$1,25(OH)_2D_3$ in human liver and intestine, suggesting negative feedback control of $1,25(OH)_2D_3$ action.[6]

The *VDR* gene consists of 6 exon 1's (exons 1f, 1e, 1a, 1d, 1b, and 1c in this order) and 8 other exons (exons 2 to 9), and spans approximately 100 kb at chromosome 12q13.11.[7] The VDR protein is expressed in many tissues including kidney, skin and liver.[1] Alternative usage of exon 1's yields 14 transcripts including 3 major types: exon 1a-1c, exon 1d-1c or exon 1f-1c combinations, followed by common exons 2–9.[7] A translational initiation codon in exon 2 is used in transcripts starting from exon 1a or 1f. In the transcript from exon 1d (1d transcript), translation could initiate from the ATG codon in exon 1d, generating an active protein 50 amino acids longer than that from the exon 1a- or 1f-containing transcript (1a or 1f transcript).[7,8] However, the 1d and 1f transcripts are assumed to be minor (expression levels were less than 10 and 20%, respectively, of the 1a transcript).[7]

Genetic polymorphisms of transcriptional factors involved in induction of drug metabolizing enzymes could influence their expression levels, and as a result drug pharmacokinetics/pharmacodynamics. As for *VDR*, several polymorphisms with functional significance have been reported.[9] For example, the single nucleotide polymorphism (SNP) 2T>C (*Fok*I polymorphism) in the first ATG codon of 1a and 1f transcripts results in generation of a three amino acid shorter protein, which has a significantly higher transcriptional activity than the longer transcript.[9,10] Another SNP -29649G>A upstream of exon 1e (thus exons 1a and 1d) is located in the Cdx2 (an intestinal transcriptional factor)-binding element. This nucleotide change facilitates Cdx2 binding, resulting in increased *VDR* transcription.[9,11]

Recently, Nejentsev *et al.* resequenced *VDR* and found 245 genetic variations in Caucasians (Britons).[12] However, no comprehensive screening of *VDR* polymorphisms has been reported for Asian populations including Japanese. In this study, we searched for *VDR* variations by resequencing the promoter regions, all 14 exons and their surrounding introns from 107 Japanese subjects.

Materials and Methods

Human genomic DNA samples: One hundred and seven Japanese cancer patients administered paclitaxel were analyzed. Written informed consent was obtained from all subjects. The ethical review boards of the National Cancer Center and the National Institute of Health Sciences approved this study. DNA was extracted from whole blood, which was collected from the patients prior to paclitaxel administration.

DNA sequencing

Amplification of exon 1's, and exons 2 to 6: First, three sets of PCRs were separately performed to amplify exons 1f to 1b (Mix 1 primer set), the Cdx2 region (Mix 2), and exons 1c to 6 (Mix 3) from 50 ng of genomic DNA using 1.25 units of Z-Taq (Takara Bio Inc., Shiga, Japan) with 2 μM of each primer designed in the intronic regions (**Table 1**, 1st PCR). The first PCR conditions were 30 cycles of 98°C for 5 sec, 55°C for 5 sec, and 72°C for 190 sec. Next, exon 1's and exon 2 with high GC content were amplified separately from the 1st PCR product as a template with 2.5 units of LA-Taq (Takara Bio Inc.) in GC buffer I and 1 μM of each primer (2nd PCR in **Table 1**). For the Cdx2 region and exons 3 to 6, PCR reactions were performed using Ex-Taq (1 unit, Takara Bio Inc.) and 0.4 μM of primers in **Table 1**. The second PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension for 7 min at 72°C.

Amplification of exons 7 to 9: These exons were directly amplified from 50 ng DNA. Amplification was performed with Ex-Taq for exons 7 to 8, 9-1 and 9-3, and with LA-Taq in GC buffer I for exon 9-2. Primer concentrations, polymerase units and PCR conditions were the same as described above for the second round PCR.

Sequencing: PCR products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA, USA) with sequencing primers listed in **Table 1** (Sequencing). Excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany) and the eluates were analyzed on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). All novel SNPs were verified by sequencing PCR products obtained by a new genomic DNA amplification. Under the conditions used, up to 1,640 bases upstream of exon 1f, up to 4,090 (4,460) bases upstream of exon 1a (1d), all exons and their flanking introns were successfully sequenced for all subjects. *VDR* genomic sequence (NT_029419.11) and cDNA sequence (exon 1a-1c transcript, NM_000376.2) obtained from GenBank were used as reference sequences. Nucleotide positions based on cDNA sequence were numbered from the adenine of the translational start site (10416202 in NT_029419.11 and 161 in NM_000376.2) or the nearest exons (for introns 2 to 8).

Other analysis: Hardy-Weinberg equilibrium and linkage disequilibrium (LD) analyses were performed with SNPAlyze version 3.1 (Dynacom Co., Yokohama, Japan), and pairwise LDs between variations were obtained for rho square (r^2) values. P-values for Hardy-Weinberg equilibrium were corrected by false-discovery

Table 1. Primers used for sequencing *VDR*

		Amplified or sequensed region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified length (bp)
1st PCR	Mix 1	Exon 1f	TTGTTGACTCTCCTGGCTTTATCAG	CCAGTCACTTTGAAGAGAAACCTGC	3,823
		Exon 1e, 1a, 1d, 1b	TGGTCCCTTCTGTCTTTCTAACTCC	TTGGGAGGATGTAGAACCGTGGGAT	10,421
	Mix 2	Cdx2	ACTGGTGGTGCCCTACTCTTTCT	TTGGGAGGATGTAGAACCGTGGGAT	11,835
	Mix 3	Exon 1c to Exon 2	GCTAAAAGTGGCAGAAAACATCCTG	TTGTGTTGACAGAGAGACCCCAAGT	5,476
		Exon 3	TAGCATCCTTACTTCTCATAGCGGC	CCTGTTCTGTGACTTATCCTCTGTC	2,556
		Exons 4 to 6	TGGTGTGGCTGGCAGAAACAGGTCT	TCTCTCAAAGTGTTGGGATAGGCAT	4,847
		Exons 7 to 8	AACACTCTTGTCCCTTCCAG	TCTGTGTCTCCTTTTGCTAC	753
		Exon 9-1	GTCAGCAGTCATAGAGGGGT	CAGATGGAGAAGATGCGGCT	1,327
		Exon 9-2	AGAGGGTCTGGAGAAGCAGT	AATGAGGGGATTGACTCGTT	1,012
		Exon 9-3	TCTTCAGTGGGAGAAAACAC	CTGTCAAATGGGGGTAATAA	1,417
2nd PCT		Exon 1f	GAACCCTTTTCTCTGCCCTCACCTT	AGACAGAGGACTGGAGAAGGAGATA	606
			ACTCTGCTCCTTCACCACCTCTACA	AAACTGAGTGCGTGTGAGTGAGAGA	628
			GCTGGAGGTGTTGAACTGGTTGCTT	CGCATACCCGACACTTGTTCACCTC	851
		Cdx 2	GTAATCCTTCCACCTCAACTTCCTA	CCACAGAGTCCAAAGAAAGGCAGGG	1,148
		Exon 1e	AGAGGAGAGGGTCTGGAGAG	TTCCAACCACCAATACCTTG	640
		Exon 1e to 1a	GCAGAGAATGTCCCAAGGTA	GACCGTCGTCCATAGGGCAA	1,411
		Exon 1a to 1d	AGAAGCGTGCCTTGCCCTAT	CTGAACATCTATTGACAGGC	871
		Exon 1b	CTGGAAGGCAAATAGGAAAC	GCCGTGTAAAGCAGTGGTTA	368
		Exon 1c	GCTGTGAGAGGAGAAGGAGT	GCAAAATCCTGGGTGGTATC	517
		Exon 2	GTGAACCACTAAACCCAAAT	GAAGGAGATGTGAAAAATGC	528
		Exon 3	TCTGTTGGAGAAATGGAGAC	GCCTCTGACACCAACACACA	478
		Exons 4 to 5	TGCCTTCCTTTTCACCATAG	AGAGGGGCTGTTGTGAAGAC	912
		Exon 6	TGAAAGAGGCAGAGAGAGTC	GGTTATAGTGAGCCAAGATAGTG	719
Sequencing		Exon 1f	GAACCCTTTTCTCTGCCCTCACCTT	AGACAGAGGACTGGAGAAGGAGATA	
			ACTCTGCTCCTTCACCACCTCTACA	AAACTGAGTGCGTGTGAGTGAGAGA	
			GCTGGAGGTGTTGAACTGGTTGCTT	CAGGACAGCAGGACCTTCAGGGAAC	
			CACTGACTCTCACCTTCCTTTCCTC	CGCATACCCGACACTTGTTCACCTC	
			GGTGGCTCCCTCCTGCTGTGTGG		
		Cdx2	AGGAAGGAAGGAAAAGAGGAT	TGGAGTTAGAAAGACAGAAGGGACC	
		Exon 1e	TGTTTTTTAGAGGCAGCAT	TTCCAACCACCAATACCTTG	
			TAGAGGCAGCATGAAACAGT	GATGGATGGATTCTCTACCT	
		Exon 1e to 1a	GCAGAGAATGTCCCAAGGTA	CTCACAAAATCATCCAGCAG	
			CTGGCTAAAGGAGGTCATCG	CGCTCGCCAACCTGTTACTG	
			CAGTAACAGGTTGGCGAGCG	GACCGTCGTCCATAGGGCAA	
			CGACTGCTGGATGATTTTGT	CGATGATTATAGGTGCGGAT	
		Exon 1a	AGCGTGCCTTGCCCTATGGA	TTTACCGCTGAGACTTAGAC	
		ERxon 1d	AAACTTGGCTACTGAGGTCC	TAAAAGACCCAACTCCACC	
		Exon 1b	CTGGAAGGCAAATAGGAAAC	GCCGTGTAAAGCAGTGGTTA	
		Exon 1c	CTTCCCACTGCTCCTGCTAC	GGTGGTATCCCTTCCTTCCC	
		Exon 2	GTGAACCACTAAACCCAAAT	GAAGGAGATGTGAAAAATGC	
		Exon 3	TCTGTTGGAGAAATGGAGAC	GCCTCTGACACCAACACACA	
		Exon 4	TGCCTTCCTTTTCACCATAG	CGTCCCTACCCCAGTTCTGT	
		Exon 5	ACAGAACTGGGGTAGGGACG	AGAGGGGCTGTTGTGAAGAC	
		Exon 6	TGAAAGAGGCAGAGAGAGTC	GAAGTTTCTTACCTGAATCCTGG	
		Exon 6	CACTCATCCACCACTTCTTT	TGGTGTCTGGTGCCTGTAATCCC	
		Exons 7 to 8	AACACTCTTGTCCCTTCCAG	TCTGTGTCTCCTTTTGCTAC	
		Exon 9-1	GGTGCTGCCGTTGAGTGTCT	ACATCAGTCAGCAGCCACTT	
			AAGTGGCTGCTGACTGATGT	GAAGATGCGGCTCACTGCTT	
		Exon 9-2	AGCCGCATCTTCTCCATCTG	GCCGCATTCCCCAAACTCAA	
			TTGAGTTTGGGGAATGCGGC	AATGAGGGGATTGACTCGTT	
		Exon 9-3	TCTTCAGTGGGAGAAAACAC	ATGTTGGTCAGGTTGGTCTC	
			GGAGGCTGAGGCAGAAGAAT	CCAGGGCTGAGTAACTGATA	
			CCTGCCTTCTTCGGGGAACT	CTGTCAAATGGGGGTAATAA	

Table 2. Summary of *VDR* variations detected in this study

SNP ID		Location	Position		Nucleotide change	Amino acid change	Allele frequency (n=214)
This Study	dbSNP (NCBI)		NT_029419.11	From the translational start site[d] or from the end of the nearest exon			
MPJ6_VDR_001	rs4547172	5'-flanking	10481728	-65526	tccatatttatcT/Cttttatacttct		0.238
MPJ6_VDR_002	rs4583039	5'-flanking	10481720	-65518	tatccttttataC/Tttctttcaagt		0.252
MPJ6_VDR_003	rs7970376	5'-flanking	10481502	-65300	cattaaacagatG/Atatcatcatctg		0.037
MPJ6_VDR_004	rs4237856	5'-flanking	10481356	-65154	cccaggctcagcT/Ggccctttgacat		0.196
MPJ6_VDR_005[a]		5'-flanking	10480937	-64735	tgtgccaggtagG/Ccgtggtgccccc		0.028
MPJ6_VDR_006	rs3923693	5'-flanking	10480935	-64733	tgccaggtaggcG/Atggtgcccccgc		0.327
MPJ6_VDR_007	rs4073726	5'-flanking	10480623	-64421	cgtcctccctggG/Ccaagccatctcc		0.037
MPJ6_VDR_008[a]		5'-flanking	10480382	-64180	cttctggtgcccC/Gcctgctccctca		0.061
MPJ6_VDR_009	rs4073729	5'-flanking	10480375	-64173	tgccccctgctC/Tctcatggccag		0.229
MPJ6_VDR_010	rs11168297	inton 1f-1e[b]	10446202	-30000	gtactgggattaC/Taggcctgagcca		0.028
MPJ6_VDR_011	rs17880972	inton 1f-1e[b]	10446166	-29964	aggatttttaatA/Gtgtattttggg		0.028
MPJ6_VDR_012	rs11568820	inton 1f-1e[b]	10445851	-29649 (Cdx2)	aactaggtcacaG/Ataaaaacttatt		0.430
MPJ6_VDR_013[a]		inton 1f-1e[b]	10445734	-29532	cattctatttttA/Gccatctggaaca		0.005
MPJ6_VDR_014[a]		inton 1f-1e[b]	10445338	-29136	catggggacaggG/Atctctgggagac		0.005
MPJ6_VDR_015	rs11614332	inton 1f-1e[b]	10445204	-29002	gatgaggacaggC/Ttgcagtcctgtg		0.028
MPJ6_VDR_016[a]		inton 1e-1a[b]	10443873	-27671	aacaaaggctgtG/Aaaaaaagactaa		0.028
MPJ6_VDR_017[a]		inton 1e-1a[b]	10443771	-27569	gtcaagcaccacA/Taaagtgacttct		0.028
MPJ6_VDR_018	rs7139166	inton 1e-1a[b]	10443640	-27438	tagctttcccacG/Catgctttgggca		0.028
MPJ6_VDR_019[a]		inton 1e-1a[b]	10443419	-27217	cagtgcttaacaA/Gacttcactgttg		0.005
MPJ6_VDR_020	rs4516035	inton 1e-1a[b]	10443132	-26930 (GATA)	gcgaatagcaatA/Gtcttccctggct		0.028
MPJ6_VDR_021	rs11574006	inton 1e-1a[b]	10442825	-26623	tgccacggggcgG/Tgggggaaggcg		0.023
MPJ6_VDR_022	rs11574007	inton 1e-1a[b]	10442819_10442818	-26617_-26616	gggcgggggggg/insG/aaggcggaactc		0.014
MPJ6_VDR_023	rs11574007	inton 1e-1a[b]	10442819_10442818	-26617_-26616	gggcgggggggg/insGG/aaggcggaactc		0.023
MPJ6_VDR_024[a]		inton 1e-1a[b]	10442795	-26593	cgggaccagggaC/Tcagggaagctga		0.028
MPJ6_VDR_025[a]		inton 1e-1a[b]	10442587	-26385	tggcgagcggagC/Tccgggatttccc		0.028
MPJ6_VDR_026[a]		inton 1e-1a[b]	10442542	-26340	cggtgccagtcgG/Tcaggcgcccccc		0.023
MPJ6_VDR_027	rs11574012	inton 1d-1b[b]	10441601	-25399	ggtcacctgtgaT/Cggtggagttggg		0.093
MPJ6_VDR_028	rs11168293	exon 1b (5'-UTR)	10437022	-20820	agcccagctggaC/Aggagaaatggac		0.028
MPJ6_VDR_029[a]		exon 1b (5'-UTR)	10436972	-20770	ccaggccccgtgC/Tacattgctttgc		0.014
MPJ6_VDR_030	rs11168292	inton 1b-1c[b]	10436911	-20709	aagtacagttatG/Cttctctagcagg		0.028
MPJ6_VDR_031	rs10735810	exon 2	10416201	2 (*FokI*)	ttcttacagggaT/Cggaggcaatggc	Translational start site change	0.636
MPJ6_VDR_032	rs10783218	intron 2	10416049	IVS2+8	ttcaggtgagccC/Ttcctcccaggct		0.065
MPJ6_VDR_033[a]		intron 2	10415986	IVS2+71	tccatgaagggaG/Acccttgcatttt		0.005
MPJ6_VDR_034[a]		exon 3	10402259	154	ggcaggcgaagcA/Gtgaagcggaagg	Met52Val	0.005
MPJ6_VDR_035	rs11168267	intron 3	10394848	IVS3-71	ggacctttacccC/Tcaacgcaggag		0.151
MPJ6_VDR_036	rs11168266	intron 3	10394839	IVS3-62	cccccaaccgcaG/Agaggaaggtttc		0.360
MPJ6_VDR_037[a]		intron 3	10394802	IVS3-25	ctggccagccctC/Tctgactcccctt		0.005
MPJ6_VDR_038[a]		intron 4	10394565	IVS4+28	gggaggatgagcC/Tggtccagaggag		0.014
MPJ6_VDR_039[a]		exon 5	10394225	576	tcactgtatcacC/Ttcttcaggtaag	Thr192Thr	0.005
MPJ6_VDR_040[a]		intron 6	10392586	IVS6+133	cttgctctgtcaC/Tgcaggctggagt		0.014
MPJ6_VDR_041	rs11168265	intron 6	10392565	IVS6+154	gagtgcagtggcG/Acgatctcggctc		0.150
MPJ6_VDR_042	rs11574093	intron 6	10392429_10392428	IVS6+290_291	atttatttattt/insATTT/ttatttttttttc		0.187
MPJ6_VDR_043[a]		exon 7	10383807	846	cttcaccatggaC/Tgacatgtcctgg	Asp282Asp	0.005
MPJ6_VDR_044	rs11574113	intron 8	10382206	IVS8-112	catagaggggtgG/Ccctaggggtgc		0.154
MPJ6_VDR_045[a]		intron 8	10382176	IVS8-82	ttgagtgtctgtG/Atgggtgggggt		0.047
MPJ6_VDR_046	rs7975232	intron 8	10382143	IVS8-49 (*ApaI*)	tgagcagtgaggG/Tgcccagctgaga		0.318
MPJ6_VDR_047	rs731236	exon 9	10382063	1056 (*TaqI*)	cgccgcgctgatT/Cgaggccatccag	Ile352Ile	0.164
MPJ6_VDR_048[a]		exon 9	10381988	1131	cccgggcagccaC/Tctgctctatgcc	His377His	0.005
MPJ6_VDR_049	rs2229829	exon 9	10381913	1206	caagcagtaccgC/Atgcctctcctte	Arg402Arg	0.014
MPJ6_VDR_050	rs739837	3'-UTR	10381527	1592 (*308)[c]	ctccaccgctgcC/Ataagtggctgct		0.318
MPJ6_VDR_051	rs3847987	3'-UTR	10381374	1745(*461)[c]	gataaataatcgG/Tcccacagctccc		0.154
MPJ6_VDR_052[a]		3'-UTR	10381348	1771 (*487)[c]	ccccaccccctC/Tagtgcccaccaa		0.005
MPJ6_VDR_053[a]		3'-UTR	10381047	2072 (*788)[c]	cgacctcgtcctC/Acccctgccagtg		0.005
MPJ6_VDR_054	rs11574125	3'-UTR	10381042	2077 (*793)[c]	tcgtcctccccc/delT/gccagtgcctta		0.318
MPJ6_VDR_055[a]		3'-UTR	10380870	2249 (*965)[c]	gaagaattttcaG/Caccccagcggct		0.005
MPJ6_VDR_056	rs11574129	3'-UTR	10380609	2510 (*1226)[c]	tcaagtgcatgcT/Cctctgcagccag		0.126
MPJ6_VDR_057	rs11574131	3'-UTR	10380516	2603 (*1319)[c]	aggtgtgcgggaC/Tcggtacagaaag		0.042
MPJ6_VDR_058	rs9729	3'-UTR	10379929	3190 (*1906)[c]	aatcccctcattC/Aaggaaaactgac		0.322
MPJ6_VDR_059[a]		3'-UTR	10379840	3279 (*1995)[c]	gcggtggctcacG/Acctgtaatccca		0.005
MPJ6_VDR_060	rs2853562	3'-UTR	10379692	3427 (*2143)[c]	catggtggcgcaA/Tgcctgtaatccc		0.322
MPJ6_VDR_061[a]		3'-UTR	10379608	3511 (*2227)[c]	tgagatcgtgccG/Attactctccaac		0.037

[a]Novel variations detected in this study.
[b]The intronic region between the two exon 1's indicated.
[c]The position with an asterisk in parenthesis is numbered from the translational termination codon TGA.
[d]The translational start site in exon 2 was used for numbering according to NM_000376.2.

rate methods.[13]

Results and Discussion

We found 61 genetic variations, including 25 novel ones, from 107 Japanese subjects (**Table 2**). Of them, 9 were located in the 5′-flanking region of exon 1f, 19 in the introns between the exon 1's, 2 in the 5′-untranslated region (UTR), 7 in the coding exons (5 synonymous and 2 nonsynonymous variations), 12 in the 3′-UTR, and 12 in introns 2 to 8. All observed allele frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$) after correction for multiple comparison, except for 3 linked variations -27671G>A, -26593C>T and -26385C>T. Deviations from equilibrium were derived from an unexpected occurrence of one minor allele homozygote in these low frequency variations. However, the occurrence at these positions was confirmed by repeated sequencing.

One novel nonsynonymous variation, 154A>G (Met52Val), was found heterozygously with an allele frequency of 0.005. The Met52Val is found in the nuclear localization signal between two zinc finger regions.[14] Furthermore, the precedent amino acid Ser51 is phosphorylated by protein kinase C, which could modulate the VDR binding to the vitamin D responsive-element and transactivation activity.[15] Thus, functional significance of this Met52Val variation should be clarified in future studies.

One known nonsynonymous SNP, 2T>C (the *Fok*I polymorphism) that results in increased transcriptional activity, was also detected at a 0.636 allele frequency, which is similar to frequencies reported for Japanese women (0.59)[10] and Caucasians (0.62),[16] but slightly higher than that in Chinese (0.53)[17] and lower than that in African-Americans (0.79).[16]

Other functionally relevant SNPs are -29649G>A and -26930A>G. The SNP -29649G>A, leading to higher affinity for Cdx2 and increased *VDR* transcription, was detected at a 0.430 frequency, which is higher and lower than those in Caucasians (0.17) and Africans (0.75), respectively.[18] By luciferase reporter and electrophoretic mobility shift assays, the other SNP -26930A>G was shown to two-fold reduce *VDR* transcription with decreased binding affinity to a GATA protein.[18] The allele frequency of this variation was 0.028, which is comparable to that in Africans (0.06) but considerably lower than that in Caucasians (0.43).[18] Since GATA family proteins are expressed in many tissues,[19] this variation might be important for interindividual differences in VDR expression levels.

Fang *et al.* (2005) reported that the 3'-UTR SNPs 1592(*308)C>A, 2077(*793)delT, 3190(*1906)C>A, 3387(*2103)_3406(*2122)A_{20}>$A_{13\text{-}24}$ and 3427(*2143)A>T were in high LD in Caucasians, and that the major haplotype 1592(*308)C-2077(*793)T-3190(*1906)C-3387(*2103)_3406(*2122)A_{22}-3427(*2143)A showed a 15% lower level and 30% faster decay of VDR mRNA than the minor reciprocal haplotype.[18] However, the variation responsible for these functional alterations has not been identified. In our hands, the polymorphism 3387(*2103)_3406(*2122)A_{20}>$A_{13\text{-}24}$ could not be precisely genotyped by direct sequencing and thus is not listed in **Table 2.** However, the four other SNPs were in very high LD ($r^2 \geq 0.979$) also in our Japanese population. Note that IVS8-49G>T (known as the *Apa*I polymorphism)[9] also linked to these four variations ($r^2 \geq 0.979$). Thus, VDR mRNA stability is probably influenced by this haplotype also in Japanese. The tagging SNP 1592(*308)C>A (or IVS8-49G>T) was detected at a 0.318 frequency, which is lower than that in Caucasians (0.55).[18] Moreover, it was shown that this SNP was also closely linked to IVS8+283G>A (the *Bsm*I polymorphism).[18]

In conclusion, we identified 61 genetic variations, including 25 novel ones, from 107 Japanese subjects in *VDR*. One novel variation results in an amino acid substitution. Close associations of 5 SNPs [(IVS8-49G>T, 1592 (*308) C>A, 2077 (*793) delT, 3190 (*1906)C>A, and 3427(*2143)A>T] were also shown in Japanese. This information would be useful for pharmacogenetic studies to investigate the associations of *VDR* variations with interindividual differences in drug metabolism catalyzed by VDR-regulated drug metabolizing enzymes in Japanese.

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References

1) Malloy, P. J., Pike, J. W. and Feldman, D.: The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets. *Endocr. Rev.*, **20**: 156-188 (1999).
2) Pascussi, J. M., Gerbal-Chaloin, S., Drocourt, L., Maurel, P. and Vilarem, M. J.: The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. *Biochim. Biophys. Acta.*, **1619**: 243-253 (2003).
3) Makishima, M., Lu, T. T., Xie, W., Whitfield, G. K., Domoto, H., Evans, R. M., Haussler, M. R. and Mangelsdorf, D. J.: Vitamin D receptor as an intestinal bile acid sensor. *Science*, **296**: 1313-1316 (2002).
4) Drocourt, L., Ourlin, J. C., Pascussi, J. M., Maurel, P. and Vilarem, M. J.: Expression of *CYP3A4*, *CYP2B6*, and *CYP2C9* is regulated by the vitamin D receptor pathway in primary human hepatocytes. *J. Biol. Chem.*, **277**: 25125-25132 (2002).
5) Thompson, P. D., Jurutka, P. W., Whitfield, G. K., Myskowski, S. M., Eichhorst, K. R., Dominguez, C. E., Haussler, C. A. and Haussler, M. R.: Liganded VDR in-

duces CYP3A4 in small intestinal and colon cancer cells *via* DR3 and ER6 vitamin D responsive elements. *Biochem. Biophys. Res. Commun.*, **299**: 730–738 (2002).
6) Xu, Y., Hashizume, T., Shuhart, M. C., Davis, C. L., Nelson, W. L., Sakaki, T., Kalhorn, T. F., Watkins, P. B., Schuetz, E. G. and Thummel, K. E.: Intestinal and hepatic CYP3A4 catalyze hydroxylation of 1α,25-dihydroxyvitamin D_3: implications for drug-induced osteomalacia. *Mol. Pharmacol.*, **69**: 56–65 (2006).
7) Crofts, L. A., Hancock, M. S., Morrison, N. A. and Eisman, J. A.: Multiple promoters direct the tissue-specific expression of novel N-terminal variant human vitamin D receptor gene transcripts. *Proc. Natl. Acad. Sci. USA*, **95**: 10529–10534 (1998).
8) Sunn, K. L., Cock, T. A., Crofts, L. A., Eisman, J. A. and Gardiner, E. M.: Novel N-terminal variant of human VDR. *Mol. Endocrinol.*, **15**: 1599–1609 (2001).
9) Uitterlinden, A. G., Fang, Y., Van Meurs, J. B., Pols, H. A. and Van Leeuwen, J. P.: Genetics and biology of vitamin D receptor polymorphisms. *Gene*, **338**: 143–156 (2004).
10) Arai, H., Miyamoto, K., Taketani, Y., Yamamoto, H., Iemori, Y., Morita, K., Tonai, T., Nishisho, T., Mori, S. and Takeda, E.: A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *J. Bone Miner. Res.*, **12**: 915–921 (1997).
11) Arai, H., Miyamoto, K., Yoshida, M., Yamamoto, H., Taketani, Y., Morita, K., Kubota, M., Yoshida, S., Ikeda, M., Watabe, F., Kanemasa, Y. and Takeda, E.: The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. *J. Bone Miner. Res.*, **16**: 1256–1264 (2001).
12) Nejentsev, S., Godfrey, L., Snook, H., Rance, H., Nutland, S., Walker, N. M., Lam, A. C., Guja, C., Ionescu-Tirgoviste, C., Undlien, D. E., Ronningen, K. S., Tuomilehto-Wolf, E., Tuomilehto, J., Newport, M. J., Clayton, D. G. and Todd, J. A.: Comparative high-resolution analysis of linkage disequilibrium and tag single nucleotide polymorphisms between populations in the vitamin D receptor gene. *Hum. Mol. Genet.*, **13**: 1633–1639 (2004).
13) Benjamini, Y. and Hochberg, Y.: Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. B.*, **57**: 289–300 (1995).
14) Hsieh, J. C., Shimizu, Y., Minoshima, S., Shimizu, N., Haussler, C. A., Jurutka, P. W. and Haussler, M. R.: Novel nuclear localization signal between the two DNA-binding zinc fingers in the human vitamin D receptor. *J. Cell. Biochem.*, **70**: 94–109 (1998).
15) Hsieh, J. C., Jurutka, P. W., Nakajima, S., Galligan, M. A., Haussler, C. A., Shimizu, Y., Shimizu, N., Whitfield, G.K. and Haussler, M. R.: Phosphorylation of the human vitamin D receptor by protein kinase C. Biochemical and functional evaluation of the serine 51 recognition site. *J. Biol. Chem.*, **268**: 15118–15126 (1993).
16) Oakley-Girvan, I., Feldman, D., Eccleshall, T. R., Gallagher, R. P., Wu, A. H., Kolonel, L. N., Halpern, J., Balise, R. R., West, D. W., Paffenbarger, R. S. Jr. and Whittemore, A. S.: Risk of early-onset prostate cancer in relation to germ line polymorphisms of the vitamin D receptor. *Cancer Epidemiol. Biomarkers Prev.*, **13**: 1325–1330 (2004).
17) Wong, H .L., Seow, A., Arakawa, K., Lee, H. P., Yu, M. C. and Ingles, S. A.:Vitamin D receptor start codon polymorphism and colorectal cancer risk: effect modification by dietary calcium and fat in Singapore Chinese. *Carcinogenesis*, **24**: 1091–1095 (2003).
18) Fang, Y., van Meurs, J. B., d'Alesio, A., Jhamai, M., Zhao, H., Rivadeneira, F., Hofman, A., van Leeuwen, J. P., Jehan, F., Pols, H. A. and Uitterlinden, A. G.: Promoter and 3'-untranslated-region haplotypes in the vitamin D receptor gene predispose to osteoporotic fracture: The Rotterdam study. *Am. J. Hum. Genet.*, **77**: 807–823 (2005).
19) Viger, R. S., Taniguchi, H., Robert, N. M. and Tremblay, J. J.: Role of the GATA family of transcription factors in andrology. *J. Androl.*, **25**: 441–452 (2004).

Outcome of 93 patients with relapse or progression following allogeneic hematopoietic cell transplantation

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Relapse/progression after allogeneic hematopoietic cell transplantation (allo-HCT) remains the major cause of treatment failure. In this study, the subsequent clinical outcome was overviewed in 292 patients with leukemia/myelodysplastic syndrome who received allo-HCT. Among them, 93 (32%) showed relapse/progression. Cohort 1 was chosen to receive no interventions with curative intent (n = 25). Cohort 2 received reinduction chemotherapy and/or donor lymphocyte infusion (n = 48), and Cohort 3 underwent a second allo-HCT (n = 20). Sixty-three patients received reinduction chemotherapy, and 27 (43%) achieved subsequent complete remission (CR). The incidence of nonrelapse mortality (NRM) was similar among the three cohorts (4, 15, and 5%). The 1-year overall survival (OS) after relapse was significantly better in patients with a second HCT (58%) than in others (14%, Cohorts 1 and 2; P < .001). However, the 2-year OS did not differ between the two groups, which suggests that it is difficult to maintain CR after the second HCT. Multivariate analysis showed that reinduction chemotherapy, CR after intervention, second HCT, and longer time to post-transplant relapse were associated with improved survival. In conclusion, for patients with relapse after allo-HCT, successful reinduction chemotherapy and a second HCT may be effective for prolonging survival without excessive NRM. However, effective measures to prevent disease progression after a second HCT clearly need to be developed. Am. J. Hematol. 84:815–820, 2009. © 2009 Wiley-Liss, Inc.

Introduction

Relapse or progression of leukemia occurring after allogeneic hematopoietic cell transplantation (allo-HCT) remains the major cause of post-transplantation mortality, with a median postrelapse survival of 1.6–6 months when aggressive intervention is suspended [1–6]. The optimal treatment strategy for these patients has not yet been established. Although some patients can be reinduced into complete remission (CR) with conventional chemotherapy, only a few become long-term survivors while maintaining conventional chemotherapy [4–6], and the benefit of donor lymphocyte infusion (DLI) for acute leukemia is limited [1,3,7].

Several studies have shown that a second allo-HCT improved survival after relapse and represents a potential therapeutic option, which may increase the duration of leukemia-free survival (6–25 months) [1,6,8–14]. However, this is associated with a high rate of nonrelapse mortality (NRM) (24–75%) [8–13,15]. In many studies, the results regarding a second HCT are generally represented by heterogeneous cohorts of patients or series with relatively few patients carrying variable backgrounds. Furthermore, most studies have not compared the outcome of a second HCT with that of other interventions in the modern treatment era.

To identify the factors that influence the outcome of patients with relapse after various salvage therapies, including second HCT, we performed a retrospective single-center analysis of consecutive 292 patients.

Patients and Methods

Patients. Between January 2000 and December 2006, a total of 292 patients with leukemia or myelodysplastic syndrome (MDS) underwent allo-HCT at the National Cancer Center Hospital. Recipients of haploidentical transplants from related donors and patients aged 15 or under were not included in this study. The characteristics of the patients and transplantations are summarized in Table I. The underlying diseases were AML (n = 142), MDS (n = 73), CML (n = 34), and ALL (n = 43). The median age at the initial HCT was 50 years (range: 16–68). Of the 292 patients, 148 received an initial HCT with myeloablative conditioning (cyclophosphamide plus fractionated TBI or busulfan), and the remaining 144 received reduced-intensity conditioning (RIC; fludarabine- or cladribine-based).

Definitions. Relapse/progression after transplantation was defined as the presence of or increase in leukemic blasts as detected by morphology either in bone marrow or peripheral blood. Detection of minimal residual disease by flow cytometry, PCR, or decreasing donor chimerism did not constitute evidence of recurrence in the absence of morphological abnormalities. CR was defined as normocellular bone marrow with less than 5% blasts along with the absence of blasts in the peripheral blood [16]. Postrelapse overall survival (OS) was measured from the date of relapse or progression to the time of death or censored date of last contact. Withdrawal of immunosuppression (WIS) was defined as the cessation of immunosuppression at the diagnosis of relapse or progression. Chemotherapy was categorized into two groups: reinduction chemotherapy and less-intensive chemotherapy intended for palliative treatment. Disease-specific reinduction chemotherapy included high-dose cytarabine, idarubicin + cytarabine, aclarubicin + low-dose cytarabine [17,18], and other remission-induction therapies for myeloid and lymphoid leukemia. Imatinib mesylate for CML, all-trans retinoic acid or arsenic trioxide for acute promyelocytic leukemia (APL), gemtuzumab ozogamicin for CD33-positive AML, and intrathecal chemotherapy alone for isolated central nervous system (CNS) relapse were also included in the reinduction chemotherapy group. Less-intensive chemotherapy included oral hydroxyurea, cytarabine or 6-mercaptopurine, and the sole intravenous administration of aclarubicin or vincristine, which are not thought to be intensive enough to achieve remission, but are aimed at palliation. NRM was defined as death from toxicities related to therapy without disease recurrence.

Interventions were categorized into three cohorts: Cohort 1, WIS or less-aggressive chemotherapy; Cohort 2, reinduction chemotherapy and/or DLI; Cohort 3, second allo-HCT.

Statistical analysis. Data were retrospectively reviewed and analyzed as of August 2007. The primary endpoint of the study was OS following relapse/progression. OS was estimated by the Kaplan-Meier method.

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Conflict of interest: Nothing to report.

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The log-rank test and generalized Wilcoxon test were used to compare the probabilities of survival over time across patient subgroups. Multiple cox regression models were used for multivariate risk-factor analysis for OS following relapse/progression. The clinical factors evaluated were diagnosis, patient age at the initial HCT, gender, conditioning in the initial HCT (myeloablative or RIC), donor in the initial HCT (HLA-matched related or others), disease status at the initial HCT, interval from the initial HCT to relapse/progression, interventions that were chosen after relapse (Cohorts 1–3), and the response to the initial intervention. We considered two-sided *P*-values of <0.05 to be statistically significant. Statistical analyses were performed with the SPSS statistics and SAS version 8.2 (SAS, Cary, NC).

TABLE I. Patient and Transplanation Characteristics

Characteristics	All patients	Relapsed patients %[a]
No. of patients	292	93 (32)
Age, year, median (range)	50 (16–68)	47 (16–68)
Diagnosis[b]		
AML	142	57 (40)
MDS	73	13 (9)
CML	34	5 (4)
ALL	43	18 (13)
Gender		
Male	173	49 (35)
Female	119	44 (31)
Matched related donor		
Yes	125	44 (31)
No	167	49 (35)
Conditioning regimen		
Myeloablative		
TBI-based	90	38 (27)
BU/CY-based	58	21 (15)
RIC	144	34 (24)
Stem cell source		
BM	125	37 (26)
PBSC	149	49 (35)
CB	18	7 (5)
Disease status at first HCT		
CR	150	42 (30)
non-CR	142	51 (36)
GVHD prophylaxis		
CSP-based	243	77 (54)
TAC-based	49	16 (11)

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoid leukemia; TBI, total body irradiation; BU/CY, busulfan/cyclophosphamide; RIC, reduced-intensity conditioning; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; CR, complete remission; GVHD, graft-versus-host disease; CSP, cyclosporin; TAC, tacrolimus.

[a] The percentage shown here indicates the proportion to relapsed patients among each category.

[b] MDS overt leukemia was categorized into AML.

Results

Relapse or progression

The characteristics of all patients and relapsed patients are shown in Table I. Overall, 93 of the 292 patients (32%) relapsed or progressed at a median of 154 days (range; 15–1,211) after the initial HCT (AML, $n = 57$; MDS, $n = 13$; CML, $n = 5$; ALL, $n = 18$). The interval from the initial HCT to relapse/progression was less than 100 days in 34 patients, 100 days to 1 year in 39 patients, and more than 1 year in 20 patients.

TABLE II. Outcomes of Interventions after Relapse

Therapy	*n*	CR (%)	NRM (%)	OS after relapse, day, median, (range)
Total	93	34 (37)	9 (10)	184 (5–1456)
No aggressive Tx	25	1 (4)	1 (4)	61 (5–245)
No therapy	7	0	0	56 (22–166)
WIS alone	10	1	1	60 (5–245)
Less- int. CTx	8	0	0	74 (12–203)
Chemotherapy/DLI	48	18 (38)	7 (15)	194 (19–1,456)
Reinduction CTx	31	9 (29)	2 (6)	167 (19–1,456)
CTx + DLI	14	7 (50)	4 (29)	194 (52–1,254)
DLI alone	3	2 (67)	1 (33)	240 (32–243)
second HCT	20	15 (75)	1 (5)	502 (66–997)

CR, complete remission; NRM, nonrelapse mortality; OS, overall survival; Tx, therapy; WIS, withdrawal of immunosuppression; Less-int. CTx, less-intensive chemotherapy; DLI, donor lymphocyte infusion; HCT, hematopoietic cell transplantation.

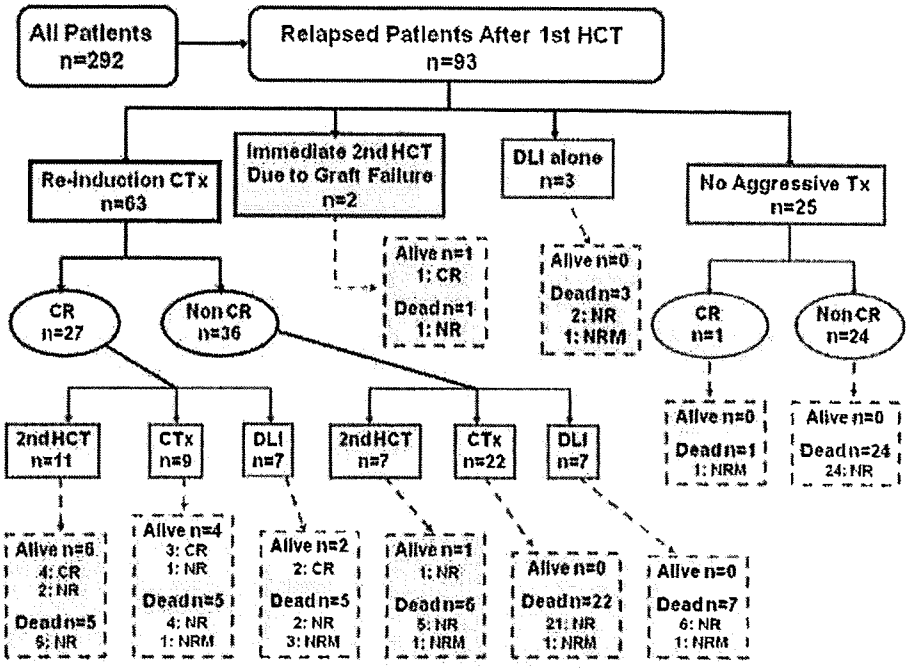

Figure 1. Summary of interventions after relapse. Abbreviations: HCT, hematopoietic cell transplantation; CTx, chemotherapy; Tx, therapy; CR, complete remission; DLI, donor lymphocyte infusion; NR, nonremission; NRM, nonrelapse mortality. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE III. Patient Characteristics of Intervention Group

Characteristics	No aggressive Tx (%)	CTx and/or DLI (%)	Second HCT (%)	P
Total no. of patients	25	48	20	
Diagnosis				0.053
AML	10 (40)	32 (67)	15 (75)	
MDS	7 (28)	3 (6)	3 (15)	
CML	2 (8)	3 (6)	0 (0)	
ALL	6 (24)	10 (21)	2 (10)	
Age				0.333
<50	11 (44)	28 (58)	13 (65)	
≥50	14 (56)	20 (42)	7 (35)	
Matched related donor				0.143
Yes	8 (32)	27 (56)	9 (45)	
No	17 (68)	21 (44)	11 (55)	
Disease status at first HCT				0.105
CR	7 (28)	26 (54)	9 (45)	
non-CR	18 (72)	22 (46)	11 (55)	
Time from first HCT to relapse				0.938
≥100 days	16 (64)	31 (65)	12 (60)	
<100 days	9 (36)	17 (35)	8 (40)	

Tx, therapy; CTx, chemotherapy; HCT, hematopoietic cell transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoid leukemia; CR, complete remission.

Interventions after relapse/progression

After the diagnosis of relapse or progression, the need for salvage therapy was determined at a multiprofessional conference, at which the clinical circumstances and the opinions of physicians and patients were weighed. The various therapeutic options used after the diagnosis of relapse are summarized in Table II and Fig. 1.

At the diagnosis of relapse or progression, 70 patients had been receiving immunosuppression (median days after initial HCT, 125; range 15–705) and 63 of them had it withdrawn before receiving any other therapies.

After the diagnosis of relapse or progression, 63 patients received reinduction chemotherapy with disease-specific regimens, which included imatinib mesylate (CML, $n = 4$), all trans-retinoic acid and arsenic trioxide (APL, $n = 1$), gemtuzumab ozogamicin (AML, $n = 3$), and intrathecal chemotherapy alone for isolated CNS relapse (AML, $n = 3$; ALL, $n = 1$; CML, $n = 1$). Overall, 27 of the 63 patients who received reinduction chemotherapy achieved CR (43%). Among the 27 patients who achieved CR, 18 proceeded to DLI ($n = 7$) or second HCT ($n = 11$). The remaining nine received no further therapy other than chemotherapy; three patients with CNS relapse were in remission, and the remaining six patients subsequently progressed. Among the 36 patients who did not achieve CR, 14 proceeded to DLI ($n = 7$) or second HCT ($n = 7$), and the remaining 22 did not receive further treatment because of various reasons (disease progression, $n = 15$; infection and/or graft-versus-host disease (GVHD), $n = 4$; refusal, $n = 3$). Two other patients proceeded to second HCT directly after disease relapse with concomitant graft failure.

To compare the outcomes of the interventions after relapse/progression, we divided the 93 patients into three cohorts according to the intervention, that is, no aggressive therapy (Cohort 1, $n = 25$), reinduction chemotherapy and/or DLI without second HCT (Cohort 2, $n = 48$), and second HCT (Cohort 3, $n = 20$). There were no significant differences among the three groups in clinical characteristics such as patient age at the initial HCT, diagnosis, donor in the initial HCT, disease status at the initial HCT, and interval from the initial HCT to relapse (Table III).

No aggressive therapy (Cohort 1)

Among the 93 patients who relapsed, 25 (27%) received no aggressive therapy with curative intent other than WIS or less-intensive chemotherapy, mostly because of comorbidities and/or refractoriness of leukemia/MDS. Among the 10 patients who received WIS alone, only one achieved CR, but this patient subsequently died of bronchiolitis obliterans. All of the remaining eight patients who were given less-intensive chemotherapy alone and seven who received no therapy after relapse/progression died of disease progression without achieving CR. The median OS of the patients in Cohort 1 was 61 days after relapse/progression and the cause of death was primarily disease progression.

Reinduction chemotherapy and/or DLI without second HCT (Cohort 2)

Of the 63 patients who received reinduction chemotherapy after relapse, 45 patients did not receive a second HCT; these 45 patients with or without subsequent DLI and three other patients who received DLI without preceding chemotherapy were placed in Cohort 2.

Overall, 16 (36%) of the 45 patients achieved CR as the best response after reinduction chemotherapy. All three patients with isolated CNS relapse were alive in remission, whereas 11 of 13 patients who had marrow relapse eventually relapsed.

After reinduction chemotherapy, 14 patients (AML, $n = 9$; MDS, $n = 1$; ALL, $n = 3$; CML, $n = 1$) received DLI from the same donor as in the initial HCT. The initial CD3-positive cell dose of DLI ranged from 0.03 to 161 $\times$ 10^8/kg (median: 2.9 $\times$ 10^8/kg), and the number of courses of DLI was one to four, which were chosen according to the donor source or the disease status of patients at the discretion of physicians. Although the remission rate of patients who received DLI after chemotherapy was 50%, the incidence of NRM was also rather high (29%, GVHD with or without infection). The median OS of patients who received DLI after relapse/progression was 194 days (range: 52–1,254), which was similar to that of patients without DLI (167 days, range; 19–1,456).

Among the three patients who received DLI without preceding chemotherapy (AML, 1; MDS, 2), two achieved CR but all of them eventually died: one with toxicity and two with disease progression.

Second HCT (Cohort 3)

Table IV summarizes the profiles of 20 patients who underwent a second HCT. The median age at the initial HCT was 38 years (21–66 years) and 65% of the patients were younger than 50 years. The median time from the initial HCT to relapse/progression was 152 days (range: 21–1,211), and the median interval between the initial HCT and the second HCT was 325 days (range: 126–1,310). Six patients received HCT from the same donor as in the initial HCT (HLA-matched related donor, $n = 5$; unrelated bone marrow donor, $n = 1$), and the remaining 14 received the second HCT from a different donor (unrelated bone marrow donor, $n = 7$; cord blood, $n = 6$; haploidentical related do-

TABLE IV. Characteristics of Second Transplantation

Characteristics	No of patients second HCT (%)
Total	20
Age	
<50	13 (65)
>50	7 (35)
Diagnosis	
AML	15 (75)
MDS	3 (15)
CML	0 (0)
ALL	2 (10)
Gender	
Male	9 (45)
Female	11 (55)
Time from first HCT to relapse	
<100 days	8 (40)
≥100 days	12 (60)
Time from first HCT to second HCT	
<1 year	12 (60)
≥1 year	8 (40)
Donor for first/second HCT	
Same	6 (30)
MRD-MRD	5
UBM-UBM	1
Different	14 (70)
UBM-UBM	4
MRD/CB-UBM	3
MRD/UBM-CB	6
Other	1
Conditioning for first/second HCT	
Myeloablative	8 (40)
Myeloablative-RIC	7 (35)
RIC-RIC	5 (25)
Stem cell source	
BM	8 (40)
PBSC	6 (30)
CB	7 (35)
Remission at second HCT	
No	9 (45)
yes	11 (55)
GVHD prophylaxis	
CSP-based	8 (40)
TAC-based	3 (15)
Others	3 (15)
GVHD	
No	10 (50)
Yes	10 (50)

HCT, hematopoietic cell transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoid leukemia; MRD, matched-related donor; UBM, unrelated bone marrow; CB, cord blood; RIC, reduced-intensity conditioning; PBSC, peripheral blood stem cell; CSP, cyclosporin; TAC, tacrolimus.

nor, $n = 1$). Among the 15 patients who had received myeloablative conditioning for the initial HCT, eight received myeloablative conditioning and seven received RIC for the second HCT. The remaining five patients received both HCT with RIC. Although the 1-year OS after relapse was better in patients who received myeloablative conditioning for the second HCT than in patients who received RIC (100 vs. 37%, $P = 0.015$), patients who received myeloablative conditioning for the second HCT were younger and had a longer interval between the initial and the second HCT than those who received RIC ($P < 0.001$ and $P = 0.006$, respectively). There was no difference in OS between patients who received a second HCT from the same donor and those who had a different donor (1-year OS: 44 vs. 60%, $P = 0.48$).

Two patients underwent immediate HCT after relapse with concomitant graft failure. Among the other 18 patients who received reinduction chemotherapy before the second HCT, 11 had achieved CR at the second HCT and seven were not in CR. Four of the nine patients with nonremission disease at the second HCT, including two patients who did not receive reinduction chemotherapy, subsequently achieved CR; only one of the nine patients is currently alive in CR.

Of the 20 patients who underwent a second HCT, eight are alive with a median follow-up after relapse of 335 days (range: 181–997); five are in CR and three have recurrent disease.

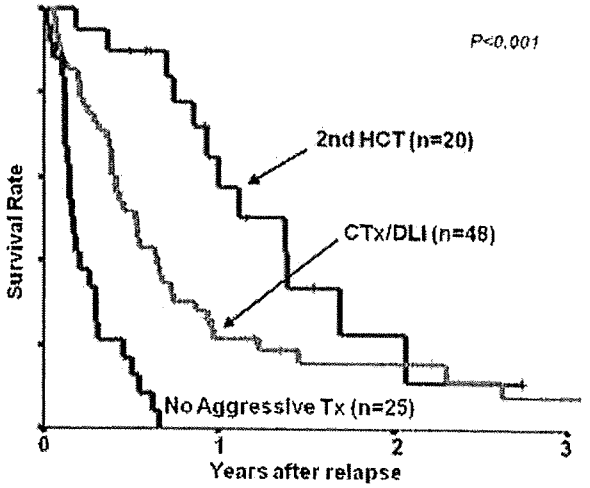

Figure 2. Overall survival. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

GVHD was newly diagnosed or interpreted to progress after the second HCT in 10 of the 20 patients. The median OS after relapse in patients with GVHD after the second HCT was 422 days (range: 181–997), and all of these patients achieved CR as a best response. The median OS after relapse for the remaining 10 patients without GVHD was 314 days (range: 66–757), and five of them failed to achieve CR as a best response.

Comparison of CR, NRM, and OS after relapse following the initial HCT

The median OS after the development of relapse in the 93 patients who had relapse/progression was 184 days (range: 5–1,456). Overall, 15 patients (16%) are currently alive with a median follow-up of 346 days (range; 33–1,456 days), and 10 of these patients are still in CR. Among the 78 patients who died, 69 died of disease progression and nine died of NRM (10%). The causes of NRM were GVHD and/or infection in eight (Cohort 1, one patient; Cohort 2, seven patients), and one early death after the second HCT with hepatic failure, which accounts for the one case of NRM for second HCT (Table II).

We compared the rate of CR, NRM, and OS after relapse among the three different cohorts (Table II). As the maximum response, the probabilities of achieving CR were 4% in Cohort 1, 38% in Cohort 2, and 75% in Cohort 3. The NRM rates were 4, 15, and 5% for each group, respectively. The median duration of remission after achieving CR was 177 days (range, 17–1,167). The median OS after relapse/progression in patients who underwent a second HCT (Cohort 3, 502 days) was significantly longer than those in Cohort 1 (61 days) and Cohort 2 (194 days, $P < .001$, Fig. 2). The 1-year OS after relapse was significantly better in patients with a second HCT (Cohort 3) than in the other patients (Cohorts 1 and 2) (58 vs. 14%). However, there was no significant difference in the 2-year OS, which suggests that it is difficult to maintain CR after a second HCT.

A multivariate analysis showed that CR after intervention (HR 3.83, 95% CI 2.06–7.11, $P < .001$), reinduction chemotherapy (HR 2.83, 95% CI 1.65–4.86, $P < .001$), a second HCT (HR 3.02, 95% CI 1.58–5.79, $P < .001$), and a longer time from the initial HCT to relapse (HR 1.99, 95% CI 1.21-3.28, $P = 0.007$) were associated with an improved OS after relapse/progression (Table V). Diagnosis, patient age at initial HCT, gender, conditioning regimen, or donor in the initial HCT and DLI were not significant factors.

TABLE V. Univariate and Multivariate Analysis of risk Factors for OS after Relapse

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P	HR (95%CI)	P
Diagnosis			–	–
CML	1.00			
AML	2.03 (0.62–6.65)	0.241		
ALL	2.54 (0.71–9.00)	0.150		
MDS	3.39 (0.94–12.24)	0.062		
Age			–	–
<50	1.00			
≥50	1.53 (0.98–2.41)	0.063		
Gender			–	–
Male	1.00			
Female	0.92 (0.59–1.43)	0.701		
Conditioning			–	–
Myeloablative	1.00			
RIC	1.34 (0.84–2.12)	0.216		
Donor			–	–
MRD	1.00			
Others	1.26 (0.80–1.97)	0.322		
Disease Status at first HCT				
Standard	1.00			
High	1.23 (0.70–2.12)	0.465		
Time from first HCT to relapse				
≥100 days	1.00		1.00	
<100 days	1.74 (1.09–2.79)	0.020	1.99 (1.21–3.28)	0.007
Reinduction CTx				
Yes	1.00		1.00	
No	3.79 (2.24–6.40)	<.001	2.83 (1.65–4.86)	<.001
CTx Intensity			–	–
Reinduction	1.00			
Less Intensive	4.44 (2.00–9.88)	<.001		
DLI			–	–
Yes	1.00			
No	1.00 (0.57–1.72)	0.968		
Second HCT				
Yes	1.00		1.00	
No	2.89 (1.55–5.38)	<.001	3.02 (1.58–5.79)	<.001
CR after Interventions				
Yes	1.00		1.00	
No	3.54 (2.06–6.09)	<.001	3.83 (2.06–7.11)	<.001

OS, overall survival; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; MDS, myelodysplastic syndrome; RIC, reduced-intensity conditioning; MRD, matched-related donor; HCT, hematopoietic cell transplantation; CTx, chemotherapy; DLI, donor lymphocyte infusion; CR, complete remission.

Discussion

With this retrospective single-center survey in which we compared the outcomes of interventions for relapse/progression after allo-HCT, we showed that a second HCT significantly improved the remission rate and survival. In contrast to previous reports (8–13, 15), NRM after a second HCT was observed in an acceptable percentage of patients (5%), even though 40% of the patients received myeloablative conditioning regimen for the second HCT.

As salvage interventions for leukemia/MDS relapsing after allo-HCT, chemotherapy, DLI either alone or in combination, and second HCT have been considered with different degrees of success. Consistent with reports from other groups [1,4–6], we found that patients who did not undergo intensive chemotherapy had significantly shorter survival. Even though 43% of the patients who were given reinduction chemotherapy achieved CR, all of the relapsed patients who did not receive further intervention eventually relapsed unless relapse is isolated to CNS, and all but one patient died. Prior reports have also suggested that, instead of a certain probability of obtaining remission with reinduction chemotherapy, subsequent relapse is frequently observed and the prognosis is poor when further immunotherapy is suspended [1,4,6,19].

Although DLI has been recognized as an effective treatment for relapsed CML, the efficacy of DLI for relapsed acute leukemia is rather discouraging [3,7,20–22]. Although the remission rate has been reported to be 15–42%, the survival rate has not improved (3-year OS less than 20%), mostly because of a high incidence of uncontrolled GVHD (10–50%). In our cohorts, survival was not improved by adding DLI after chemotherapy, although half of the patients had achieved transient remission. The incidence of NRM after DLI was 29%, which was mostly explained by GVHD. Compared to DLI, a second HCT yielded an even better remission rate and lower NRM in our cohort, which could be respectively explained by the efficacy of the use of conditioning radiochemotherapy and GVHD prophylaxis in the second HCT.

In our data, a second HCT significantly improved the remission rate and survival compared to other interventions, as proven by a multivariate analysis. Although Arellano et al. [1] indicated that immunotherapy including a second HCT was effective compared to chemotherapy or supportive care, other reports that compared interventions after relapse following initial HCT failed to show the advantage of a second HCT [2,6,22]. Prior reports that focused on a second HCT have also expressed concerns about the negative impact of NRM, which has ranged from 24 to 75% (8–13, 15). In contrast, our data revealed a 5% incidence of NRM after a second HCT, which led to improved OS. This unexpectedly low incidence of NRM may reflect the advances in GVHD prophylaxis and supportive care over the past several years. Another possible explanation would be a selection bias of fitter patients that led to less NRM after the second HCT, although there were no significant differences in available characteristics of patients in each intervention group.

Concerning the conditioning regimen for the second HCT, we found that patients who received myeloablative conditioning had a better OS than patients who received

RIC. Eapen et al. [9] indicated the importance of a tumor-killing effect of myeloablative conditioning for the second HCT compared to RIC. Other groups also reported a superior outcome of TBI-based myeloablative conditioning in the second HCT [8,11]. On the other hand, several recent reports have shown that RIC offers a toxicity-reducing benefit in the second HCT [10]. In our cohort, patients who received myeloablative conditioning for the second HCT were younger and had a longer interval from the initial HCT to the second HCT, which could reflect a selection bias in the choice of myeloablative conditioning. Therefore, myeloablative conditioning for the second HCT could be considered beneficial for selected patients.

Consistent with several previous reports, we demonstrated that remission status [4,6,8–12,14,22,23], the use of reinduction chemotherapy [2,6], and a longer interval from the initial HCT to relapse [1,2,4,8–12,14,15,19,22–24] were associated with improved OS after relapse by multivariate analysis. Most prior reports have shown that an interval of 6 months or longer was associated with better OS. We found that patients who relapsed after 100 days following the initial HCT had better OS. However, relapses after intervals of 6 months or 1 year were not significantly associated with improved OS (data not shown).

Prior reports have also suggested that the development of GVHD after a second HCT [2,7–9,13,15,24] and the use of a different donor for the second HCT were associated with a better outcome after the second HCT [10]. Our data showed that both the remission rate and OS tended to be improved in patients who developed newly diagnosed GVHD after the second HCT. However, the use of a different donor for the second HCT did not appear to offer any advantage. Nevertheless, the small number of patients who received a second HCT in our study limits our ability to draw definite answers.

Although the 1-year OS after the second HCT was significantly better than that with other interventions (58 vs. 14%), there was no significant difference in 2-year OS (22 vs. 10%). The substantial decline in the survival curve in the second HCT group after 1 year from relapse was clearly related to recurrence of the underlying diseases. Previous reports also showed a decline in survival in the later period (<30% at 3–5 years from the second HCT) and a substantial relapse rate after the second HCT (>40%) [9–11]. This evidence suggests the need for the effective management of disease recurrence after the second HCT.

Our study is limited by several inherent selection biases. Most importantly, this is a retrospective study that compared the outcomes of interventions that were chosen at the discretion of physicians, although there were no significant differences in patient characteristics among the three cohorts. For example, patients who successfully received intensive intervention such as a second HCT had to survive long enough after relapse to be able to undergo adequate salvage chemotherapy with a rather controlled disease and less comorbidity. Other limitations include the small number of patients, a short follow-up period, and other transplant variables that may have affected the outcomes. Nevertheless, the present data in a consecutive-case series from a single center that reviewed various interventions after relapse allowed us to identify the factors that influenced the prognosis of patients with relapse/progression after allo-HCT.

In summary, these observations may have important implications for the selection of interventions in patients who relapse after allo-HCT. Our data indicated that reinduction chemotherapy with curative intent is required for prolonged survival, if feasible. However, when CR is not available with chemotherapy, long-term survival may be unlikely even with a second HCT. The second HCT may produce improved survival without excessive toxicity. However, the substantial incidence of a later relapse after the second HCT was revealed to be a major concern. Further studies are warranted to identify innovative post-transplant strategies to reduce disease recurrence, including immunotherapy such as a vaccination strategy.

References

1. Arellano ML, Langston A, Winton E, et al. Treatment of relapsed acute leukemia after allogeneic transplantation: A single center experience. Biol Blood Marrow Transplant 2007;13:116–123.
2. Bethge WA, Storer BE, Maris MB, et al. Relapse or progression after hematopoietic cell transplantation using nonmyeloablative conditioning: Effect of interventions on outcome. Exp Hematol 2003;31:974–980.
3. Collins RH Jr, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol 1997;15:433–444.
4. Frassoni F, Barrett AJ, Granena A, et al. Relapse after allogeneic bone marrow transplantation for acute leukaemia: A survey by the E.B.M.T. of 117 cases. Br J Haematol 1988;70:317–320.
5. Mortimer J, Blinder MA, Schulman S, et al. Relapse of acute leukemia after marrow transplantation: Natural history and results of subsequent therapy. J Clin Oncol 1989;7:50–57.
6. Oran B, Giralt S, Courlel D, et al. Treatment of AML and MDS relapsing after reduced-intensity conditioning and allogeneic hematopoietic stem cell transplantation. Leukemia 2007;21:2540–2544.
7. Dazzi F, Fozza C. Disease relapse after haematopoietic stem cell transplantation: Risk factors and treatment. Baillieres Best Pract Res Clin Haematol 2007;20:311–327.
8. Bosi A, Laszlo D, Labopin M, et al. Second allogeneic bone marrow transplantation in acute leukemia: Results of a survey by the European Cooperative Group for Blood and Marrow Transplantation. J Clin Oncol 2001;19:3675–3684.
9. Eapen M, Giralt SA, Horowitz MM, et al. Second transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant. Bone Marrow Transplant 2004;34:721–727.
10. Hosing C, Saliba RM, Shahjahan M, et al. Disease burden may identify patients more likely to benefit from second allogeneic hematopoietic stem cell transplantation to treat relapsed acute myelogenous leukemia. Bone Marrow Transplant 2005;36:157–162.
11. Michallet M, Tanguy ML, Socie G, et al. Second allogeneic haematopoietic stem cell transplantation in relapsed acute and chronic leukaemias for patients who underwent a first allogeneic bone marrow transplantation: A survey of the Societe Francaise de Greffe de moelle (SFGM). Br J Haematol 2000;108:400–407.
12. Mrsic M, Horowitz MM, Atkinson K, et al. Second HLA-identical sibling transplants for leukemia recurrence. Bone Marrow Transplant 1992;9:269–275.
13. Radich JP, Sanders JE, Bucknor CD, et al. Second allogeneic marrow transplantation for patients with recurrent leukemia after initial transplant with total-body irradiation-containing regimens. J Clin Oncol 1993;11:304–313.
14. Wagner JE, Vogelsang GB, Zehnbauer BA, et al. Relapse of leukemia after bone marrow transplantation: Effect of second myeloablative therapy. Bone Marrow Transplant 1992;9:205–209.
15. Kishi K, Takahashi S, Gondo H, et al. Second allogeneic bone marrow transplantation for post-transplant leukemia relapse: Results of a survey of 66 cases in 24 Japanese institutes. Bone Marrow Transplant 1997;19:461–466.
16. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 2003;21:4642–4649.
17. Saito K, Nakamura Y, Aoyagi M, et al. Low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor (CAG regimen) for previously treated patients with relapsed or primary resistant acute myelogenous leukemia (AML) and previously untreated elderly patients with AML, secondary AML, and refractory anemia with excess blasts in transformation. Int J Hematol 2000;71:238–244.
18. Yamada K, Furusawa S, Saito K, et al. Concurrent use of granulocyte colony-stimulating factor with low-dose cytosine arabinoside and aclarubicin for previously treated acute myelogenous leukemia: A pilot study. Leukemia 1995;9:10–14.
19. Pollyea DA, Artz AS, Stock W, et al. Outcomes of patients with AML and MDS who relapse or progress after reduced intensity allogeneic hematopoietic cell transplantation. Bone Marrow Transplant 2007;40:1027–1032.
20. Kolb HJ, Schmid C, Buhmann R, et al. DLI: Where are we know? Hematology 2005;10(Suppl 1):115–116.
21. Kolb HJ, Schmid C, Weisser M, et al. Cytoreduction, DLI, or mobilized peripheral blood progenitors. Ann Hematol 2002;81(Suppl 2):S30–S33.
22. Mielcarek M, Storer BE, Flowers ME, et al. Outcomes among patients with recurrent high-risk hematologic malignancies after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2007;13:1160–1168.
23. Levine JE, Braun T, Penza SL, et al. Prospective trial of chemotherapy and donor leukocyte infusions for relapse of advanced myeloid malignancies after allogeneic stem-cell transplantation. J Clin Oncol 2002;20:405–412.
24. Barrett AJ, Locatelli F, Treleaven JG, et al. Second transplants for leukaemic relapse after bone marrow transplantation: High early mortality but favourable effect of chronic GVHD on continued remission. A report by the EBMT Leukaemia Working Party. Br J Haematol 1991;79:567–574.

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ORIGINAL ARTICLE

Intensive glucose control after allogeneic hematopoietic stem cell transplantation: a retrospective matched-cohort study

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Some studies have shown that intensive glucose control (IGC) improves outcome in the intensive care unit setting. However, it is the benefit of IGC in hematopoietic SCT (HSCT) that is not well defined. Between June 2006 and May 2007, IGC was maintained prospectively after allogeneic HSCT and clinical outcomes were compared with a cohort matched for conditioning regimen, source of stem cells, age and relation to donor. A stratified Cox regression model was used. There were no significant differences in baseline clinical characteristics. The median age was 43.5 years in both groups. The primary diagnosis was a hematologic malignancy. Patients in the IGC group had a lower glucose level (least-square mean, 116.4 vs 146.8 mg per 100 ml, $P<0.001$) compared to the standard glucose control group. The incidences of documented infections and bacteremia were significantly lower in the IGC group (14 vs 46%, $P=0.004$, 9 vs 39%, $P=0.002$, respectively). IGC tended to reduce the incidence of renal dysfunction (19 vs 37%, $P=0.36$) and the elevation of C-reactive protein (18 vs 38%, $P=0.13$). This study suggests that IGC has may have a beneficial effect after HSCT. IGC should be evaluated further in a large prospective, randomized study.

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Introduction

Previous studies showed that intensive glucose control (IGC), in which the target blood glucose level was set within 80–110 mg per 100 ml, reduced infections, dysfunction of organs including the liver and kidney and mortality compared to patients who received standard glucose control.[1–3] Although these results have been confirmed in several subsequent studies,[4–7] the precise mechanism that underlies this association is unclear. In animal models, it has been shown that insulin itself has a direct inhibitory effect on the inflammation process.[8,9] However in human studies, it has been suggested that these benefits could be directly attributed to IGC rather than to any pharmacological activity of administered insulin *per se*.[3,4]

Recipients of allogeneic hematopoietic SCT (HSCT), which is the most drastic therapeutic modality in patients with hematological malignancies, often suffer from serious complications including infectious diseases, GVHD and multiple organ failure. They are also at higher risk of hyperglycemia because of the use of steroids for the treatment of GVHD, the use of total parenteral nutrition (TPN), immunosuppressive drugs and infectious complications,[10,11] which makes them further susceptible to numerous serious complications including infectious diseases and multiple organ failure.[12–14] Our group previously reported that hyperglycemia during neutropenia was associated with an increased risk of acute GVHD and nonrelapse mortality (NRM) after myeloablative allogeneic HSCT,[15] and that hyperglycemia during neutropenia was associated with a higher incidence of subsequent acute GVHD. It is well known that an increase in the levels of circulating cytokines may aggravate hyperglycemia, and hyperglycemia itself could increase the levels of cytokines. This vicious cycle could lead to elevated cytokine levels, which could lead to subsequent acute GVHD. With this background, it can be hypothesized that IGC would reduce the incidence of infectious diseases, acute GVHD and organ dysfunctions after allogeneic HSCT. Therefore, we prospectively investigated the effect of IGC after allogeneic HSCT, and compared the clinical outcomes to those in a matched cohort to address whether IGC following allogeneic HSCT could improve the clinical course of patients, that is, reduction of infectious diseases and organ dysfunction, as has been shown in the intensive care unit (ICU) setting.

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Patients and methods

Patients

From June 2006 to May 2007, a total of 73 patients received allogeneic HSCT at the National Cancer Center Hospital (Tokyo, Japan); 60 patients were eligible for participation in this trial. Finally, 22 patients (36.7%) were enrolled in this IGC study to keep the blood glucose level at 80–110 mg per 100 ml, as shown in Figure 1.

Study center and organization

The National Cancer Center Hospital in Tokyo holds 600 beds. The transplant team consists of 4 full-time physicians and 26 nursing staff who oversee 26 beds in the HSCT, and the entire ward is covered by high-efficiency particulate air-filters. We regularly perform 90–120 transplants per year: 80% allogeneic and 20% autologous.

Study design

This was a case–control study to investigate the clinical benefits of comprehensive nutritional support including IGC and parenteral nutrition (PN) management, which was approved by the Institutional Review Board. A matching control group was selected among patients who received HSCT from January 2002 to March 2007 (ratio of 1:2 compared to the study group) according to the following criteria: (1) conditioning regimen (conventional myeloablative or reduced intensity), (2) source of stem cells (BM, peripheral blood or cord blood), (3) age and (4) source of donor (related or unrelated). Criteria (1–4) were essential for inclusion. As a result, 42 matched controls were selected, and a total of 64 patients were subjected to further analysis (Table 1).

Exclusion criteria

Exclusion criteria were as follows: (1) patients who received a reduced-intensity conditioning regimen for an HLA-matched related donor, as we applied GVHD prophylaxis without short-term MTX in this setting, and they had much less need for TPN and less need for intense glucose control,[16] (2) those with a poor performance status (Eastern Cooperative Oncology Group) $\geqslant 2$, (3) those with uncontrolled infectious diseases at the beginning of the conditioning regimen and (4) those with preexisting neutropenia. We previously reported that the incidence of severe stomatitis (Common Terminology Criteria for Adverse Events (CTCAE) grade (3) was 0% after reduced-intensity SCT (RIST) from a related HLA-matched donor.[16] In this situation, the need for TPN and the incidence of hyperglycemia were quite low, compared to RIST from an unrelated donor, which included additional low-dose TBI or antithymocyte globulin (ATG) and short-term MTX or conventional SCT with a myeloablative regimen. Hence, we only included patients who received a RIST regimen from an unrelated donor, who had a higher probability of glucose-control intervention, to evaluate the beneficial effects of IGC.

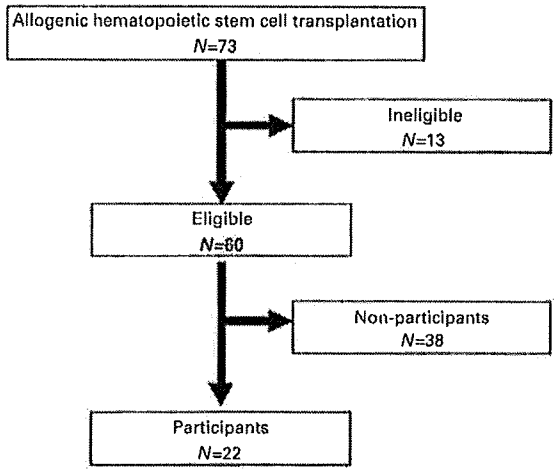

Figure 1 Trial profile.

Table 1 Patients' characteristics

Variable	N *(%)/median (range)*		P-*value*
	Intensive glucose control (n = *22*)	*Standard glucose control* (n = *42*)	
Age (years)	43.5 (17–64)	43.5 (20–66)	
<40	8 (36)	18 (43)	0.62
⩾40	14 (64)	24 (57)	
Sex			
Male	9 (41)	22 (52)	0.38
Female	13 (59)	20 (48)	
Disease risk[a]			
Standard	6 (27)	16 (38)	0.39
High	16 (73)	26 (62)	
Conditioning			
CST	14 (64)	27 (64)	
BU/CY	9 (40)	18 (43)	
CY/TBI (12 Gy)	4 (18)	6 (14)	
Other	1 (5)	3 (7)	
RIST	8 (36)	15 (36)	0.96
2CdA/BU	1 (5)	1 (2)	
Flu/BU	7 (32)	14(33)	
Low-dose TBI (2–4 Gy)	3 (14)	7 (17)	
Low-dose ATG	5 (23)	10 (24)	0.92
GVHD prophylaxis			
Cyclosporin-based	7 (32)	27 (64)	
Tacrolimus-based	15 (68)	15 (36)	0.01
Short-term MTX (+)	22 (100)	40 (95)	0.30
Relation to donor			
Related	6 (27)	12 (29)	
Unrelated	16 (73)	30 (71)	0.91
Stem cell source			
Bone marrow	15 (68)	30 (71)	
PBSC	5 (23)	10 (24)	
Cord blood	2 (9)	2 (5)	0.19
HLA match			
Match	11 (50)	28 (67)	
Mismatch	11 (50)	14 (33)	0.19

Abbreviations: ATG = antithymocyte globulin; 2CdA = cladribine; CST = conventional stem cell transplantation; Flu = fludarabine; RIST = reduced-intensity stem cell transplantation.
[a]Standard-risk patients included those with acute leukemia in first complete remission, chronic leukemia in first chronic phase, MDS in refractory anemia and NHL in complete remission, and the remaining patients were categorized as high risk.

Transplantation procedures

Forty-one patients received a myeloablative conditioning regimen that included BU (orally 4 mg/kg per day × 4 days or i.v. 3.2 mg/kg per day × 4 days) plus CY (60 mg/kg per day × 2 days, $n=27$), CY plus 12 Gy TBI ($n=10$) or other ($n=4$). Twenty-three patients received a reduced-intensity conditioning regimen that included fludarabine (30 mg/m^2 per day × 6 days) or cladribine (0.11 mg/kg per day × 6 days) plus BU (oral 4 mg/kg per day × 2 days or i.v. 3.2 mg/kg per day × 2 days). Low-dose TBI (2 or 4 Gy, $n=10$) and/or low-dose ATG (total dose 5–10 mg/kg ATG-F or 5 mg/kg thymoglobulin, $n=15$) were added. GVHD prophylaxis included CYA- ($n=13$) and tacrolimus-based regimens ($n=51$), with an additional short course of MTX. G-CSF was administered in all patients from day +6 after transplantation until engraftment. Most patients received ciprofloxacin (200 mg orally three times daily) for bacterial prophylaxis after the beginning of the conditioning regimen until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis after the beginning of the conditioning regimen. Low-dose acyclovir was given for prophylaxis against herpes simplex virus and VZV after the beginning of the conditioning regimen until immunosuppressive agents were discontinued. Prophylaxis against *Pneumocystis jiroveci* infection consisted of trimethoprim-sulfamethoxazole (400 mg of sulfamethoxazole once daily) from the first day of conditioning to day −3 of transplantation, and from day +28 until day +180 or the cessation of immunosuppressive agents. Patients who developed fever during the neutropenic period were treated with cefepime or other cephalosporin, and additional agents including vancomycin, aminoglycosides and amphotericin B were given as clinically indicated. Neutrophil engraftment was defined as the first of 3 consecutive days after transplantation that the ANC exceeded 0.5 × 10^9 per l.

Glucose management protocol

In the IGC group, the blood glucose level was routinely tested every morning to adjust the dose of insulin so as to keep the level within the range of 80–110 mg per 100 ml. Owing to the presence of fewer nursing staff in the HSCT unit than in the ICU, we replaced the continuous infusion of insulin with the addition of Humulin R to the bottle of PN to control the glucose level within the target range. In TPN, we universally added at least 1 unit of Humulin R per 10 g glucose. In patients who had an elevated blood glucose level, we also added Humulin R to the bottle of PN. We monitored the glucose level at least once a day in the morning as long as the level remained within the target range of 80–110 mg per 100 ml. When the glucose level became elevated, we increased the frequency of monitoring up to 2–4 times daily. In most patients, we adjusted the dose of insulin added to the bottle of PN as described in Table 2. When the blood glucose level was >180 mg per 100 ml or the dose of insulin was high, we manually adjusted the dose of Humulin R and administered insulin subcutaneously according to the attending physician's discretion. S.c. insulin administration usually consisted of 3–5 units at the beginning, and, if this was insufficient, the dose was manually adjusted by 2–4 units. When the patients received high-dose systemic steroid such as methylprednisolone 1–2 mg/kg per day for GVHD, we used the preprandial s.c. injection of insulin Aspart (NovoRapid) three times daily to avoid postprandial hyperglycemia and adjusted the dose according to the amount of food intake and the postprandial glucose level. When patients exhibited nausea, anorexia or vomiting, the amount of food intake became unstable. In such situations, insulin Aspart was injected immediately after the meal. When food intake was <50%, the dose was reduced or discontinued. Routine glucose monitoring was continued until PN was stopped, whereas the blood glucose level was maintained within the target range. Daily caloric intake was calculated by the dietitians. We tried to maintain oral intake as much as possible by using a suitable diet in jelly or liquid form. A dietitian adjusted the dose of supplemental PN to maintain the total caloric intake over 1.0 × basal energy expenditure (BEE), and if the glucose level was stable, the nutritional intake could be increased up to 1.5 × BEE. The glucose concentration in PN was usually started at 7.5% glucose as supplemental PN. The concentration was gradually increased to 12%, and, if necessary, this was further increased up to 18% to meet the target caloric intake. A lipid emulsion was also used to supply 10–30% of total caloric intake. The minimal total nutritional intake was set at 1.0 × BEE because a retrospective analysis at our institute showed that caloric intake of more than 1.0 × BEE was not associated with clinically significant wt loss.[17] To improve the glucose control, this level was set to be slightly lower

Table 2 Protocol for adjustment of Humulin R

Glucose level (mg per 100 ml)	*Adjustment of Humulin R*
BS ≤ 40	i.v. 50% glucose 20 ml and recheck the glucose level
	Reduce the dose of Humulin R to 40–60% of the original dose
40 ≤ BS < 60	i.v. 50% glucose 20 ml and recheck the glucose level
	Reduce the dose of Humulin R to 60–80% of the original dose
60 ≤ BS < 80	i.v. 50% glucose 20 ml and recheck the glucose level
	Reduce the dose of Humulin R to 70–90% of the original dose
80 ≤ BS ≤ 110	No change
110 < BS < 130	Increase the dose of Humulin R to 110–120% of the original dose
130 ≤ BS < 150	Increase the dose of Humulin R to 120–130% of the original dose
150 ≤ BS < 180	Increase the dose of Humulin R to 130–150% of the original dose
BS ≥ 180	Manually adjust the dose of Humulin R combined with sliding subcutaneous insulin administration

Abbreviation: BS = blood sugars.

than the recommendation in the HSCT setting (1.3–1.5 × BEE[18]). There are two beneficial aspects of this protocol: we could maintain the minimal caloric intake with supplemental PN and we could immediately start insulin as required after the introduction of PN. The SGC group was managed without a specific protocol for nutrition practice and glucose control, although we routinely monitored blood glucose at least three times weekly to avoid severe hyperglycemia (blood glucose >200 mg per 100 ml).

Outcome measures

Serially monitored glucose values were compared between the IGC group and the SGC group. We also analyzed the association between the mean glucose level during monitoring and the infection rate in both the SGC group and IGC group. Mean glucose levels were estimated for each patient and were categorized as follows: 80–110, 111–140, 141–179 and >180. Glycemic variability, defined as the s.d. of the mean glucose value, was also analyzed. The outcome measures were time to the occurrence of documented infectious complications within 100 days after HSCT, time to each organ dysfunction defined as described below, time to grades II–IV and grades III–IV acute GVHD and time to NRM. These were calculated from the date of the start of the conditioning regimen. Organ dysfunction was defined with reference to van den Berghe[5–7] as follows: (1) hypercreatininemia; serum creatinine level ⩾2.0 mg per 100 ml or more than twice the baseline, (2) hyperbilirubinemia; serum total bilirubin level ⩾2.0 mg per 100 ml and (3) increased inflammatory markers; serum C-reactive protein (CRP) level ⩾15 mg per 100 ml. In our institute, the CRP level was routinely monitored at least three times a week, as we previously reported that the preengraftment CRP level may predict a subsequent occurrence of acute GVHD and NRM after allogeneic HSCT.[19] These results suggested that CRP might be useful not only as a marker of infectious diseases but also as a surrogate marker for produced cytokines. Therefore, the serial changes of CRP level were compared between the two groups. Acute GVHD was graded by the consensus criteria.[20]

Statistical analyses

Baseline characteristics were summarized using descriptive statistics. The Student's t, χ^2 and Wilcoxon rank-sum tests were used to compare clinical and patient characteristics. The probability of documented infectious complications and organ dysfunction were calculated using Kaplan–Meier estimates. A stratified Cox regression model, which accounts for the matched-cohort design, was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). On the basis of 64 patients, the study has an approximately 80% power to detect a HR of 0.5 for documented infections. The glucose values, measured repeatedly, were compared between groups using a repeated-measure analysis with a linear mixed-effect model. A level of $P<0.05$ was defined as statistically significant. All P-values are two-sided. All analyses were performed using SAS version 9.1.3 (Cary, NC, USA).

Results

Patient characteristics

Table 1 lists the patients' clinical and transplantation characteristics. Patients and transplantation characteristics were well balanced with the application of matching criteria. Nevertheless, in the IGC group, more patients received tacrolimus for GVHD prophylaxis (68 vs 36%, $P=0.01$) and more had a previous transplantation (32 vs 7%, $P=0.01$). The median duration of follow-up in surviving patients was 299 days (range, 78–607 days) in the IGC group and 1146 days (range, 329–1774 days) in the SGC group.

Glycemic control

Duration of monitoring and number of tests. The median duration of glucose monitoring and intervention in the IGC group was 38 days (range, 24–70 days) after the start of the conditioning regimen. The total number of glycemic monitorings was 867 and 1094 in the SGC group and IGC group, respectively.

Mean values and distribution of values. Patients in the IGC group had a lower glucose level (least-square mean, 116.4 vs 146.8 mg per 100 ml, $P<0.001$) than the SGC group. The trend of the glucose value is shown in Figure 2a. All glycemic results for the SGC and IGC groups were stratified into six levels: <40, 40–79, 80–110, 111–140, 141–179 and ⩾180, as shown in Figure 2b.

Hypoglycemia

In the IGC group, the incidence of mild hypoglycemia (CTCAE grades 1–2, glucose level 40–69 mg per 100 ml) was significantly higher than that in the SGC group (11 vs 3 patients, $P<0.001$). Although one patient (4.5%) in the IGC group who was diagnosed as type 2 diabetes mellitus developed severe hypoglycemia (CTCAE grade 3, glucose level 30–39 mg per 100 ml) with faintness, no patient developed seizure or loss of consciousness.

Glycemic variability

The mean glycemic variability in the SGC group and IGC group was 37.2 mg per 100 ml (range, 10.1–121.7 mg per 100 ml) and 27.5 mg per 100 ml (range, 11.3–46.6 mg per 100 ml), respectively, and glycemic variability in the IGC group tended to be lower than that in the SGC group ($P=0.07$).

TPN and insulin dosing

The percentage of patients who received TPN was 60% (25 patients) and 77% (17 patients) in the SGC group and the IGC group, respectively. The mean duration of TPN was 9 days (range, 0–35) and 13 days (range, 0–38) in the SGC group and IGC group, respectively. There was a tendency for more patients in the IGC group to receive TPN compared to the SGC group, but this difference was not statistically significant. The mean maximal dose of insulin (median (range), 51 (0–100) vs 2 (0–110) IU, $P<0.001$) and the mean maximal dose of insulin per 1 g parenteral glucose

were significantly higher in the IGC group (median (range), 0.22 (0–0.71) vs 0.003 (0–0.4) IU/g glucose, $P<0.001$).

Infections

Table 3 summarizes the results. In the IGC group, dramatically fewer patients developed documented infections within 100 days compared to the SGC group, as shown in Figure 3.

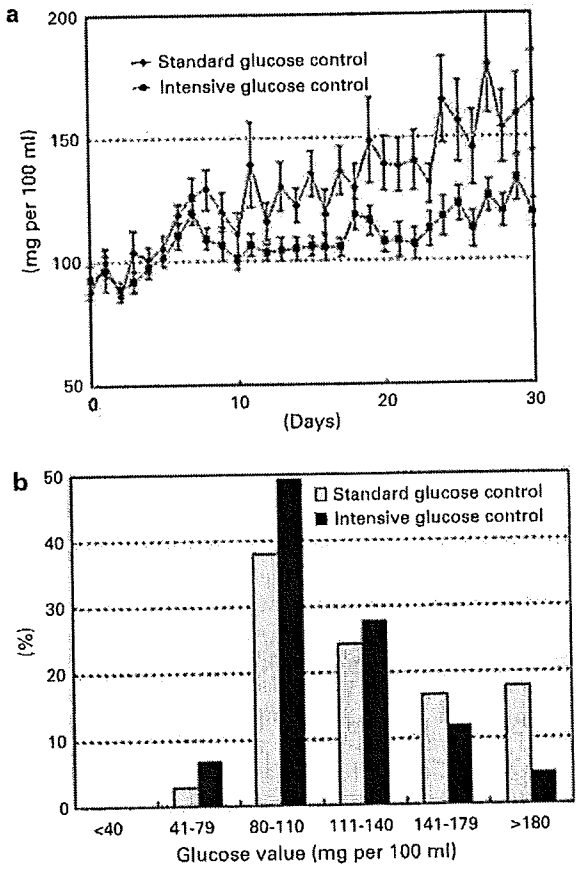

Figure 2 Serial changes in the mean glucose level in the intensive glucose control (IGC) and standard glucose control (SGC) groups. Values are mean + s.e. (a). The distribution of the glucose values in IGC and SGC is shown as a histogram (b).

Relation to mean glucose level

We also analyzed the association between the mean glucose level during monitoring and the infection rate in both the SGC and IGC groups. The incidence of infection was 34, 17, 67 and 40%, respectively, with mean glucose levels of 80–110, 111–140, 141–179 and ⩾180. When we compared a lower glucose-level group (mean glucose level of 80–140) with a higher glucose-level group (mean glucose level of >140), the incidence of infection was significantly higher in the latter group (28 vs 57%, $P=0.042$). When we assessed only patients with a lower glucose level, the IGC group tended to show a lower incidence of infectious diseases than the SGC group (14 vs 41%, $P=0.061$).

Relation to glycemic variability

We also analyzed the association between glycemic variability and the infection rate. The mean glycemic variability in patients with and without infection was 34.6 mg per 100 ml (range, 10.5–121.7 mg per 100 ml) and 33.3 mg per 100 ml (range, 10.1–110.6 mg per 100 ml), respectively, with no significant difference. As the importance of glycemic variability could vary among patients

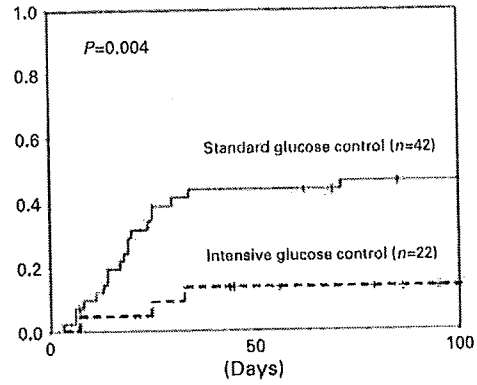

Figure 3 Probability of documented infections in the IGC and SGC groups.

Table 3 Incidence of infectious diseases and organ dysfunction

Variable	N *(%)/median (range)*			
	Intensive glucose control n = *22 (%)*	*Standard glucose control* n = *42 (%)*	*HR (95% CI)*	P-*value*
Documented infection	13	46	0.17 (0.04–0.75)	0.004
Bacteremia	9	39	0.10 (0.01–0.74)	0.002
Organ dysfunction				
Hypercreatininemia[a]	19	37	0.60 (0.19–1.88)	0.36
Hyperbilirubinemia[b]	28	31	1.05 (0.38–2.91)	0.93
Increased inflammatory markers[c]	18	38	0.45 (0.15–1.37)	0.13

Abbreviations: CI = confidence interval.
[a]Serum creatinine level ⩾2.0 mg per 100 ml or more than twice of baseline.
[b]Serum bilirubin level ⩾2.0 mg per 100 ml.
[c]Serum C-reactive protein level ⩾15 mg per 100 ml.

with different mean glucose levels,[21] we divided the patients into two groups based on mean glucose level 80–140 or 140+ and then determined whether glycemic variability was associated with an increased incidence of infections. However, there was no significant association between glycemic variability and the incidence of infections in both groups.

CRP levels

Figure 4 shows serial changes in the CRP level. Even though there was no difference in the CRP level between the two groups at the beginning of the conditioning regimen, the CRP level was significantly elevated in the SGC group compared to that in the IGC group 15 days after the beginning of the conditioning regimen, and this trend continued up to 40 days ($P<0.05$). The maximal CRP level during the neutropenic period in the IGC group was significantly lower than that in the SGC group (median (range), 6.9 (0.9–16.3) vs 11.5 (1.6–37.3), $P=0.007$).

Other clinical outcomes

The probability of grades II–IV acute GVHD within 100 days was 28 and 37% in the IGC and SGC groups (HR 1.05, 95% CI 0.38–2.91, $P=0.93$). The incidences of grades III–IV acute GVHD and NRM within 100 days were low in both groups (one and two patients, and one and one patient, in the IGC and SGC groups, respectively).

Discussion

This is the first study to evaluate the outcomes in allogeneic HSCT patients who were treated with a glucose management protocol. A salient finding of this study is that the incidence of documented infections, especially the incidence of bacteremia, was significantly lower in the IGC group than in the SGC group, as in a previous report in the ICU setting.[1] Moreover, there tended to be fewer organ dysfunctions in the IGC group, albeit this difference was not statistically significant. Furthermore, the CRP level, which might be a surrogate marker for produced cytokines,[19] was significantly lower in the IGC group than in the SGC group, as shown in Figure 4. Even though this study did not have enough power to detect a decrease in acute GVHD and NRM, it could be anticipated that IGC could reduce the CRP level, which would lead to a reduced incidence of acute GVHD and NRM.

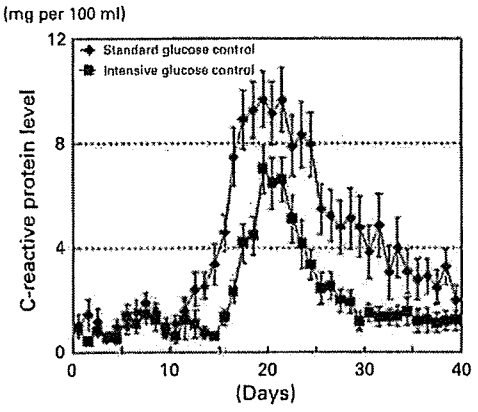

Figure 4 Serial change in the CRP level in the IGC and SGC groups. Values are mean + s.e.

This study has several limitations. One limitation is that only 64 patients were analyzed with no sufficient power to demonstrate any statistically significant changes in the incidences of organ dysfunctions, which was similar to the result in a previous report in the ICU.[1,2] An additional limitation was that the control of the glucose level could be suboptimal. This could be because of the glucose control protocol, which included monitoring of glucose level and the administration of insulin. With regard to the administration of insulin, we replaced the continuous infusion of insulin with the addition of Humulin R to the bottle of PN to control the glucose level within the target range because of the presence of fewer nursing staff in the HSCT unit than in the ICU. This could delay the normalization of hyperglycemia. Even though severe hyperglycemia (>180 mg per 100 ml) was reduced, a glucose value within the normal range (80–110 mg per 100 ml) could be achieved in only 49% of the IGC group as shown in Figure 1b. From a methodological point of view, it might be inappropriate to simply count the number of glucose value measurements, as patients with hyperglycemia were monitored more frequently, as defined in this protocol. Furthermore, as the mode of glucose monitoring was quite different between the IGC group and the SGC group, it could be inappropriate to compare the glucose values. A future protocol should include a more appropriate monitoring of glucose level and administration of insulin system that assures the fine tuning of glucose levels within the target range. Finally, there was a possible selection bias that may have affected the results, as this study was not a randomized-control study and there were many nonparticipants. However, the incidence of documented infections in nonparticipants within 100 days after allogeneic HSCT was 42%. Therefore, the reduction in the incidence of documented infections in the IGC group could not simply be explained by other causes such as the selection of antibiotics or catheter management.

With these limitations in mind, we took several steps to improve the quality of the study. First, we carefully matched patients and transplantation characteristics. Second, the IGC strategy was applied prospectively. Third, the low rate of patients who developed clinically significant hypoglycemia should be emphasized. As previously reported, the IGC procedure becomes very difficult in the medical ICU, especially in patients who have sepsis, a high APACHE score or mechanical ventilation.[1,2,22,23] The low rate of hypoglycemia could be because the medical acuity of our patients were relatively mild compared to those of patients in the medical ICU. Moreover, patients undergoing HSCT are younger and might have better β-cell function. The low rate of hypoglycemia could be important for maximizing the benefit of IGC because severe hypoglycemia could be associated with an increased risk of mortality.[23]

The biological plausibility of the intervention should be discussed. The reduction in infectious diseases by IGC may reflect the deleterious effects of hyperglycemia on macrophage or neutrophil function or insulin-induced protective effects on mucosal and skin barriers.[24–27] The improvement of innate immunity could be quite important, especially during the period of granulocytopenia after allogeneic HSCT. The protection of mucosal tissues could reduce bacterial translocation, which might lead to a reduced incidence of sepsis.

In conclusion, our results suggest that prospective IGC reduced the incidences of infectious diseases and organ dysfunction after allogeneic HSCT. To confirm these findings, a larger, prospective randomized-controlled trial is warranted.

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References

1 Van den Berghe G, Wouters P, Weekwrs F, Verwaest C, Bruyninckx F, Schetz M *et al.* Intensive insulin therapy in the critically ill patients. *N Engl J Med* 2001; **345**: 1359–1367.
2 Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I *et al.* Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006; **354**: 449–461.
3 Van den Berghe G, Wouters PJ, Bouillon R, Weekers F, Verwaest C, Schetz M *et al.* Outcome benefit of intensive insulin therapy in the critically ill: insulin dose versus glycemic control. *Crit Care Med* 2003; **31**: 359–366.
4 Krinsley JS. Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients. *Mayo Clin Proc* 2003; **78**: 1471–1478.
5 Krinsley JS. Effect of an intensive glucose management protocol on the mortality of critically ill adult patients. *Mayo Clin Proc* 2004; **79**: 992–1000.
6 Vogelzang M, Nijboer JM, van der Horst IC, Zijlstra F, ten Duis HJ, Nijsten MW. Hyperglycemia has a stronger relation with outcome in trauma patients than in other critically ill patients. *J Traum* 2006; **60**: 873–877.
7 Ingels C, Debaveye Y, Milants I, Buelens E, Peeraer A, Devriendt Y *et al.* Strict blood glucose control with insulin during intensive care after cardiac surgery: impact on 4-years survival, dependency on medical care, and quality-of-life. *Eur Heart J* 2006; **27**: 2716–2724.
8 Jeschke MG, Klein D, Bolder U, Einspanier R. Insulin attenuates the systemic inflammatory response in endotoxemic rats. *Endocrinology* 2004; **145**: 4084–4093.
9 Brix-Christensen V, Andersen SK, Andersen R, Mengel A, Dyhr T, Andersen NT *et al.* Acute hyperinsulinemia restrains endotoxin-induced systemic inflammatory response: an experimental study in a porcine model. *Anesthesiology* 2004; **100**: 861–870.
10 Sheean PM, Freels SA, Helton WS, Braunschweig CA. Adverse clinical consequences of hyperglycemia from total parenteral nutrition exposure during hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006; **12**: 656–664.
11 Sheean PM, Braunschweig C, Rich E. The incidence of hyperglycemia in hematopoietic stem cell transplant recipients receiving total parenteral nutrition: a pilot study. *J Am Diet Assoc* 2004; **104**: 1352–1360.
12 Fietsam Jr R, Bassett J, Glover JL. Complications of coronary artery surgery in diabetic patients. *Am Surg* 1991; **57**: 551–557.
13 Ortiz A, Ziyadeh FN, Neilson EG. Expression of apoptosis-regulatory genes in renal proximal tubular epithelial cells exposed to high ambient glucose and in diabetic kidney. *J Invest Med* 1997; **45**: 50–56.
14 Vanhorebeek I, De Vos R, Mesotten D, Wouters PJ, De Wolf-Peeters C, Van den Berghe G. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. *Lancet* 2005; **365**: 53–59.
15 Fuji S, Kim SW, Mori S, Fukuda T, Kamiya S, Yamasaki S *et al.* Hyperglycemia during the neutropenic period is associated with a poor outcome in patients undergoing myeloablative allogeneic hematopoietic stem cell transplantation. *Transplantation* 2007; **84**: 814–820.
16 Saito AM, Kami M, Mori SI, Kanda Y, Suzuki R, Mineishi S *et al.* Prospective phase II trial to evaluate the complications and kinetics of chimerism induction following allogeneic hematopoietic stem cell transplantation with fludarabine and busulfan. *Am J Hematol* 2007; **82**: 873–880.
17 Fuji S, Kim S, Fukuda T, Kamiya S, Kuwahara S, Takaue Y. Positive impact of maintaining minimal caloric intake above 1.0 × basal energy expenditure on nutritional status of patients undergoing allogeneic hematopoietic stem cell transplantation. *Am J Hematol* 2008; **84**: 63–64.
18 Muscaritoli M, Grieco G, Capria S, Iori AP, Rossi Fanelli F. Nutritional and metabolic support in patients undergoing bone marrow transplantation. *Am J Clin Nutr* 2002; **75**: 183–190.
19 Fuji S, Kim SW, Fukuda T, Mori S, Yamasaki S, Morita-Hoshi Y *et al.* Pre-engraftment serum C-reactive protein (CRP) value may predict acute graft-versus-host disease and non-relapse mortality after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2008; **14**: 510–517.
20 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
21 Ali NA, O'Brien Jr JM, Dungan K, Phillips G, Marsh CB, Lemeshow S *et al.* Glucose variability and mortality in patients with sepsis. *Crit Care Med* 2008; **36**: 2316–2321.
22 Van Cromphaut S, Wilmer A, Van den Berghe G. Management of sepsis. *N Engl J Med* 2007; **356**: 1179–1181.
23 Krinsley JS, Grover A. Severe hypoglycemia in critically ill patients: risk factors and outcomes. *Crit Care Med* 2007; **35**: 2262–2267.
24 Rayfield EJ, Ault MJ, Keusch GT, Brothers MJ, Nechemias C, Smith H. Infection and diabetes: the case for glucose control. *Am J Med* 1982; **72**: 439–450.
25 Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol Med Microbiol* 1999; **26**: 259–265.
26 Rassias AJ, Marrin CA, Arruda J, Whalen PK, Beach M, Yeager MP. Insulin infusion improves neutrophil function in diabetic cardiac surgery patients. *Anesth Analg* 1999; **88**: 1011–1016.
27 Losser MR, Bernard C, Beaudeux JL, Pison C, Payen D. Glucose modulates hemodynamic, metabolic, and inflammatory responses to lipopolysaccharide in rabbits. *J Appl Physiol* 1997; **83**: 1566–1574.

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Preengraftment Serum C-Reactive Protein (CRP) Value May Predict Acute Graft-versus-Host Disease and Nonrelapse Mortality after Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

In a mouse model, inflammatory cytokines play a primary role in the development of acute graft-versus-host disease (aGVHD). Here, we retrospectively evaluated whether the preengraftment C-reactive protein (CRP) value, which is used as a surrogate marker of inflammation, could predict posttransplant complications including GVHD. Two hundred twenty-four adult patients (median age, 47 years; range: 18-68 years) underwent conventional stem cell transplantation (CST, n = 105) or reduced-intensity stem cell transplantation (RIST, n = 119). Patients were categorized according to the maximum CRP value during neutropenia: the "low-CRP" group (CRP < 15 mg/dL, n = 157) and the "high-CRP" group (CRP ≥ 15 mg/dL, n = 67). The incidence of documented infections during neutropenia was higher in the high-CRP group (34% versus 17%, $P = .004$). When patients with proven infections were excluded, the CRP value was significantly lower after RIST than after CST ($P = .017$) or after related than after unrelated transplantation ($P < .001$). A multivariate analysis showed that male sex, unrelated donor, and HLA-mismatched donor were associated with high CRP values. The high-CRP group developed significantly more grade II-IV aGVHD ($P = .01$) and nonrelapse mortality (NRM) ($P < .001$), but less relapse ($P = .02$). The present findings suggest that the CRP value may reflect the net degree of tissue damage because of the conditioning regimen, infection, and allogeneic immune reactions, all of which lead to subsequent aGVHD and NRM.

KEY WORDS

C-reactive protein • Allogeneic transplantation • Acute graft-versus-host disease • Nonrelapse mortality

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is associated with high treatment-related mortality (TRM) because of acute graft-versus-host disease (aGVHD) and infections [1,2]. Inflammatory cytokines, for example, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6 [3-11], are produced following conditioning and play a primary role in activating T cells, leading to GVHD and resultant target tissue destruction [12,13]. An acute-phase protein, C-reactive protein (CRP), is produced by hepatocytes downstream of IL-6 [14] and is widely used as a reliable surrogate marker of infectious diseases [15-19]. This process is further stimulated by other cytokines including TNF-α [12,13]. After allogeneic HSCT, the elevation of CRP was observed with infectious complications, but not in uncomplicated aGVHD [8,20]. On the other hand, elevation of CRP has been shown to be associated with TRM [21-24]. Nevertheless, these previous studies adopted the sporadic measurement of CRP and mostly focused on patients undergoing conventional HSCT (CST) with a myeloablative regimen. It has been hypothesized that recently developed reduced-intensity HSCT (RIST) decreases regimen-related toxicities and, hence, may reduce inflammation

that augments the subsequent allogeneic immune reaction to induce GVHD and nonrelapse mortality (NRM).

In this study, the correlation between the preengraftment CRP value and subsequent clinical events was analyzed to test whether high CRP reflected the degree of tissue damage because of the conditioning regimen, infections, and allogeneic immune reactions and/or inflammation, all of which could contribute to subsequent aGVHD and NRM.

MATERIALS AND METHODS

Patient Characteristics

The data from a cohort of 224 consecutive adult patients with hematologic malignancies, who were treated between January 2002 and July 2006 at the National Cancer Center Hospital (NCCH, Tokyo, Japan), were reviewed retrospectively. Patients who developed graft failure or who had previous allogeneic transplantation were excluded. Their characteristics are listed in Table 1. The median age of the patients was 47 years (range: 18-68 years), and their diagnosis included acute myeloid leukemia (AML, n = 94), acute lymphoblastic leukemia (ALL, n = 23), non-Hodgkin lymphoma (NHL, n = 62), myelodysplastic syndrome (MDS, n = 27) and chronic myeloid leukemia (CML, n = 12). Standard risk included acute leukemia in first complete remission, chronic leukemia in the first chronic phase, MDS in refractory anemia, and NHL in complete remission, with the rest of the patients categorized as a high-risk group. Stem cell sources used for transplantation included bone marrow (BM, n = 108), peripheral blood stem cells (PBSC, n = 98) and cord blood cells (CB, n = 18). One-hundred five patients received a CST regimen including total-body irridiation (TBI)-based (n = 50) and non-TBI-based busulfan-containing regimens (n = 55), whereas 119 patients received a RIST regimen including fludarabine or cladribine plus busulfan or melphalan (Table 1). CMV serostatus was positive in 157 patients and negative in 67 patients. The median age of the patients was 49 years in the high-CRP group (range: 19-67) and 47 years in the low-CRP group (range: 18- 68). Written informed consent was obtained according to the Declaration of Helsinki.

Table 1. *Patients' Characteristics*

	N (%)/ Median		
Variable	Low CRP Group CRP < 15 mg/dL n = 157	High CRP Group CRP ≥ 15 mg/dL n = 67	P Value
Age (year)	47 (18-68)	49 (19-67)	.85
<40	53 (34)	26 (39)	
≥40	104 (66)	41 (61)	.47
Patient sex			
Male	84 (54)	48 (72)	
Female	73 (46)	19 (28)	.01
Donor sex			
Male	81 (52)	30 (45)	
Female	76 (48)	37 (55)	.35
CMV serostatus			
Positive	140 (89)	64 (96)	
Negative	17 (11)	3 (4)	.20
Disease risk			
Standard	35 (22)	17 (25)	
High	122 (78)	50 (75)	.62
Conditioning			
CST	72 (47)	33 (50)	
RIST	85 (53)	34 (50)	.64
GVHD prophylaxis			
Cyclosporin-based	122 (78)	52 (78)	
Tacrolimus-based	35 (22)	15 (22)	.99
Short term MTX (+)	107 (68)	58 (87)	.004
Relation to donor			
Related	94 (60)	13 (19)	
Unrelated	63 (40)	54 (81)	<.001
Stem cell source			
Bone marrow	63 (40)	45 (67)	
PBSC	87 (55)	11 (16)	
Cord blood	7 (5)	11 (16)	<.001

CRP indicates C-reactive protein; CMV, cytomegalovirus; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; GVHD, graft-versus-host disease; MTX, methotrexate; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen.

Transplantation Procedures

GVHD prophylaxis included cyclosporine- (n = 174) and tacrolimus-based regimens (n = 50), with an additional short course of methotrexate (MTX) in 165 patients. Granulocyte colony-stimulating factor (G-CSF) was administered in all patients from day +6 of transplantation until engraftment was confirmed. Most patients received ciprofloxacin (200 mg orally 3 times daily) for bacterial prophylaxis until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis. Low-dose acyclovir was given for prophylaxis against herpes simplex virus and varicella zoster virus until the cessation of immunosuppressive agents. Prophylaxis against *Pneumocystis jiroveci* infection was provided with trimethoprim-sulfamethoxazole (400 mg of sulfamethoxazole once daily) from the first day of conditioning to day −3 of transplantation, and from day +28 until day +180 or the discontinuation of immunosuppressive agents. Patients with fever during the neutropenic period were treated with cefepime, and additional agents including vancomycin and aminoglycosides, and amphotericin B were given as clinically indicated. Neutrophil engraftment was defined as the first of 3 consecutive days after transplantation that the absolute neutrophil count exceeded 0.5×10^9/L. In our institute, the CRP level was serially measured as part of our routine checkup at least 3 times a week. Hence, all serially admitted patients were subjected to this analysis. Every patient had started CRP measurement