	No-HC (n = 224)	*P* Value	
					ADV versus No	BKV versus No
Age, median (range)	48 (16-69)	48.5 (17-69)	52 (29-63)	46.5 (16-68)	.34	.21
Sex, male/female	152/114	17/9	11/5	124/100	.33	.3
Underlying disease					***.004***	.27
MDS/AML	106	5	10	91		
CML	11	1	1	9		
ALL	33	1	1	31		
ML	80	11	2	67		
AA	14	4	0	10		
Others	22	4	2	16		
Disease status at transplantation					.9	***.011***
Standard risk	123	13	1	109		
High risk	143	13	15	115		
Conditioning regimen					.93	.58
Conventional	134	13	7	114		
Reduced intensity	132	13	9	110		
Stem cell source					.66	.55
Related PB	69	6	6	57		
Related BM	14	3	0	11		
Unrelated BM	105	8	5	92		
Unrelated CB	64	7	5	52		
Haploidentical PB/BM	14	2	0	12		
Cycles of prior therapies, median (range)	5 (0-23)	4 (0-23)	4.5 (0-12)	5 (0-19)	.68	.79
Times of HSCT					.14	.5
1st	190	22	10	158		
≥2nd	76	4	6	66		
HLA matching					.6	.17
Full-matched	143	13	6	124		
Mismatched	123	13	10	100		
GVHD prophylaxis*					.61	.5
CsA-based	126	11	9	106		
FK-based	139	15	7	117		
In vivo T cell purging					***.025***	.8
Yes	19	5	0	14		
No	247	21	16	210		
IgG-antibody for ADV (titer)					.84	.26
≤ ×4	159	15	11	133		
×8	13	1	0	12		
×16	32	3	1	28		
Unknown	62	7	4	51		

MDS/AML indicates myelodysplastic syndrome/acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; AA, aplastic anemia; PB, peripheral blood; BM, bone marrow; CB, cord blood; HSCT, hematopoietic stem cell transplantation; CsA, cyclosporine; FK, tacrolimus; GVHD, graft-versus-host disease; ADV, adenovirus; BKV, BK virus.
*One case that used only mPSL (methylprednisolone) was excluded.

PATIENTS AND METHODS

Patients

The medical records of 266 patients (152 men and 114 women; median age = 48 years), who underwent allo-HSCT at Kyushu University Hospital between January 2002 and June 2010, were reviewed; a subset of these patients has been described earlier [11]. Patient characteristics are listed in Table 1. Primary diseases included myelodysplastic syndrome (MDS)/ acute myeloid leukemia (AML; n = 106), chronic myelogenous leukemia (n = 11), acute lymphoblastic leukemia (ALL; n = 33), malignant lymphoma (n = 80), aplastic anemia (n = 14), and others (n = 22). Patients with any of the following conditions were classified as standard risk: acute leukemia (AML or ALL) in remission; chronic myelogenous leukemia in chronic phase; MDS classified as refractory anemia orrefractory anemia with ringed sideroblasts. All others (n = 143) were categorized as high risk. This study was approved by the institutional review board of Kyushu University Hospital.

Transplantation Procedures

A total of 134 patients received conventional preparative regimens, either 12 Gy total body irradiation/CY (n = 94) or BU/CY (n = 40). The remaining 132 cases received purine analog-based reduced-intensity conditioning consisting of either fludarabine (Flu)/CY (n = 25), Flu/BU (n = 69), or Flu/melphalan (n = 38). Low-dose total body irradiation (2-4 Gy), antithymocyte globulin (ATG), and alemtuzumab were administered in 73, 14, and 4 cases, respectively (Table 1). The sources of stem cells included related granulocyte colony-stimulating factor-mobilized peripheral blood (n = 82), related bone marrow (n = 15), unrelated

bone marrow (n = 105), or unrelated cord blood (n = 64). Human leukocyte antigen (HLA)-matching varied from haploidentical (3 of 6) to identical (6 of 6). Of 266 patients, 126 and 139 received cyclosporine- or tacrolimus-based GVHD prophylaxis, respectively; the remaining 1 patient received methylprednisolone alone. A total of 76 patients had received at least 1 prior autologous (n = 28) or allogeneic (n = 48) HSCT, and the reason of second or more transplantations was either relapse (n = 65) or graft failure (n = 11) (Table 1). Acyclovir was given as prophylaxis against herpes simplex virus reactivation, 1000 mg/day orally from days −7 to 35 after HSCT.

Diagnosis and Treatment of Viral HC

Urinalysis was routinely performed at least once a week beginning with the initiation of preparative regimens until discharge or when clinical signs of cystitis appeared after that. If microscopic or macroscopic hematuria and/or bladder irritation existed, urine was further analyzed by rapid immunochromatography and PCR method to detect ADV antigen [11] or ADV, as well as BKV and JCV, viral DNA. Only patients with viruria confirmed by PCR were diagnosed with viral HC and included in our analysis.

All patients with viral HC were treated by supportive modalities including hyperhydration, forced diuresis, and/or blood transfusions. In addition, continuous bladder irrigation and/or administration of antiviral agents were performed based on each physician's decision. According to previous reports with a minor modification [9,10], the response criteria were defined as follows: complete response (CR), the complete resolution of HC symptoms accompanied by eradication of ADV or at least a 2-log reduction of BKV viral load; partial response (PR), a significant improvement of HC symptoms accompanied by persisting microhematuria or continued detection of ADV or BKV in the urine samples; and no change, no improvement or worsening of HC.

Statistical Analysis

The aim of this study was to identify factors correlating with the development of viral HC. Chi-square tests were used for univariate comparisons to examine categoric variables, including sex, underlying diseases, disease status, conditioning regimen, stem cell source, HLA matching, GVHD prophylaxis, and prior HSCT. A numerical variable (age) was compared using the Mann-Whitney test. Odds ratios (ORs) were calculated using a logistic regression analysis, and variables were analyzed using a multivariate stepwise logistic regression model. Survival following allo-HSCT was measured from the date of stem cell infusion until the date of death. The survival period was calculated using the Kaplan-Meier method. P values <.05 were considered statistically significant. All statistical analyses were performed using SPSS 17.0 software (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Incidence of Viral HC

In our series, a total of 42 of 266 allo-HSCT recipients (15.8%) developed viral HC. Of these, 26 (9.8%) were diagnosed with ADV-HC, including coinfection with BKV (n = 3) or JCV (n = 1), and 16 (6.0%) with BKV-HC alone. The immunochromatography assay for ADV antigen was positive in 20 of 24 tested urine samples of ADV-HC patients, although false-positive results were obtained in 4 of 13 BKV-HC patients, confirming the reliability of this assay for diagnosing ADV-HC [11].

ADV-HC has predominantly been reported from Japanese transplantation centers [11-17], whereas BKV-HC is frequently seen worldwide [5-10]. Because the role of BKV in HC pathogenesis remains unclear, because it is commonly found in the urine of unaffected patients, we analyzed ADV-HC and BKV-HC separately and compared it to patients without HC (n = 224).

Pretransplantation Characteristics of Patients with ADV-HC and BKV-HC

Six of 128 (4.7%) patients who underwent HSCT for acute leukemia (MDS/AML and ALL) developed ADV-HC, which was significantly less frequent than in the 20 of 122 (16.4%) patients suffering from other disorders (P = .004). A high incidence of ADV-HC was found in patients who received T cell purging using ATG or alemtuzumab (26.3%; 5 of 19) compared with those who did not (9.1%; 21 of 231; P = .025). Some studies have reported a close association between positive results of anti-ADV antibody and the development of ADV-HC [12,16], whereas another group [14] and our study could not detect such a relationship between them (Table 1). In contrast, BKV-HC was closely related to the status of underlying diseases at HSCT: high-risk patients developed BKV-HC more frequently than standard-risk patients (11.5%, n = 130 versus 0.9%, n = 110; P = .011). There was no association among viral HC with sex, stem cell source, or HLA matching. Moreover, the incidence of viral-HC was not affected by the usage of BU (BU-containing, 12.8%, n = 109 versus non-BU, 17.8%, n = 157; P = 0.27), usage of CY (CY-containing, 16.4%, n = 159 versus non-CY, 15.0%, n = 107; P = 0.76), or prior history of treatment (number of cycles of pretransplantation therapy) (Table 1).

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Table 2. Clinical and Laboratory Manifestations of Viral-Associated HC

Case	Diagnosis	Graft	Conditioning	Preengraftment Allo-reaction	Maximum Grade of GVHD	Immunosuppressive Agents at HC Onset	Onset (Day)	Hematuria	Bladder Irritation	ADV-IC	Viruria (PCR)	Antiviral Agents	Response	CMV Reactivation	VZV Reactivation	Outcome
1	LPL	UBM	Conv	(−)	(−)	FK	2	macro	No	(+)	ADV	CDV	CR	(−)	(−)	survive
2	AA	RBM	RIC (ATG)	NA	NA	FK	4	macro	Yes	(+)	ADV	CDV	CR	NA	(−)	dead by infection
3	AA	RBM	RIC (Campath)	(−)	(−)	CsA	7	OB	No	(+)	ADV	CDV	CR	antigenemia	(+)	survive
4	ATL	UCB	Conv	NA	NA	CsA	7	macro	Yes	(−)	ADV	CDV + FCV	PR (viruria+)	NA	(−)	ATL relapse
5	ATL	UCB	RIC	HPS	(−)	FK	11	macro	No	(+)	ADV + BKV	CDV	PR (viruria+)	(−)	(−)	dead by bleeding
6	HPS	UCB	RIC (ATG)	(−)	(−)	FK/PSL	13	macro	Yes	(+)	ADV + BKV	CDV	PR (viruria+)	antigenemia	(−)	survive
7	ATL	RPB	Conv	PIR	acute(**IV**)	CsA/mPSL	19	OB	Yes	(+)	ADV	CDV	PR (OB+)	antigenemia	(−)	dead by infection
8	NK leukemia	UCB	Conv	(−)	(−)	CsA/PSL	22	macro	Yes	(+)	ADV	CDV + ribavirin	NC	antigenemia	(−)	survive
9	AA	haplo-BM	Conv	(−)	acute(**II**)	FK/PSL	25	macro	No	(+)	ADV	CDV	CR	antigenemia	(−)	survive
10	PTCL-u	UCB	RIC	(−)	(−)	FK	29	OB	Yes	(+)	ADV	GCV	CR	gastritis	(+)	survive
11	AA	UBM	RIC (ATG)	PIR	(−)	CsA	30	macro	Yes	(+)	ADV	CDV	PR (OB+)	antigenemia	(−)	dead by bleeding
12	AML	UBM	Conv	HPS	acute(**II**)	FK/PSL	31	macro	No	(+)	ADV	None	CR	antigenemia	(+)	dead by PD
13	NK lymphoma	RPB	RIC	PIR	acute(**IV**)	FK/mPSL	47	macro	Yes	(+)	ADV	CDV	CR	colitis	(−)	dead by TMA
14	MM	RPB	RIC	(−)	chronic(extensive)	CsA/PSL	79	OB	Yes	(+)	ADV	CDV	CR	antigenemia	(−)	survive
15	ATL	haplo-PB	RIC (ATG)	(−)	(−)	FK/PSL	120	macro	Yes	(+)	ADV	CDV	CR	antigenemia	(−)	survive
16	AML	UBM	Conv	(−)	(−)	FK	144	macro	Yes	(−)	ADV	CDV	CR	antigenemia	(−)	survive
17	AITL	UCB	RIC	PIR	acute(**III**)	CsA/PSL/MMF/ basiliximab	149	macro	No	NA	ADV	CDV	CR	antigenemia	(−)	dead by infection
18	MDS/AML	RPB	RIC	PIR	acute(**II**)	CsA/PSL	183	macro	Yes	(+)	ADV	CDV	PR (OB+)	antigenemia	(−)	dead by PD
19	MF	RPB	Conv	(−)	chronic (extensive)	CsA/PSL	184	macro	Yes	(+)	ADV	CDV	CR	(−)	(+)	survive
20	HCL	RPB	Conv	PIR	acute(**II**), chronic(limited)	CsA/PSL	265	OB	Yes	(+)	ADV	CDV	CR	(−)	(−)	survive
21	AML	RBM	Conv	(−)	acute(**I**), chronic (extensive)	CsA/PSL	266	macro	Yes	NA	ADV	None	CR	(−)	(−)	survive
22	CML	RBM	Conv	(−)	chronic(extensive)	PSL	281	macro	Yes	(+)	ADV	CDV	CR	antigenemia	(−)	survive
23	MF	UBM	RIC	(−)	(−)	FK/PSL	368	macro	Yes	(+)	ADV	CDV	CR	antigenemia	(−)	survive
24	AML	UBM	Conv	(−)	(−)	(−)	455	macro	Yes	(−)	ADV + JCV	None	PR (viruria+)	gastritis/colitis	(−)	dead by PD
25	ALL	UCB	RIC	(−)	(−)	(−)	484	macro	No	(+)	ADV	CDV	CR	(−)	(−)	survive
26	DLBCL	UBM	Conv	PIR	chronic(limited)	FK	875	macro	Yes	(−)	ADV + BKV	CDV	CR	antigenemia	(+)	survive
27	AML	UCB	RIC	HPS	(−)	CsA	6	OB	Yes	(+)	BKV	None	CR	antigenemia	(−)	dead by PD
28	ALL	UBM	Conv	PIR	acute(**II**)	FK	7	OB	Yes	(−)	BKV	None	PR (OB+)	antigenemia	(−)	dead by PD
29	Gastric Ca	RPB	RIC	HPS	NA	CsA/PSL	11	macro	No	(−)	BKV	None	NC	NA	(−)	dead by infection
30	DLBCL	UCB	RIC	PIR	acute(**III**)	CsA/PSL	29	macro	Yes	NA	BKV	None	NC	(−)	(−)	dead by PD
31	MDS/AML	UBM	Conv	(−)	acute(**III**)	FK/mPSL/MMF/ basiliximab	40	macro	No	NA	BKV	None	NC	antigenemia	(−)	dead by GVHD
32	ATL	UCB	RIC	PIR	acute(**II**)	CsA	42	macro	Yes	(−)	BKV	None	CR	antigenemia	(−)	dead by PD
33	MDS/AML	RPB	Conv	PIR	acute(**II**)	CsA/mPSL	44	OB	Yes	(−)	BKV	None	CR	antigenemia	(+)	dead by PD
34	MDS/AML	RPB	RIC	(−)	acute(**II**)	CsA/mPSL	49	macro	Yes	(+)	BKV	None	CR	antigenemia	(−)	dead by PD
35	MDS/AML	UBM	RIC	PIR	acute(**II**)	FK/mPSL	50	OB	Yes	(−)	BKV	None	CR	antigenemia	(−)	dead by PD

36	AML	UBM	Conv	PIR	acute(**IV**)	FK/PSL/MMF/ etanercept	52	macro	No	(+)	BKV	CDV	PR (viruria+)	(−)	(−)	dead by GVHD
37	AML	UCB	RIC	PIR	acute(**III**)	CsA/PSL	61	macro	Yes	(−)	BKV	CDV	PR (OB+)	antigenemia	(−)	AML relapse
38	AML	UCB	Conv	PIR	(−)	CsA	66	macro	Yes	(−)	BKV	None	PR (OB+)	antigenemia	(−)	dead by infection
39	MDS/AML	RPB	RIC	PIR	acute(**II**)	CsA/PSL	86	macro	No	(−)	BKV	CDV	PR (viruria+)	antigenemia	(−)	survive
40	AML	RPB	Conv	HPS	chronic(limited)	CsA/PSL	122	macro	No	NA	BKV	CDV + Ara-A	PR (viruria+)	antigenemia	(−)	dead by PD
41	Thymic Ca	RPB	RIC	(−)	acute(**II**), chronic(extensive)	CsA/PSL	134	macro	No	(−)	BKV	None	NC	(−)	(−)	dead by PD
42	CML	UBM	Conv	PIR	chronic(extensive)	FK/PSL	443	macro	No	(+)	BKV	None	NC	antigenemia	(−)	dead by renal failure

LPL indicates lymphoplasmacytic lymphoma; AA, aplastic anemia; ATL, adult T cell lymphoma; HPS, hemophagocytic syndrome; PTCL-u, peripheral T cell lymphoma unclassified; AML, acute myelogenous leukemia; MM, multiple myeloma; AITL, angioimmunoblastic T cell lymphoma; MDS, myelodysplastic syndrome; MF, myelofibrosis; HCL, hairy cell leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; DLBCL, diffuse large B cell lymphoma; UBM, unrelated bone marrow; RBM, related bone marrow; UCB, unrelated cord blood; RPB, related peripheral blood; Conv, conventional conditioning; RIC, reduced-intensity conditioning; ATG, antithymocyte globulin; NA, not assessed; PIR, preengraftment immune reaction; FK, tacrolimus; CsA, cyclosporin A; PSL, prednisolone; mPSL, methylprednisolone; MMF, mycophenolate mofetil; OB, occult blood; CDV, cidofovir; FCV, foscarnet; GCV, gancyclovir; CR, complete remission; PR, partial remission; NC, no change; VZV, varicella zoster virus; PD, progressive disease; TMA, thrombotic microangiitis; GVHD, graft-versus-host disease.

Clinical Findings of ADV-HC and BKV-HC

The clinical features of patients developing viral HC are shown in Table 2. The median onset of clinical symptoms was 49.5 days (range: 2-875 days), consistent with previous reports [13-15]. Most of the patients with ADV-HC (53.8%) and BKV-HC (75%) presented their symptoms more than 1 month after transplantation, indicating that viral HC was caused by the reactivation of latent infection rather than acute infection during the transplantation course. The frequency of macrohematuria or bladder irritation was similar between patients with ADV-HC and those with BKV-HC; however, the symptoms were more severe in ADV-HC.

At the onset of viral HC, almost all the patients were receiving immunosuppressive agents as either prophylaxis or treatment of GVHD or hemophagocytic syndrome (HPS). Interestingly, the incidence of allogeneic immune reactions was significantly different between patients with ADV-HC and BKV-HC. Acute GVHD (aGVHD) (maximum; grade II to IV) throughout the clinical course was marginally less frequent in ADV-HC patients than in patients without viral HC (P = .054; Table 3), possibly because of T cell purging. In contrast, patients with BKV-HC were more likely to have developed either noninfectious fever before engraftments known as a preengraftment immune reaction (PIR) [18] or HPS (80%) or postengraftment aGVHD (grade II-IV; 73.3%) than patients without viral HC (43.8%, P = .013 and 50.5%, P = .099, respectively; Table 3).

Finally, we found a significant or marginal increase of proven cytomegalovirus diseases (e.g. gastritis, colitis) or varicella zoster virus reactivation in ADV-HC patients compared with non-HC individuals (12.5% versus 3.6%, P = .05, and 20.8% versus 12.9%, P = .29, respectively).

Treatment and Outcome of Viral HC

In addition to supportive treatments, 8 of 46 patients with HC required continuous bladder irrigation. Twenty-two of 26 patients with ADV-HC and 4 of 16 patients with BKV-HC were treated with low-dose cidofovir (CDV; 1 mg/kg/day, 3 times a week), as previously reported [11]. In addition to CDV treatment, foscarnet was subsequently administered for 1 patient, ribavirin for 1, and Ara-A for the other because of their inadequate responses. Clinical features were relieved in all except 1 ADV-HC patient; 18 patients obtained CR, 7 obtained PR with persistent viruria (n = 4) or microhematuria (n = 3) without bladder irritation, and in the remaining case, autologous stem cell rescue for the primary graft failure improved his symptoms (case 8). Of the 16 patients with BKV-HC, symptoms persisted in 6 patients, although 5 of them did not receive antiviral agents because of either relapse of their

Table 3. Posttransplantation Characteristics of the 233 Engrafted Recipients

Characteristics	Total	ADV-HC (n = 24)	BKV-HC (n = 15)	No-HC (n = 194)	*P* Value ADV versus No	*P* Value BKV versus No
PIR and/or HPS					.56	*.013*
Yes	106	9	12	85		
No	127	15	3	109		
aGVHD*					*.054*	.099
No	89	16	4	69		
Grade I	28	1	0	27		
Grade II	79	4	7	68		
Grade III	25	1	3	21		
Grade IV	12	2	1	9		
CMV reactivation					.69	.47
Yes (antigenemia/diseases)	158/10	15/3	12/0	131/7		
No	65	6	3	56		
VZV reactivation					.29	.49
Yes	31	5	1	25		
No	202	19	14	169		

PIR indicates preengraftment immune reaction; HPS, hemophagocytic syndrome; aGVHD, acute graft-versus-host disease; CMV, cytomegalovirus; VZV, varicella zoster virus.
*Maximum grade throughout the observation period.
Statistically significant differences were indicated by italics.

underlying diseases, low performance status, or insufficient organ function (Table 2).

Nine of 26 patients with ADV-HC died because of infection (n = 4), disease progression (n = 3), and bleeding (n = 2). Of the surviving patients, 1 relapsed and 16 remained disease-free. In contrast, 14 of 16 patients with BKV-HC died because of disease progression (n = 9), other infection (n = 2), GVHD (n = 2), or renal failure (n = 1), and 1 patient relapsed, leaving a single disease-free survivor from the patients with this complication. In our study, the 1-year overall survival after HSCT was only 16.1% ± 10.2% for patients with BKV-HC, significantly lower than that of patients without HC (52.5% ± 3.4%) or with ADV-HC (63.7% ± 10.4%).

Risk Factors for ADV-HC and BKV-HC

Univariate analysis using logistic regression identified strong associations between ADV-HC and (1) the underlying disease (acute leukemia versus others; *P* = .004), (2) T cell purging (*P* = .025), and (3) less occurrence of severe aGVHD (grade II-IV versus grade 0-I; *P* = .054). Multivariate logistic regression analysis confirmed that all 3 factors had significant or marginal association with developing ADV-HC; ORs were 4.488 (95% confidence interval (CI) = 1.625-12.40; *P* = .004), 4.176 (95% CI = 0.942-18.50; *P* = .06), and 0.390 (95% CI = 0.148-1.025; *P* = .054), respectively (Table 4).

Similar analysis of BKV-HC patients identified a significant relationship with disease status at HSCT (high risk versus standard risk; *P* = .011), PIR and/or HPS (*P* = .013), and marginal to aGVHD (grade II-IV versus grade 0-I; *P* = .099). Multivariate analysis showed that high-risk disease status (OR = 14.34; 95% CI = 1.81-113.4; *P* = .012) and presence of PIR and/or HPS (OR = 4.13; 95% CI = 1.06-16.14; *P* = .041) were the risk factors for BKV-HC (Table 4).

DISCUSSION

BKV is frequently isolated from asymptomatic patients before or following HSCT [5-8], and even from healthy individuals [19-21], indicating that the presence of BKV in urine samples is not always associated with HC. In contrast, ADV is almost exclusively detected in patients with HC, indicating a likely causative role [11-17,22]. BKV is more

Table 4. Results of Multivariate Logistic Regression Analysis

Characteristics		Odds Ratio (95% CI)	*P* Value
ADV-HC			
Underlying disease	Others versus acute leukemia	4.488 (1.625-12.40)	*.004*
In vivo T cell purging	Yes versus no	4.176 (0.942-18.50)	.06
aGVHD	grade II to IV versus 0 to I	0.390 (0.148-1.025)	.054
BKV-HC			
Disease status at transplantation	High risk versus standard risk	14.34 (1.812-113.4)	*.012*
PIR and/or HPS	Yes versus no	4.132 (1.058-16.14)	*.041*

Odds ratio were calculated by the backward or the forward stepwise selection methods.
Statistically significant differences were indicated by italics.

frequently detected in the urine of the posttransplantation patients compared with ADV, increasing from 7% to 47% for BKV and by 4% for ADV [6]. Despite a high BKV reactivation rate, HC occurs in only a fraction of patients with sustained BK viruria, whereas the majority of HSCT recipients with ADV viruria progress to HC [6,14,23]. In our study, we retrospectively analyzed 266 patients to identify the typical clinical features of Japanese adult allogeneic HSCT recipients who develop viral HC. The cumulative incidence of viral HC was 15.8% overall in our study group, 9.8% because of ADV and 6.0% because of BKV. We found that the factors associated with ADV- or BKV-HC were significantly different.

In the present study, approximately one-half of the ADV-HC cases were early onset (<1 month post-HSCT) and were closely associated with the underlying diseases including lymphoid malignancies and usage of ATG or alemtuzumab as a part of conditioning for AA patients, consistent with previous reports [22,24-26]. In contrast, late-onset ADV-HC (>1 month post-HSCT) was associated with chronic GVHD (6 of 14 patients) and the administration of prolonged immunosuppressants (12 of 14 patients; Table 2). In addition, 15 of 24 patients with ADV-HC were positive for the cytomegalovirus antigen test throughout HSCT, possibly indicating a general impairment of immune protection against viral reactivation. This suggests that profound immune suppression, such as T cell depletion or persistent GVHD and the resultant prolonged administration of immunosuppressants, may be a critical factor in the etiology of ADV-HC.

Only a subset of HSCT recipients with BK viruria progress to clinical BKV-HC, suggesting that other factors may be involved in this complication. Previous reports have shown that BKV-HC is extremely rare in autologous HSCT recipients [14,27,28], although their intensity of myeloablative preparative regimens, as well as the level and incidence of BK viruria, were similar among patients with autologous and allogeneic HSCT [27]. Here we have identified a significant association between occurrence of BKV-HC and aGVHD or non-T cell purging, consistent with previous reports from others [6,7,9,22,29]. Ten of 16 (62.5%) cases with BKV-HC developed their symptoms between engraftment and 100 days post-HSCT, in which aGVHD were frequently occurred, suggesting that immune reactions mediated by donor T cells may be an important contributing factor for developing BKV-HC. In addition, PIR and/or HPS, which are also indicative of excessive allogeneic immune reactions, were more frequently observed in patients with BKV-HC (80%, 12 of 15 patients) than among those without viral HC (43%, 85 of 194 patients). The lower frequency of severe aGVHD among Japanese HSCT recipients than in Western countries [30,31] may, in part, account for the lower incidence of BKV-HC in Japan.

Our findings indicate that ADV- and BKV-HC may develop because of different mechanisms in allo-HSCT recipients. Although under normal circumstances, BKV and ADV remain latent in the urinary tracts following primary infection, analysis of urine samples using PCR indicates that BKV is able to replicate in healthy adults [19-21], although it does not typically lead to HC. BKV-HC was frequently found in patients with excessive allogeneic immune reactions such as GVHD, PIR, and HPS. Because BKV is usually not sufficient to cause HC, BKV might cooperate with excessive immune reactions to cause HC, although it remains unclear whether this immune attack can target the uroepithelium or not. In contrast, ADV is usually undetectable in the urine of healthy adults, indicating that ADV does not replicate under the normal immune status [32]. In the allo-HSCT recipients, ADV-HC was associated with T cell purging and the underlying disease. It is conceivable that severe immune suppression allows ADV replication to occur in the urinary tract, leading to local inflammation and subsequent development of HC. Because BKV viruria may be asymptomatic, it is likely that ADV is more virulent than BKV for developing HC.

The influence of HC on the outcome of HSCT remains controversial. In our study, 22 of 26 patients developing ADV-HC were promptly initiated low-dose CDV, as previously reported [11], resulting in CR in 15 patients and PR in 6 others, and the 1-year overall survival in patients with ADV-HC was similar to those without ADV-HC (63.7% versus 52.5%). In contrast, patients with BKV-HC had a very low probability of survival (<20%), although others have reported that the clinical course of BKV-HC was less severe than ADV-HC [6]. In these patients, the main cause of death was not the BKV-HC but the progression of the underlying diseases; 15 of 16 cases with BKV-HC underwent allo-HSCT against the uncontrolled diseases. Irrelevant immune reactions and the resultant administration of immunosuppressants might contribute to the reduction of the graft-versus-leukemia effect.

In conclusion, we have identified different related factors in HSCT recipients to develop either ADV-HC or BKV-HC, although there are the limitations to a retrospective, single-center analysis. Severe immunosuppression might play a pivotal role for ADV reactivation and subsequent development of ADV-HC, whereas an excessive immune reaction might be critical for the development of BKV-HC. Earlier diagnosis and intervention for ADV-HC with low-dose CDV therapy may provide a survival benefit. It will be interesting to see if these associations are found in other adult populations.

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ORIGINAL ARTICLE

Validation of pretransplantation assessment of mortality risk score in the outcome of hematopoietic SCT in non-Caucasians

Y Mori[1,2], T Teshima[2], K Kamezaki[1], K Kato[1], K Takenaka[1], H Iwasaki[2], T Miyamoto[1], K Nagafuji[1], T Eto[3] and K Akashi[1,2]

The Pretransplantation Assessment of Mortality (PAM) score is a risk score for mortality after allogeneic hematopoietic SCT (HSCT). Ethnicity is a genetically determined factor that correlated with immune-mediated outcomes of allogeneic HSCT. We evaluated the predictive value of the PAM score for transplant outcome in 276 Japanese populations in which transplant-related complications occur less frequently than Caucasians. The PAM score effectively risk-stratified these patients for survival; overall survival (OS) at 2 years was 100%, 80.2%, 49.4%, and 13.9% in the categories 1, 2, 3, and 4, respectively, showing a clear distinction of OS by categories ($P<0.001$). In addition, the PAM score is useful for the prediction of transplant outcomes both in patients with standard-risk underlying diseases and those with high-risk diseases. The PAM score developed in Caucasian populations is thus useful in non-Caucasian populations.

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Keywords: PAM score; hematopoietic SCT; prognosis; Japanese

INTRODUCTION

Allogeneic hematopoietic SCT (HSCT) is a curative but toxic treatment for hematological disorders. Its indication is expanding with the greater use of alternative donors and stem cell sources, such as PBSC and umbilical cord blood. Moreover, the advent of non-myeloablative conditioning regimens has led to an increasing use of transplantation for older, having comorbidities, and more heavily pretreated patients. In these patients, estimation of benefits and risks of this high-risk procedure is particularly crucial for decision making.

Recent studies have made great efforts to develop reliable and reproducible predictive models in this setting: The Pretransplantation Assessment of Mortality (PAM) score was developed at the Fred Hutchinson Cancer Research Center (FHCRC) to predict all-cause mortality within 2 years after allogeneic HSCT.[1] It is calculated by using multiple transplant-related variables, including patients' ages, donor type and the conditioning regimen as well as patients' comorbidities. However, predictive values of the PAM score remain unclear at other centers, in particular outside the Western countries.[2,3] It has been well recognized that ethnicity is one of the factors, which may affect the transplant outcome.[4-6] In this study, we evaluated whether the PAM score could be predictive for transplant outcome also in Japanese patients.

PATIENTS AND METHODS

Patients

The medical records of 276 patients who underwent their first allogeneic HSCT at the Kyushu University Hospital or Hamanomachi Hospital between January 2004 and March 2009 were reviewed. Primary diseases included myelodysplastic syndrome/AML; $n=125$, CML; $n=12$, ALL; $n=43$, malignant lymphoma (ML; $n=76$), aplastic anemia ($n=10$), solid tumors ($n=4$) and others ($n=6$). A total of 148 cases were categorized into the standard-risk (SR) group defined as follows: acute leukemia (AML or ALL) in CR; CML in chronic phase; myelodysplastic syndrome classified as refractory anemia or refractory cytopenia with multilineage dysplasia, ML in at least PR and non-malignant hematological disorders. All others ($n=128$) were defied as high-risk (HR) group. This study was approved by the Institutional Review Board.

Transplantation procedures

A total of 180 patients had received myeloablative preparative regimens, TBI/CY ($n=142$), BU/CY ($n=31$) or others ($n=7$). The remaining 96 cases had received fludarabine (Flu)-based reduced intensity conditioning regimen with either CY ($n=4$), BU ($n=71$) or melphalan ($n=21$; Table 1). Low-dose TBI (2-4 Gy) and alemtuzumab were included into the above reduced intensity conditioning regimen in 76 and 3 patients, respectively. The sources of stem cells were PBSC from a related donor ($n=53$), BM from a related ($n=23$) or unrelated donor ($n=161$) or cord blood ($n=39$). HLA matching varied from haploidentical (3/6) to identical (6/6). GVHD prophylaxis was calcineurin inhibitor (CI) plus either short-term MTX ($n=268$) or mycophenolate mofetil ($n=7$), and CI alone ($n=1$). A total of 22 patients had a previous history of autologous PBSCT.

Risk status assessment

PAM score components, including age at transplantation, disease risk, donor type, conditioning regimen and liver (serum level of alanine aminotransferase), kidney (serum level of creatinine), and lung (one second forced expiratory volume and diffusing capacity for carbon monoxide) function, were collected and scored as originally described[1] using automatic calculation system on web site (http://cdsweb.fhcrc.org/pam/).

The variables used to calculate the European Group for Blood and Marrow Transplantation (EBMT) risk score were age, interval from diagnosis to transplant, disease status, donor-recipient sex match and donor type. The risk-score for an individual patient is simply the sum of the scores of each risk factor, as originally reported.[7]

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Table 1. Characteristics of the 276 allogeneic HSCT recipients

Characteristics	*Total (n = 276)*	*Disease status at HSCT*		P-*value*
		*SR** (n = 148)*	*HR (n = 128)*	
Age, median (range)	47.5 (16-68)	45.5 (16-68)	49 (17-68)	NS
Sex				NS
Male	155	81	74	
Female	121	67	54	
Underlying disease				0.11
MDS/AML	125	63	62	
CML	12	5	7	
ALL	43	31	12	
ML (ATL)	76 (35)	38 (23)	38 (12)	
Others*	20	11	9	
Conditioning regimen				***0.02***
Myeloablative	180	106	74	
Reduced intensity	96	42	54	
Stem cell source				0.13
BM	184	106	78	
PB	53	26	27	
CB	39	16	23	
Donor type				***0.02***
Matched related	65	42	23	
Matched unrelated	105	60	45	
Mismatched	106	46	60	
Prior autologous HSCT				*0.03*
Yes	22	7	15	
No	254	141	113	
GVHD prophylaxis				NS
CsA based	103	58	45	
FK based	173	90	83	
PAM score				***<0.001***
Category 1 (8-16)	13	13	0	
Category 2 (17-23)	44	40	4	
Category 3 (24-30)	182	90	92	
Category 4 (31-50)	37	5	32	
*EBMT scoring***				***<0.001***
Risk 0	0	0	0	
Risk 1	30	30	0	
Risk 2	47	47	0	
Risk 3	61	43	18	
Risk 4	78	18	60	
Risk 5	46	6	40	
Risk 6-7	6	0	6	

Abbreviations: ATL = adult T-cell leukemia; CB = cord blood; FK = tacrolims; MDS/AML = myelodysplastic syndrome/AML; ML = malignant lymphoma; SR = standard risk; NC = not calculated; PB = peripheral blood. *Others included aplastic anemia, solid tumors and hemophagocytic syndrome. **SR included AML or ALL in remission, CML in chronic phase, MDS classified as refractory anemia or refractory cytopenia with multilineage dysplasia, ML in at least PR and nonmalignant hematological disorders. All others were categorized into HR (high risk). ** Analyzed in 268 patients. Bold italic values Indicate statistical significance.

Statistical analysis

The main aim of this retrospective analysis was to assess the predictive value of the PAM score on 2-year overall survival (OS) in allogeneic HSCT recipients. OS was defined as the time from the day of stem cell infusion to death with any cause. The probability of OS was estimated using Kaplan-Meier curves and compared using the log-rank tests. For univariate comparisons, we examined variables, including age, sex, underlying diseases, disease status, conditioning regimen, stem cell source, HLA matching, GVHD prophylaxis and prior HSCT using χ^2 test or Mann-Whitney test. *P*-values <0.05 were considered to indicate statistical significance. The diagnostic reliability of these models was compared by using the c-statistics. All statistical analyses used SPSS 17.0 program (SPSS Japan Inc., Tokyo, Japan) or SAS ver9.2.

RESULTS

Patient characteristics

There were 155 men and 121 women with a median age of 47. 5 years. Patients' characteristics are listed in Table 1. A total of 148 patients (54%) had SR diseases and 128 (46%) had HR diseases at

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transplantation. As compared with SR disease patients, patients with HR disease were less likely to have ALL ($P = 0.025$), but more likely to receive CBT ($P = 0.089$) or reduced intensity conditioning regimen ($P = 0.016$). HSCT recipients for HR disease were significantly associated with HLA-mismatched donor ($P = 0.042$) as a reflection of more frequent use of CBT in HR patients than SR patients.

PAM score

Among 276 patients, 13 (5%), 44 (16%), 182 (66%) and 37 (13%) patients were classified into categories 1, 2, 3 and 4, respectively, based on the categorization of PAM score. The median PAM score was 26 (range, 11–38) and the distribution of the score was shown in Figure 1a. Because disease status was one of the factors for scoring, patients with HR disease tended to have higher PAM scores compared with those with SR disease; numbers of patients fit into categories 1, 2, 3 and 4 were 0 (0%), 4 (3%), 92 (72%) and 32 (25%) in HR patients, whereas 13 (9%), 40 (27%), 90 (61%) and 5 (3%) in SR patients ($P < 0.001$). Hepatic dysfunction was the most common comorbidity (44 out of 276, 16%) in our cohort (Figure 1b). The frequency of other comorbidities were as follows: 10% with low one second forced expiratory volume, 10% with low diffusing capacity for carbon monoxide and 3% with increased serum creatinine level, consistent with the original study at FHCRC.[1] The proportion of patients with low one second forced expiratory volume was significantly greater in patients with HR disease than those with SR disease (14.1% vs 6.8%; $P = 0.05$, Figure 1b). Univariate analysis revealed that these components of PAM score, except for hepatic dysfunction, were independent prognostic factors in this study (data not shown).

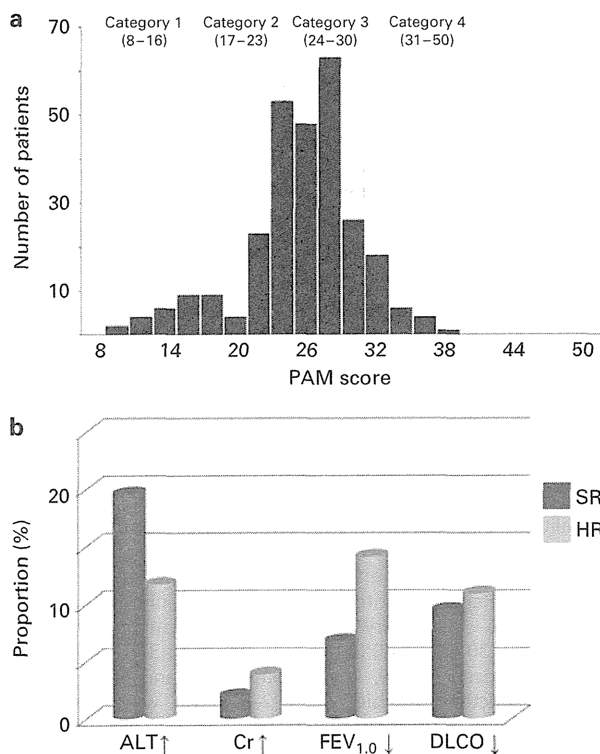

Figure 1. Risk assessment of 276 HSCT recipients. (**a**) Histogram for distribution of the PAM scores. The median score is 26 (range; 11–38). (**b**) Prevalence of comorbidities according to PAM score in patients stratified by disease status at HSCT.

Probability of 2-year OS by PAM score categories

The median follow-up of surviving patients is 45.4 months (range, 10.3–81.5 months); OS at 2 years was 51.8% (Figure 2a). There was a stepwise relationship between PAM score category and survival; The 2-year probability of OS was 100%, 80.2%, 49.4% and 13.9% in patients with category 1, 2, 3 and 4, respectively ($P < 0.001$), showing a clear distinction of OS by categories (Figure 2b). Although this study was designed to evaluate 2-year survival, it appeared that PAM score was also predictive for long-term survival beyond 2 years after transplant.

During the observation period, 64 patients (23.2%) died of disease progression; 0%, 9.1%, 26.4% and 32.4% in category 1, 2, 3 and 4, respectively, whereas 73 (26.4%) patients died of non-relapse mortality (NRM); 0%, 11.4%, 26.4% and 54.1% in category 1, 2, 3 and 4, respectively. The most common causes of NRM were infections ($n = 27$) and GVHD ($n = 21$).

We next compared the predictive value of the PAM score with that of the EBMT risk score, which is another well-established prognostic model for transplant outcome.[7,8] Among 268 patients, 0 (0%), 30 (11%), 47 (18%), 61 (23%), 78 (29%), 46 (17%) and 6 (2%) patients were classified into risk score 0, 1, 2, 3, 4, 5 and 6–7, respectively (Table 1). The distribution of patients was relatively skewed into higher risk group, when compared with previous reports,[7,8] mainly due to more advanced disease status and older age in our cohort. Higher risk score in the EBMT risk model was associated with HR patients ($P < 0.001$; Table 1) and higher PAM score (data not shown). We found that the EBMT risk score was also useful for a risk stratification in our cohort ($P < 0.001$, shown in Figure 2c). However, its predictive value for transplant outcome determined by c-statistics was slightly lower than that of the PAM score (0.63 vs 0.70; Table 2).

Impact of PAM scores on survival according to disease status

We then evaluated the impact of PAM scores on OS in HR patients ($n = 128$) and SR patients ($n = 148$) separately. At 2 years, patients with SR diseases had significantly better OS compared with those with HR diseases (64.2% vs 37.3%, $P < 0.001$). For patients with SR diseases, 2-year OS was 100%, 80.8%, 56.0% and 0% in categories 1, 2, 3 and 4, respectively (Figure 3a), with a statistically significant difference among 4 groups ($P < 0.001$). There were also statistical or marginal differences either in category 1 vs 2 ($P = 0.10$), category 2 vs 3 ($P = 0.007$) or in category 3 vs 4 ($P < 0.001$).

In patients with HR diseases, no case was classified into category 1. In all, 2-year OS was 75.0%, 43.0% and 16.2% in category 2, 3 and 4 groups, respectively ($P < 0.001$; Figure 3b). In this subgroup analysis, there was no significant difference between categories 2 and 3 ($P = 0.23$), possibly due to the low numbers of patients categorized in category 2, whereas there was a significant difference between categories 3 and 4 ($P < 0.001$).

Impact of PAM scores on survival according to graft source

We next evaluated predictive values of PAM scores for transplant outcome after CBT ($n = 39$) vs BMT/PBSCT ($n = 237$). CBT was preferentially performed in HR patients than in SR patients due to rapid availability of cord blood and less stringent requirement for HLA matching. CBT recipients had more HR diseases (59.0% vs 44.3%, $P = 0.09$), higher PAM score (mean score; 28.3 vs 25.4, proportion of patients classified into category 3 or 4; 92.3% vs 77.2%, $P = 0.07$) and worse 2-year OS (35.8% vs 54.3%, $P = 0.05$) when compared with BMT/PBSCT recipients (data not shown).

No CBT recipients were classified into category 1, and 2-year OS in category 2, 3 and 4 was 50%, 40.9% and 0%, respectively. PAM score could not distinguish the outcome of patients in category 2 from that in category 3 ($P = 0.36$; Figure 3c). In contrast, the PAM score was useful for the prediction of outcome among BMT/PBSCT recipients; 2-year OS was 100%, 81.7%, 51.0% and 16.7% in

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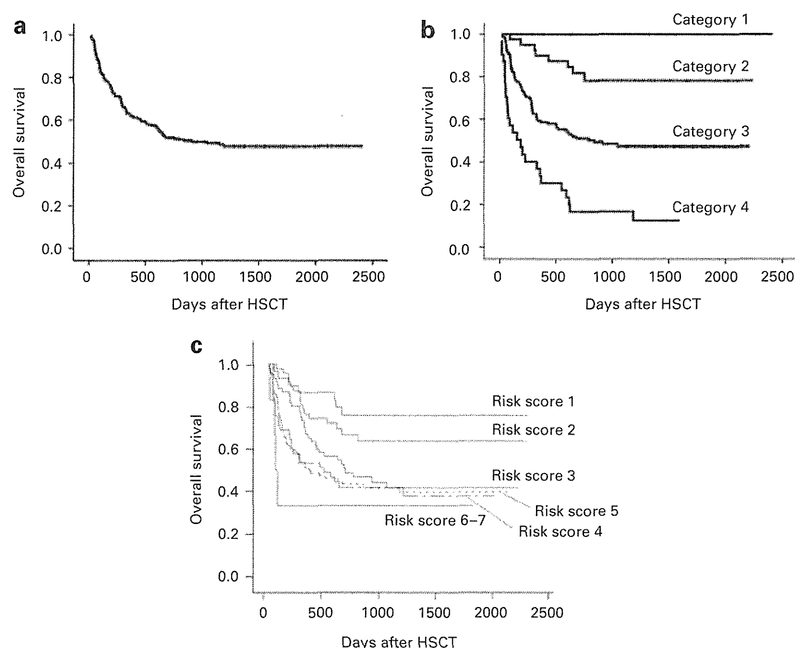

Figure 2. Comparative analysis between PAM score and EBMT risk score Probability of OS in (**a**) whole cohort, (**b**) in each PAM category and (**c**) in each EBMT risk scoring.

Table 2. Distribution of patients in each risk category

Prognostic model	*Risk category (%)*							*c-Statistics*
	1	*2*	*3*	*4*	*5*	*6*	*7*	
PAM score	5	16	66	13	–	–	–	0.70
PAM score with modified categorization	11	29	47	13	–	–	–	0.74
EBMT risk score	0	11	18	23	29	17	2	0.63

Abbreviations: EBMT = European Group for Blood and Marrow Transplantation; PAM = Pretransplantation Assessment of Mortality.

category 1, 2, 3 and 4, respectively ($P<0.001$). There were also statistical differences in category 2 vs 3 ($P=0.001$) and category 3 vs 4 ($P<0.001$) or a trend in category 1 vs 2 ($P=0.10$; Figure 3d).

Impact of PAM score according to underlying diseases

In this study, 2-year OS of patients with ML was inferior to that of non-ML patients, possibly because approximately half of ML cases were adult T-cell leukemia/lymphoma (ATL), which had a very poor prognosis.[9] Thus, we further performed subgroup analysis in patients with ATL and those with other diseases.

Characteristics of patients with ATL ($n=35$) were comparable to those without ATL ($n=241$) except for having an older age (median; 55 vs 45, $P=0.003$). Significantly inferior 2-year OS was observed in patients with ATL compared with those without ATL (28.7% vs 55.1%, $P=0.001$; data not shown). ATL patients in category 1, 2, 3 and 4 groups had 100%, 25%, 29.3% and 0% of 2-year OS, respectively (Figure 3e); The PAM score could not distinguish transplant outcome of category 2 patients from that of category 3 patients ($P=0.54$).

In contrast, the PAM score effectively risk-stratified patients with other diseases for survival: 2-year OS in category 1, 2, 3 and 4 were 100%, 81.8%, 52.6% and 15.7%, respectively ($P<0.001$; Figure 3f). There was also a statistically significant difference in OS between category 2 and 3 ($P=0.001$).

Modification of categorization of PAM score

Although this study demonstrated that the PAM score was predictive of transplant outcome in our cohort, there was a skewed distribution of patients with the majority of patients squeezed into categories 2 and 3. This makes the PAM score less useful in the clinical setting and may be related to difference in mortality in each category between our study and the original study. We therefore made a modification of categorization of PAM score to subdivide patients more equally into each category. Patients were classified into low- (score 8–19), intermediate- (20–25), high- (26–30) or very-high- (31–50) risk category. With this stratification, 30 (11%), 80 (29%), 129 (47%) and 37 (13%) patients were categorized in low, intermediate, high and very high risk group, respectively (Table 2). A 2-year OS was significantly different between the 4 groups (92.4%, 67.5%, 43.7% and 14.0%, respectively; $P<0.001$; Figure 4a), which was comparable to that in the original report. Predicitive value of the PAM score with modified categorization determined by c-statistics was better than that of the original PAM score (0.74 vs 0.70; Table 2). Notably, this flexible PAM score showed a powerful predictive value for

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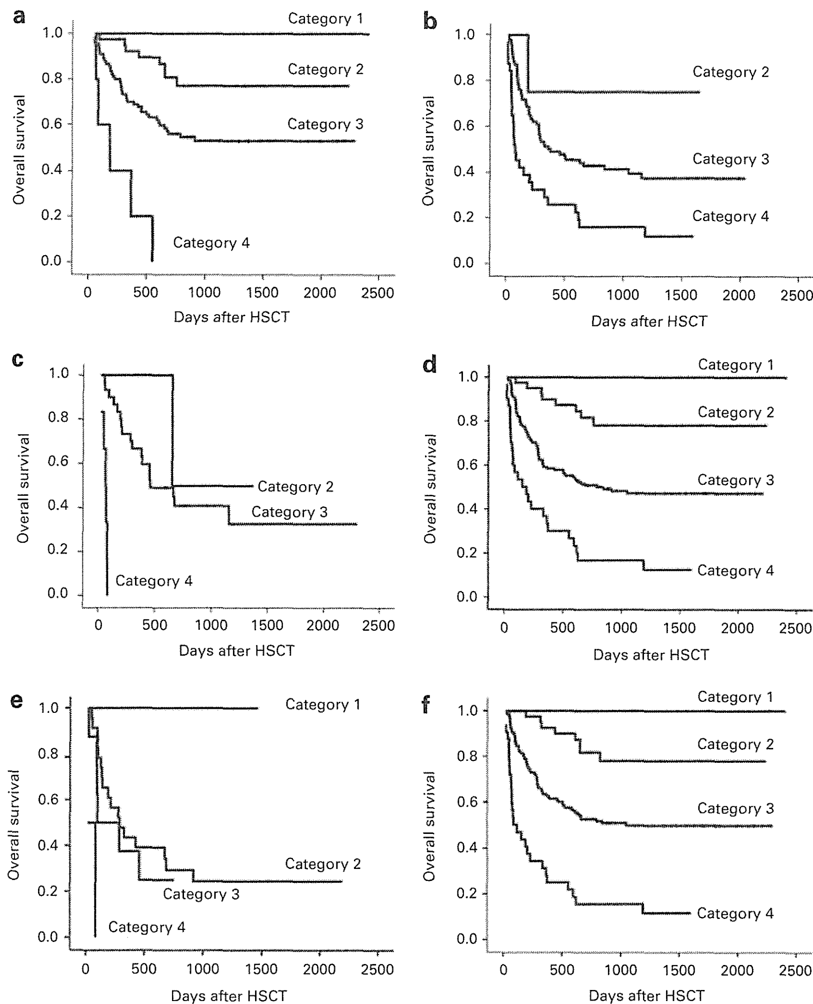

Figure 3. Subgroup analyses. Upper panels showed OS curves in patients with (**a**) standard-risk and (**b**) high-risk disease status at HSCT. Middle panels showed OS curves in recipients with (**c**) UCBT or (**d**) BMT/PBSCT. Lower panels showed OS curves in patients with (**e**) ATL or (**f**) other diseases, respectively.

transplant outcome both in patients with SR and HR diseases (Figures 4b and c).

DISCUSSION

Reliable pretransplant estimation of the transplant outcome is very important for therapeutic decision making. One of these indices is the hematopoietic cell transplantation comorbidity index (HCT-CI),[10] which is a modification of the Charslon comorbidity index[11] and primarily designed to predict NRM after HSCT. Its usefulness for the estimation of OS as well as NRM has been demonstrated in various situations.[2,12-14] EBMT risk score, originally defined as a risk score for patients with CML, has been confirmed its applicability in other hematological disorders.[8] The risk score was based on five criteria, disease status, patients' age, donor type, time from diagnosis to HSCT and donor-recipient sex combination, whereas no comorbidity covariate was considered for the scoring. A modified EBMT risk score omitted the variable time from diagnosis to HSCT from the analysis because it strongly correlated with disease stage and stem cell source, resulting in multiple sources of bias.[15,16]

The PAM score has been recently established for the estimation of 2-year all cause mortality; the PAM score includes both comorbidities and pretransplant variables such as disease status, type of donors and conditioning regimens. PAM score is highly predictive for transplant outcome in retrospective analyses of large numbers of patients at FHCRC.[1,17] However, predictive values of the PAM score remain unclear at other centers.[2,3] As ethnicity is an additional important determinant of immune-mediated outcomes of allogeneic HSCT,[6] we examined prognostic values of the PAM score in a genetically homogeneous Japanese population. We found that the PAM score was also predictive for transplant outcome in this population. Its predictive value was higher than that of EBMT risk score at least in this study, and was comparable to that of the original report from FHCRC.[1] However, 2-year OS of each category is somewhat different from those from FHCRC. One of the important differences in the variables for scoring between FHCRC studies and our study is intensity of reduced intensity conditioning regimen. FHCRC mostly used 200 cGy TBI ± fludarabine,[1] while a more intensified fludarabine-based regimen was used in this study. Thus, patients receiving reduced intensity conditioning had a high score in the 'conditioning regimen' variable in our study, and majority of these patients were classified into category 3.

For the improvement of prognostic predictive value, modified categorizations of HCT-CI and PAM score have been tested. A

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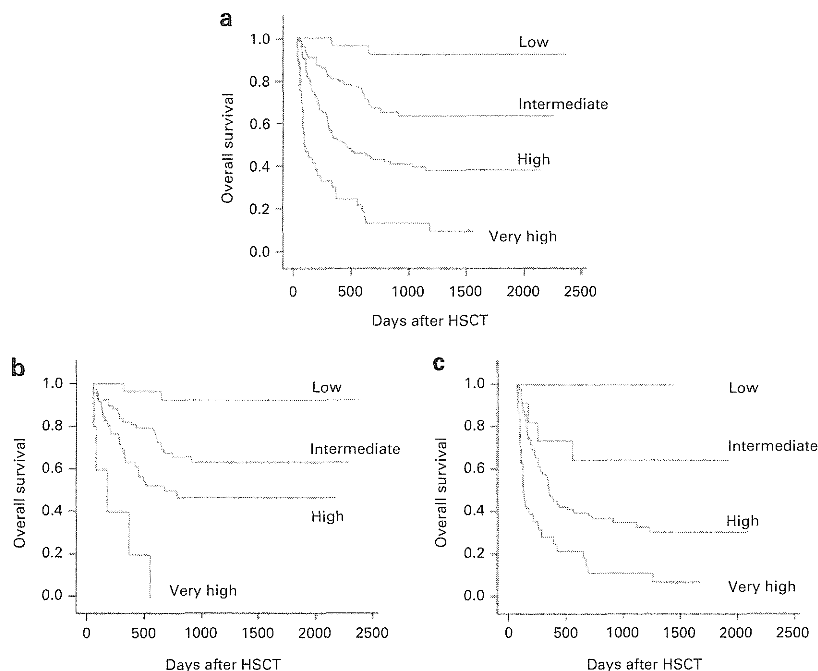

Figure 4. Predictive value of the modified PAM score. PAM score with modified categorization provided useful risk stratification (**a**) in whole cohort, (**b**) in patients with standard-risk disease and (**c**) in patients with high-risk disease.

flexible HCT-CI was predictive of OS and NRM better than PAM or Charslon comorbidity index in patients who underwent allogeneic HSCT with reduced intensity conditioning regimens,[2] and modified PAM score risk-stratified patients for NRM better than the original PAM score.[18] We also investigated whether modification of the original PAM score could risk-stratify our patients for transplant outcome. It subdivided patients more evenly into each category and was more predictive for transplant outcome than the original PAM score.

In subgroup analysis, we showed that the PAM score has prognostic impact in both SR and HR patients. PAM score, which considers disease status at transplantation as well as comorbidity, may be a powerful tool for estimation of HSCT outcomes. Nonetheless, our observation should be confirmed in a multicenter prospective study including larger numbers of patients.

On the other hand, in subgroup analyses according to stem cell source or underlying diseases, we could not find a prognostic value of PAM score in patients who underwent CBT or those with ATL. This may be due to poorer outcome of CBT than BMT/PBSCT in ATL patients than other diseases.[19] Alternately, it may be associated with a small size of the subgroup. Therefore, these observations should be confirmed in a prospective analysis of larger numbers of patients.

In conclusion, this study shows that PAM score effectively risk-stratifies Japanese patients for transplant outcome, irrespective of the risk status of underlying diseases. Accumulation of patients' data will be needed for the further subgroup analyses and development of robust risk scores for CBT and for ATL. Modifications reflecting local distinctive features of transplant indication and procedures at each region[20] may further improve its clinical usefulness.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Donor selection and release criteria of cellular therapy products

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Within the last two decades, an increasing number of transfusion services worldwide significantly expanded their activities on the development and production of cellular therapy (CT) products, including the provision of cellular therapeutics for their use in clinical trials [1]. Due to the increasing importance of CT for Transfusion Medicine, the Editorial Board of *Vox Sanguinis* decided in 2007 to establish the new section 'Cellular Therapy' as the fifth section of the journal [2]. Within the first 5 years, a yearly increasing number of manuscripts have been submitted for publication. In addition, this expanding and innovative field of Transfusion Medicine very recently leads to the foundation of a joint ISBT-AABB Working Party (WP) on Cellular Therapies with the specific purpose of developing and sharing common views on the clinical use of cellular products between the ISBT and the AABB [3]. Furthermore, the WP activities will link together scientists already involved in CT projects from both sides of the Atlantic and other parts of the world. This will provide a platform for exchanging ideas and planning joint activities. It was agreed that a good starting point would be the development of consensus articles on donor selection and product release criteria.

The International Forum (IF) on these topics of CT was initiated by members of the above mentioned joint WP and provides an overview on current procedures based on different types of cellular therapeutics which are performed in well-experienced centres of six European countries (Austria, Finland, France, Germany, Italy, UK), Japan and the USA. The questions raised in this IF mainly focus on procedures for donor selection and final product release, both for products fulfilling the criteria of Advanced Therapy Medicinal Products (ATMP) following EU regulation 1394/2007/EC (or comparably manipulated cells), and for less engineered cellular therapeutics. Specifically, the following panel of questions were developed and submitted to cellular therapy experts of transfusion services and departments:

Question 1
What kind of CT products do you prepare in your centre?

Question 2
How do you select living donors based on the respective CT product prior to the collection of CT starting material?

Question 3
What assays do you perform to characterise the quality of the starting cell/tissue material?

Question 4
How do you perform quality control testing of the final CT product prior to release?

Question 5a
Have you defined and validated acceptable ranges/thresholds for the results of your release assays?

Question 5b
What is your policy if the acceptable ranges/thresholds of your release assays are not met?

Question 6a
Do you perform specific potency assays of the final CT product prior to release to the patient?

Question 6b
If potency assays are not performed prior to release: what kind of potency assays do you perform during the product validation phase of the respective CT product?

Each of the above questions should be answered with respect to the two principally different types of cellular therapy products:

Type I products: 'Engineered' cells or tissues (for example, 'Advanced Therapy Medicinal Products' following EU regulation 1394/2007/EC): Products containing cells or tissue that have been subject of substantial manipulation, or products containing cells or tissue that are not intended

to be used for the same essential function in the recipient as in the donor.

Type II products: Non-engineered cells or tissues (for example, 'Advanced Therapy Medicinal Products' which are not "engineered" according to definitions of EU regulation 1394/2007/EC, Annex I): Products containing cells or tissue that have only been subject to non-substantial manipulations, such as cutting, shaping, centrifugation, freezing, etc.

Since the legal framework may vary between countries, the definition of type I and type II products may also vary. Therefore the answers should take into account specific legal rules in the country concerned.

The authors describe various policies and procedures regarding the production of cellular therapeutics derived from different allogeneic and autologous sources, for example, umbilical cord blood, apheresis donations or harvests from bone-marrow and adipose tissue. The panel of provided CT products cover a wide range of clinical indications, such as the use of mesenchymal stromal cells (MSC) in regenerative medicine (for example, in cardiac regeneration or treatment of critical ischemias), dendritic cells as anti-cancer vaccines or immuno-therapeutic approaches with MSC, chimeric T cells and cytokine-induced killer cells. Furthermore, valuable information is given about the different panel for quality control assays and potency assays to qualify the final CT product prior to release for clinical application.

In parallel to the mostly highly complex procedures linked to the development, processing and validation of innovative cellular therapeutics, the legal framework in different parts of the world seems to be at least as complex. Even in the European Union with its partly supra-national legislation defined by directives, regulations and guidelines, the kind of implementation of such rules in national law of the different member states and the respective responsibilities of national authorities varies significantly. The responses to some of the questions of the IF are pointing out the complexity and diversity of current regulations, and this situation might negatively influence the implementation of international clinical trials using CT products. Nevertheless, the results of this International Forum discussion are a further proof that the development, production and provision of ATMPs and other CT products will inceasingly influence and modify the scientific activities of at least academic transfusion services.

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K. Schallmoser & D. Strunk

Question 1

Type I products: engineered cells or tissues ATMP. We isolate and prepare bone marrow (BM)-derived mesenchymal stem/progenitor cells (MSPCs) in an animal component-free culture system with minimal manipulation steps for clinical application [1]. The use of human platelet-derived growth factors has been efficiently established for routine MSPC expansion protocols [2]. MSPCs are considered to be immune regulatory therapeutics in graft-versus-host disease (GvHD) and for tissue repair/bone regeneration in critical size bone defects. Additional experimental procedures have been implemented for the

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preclinical propagation of endothelial colony-forming progenitor cells (ECFCs) from the peripheral blood of healthy volunteers and patients suffering from various cardiovascular diseases [3]. ECFCs will be tested in clinical trials for their safety and feasibility in realizing therapeutic vasculogenesis. Additional experimental procedures are available to generate off-the-shelf multicellular products comprising autologous (to each other) MSPC + ECFC pairs which are propagated from the same umbilical cord and cryopreserved in conjunction with the corresponding umbilical cord blood [http://www.jove.com/video/1525/isolation-and-animal-serum-free-expansion-of-human-umbilical-cord-derived-mesenchymal-stromal-cells-mscs-and-endothelial-colony-forming-progenitor-cells-ECFCs].

Type II products: non-engineered cells or tissues. Preclinical production of human lineage-negative cell fractions from non-mobilized peripheral blood has been established based on CD2/CD19/CD56 depletion [4].

The following questions Q2–Q6B were answered regarding the CT products indicated in Q1:

Question 2

Donors have to fulfill the criteria according to the Austrian legal regulations based on European law [Directive 2004/23/EG of the European Parliament and Council, 31 March 2004 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32004L0023:DE:HTML)] and are further selected with the aim of reaching a minimized risk for the donor and the recipient of the cell therapeutics.

Detailed medical history and physical examination as well as appropriate laboratory tests are acquired at maximum 7 days prior to the stem cell collection. These comprise exclusion of infections by treponema pallidum, hepatitis B virus (HBV), hepatitis C (HCV), hepatitis A (HAV), cytomegalovirus (CMV), parvovirus B19 and human immunodeficiency virus (HIV) by syphilis screening (ELISA or TPHA test), testing for HBs-Ag, anti-HBs, anti-HBc, anti-HCV, anti-CMV and anti-HIV-1,2 antibodies, and nucleic acid-based tests (NAT) for HBV, HCV, HAV, parvovirus B19 and HIV, respectively. Infection by the human T-lymphotropic virus type I (HTLV-1) is only excluded by anti-HTLV-1-antibody testing if donors or their parents and sexual partners are living in or hail from regions with high incidence. The medical qualification for BM puncture depends furthermore on a normal full blood cell count and normal coagulation parameters, and absence of renal or hepatic diseases.

Additionally, donors are routinely tested for ABO blood groups and irregular erythrocyte allo- and autoantibodies. They receive detailed information regarding possible side effects and risks of the procedure and have to give informed consent. All diagnostic findings and relevant documents are collected, signed by the responsible physician, and saved in the patient history form for at least 30 years. The identity of the donor is confirmed again immediately before the intervention. For the application of MSPCs for bone regeneration, autologous cell therapeutics are preferred in our centre. In the immune modulatory setting treating GvHD, we use allogeneic donors (either third party or, if available, the identical donor of the haematopoietic stem and progenitor cell (HSPC) transplant).

Question 3

Quality assays as identity, purity, potency, sterility or suitability, if applicable, methods used. In our centre, BM is harvested for non-haematopoietic stem cell therapy in small aliquots of 3–5 ml per aspiration site with a maximum of 6–8 aspirations. The volume of the collected BM aspirate is determined after pooling, and a 1-ml aliquot is taken sterilely for further analyses. Then, the BM aspirate is seeded without further manipulations in culture medium supplemented with pooled human platelet lysate. Microbiological testing to exclude fungal, anaerobic and aerobic bacterial contamination is performed by an automated blood culture system (BacT/Alert, bioMérieux SA, Marcy l'Etoile, France). The leucocyte count is analysed by an automated blood cell analyzer (ADVIA 2120i, Siemens Healthcare Diagnostics GmbH, Vienna, Austria). The concentration of viable mononuclear cells (MNCs) is estimated by flow cytometry analysing the percentage of 7-amino-actinomycin D $(7\text{-AAD})^-/CD45^+/$ glycophorin A^- cells within a mononuclear cell forward and rectangular light scatter region. In parallel to the primary culture, the clonogenicity of BM-derived MSPCs is tested by colony-forming unit of fibroblast (CFU-F) assays in fivefold serial diluted seeding densities of MNCs. Quality thresholds for CFU-F frequency have not yet been established.

Question 4

In process controls. Manipulation steps during cell culture are reduced to a minimum as previously described [5]. In brief, 2–3 ml of heparinized BM is regularly re-suspended in 500 ml culture medium before seeding in one four-layered cell factory comprising 2,528 cm^2 of culture area. At day three, non-adherent cells are removed by vigorously washing with 37°C pre-warmed phosphate-buffered saline (PBS) before adding new supplemented medium. Continuous growth factor support is guaranteed by replacing 20% of the medium twice weekly with new animal protein-free medium supplemented with pooled human platelet lysate [http://www.jove.com/video/1523/

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preparation-of-pooled-human-platelet-lysate-phpl-as-an-efficient-supplement-for-animal-serum-free-human-stem-cell-cultures]. Continuous visual microscopic control and sterility testing of the conditioned as well as the fresh culture medium at each medium exchange is performed to detect a bacterial or fungal contamination as soon as possible.

After the final trypsinization step, a 1-ml aliquot of the harvested MSPCs is taken sterilely for the following quality controls and the product lot release:

Cell number and viability testing. The number of MSPCs generated in one culture passage is determined using a haemocytometer as the mean of four measurements. Viability is determined by trypan blue exclusion in the haemocytometer and 7-AAD analysis by flow cytometry, respectively.

Analysis of immune phenotype. Being aware of the heterogeneous mixture of cultured MSPC populations, we define the MSPC purity as >90% reactivity for CD73, CD90, CD105 and MHC class I and negative in >98% for CD3, CD14, CD19, CD31 and CD34. Haematopoietic cell contamination as defined by total CD45 reactivity is required to be below 10% and MHC class II reactivity of MSPCs must not exceed 10% [5]. Modified limits for $CD3^+$ T cell contamination are applied in the event that haploidentical transplantation adheres to the maximal T cell dose defined by the corresponding HSPC transplantation protocol.

Sterility testing. The final MSPC product is again tested for fungal, anaerobic and aerobic bacterial contamination by automated blood culture (BacT/Alert). A potential mycoplasma contamination is excluded using a bioluminescent reporter assay for mycoplasma-specific enzymes (MycoAlert® Lonza Basel, Switzerland) [1].

Endotoxin. As an alternative for pyrogenicity testing, endotoxin measurement is performed by a quantitative chromogenic Limulus Amebocyte Lysate (LAL) assay (Endosafe®, Charles River Laboratories International, Wilmington, MA). The LAL assay enables the microbiologic release of the cell product within two hours of harvest to circumvent the delay of testing by bacterial culture methods. According to the Food and Drug Administration (FDA) recommendations, our release criteria require endotoxin levels below 5 EU/kg recipient body weight per dose [5].

Genomic stability. Serious concerns have arisen about the occurrence of hazardous genomic aberrations in *ex vivo* expanded MSPCs. We have demonstrated genomic stability by array-comparative genomic hybridization (aCGH) analysis of clinical scale-cultured MSPCs and in association with replicative senescence without evidence for malignant transformation [1, 6]. A standardized attitude for the routine product lot release is not yet defined.

Question 5

Cell dose. Until now, there has been no standardized culture protocol and no general recommendation defined for the optimal dose of MSPCs due to the lack of appropriate dose finding clinical studies. Due to a pragmatic approach, we generate as many MSPCs in the shortest culture duration with the fewest manipulation steps possible according to our efficient cell expansion protocol. Getting a cell number of, for example, more than 8×10^8 MSPCs as routinely generated in eight four-layered cell factories on 20 224 cm^2 (Thermo Fisher, Nunc International, Naperville, IL, USA) enables us to treat an adult patient of 80 kg body weight with a maximum 10 million of MSPCs per kg body weight. Whether a single high dose i.v. infusion or a serial therapy with respective lower cell yields is more efficient as well as the optimal cell dose locally injected for tissue repair still have to be evaluated systematically in ongoing clinical trials. Based on the efficiency of our protocol, we lack experience with not meeting a lower threshold of 2 million of MSPCs/kg. Our release criteria define an additional passage in case the required cell dose was not reached. The optimal cell dose for local application (i.e. for bone repair purposes) is currently determined in animal experiments but also needs to be tested in dose finding studies.

Viability testing. For clinical application, the viability of MSPCs after trypsinization and after cryopreservation is required to be >90% in our release criteria. Only in justified cases may a reduced viability of >70% be permissible. Whenever possible we require an additional short culture passage to be performed to enrich the viable cells and to deplete the dead cell fraction by removing non-adherent cells and debris by a complete medium change 24 h after re-seeding. Furthermore, the conditions of the culture procedure should be re-assessed to identify possibilities for process optimization.

Analysis of immune phenotype. Not meeting the MSPC purity as defined by immune phenotype marker profiling may indicate either a putative contamination with haematopoietic BM-derived cells or otherwise a possible activation state of MSPCs as, for example, reflected by an elevated expression of MHC class II antigens. A further culture passage should be regularly performed to further enrich MSPCs and reduce contaminating haematopoietic cells. For further culture procedures, an optimization of the washing step removing the non-adherent cell fraction at the beginning of the primary culture passage should be executed. The clinical relevance of elevated MHC class II expression in

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particular on third party MSPCs is not yet clear and should be considered with caution. Also in this case, an additional culture passage and re-testing is recommended in our protocol.

Sterility testing and endotoxin. In our standard culture procedure, we avoid the use of antibiotics/antimycotics to detect any contamination as early as possible and to prevent patient immunization or exacerbation of pre-existing allergies to common antibiotics. Any sign or proof of a microbiological contamination of the cell product leads to the complete disposal of the product and requires decontamination of the laboratory equipment in our practice. For safety reasons, we perform an antibiotic/antimycotic reserve culture procedure in parallel in two cell factories to create a backup cell product if the standard MSPC product has to be discarded. In the event of negative microbiological testing of the standard product, this additional MSPC product is used for research purposes.

Question 6

Potency assays for MSPCs are currently not performed prior to the release of MSPCs in our centre for GvHD therapy. We are currently underway towards the establishment of appropriate protocols testing the capacity for osteogenic and chondrogenic differentiation and for the support of angiogenesis of MSPCs and ECFCs *in vitro* and in immune-deficient mice *in vivo* as well as their immune modulatory effect *in vitro*.

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Question 1

Type I products: The Advanced Cell Therapy Centre of the Finnish Red Cross Blood Service (FRCBS) currently prepares allogeneic bone marrow-derived mesenchymal stromal cells (BMMSCs) expanded in platelet-rich plasma for acute steroid-resistant GvHD.

Type II products: The FRCBS also maintain a FACT/NetCord accredited cord blood bank (the Finnish Cord Blood Bank) and a Bone Marrow Donor Registry with 20 000 prospective donors. However, we will only focus on the type I products in the subsequent questions.

Question 2

Donor eligibility is assessed by a donor eligibility questionnaire dealing with questions about common health status, possible long-term or severe diseases, previous blood transfusions, sexual behaviour, travelling, possible contraindications for the procedure (heart-disease, local anaesthesia), other risk behaviour for possible infectious diseases (tattoos, etc.), current skin diseases/infections, family history of inherited diseases.

The donors are tested for the following infectious diseases:

NAT tests: HCV-RNA, HIV-RNA, HBV-DNA, B19-DNA (Parvo), HAV-RNA.

Serologic tests: HBsAg, Anti-HCV, Anti-HIV (1+2), Anti-Hbc, Syphilis, HTLVI/II, S-CMVAb.

The blood samples for the donor screening are drawn within 30 days of the CT starting material collection. Additionally, the ABO blood group is determined and in intermediate resolution HLA screening is performed.

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