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Monitoring TIGIT/DNAM-1 and PVR/PVRL2 Immune Checkpoint Expression Levels in Allogeneic Stem Cell Transplantation for Acute Myeloid Leukemia

Norimichi Hattori*, Yukiko Kawaguchi, Yohei Sasaki, Shotaro Shimada, So Murai, Maasa Abe, Yuta Baba, Megumi Watanuki, Shun Fujiwara, Nana Arai, Nobuyuki Kabasawa, Hiroyuki Tsukamoto, Yui Uto, Kouji Yanagisawa, Bungo Saito, Hiroshi Harada, Tsuyoshi Nakamaki

Division of Hematology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan

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After allogeneic stem cell transplantation (alloSCT), several immune checkpoints play an important role in the antileukemic immune response in the bone marrow (BM) microenvironment. However, immune checkpoint expression levels in the BM have not been reported after alloSCT in patients with acute myeloid leukemia (AML). We investigated the clinical impact of immune checkpoint expression in BM samples after alloSCT for AML. Higher expression of T cell immunoreceptor with Ig and ITIM domains (TIGIT) was associated with a decreased incidence of acute graft-versus-host disease ($P = .048$) and poor overall ($P = .046$) and progression-free survival ($P = 0.024$). In addition, higher expression of TIGIT at engraftment after alloSCT was correlated with a decreased number of natural killer cells in BM ($P = .019$). Monitoring TIGIT expression in the BM could be useful for predicting outcome after alloSCT for AML. Our findings raise the possibility that blockade of TIGIT would improve survival.

INTRODUCTION

In hematologic malignancies allogeneic stem cell transplantation (alloSCT), which induces an immune-mediated response, was the first treatment with demonstrable clinical efficacy and curative potential. An antileukemic effect occurs by means of a graft-versus-leukemia (GVL) response mediated by donor-derived T and natural killer (NK) cells. However, relapse remains the major cause of treatment failure in patients with leukemia.

Blockade of immune checkpoints is 1 of the most promising cancer immunotherapies for several malignancies [1-3]. Checkpoint receptors, such as cytotoxic T lymphocyte–associated protein-4 (CTLA-4), programmed cell death protein 1 (PD-1), T cell Ig3, and lymphocyte-activation gene 3, interact with immune cells to downregulate T cell activation and effector functions. Therefore, blocking checkpoint receptors can lead to increased T cell activity and an antitumor effect. To date, several clinical trials have demonstrated prolonged remissions after solid tumors were treated with checkpoint inhibitors, including anti-CTLA-4 (ipilimumab) and anti-PD-1 (pembrolizumab and nivolumab) antibodies [4-6]. These agents have also led to antitumor immune responses in patients with hematologic malignancies such as acute myeloid leukemia (AML) [7,8]. Therefore, inhibition of immune checkpoints is a potential therapy for AML.

T cell immunoreceptor with Ig and ITIM domains (TIGIT), a known inhibitory receptor, is expressed on both NK and T cells [9,10]. Ligands for TIGIT are the poliovirus receptors (PVR; also known as CD155 and nectin-like protein 5) and PVRL2 (also known as CD112 and nectin-2). TIGIT acts by competing with the costimulatory receptors DNAM-1 (also known as CD226 and DNAX accessory molecule-1) and CD96, suppressing NK and T cell immune activity by binding to PVR. By contrast, TIGIT blockade was shown to promote NK or T cell–dependent tumor immunity in several tumors, suggesting that targeting TIGIT is a potential anticancer therapy [11-13]. Administration of TIGIT-Fc suppresses $CD8^+$ T cell activation and ameliorates acute graft-versus-host disease (aGVHD) [14]. DNAM-1, which is an activating receptor belonging to the Ig superfamily, is expressed on NK cells and T cells and triggers NK cell and T cell cytotoxicity [15-17]. Both anti-DNAM-1 antibodies and DNAM-1 knockout inhibit $CD8^+$ T cell proliferation, leading to improvement of GVHD [16,17]. PVR and PVRL2 are broadly distributed on hematopoietic cells, including myeloid cells, macrophages, and dendritic cells, and are expressed on several tumors. PVR and PVRL2 overexpression is predictive of tumor

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* Correspondence and reprint requests: Norimichi Hattori, MD, PhD, Division of Hematology, Department of Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-Ku, Tokyo 142-8666, Japan.
E-mail address: nhattor@med.showa-u.ac.jp (N. Hattori).

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progression and poor prognosis [15,18-20]. There is normally a balance between PVR/DNAM-1 as costimulatory and PVR/TIGIT as co-inhibitory factors to maintain control of NK and T cell function. This balance, however, may be disrupted in the tumor microenvironment, potentially leading to a reduced antitumor immune response. The relevance of immune checkpoints after alloSCT for AML is unclear, but given their involvement in solid tumors it is possible that these checkpoints also play a crucial role in the interaction between GVHD and the GVL effect in the bone marrow (BM) microenvironment.

We investigated the relationship between TIGIT/DNAM-1 and PVR/PVRL2 expression in BM samples from AML patients treated with alloSCT.

METHODS

Patients and Samples

Thirty-eight patients aged 19 years or older who underwent alloSCT for AML at Showa University between 2003 and 2017 had BM samples available for inclusion in our study. Our AML patients included AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, therapy-related myeloid neoplasms, and AML not otherwise specified according to the 2016 World Health Organization classification [21]. For cytogenetic risk classification, t(8;21), inv(16), and t(15;17) were grouped as favorable; complex karyotype (ie, 4 or more abnormalities) as adverse; and all others as intermediate, according to the refined disease risk index [22]. Disease status at alloSCT was classified as first complete remission (CR), second CR, induction failure, or relapse in our cohort. The graft sources were donor BM, peripheral blood stem cells, or umbilical cord blood. Donors received recombinant human granulocyte colony-stimulating factor, 5 μg/kg daily for 4 days. On the fourth day peripheral blood stem cells were harvested. When BM cells were used, they were not manipulated and were infused fresh on the day of collection. Cord blood units were matched with the recipient for at least 4 of 6 HLAs and contained $\geq 2 \times 10^7$/kg nucleated cells per unit.

Myeloablative conditioning regimens for the patients consisted of cyclophosphamide (120 mg/kg) and total body irradiation > 10 Gy or busulfan > 8 mg/kg (p.o.) or >6.4 mg/kg (i.v.). Reduced-intensity conditioning regimens were either 2 or 4 Gy of total body irradiation or busulfan at 3.2 mg/kg (i.v.). Patients who had received antithymocyte globulin conditioning regimens were excluded. aGVHD prophylaxis consisted of tacrolimus or cyclosporine with a short course of methotrexate. aGVHD was diagnosed and graded according to the Glucksberg classification [23]. The hematopoietic cell transplantation–specific comorbidity index was calculated as previously described [24]. Overall, 36 patients (94.7%) showed full donor chimerism (defined as ≥95% leukocytes of donor origin in BM samples) and 2 (5.3%) showed mixed chimerism, measured by fluorescent in situ hybridization with specific probes for the sex chromosomes or multiplex short tandem repeat PCR.This study was approved by the institutional review board of Showa University, Tokyo, Japan.

In total, 150 BM samples from the 38 AML patients had been obtained at various times during their clinical course. PVR and PVRL2 expression levels were evaluated in 46 of 150 samples obtained before alloSCT either at diagnosis and relapse (30 samples and 7 samples, respectively) or at relapse after alloSCT (9 samples) for investigating PVR and PVRL2 expression levels on leukemia cells. TIGIT and DNAM-1 expression levels were evaluated in the other 104 samples, including from patients at CR before (44 samples) alloSCT or at engraftment and at CR after (31 samples and 29 samples, respectively) alloSCT. For monitoring TIGIT and DNAM-1 expression levels, these levels at 4 time points after alloSCT (at engraftment and 6 months, 1 year, and 2 years after alloSCT) were evaluated when patients were in CR (31, 9, 10, and 10 samples, respectively) to avoid evaluating leukemia cells derived from recipients. Peripheral blood samples from 5 healthy volunteers (men, 3; women, 2; age range, 27 to 41 years) were used as control subjects. Higher or lower mRNA expression levels were defined as above or below the mean values in the BM samples from the AML patients in our cohort. For clinical parameters of overall survival (OS), progression-free survival (PFS), incidence of nonrelapse mortality (NRM), aGVHD, and relapse, PVR and PVRL2 expression levels in the BM samples at diagnosis were analyzed (30 patients), whereas TIGIT and DNAM-1 expression levels in the BM samples at engraftment after alloSCT were analyzed (31 patients).

Reverse Transcription Quantitative Real-Time PCR

Total RNA from cells was extracted with a QuickGene RNA cultured cell kit S (Fujifilm, Tokyo, Japan). First-strand cDNA was synthesized from total RNA using a cDNA Synthesis Kit for RT-PCR (Thermo Scientific Inc., Waltham, MA). Gene expression levels were measured with the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) using the KAPA SYBR FAST qPCR Kits (Kapa Biosystems, Woburn, MA). Expression of each gene was normalized to β-actin (ACTB) as a reference. The conditions for all quantitative real-time PCR reactions are as follows: 2 minutes at 95°C, followed by 15 seconds at 95°C and 30 seconds at 60°C for 40 cycles. All PCR products were confirmed by the presence of a single peak on melting curve analysis. The forward and reverse primer sequences used were as follows: ACTB (F: 5′-TTGCTGACAGGATGCAGAAG-3′ and R: 5′-AAGGGTGTAAAACGCAGCTC-3′), PVR(F: 5′-TCCTGTGGACAAACCAATCA-3′ and R: 5′-GTTACGGGACATGCCT-GAGT-3′), PVRL2 (F: 5′-CTACGATCCGAAAGCTCAGG-3′ and R: 5′-GGCCTTTCTCTGCCTTCTCT-3′), TIGIT (F: 5′-CGTGAACGATACAGGGGAGT-3′ and R: 5′-GCAATGGAATCTGGAACCTG-3′), and DNAM-1(F: 5′-GGCAGAAATTT-CACCTCCAA-3′ and R: 5′-GCAAGTAGCAGCGGTAAAGC-3′).

Flow Cytometry Analysis

Cells were analyzed using a FACSCanto II flow cytometer and analysis software (BD Biosciences, San Jose, CA) as previously described [25]. CD45-PerCP from BD Biosciences and CD3-FITC, CD4-FITC, CD8-PE, CD19-PE, CD16-FITC, and CD56-PE from Beckman Coulter (Brea, CA) were used for analysis of NK ($CD16^+$, $CD56^+$), B ($CD19^+$, $CD3^-$), CD4 T ($CD4^+$, $CD8^-$), and CD8 T ($CD4^-$, $CD8^+$) cells.

Statistical Analysis

OS was defined as the time from alloSCT to death from any cause. PFS was defined as the time from alloSCT to relapse, progression, or death from any cause. NRM was defined as the time from alloSCT to death without relapse. OS and PFS were analyzed by univariate analysis with the Kaplan-Meier method using the log-rank test. The cumulative incidence of NRM, aGVHD, and relapse was compared with competing risks with Gray's test. Variables were compared using unpaired or paired *t*-tests or Wilcoxon signed-rank test. Significance of difference was determined using the Mann-Whitney-Wilcoxon test for ordinal variables and chi-square test for nominal variables. A 2-way analysis of variance was used to compare more than 2 variables. Pearson correlation coefficients were used to evaluate correlation. Statistical significance was accepted at $P < .05$ ($^*P < .05$, $^{**}P < .01$, $^{***}P < .001$). Statistical analysis was performed using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA) and StatFlex 6.0 (Artech Co., Ltd, Osaka, Japan).

RESULTS

Patient Characteristics and Sample Data

Clinical characteristics of the 38 AML patients are listed in Table 1. Briefly, 4 AML with recurrent genetic abnormalities, 8 AML with myelodysplasia-related changes, 2 therapy-related myeloid neoplasms, and 24 AML not otherwise specified (1 AML with minimal differentiation, 7 AML without maturation, 11 AML with maturation, 3 acute myelomonocytic leukemia, and 2 acute monoblastic/monocytic leukemia) were entered in our cohort. Overall, 18 non-CR patients (induction failure or relapse, 47%) were included, and 14 patients (37%) had adverse cytogenetics. After a median follow-up of 388 days (range, 48 to 3527) 18 (47%) were alive, 13 (33%) had died from relapse, and 7 (18%) had died from transplant-related mortality. Among those with NRM, 6 died because of infections (bacterial in 4, viral in 1, idiopathic pneumonia syndrome in 1) and the other from cardiac failure. aGVHD of any grade occurred in 28 patients (74%), with 20 of the total 38 (53%) having grades II to IV aGVHD. Among the 28 with aGVHD, 8 (29%) had grade I, 18 (64%) had grade II, and 2 (7%) had grade III. Most cases of aGVHD were grade II.

The mean percentage of blast cells in the BM samples at diagnosis or at relapse was 55% ± 24%. In BM samples from patients in remission the mean percentage of lymphocytes was 16% ± 14%, and the mean percentages of CD4 T cells, CD8 T cells, B cells, and NK cells in total lymphocytes were 29% ± 17%, 38% ± 16%, 5% ± 9%, and 21% ± 14%, respectively. Comparison of PVR and PVRL1 expression levels at diagnosis or relapse (46 samples) as well as TIGIT and DNAM-1 expression levels at CR (samples) with those in peripheral blood samples from healthy donors are shown in Supplementary Figure 1.

Immune Checkpoints after Transplantation

To investigate the effects of immune checkpoints after alloSCT, we analyzed TIGIT and DNAM-1 expression after

Table 1
Characteristics of Patients with AML (N = 38)

Variable		No. of Patients or Median	Percent or Range
Median age, yr		51.5	19-72
Age, yr			
	<50	19	50.0
	≥50	19	50.0
Gender			
	Male	23	60.5
	Female	15	39.5
Disease			
	AML with RGA	4	10.5
	AML with MRC	8	21.1
	t-MN	2	5.3
	AML, NOS	24	63.2
Cytogenetics*			
	Favorable	4	10.5
	Intermediate	20	52.6
	Adverse	14	36.8
Disease status at alloSCT			
	CR1	14	36.8
	CR2	6	15.8
	Induction failure	8	21.1
	Relapse	10	26.3
HCT-CI score			
	0	26	68.4
	1 or 2	6	15.8
	3 or higher	6	15.8
Graft source			
	PBSCs	4	10.5
	BM	9	23.7
	UCB	25	65.8
Donor type			
	Related	4	10.5
	Unrelated	9	23.7
	UCB	25	65.8
HLA matching			
	Matched	8	21.1
	Mismatched	30	78.9
Conditioning regimen			
	MAC	31	81.6
	TBI-based	12	31.6
	Bu-based	19	50.0
	RIC	7	18.4
	TBI-based	5	13.2
	Bu-based	2	5.3
GVHD prophylaxis			
	CSP-based	1	2.6
	TAC-based	37	97.4

RGA indicates recurrent genetic abnormalities; MRC, myelodysplasia-related changes; t-MN, therapy-related myeloid neoplasms; NOS, not otherwise specified; PBSC, peripheral blood stem cells; UCB, umbilical cord blood; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; TBI, total body irradiation; Bu, busulfan; CSP, cyclosporine A; TAC, tacrolimus

* Classified according to the refined disease risk index.

alloSCT. In engrafted BM samples from patients in remission after alloSCT (23 patients), the mean percentage of lymphocytes was 19% ± 13%, among which the mean percentages of CD4 T, CD8 T, B, and NK cells were 25% ± 16%, 40% ± 15%, 6% ± 8%, and 22% ± 13%, respectively. In engrafted BM samples from patients who relapsed after alloSCT (13 patients) the mean percentage of lymphocytes was 19% ± 19%, among which the mean percentages of CD4 T, CD8 T, B, and NK cells were 25% ± 17%, 24% ± 14%, 9% ± 15%, and 21% ± 19%, respectively (Figure 1A). Data for 2 patients were not available. TIGIT expression in samples from patients in any remission (40 samples) after alloSCT was significantly lower than in samples from patients in relapse (19 samples; .019 ± .047 versus .10 ± .16, $P = .046$) (Figure 1B, left). In engrafted BM samples, TIGIT expression was somewhat lower in samples taken in remission (21 samples) compared with samples taken in relapse (10 samples), although not significantly so (.021 ± .06 versus .12 ± .17, $P = .089$). DNAM-1 expression did not differ between remission and relapse (.023 ± .029 versus .031 ± .033, $P = .38$) (Figure 1B, right) or when only engrafted samples were examined (.019 ± .025 versus .027 ± .027, $P = .43$).

We compared TIGIT and DNAM-1 expression between paired matched samples pre- and post-alloSCT in patients in the remission and relapsed groups. In those in the remission group (19 patients), TIGIT expression did not differ pre- and post-alloSCT ($P = .50$). In the relapse group (9 patients), however, TIGIT expression was significantly higher after than before alloSCT ($P = .027$) (Figure 1C, left). In all groups TIGIT expression did not differ pre- and post-alloSCT ($P = .20$, data not shown). DNAM-1 expression did not differ pre- and post-alloSCT in those in remission ($P = .38$) or in those in relapse ($P = .25$) (Figure 1C, right) or in all groups ($P = .88$, data not shown).

We further analyzed TIGIT and DNAM-1 expression levels at 4 time points after alloSCT in patients in remission and in those who relapsed (Figure 1D). At engraftment (21 samples), 6 months (5 samples), 1 year (7 samples), and 2 years (7 samples) after alloSCT, TIGIT expression levels in patients in remission were .032 ± .074, .0059 ± .0073, .018 ± .030, and .011 ± .019, respectively. Values in patients who relapsed at engraftment (10 samples), 6 months (4 samples), 1 year (3 samples), and 2 years (3 samples) after alloSCT were .11 ± .18, .19 ± .28, .049 ± .062, and .087 ± .14, respectively. These values were significantly higher than those from patients in remission ($P = .012$). DNAM-1 expression levels, however, did not differ significantly at the same time points between those in remission and those who had relapsed (.018 ± .026 versus .022 ± .027, .0083 ± .0056 versus .065 ± .059, .029 ± .029 versus .032 ± .036, and .030 ± .036 versus .021 ± .017, respectively; $P = .14$).

Clinical Outcome after Transplantation

The clinical characteristics of AML patients divided between higher and lower expression levels of immune checkpoints are shown in Table 2. Only higher TIGIT expression was statistically associated with disease status at alloSCT compared with lower TIGIT expression ($P = .027$).We evaluated the cumulative incidence of grades II to IV aGVHD, NRM, and relapse as well as PFS and OS between patients with higher PVR and PVRL2 expression levels versus those with lower expression levels at diagnosis (Figure 2). Patients with higher PVR expression had significantly higher incidence of relapse ($P = .006$), shorter PFS ($P = .043$), and shorter OS ($P = .046$) compared with patients with lower PVR expression (Figure 2A). Patients with higher PVRL2 expression had significantly higher incidence of relapse ($P = .041$). PVRL2 expression was not significantly associated with PFS ($P = .15$) or OS ($P = .14$), but higher PVRL2 expression appeared to be correlated with shorter PFS and OS, although not significantly so (Figure 2B).

We analyzed the cumulative incidence of grades II to IV aGVHD, of NRM, and of relapse and estimated PFS and OS in terms of TIGIT and DNAM-1 in patients with higher and lower expression levels at engraftment after alloSCT (Figure 3). Patients with higher TIGIT expression had a significantly lower incidence of grades II to IV aGVHD ($P = .048$), shorter PFS ($P = .024$), and shorter OS ($P = .046$) compared with patients with lower TIGIT expression (Figure 3A). In addition, higher TIGIT expression was associated with higher incidence of relapse; however, this was not significant ($P = .051$). DNAM-1

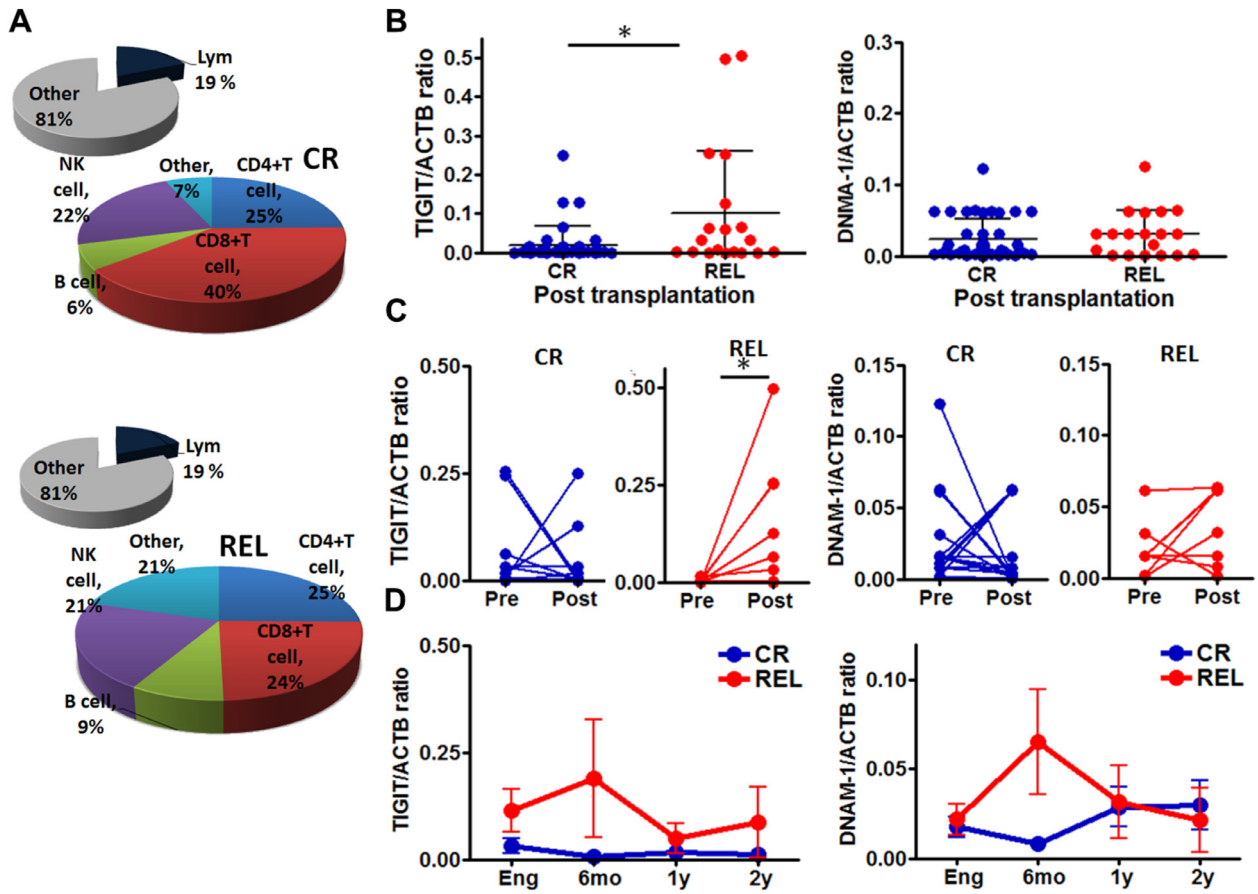

Figure 1. Lymphocyte subsets in BM at engraftment and TIGIT and DNAM-1 expression in patients in CR or relapse (REL) after alloSCT. (A) The mean percentage of lymphocytes and of CD4 T, CD8 T, B, and NK cells in the lymphocyte populations at engraftment after alloSCT. (B) TIGIT and DNAM-1 expression in patients in CR (n = 40) compared with those in REL (n = 19). (C) TIGIT and DNAM-1 expression in paired pre- and post-alloSCT samples in CR (n = 19) and REL (n = 9). (D) TIGIT and DNAM-1 expression levels in patients in CR or REL at engraftment (ENG), 6 months, 1 year, and 2 years after alloSCT. The error bars represent mean ± standard deviation. *P <.05.

expression levels were not significantly associated with these parameters (Figure 3B).

Correlation between TIGIT/ DNAM-1 Expression and Lymphocyte Subsets

Next, we evaluated higher and lower expression levels of TIGIT and DNAM-1 in patients at engraftment after alloSCT to verify their association with various absolute lymphocyte subset counts (Table 3). Patients with higher TIGIT expression demonstrated significantly decreased B cell counts and NK cell counts compared with patients having lower TIGIT expression levels (P = .015 and P = .019, respectively). However, DNAM-1 expression was not significantly associated with any absolute lymphocyte subset counts.

DISCUSSION

In the last decade immune checkpoint blockade, particularly of CTLA-4 and PD-1, has yielded impressive clinical responses in several cancer types [1-6]. For patients with hematologic malignancies after alloSCT, immune checkpoint inhibitors might also have therapeutic efficacy [7,8,26]. However, there have been no reports of immune checkpoint levels, particularly TIGIT, after alloSCT for AML. Our study found that AML patients with higher TIGIT expression in the BM after alloSCT were less likely to have aGVHD and had inferior OS and PFS. It is noteworthy that higher TIGIT expression levels were found at engraftment after alloSCT in patients who relapsed, as demonstrated over time during the clinical course. These results indicate that TIGIT binding to PVR on donor and/or recipient dendritic cells might suppress donor NK and T cell activation, leading to exhaustion of these donor cells in the BM microenvironment in the early post-alloSCT phase [9-13]. This would in turn reduce the development of aGVHD. In addition, we showed that our patients with higher TIGIT expression had lower NK cell counts in the BM after alloSCT, whereas significant differences were not found in CD8 T cells. It was suggested that TIGIT might play a crucial role for the GVL effect and GVHD to control NK cell activity and proliferation after alloSCT [12,27]. Moreover, because our study suggested that higher TIGIT expression levels in the early post-alloSCT phase are a poor prognostic factor, it is possible that early intervention to blockade TIGIT might improve the outcome of AML patients after alloSCT. TIGIT blockade alone or in combination with other immune checkpoint inhibitors has provided antitumor effects in solid tumors [12,28]. In patients with hematologic malignancies after alloSCT, current studies of CTLA-4 or PD-1 blockade have shown clinical benefit [26,29]. However, in these studies strong GVL responses were observed in association with severe GVHD because of the strong immunogenic potential in the context of alloSCT. Therefore, a TIGIT-blocking antibody for AML after alloSCT could be a promising antitumor therapy. However, careful dose titration would be required for optimal treatment. In contrast to TIGIT blockade, administration of TIGIT-Fc may be help prevent severe aGVHD [14].

Table 2
Patient Characteristics between Higher and Lower Expression Levels of Immune Checkpoints in Each Group

Variable	PVR High (n = 6)	PVR Low (n = 24)	*P*	PVRL2 High (n = 6)	PVRL2 Low (n = 24)	*P*	TIGIT High (n = 9)	TIGIT Low (n = 22)	*P*	DNAM-1 High (n = 10)	DNAM-1 Low (n = 21)	*P*
Median age, yr (range)	62.5 (31-72)	52.5 (21-69)	.36	57.5 (31-72)	52.5 (21-69)	.51	49 (19-61)	60.5 (21-72)	.23	45.5 (19-67)	54 (21-72)	.16
Age, yr												
<50	2	9	.85	2	9	.85	5	8	.33	6	7	.16
≥50	4	15		4	15		4	14		4	14	
Gender												
Male	3	15	.58	4	14	.71	4	13	.45	5	12	.68
Female	3	9		2	10		5	9		5	9	
Disease												
AML with RGA	0	2	.20	0	2	.34	1	2	.34	2	1	.69
AML with MRC	1	6		2	5		1	5		0	6	
t-MN	1	1		1	1		1	1			1	1
AML, NOS	4	15		3	16		6	14			7	13
Cytogenetics												
Favorable	0	2	.27	0	2	.31	1	2	.33	2	1	.46
Intermediate	2	15		1	16		3	13		2	14	
Adverse	4	7		5	6		5	7		6	6	
Disease status at alloSCT												
CR1	0	11	.11	0	11	.057	3	8	.027	3	7	.057
CR2	0	4		1	3		0	4		2	3	
Induction failure	5	3		4	4		3	5		2	6	
Relapse	1	6		1	6		3	5		3	5	
R-DRI												
Low	0	2	.057	0	2	.057	0	2	.11	1	1	.20
Intermediate	1	11		0	12		1	9		1	9	
High	2	5		2	5		5	6		5	6	
Very high	3	6		4	5		3	5		3	5	
Graft source												
PBSCs	0	1	.20	0	1	.20	0	4	.40	2	2	.70
BM	0	7		0	7		2	5		2	5	
UCB	6	16		6	16		7	13		6	14	
Donor type												
Related	0	1	.20	0	1	.20	0	4	.40	2	2	.70
Unrelated	0	7		0	7		2	5		2	5	
UCB	6	16		6	16		7	13		6	14	
HLA matching												
Matched	0	5	.22	0	5	.22	0	6	.08	2	4	.85
Mismatched	6	19		6	19		9	16		8	17	
Conditioning regimen												
MAC	4	20	.067	5	19	.064	9	16	.084	10	15	.063
TBI-based	1	7		1	7		2	7		3	6	
Bu-based	3	13		4	12		7	9		7	9	
RIC	2	4		1	5		0	6		0	6	
TBI-based	1	1		0	2		0	4		0	4	
Bu-based	1	3		1	3		0	2		0	2	
GVHD prophylaxis												
CSP-based	0	0	N/A	0	0	N/A	0	1	.51	0	1	.48
TAC-based	6	24		6	24		9	21		10	20	

R-DRI indicates refined disease risk index; N/A, not available.

Further clinical studies are required to establish the safety and efficacy of TIGIT blockade after alloSCT.

DNAM-1 expression in the tumor microenvironment is downregulated in several tumors, leading to a reduction in NK cell cytotoxicity [30-32]. DNAM-1 expression levels in AML treated with alloSCT have not been described. We found that DNAM-1 expression in AML patients was lower than that in healthy control subjects. However, DNAM-1 expression did not differ between patients in remission or relapse samples or before or after alloSCT. Additionally, DNAM-1 expression levels were not correlated with any clinical outcome. These results consistently showed that GVHD and GVL effect may be less likely enhanced in DNAM-1 expression than in TIGIT expression after alloSCT [33]. However, some studies have demonstrated that DNAM-1 plays an key role in the development of GVHD and that DNAM-1 blockade prevents GVHD [16,17]. Also, our findings also indicated that TIGIT has a higher binding affinity for PVR than does DNAM-1, such that the interaction of TIGIT with PVR is dominant over that of DNAM-1 [10,11]. However, BM samples without several cell sortings in our study were collected because we focused on the expression levels of several immune checkpoints in the BM microenvironment adapted to clinical course. Indeed, after transplantation DNAM-1 expression on donor regulatory T cells and effector T cells is increased in mice [16]. Further investigation is needed to evaluate the expression levels in each BM cell after alloSCT.

PVR overexpression on tumors promotes tumor cell proliferation, migration, invasion, and angiogenesis [20]. PVR expression on tumor-infiltrating myeloid cells was shown to negatively affect immune antitumor responses and contributed to immune evasion in the tumor microenvironment [34]. However, there are no reports focusing on AML patients with PVR expression who underwent alloSCT. We found that our patients with higher PVR expression in the BM at diagnosis

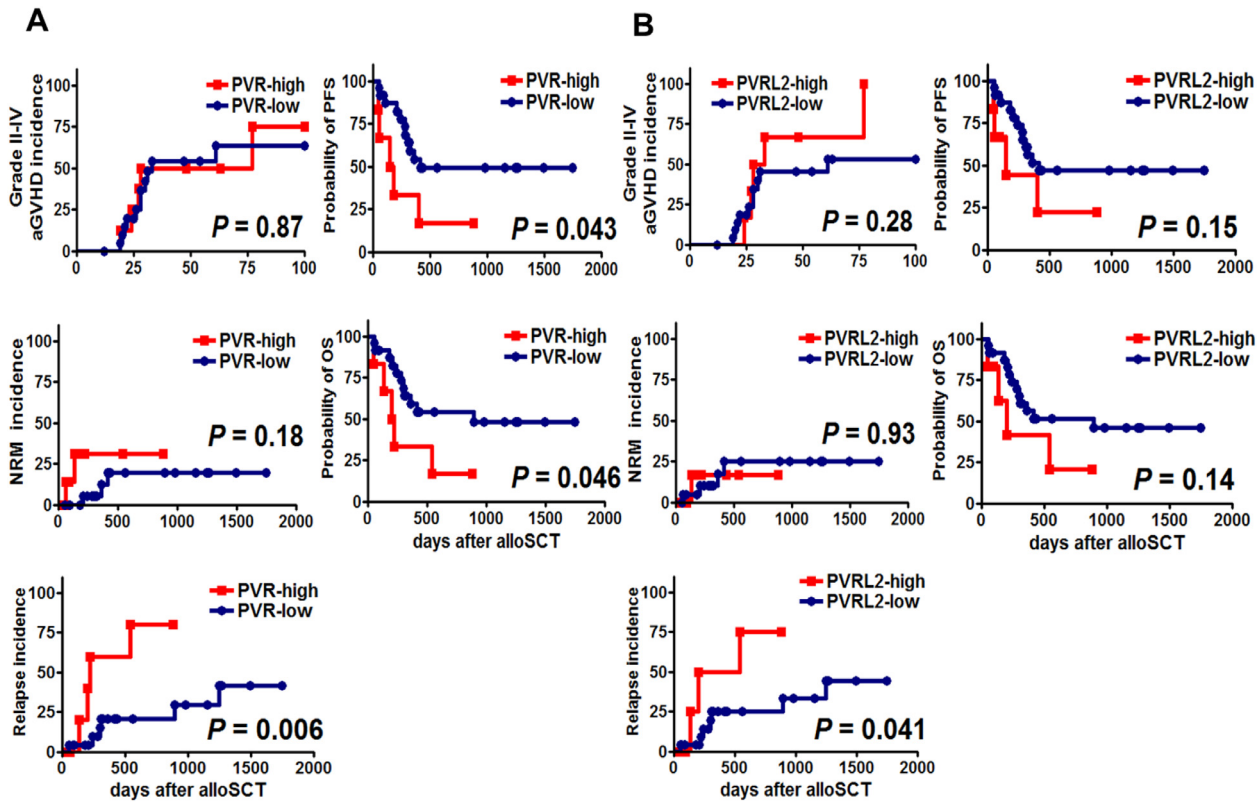

Figure 2. Clinical outcomes after alloSCT according to PVR and PVRL2 expression levels. PVR (A) and PVRL2 (B) expression levels after alloSCT in patients with AML and the cumulative incidence of grades II to IV aGVHD, NRM, and relapse as well as Kaplan-Meier estimates of PFS and OS. *Blue lines* indicate low PVR and PVRL2 and *red lines* high PVR and PVRL2 expression levels.

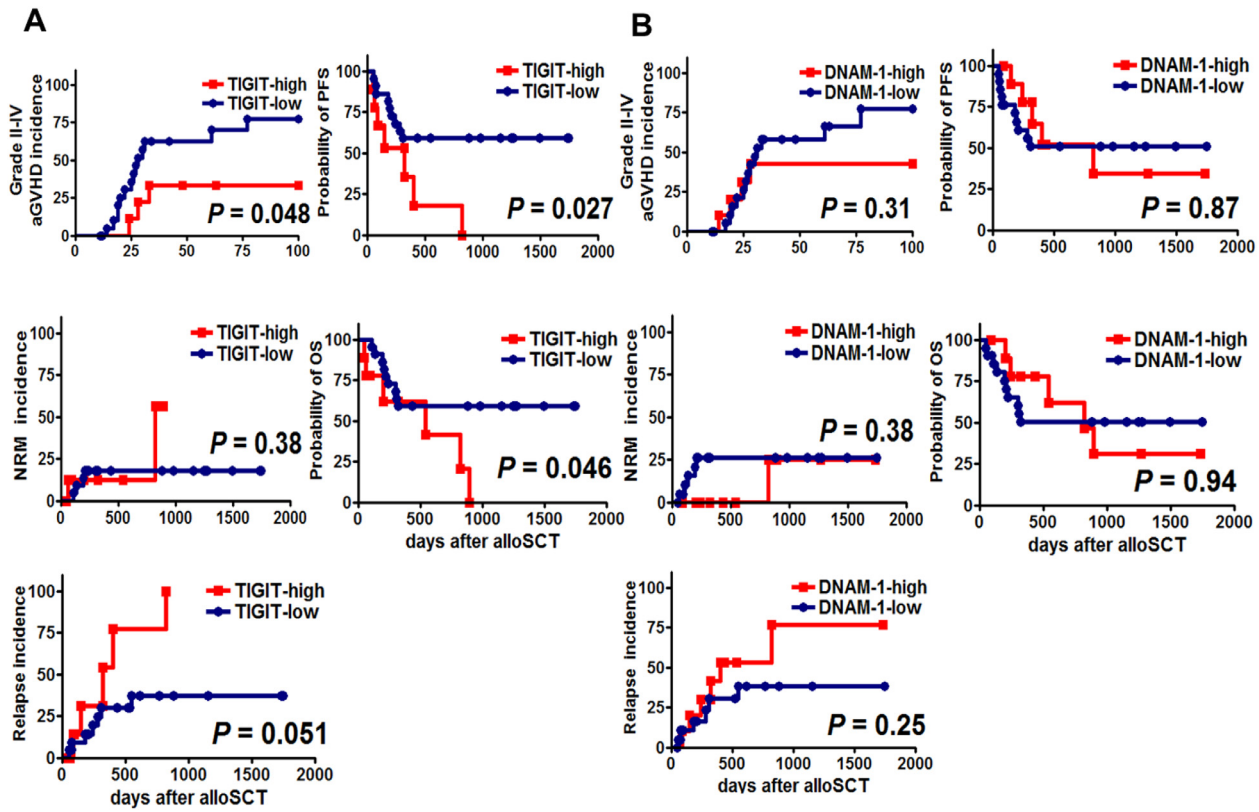

Figure 3. Clinical outcomes after alloSCT according to TIGIT and DNAM-1 expression levels. TIGIT (A) and DNAM-1 (B) expression levels after alloSCT in patients with AML and the cumulative incidence of grades II to IV aGVHD, NRM, and relapse as well as Kaplan-Meier estimates of PFS and OS. *Blue lines* indicate low TIGIT and DMAM-1 and *red lines* high TIGIT and DMAM-1 expression levels.

Table 3
Comparison between Higher and Lower Expression Levels of TIGIT/DNAM-1in Absolute Lymphocyte Subsets at Engragtment after AlloSCT

Variable	TIGIT High (n = 9)	Low (n = 22)	*P*	DNAM-1 High (n = 10)	Low (n = 21)	*P*
CD4 T cells	134.8 ± 144.9	160.7 ± 104.1	.71	135.0 ± 120.6	166.2 ± 117.2	.60
CD8 T cells	125.9 ± 194.8	303.0 ± 261.8	.14	155.1 ± 185.0	316.4 ± 284.4	.18
B cells	3.92 ± 2.16	12.66 ± 9.71	.015	5.82 ± 7.91	11.84 ± 10.03	.19
NK cells	53.8 ± 49.3	228.3 ± 201.9	.019	90.8 ± 105.8	234.1 ± 215.8	.10

Values are mean ± standard deviation.

had an inferior OS and PFS despite alloSCT. AML patients who had higher PVRL2 expression levels also appeared to have an inferior OS and PFS. Such patients need not only alloSCT but also additional therapy such as post-alloSCT TIGIT blockade. Also, antibody co-blockade of TIGIT and PD-L1 or blocking of PVR and PVRL2 combined with AMG330 may be a promising therapeutic strategy [11,19].

We evaluated expression levels of immune checkpoints including TIGIT, DNAM-1, PVR, and PVRL2 in the BM microenvironment in AML patients who underwent alloSCT. Our findings suggest that TIGIT could be a prognostic predictor after alloSCT and that blocking TIGIT might be a potent immunotherapeutic strategy to intensify the GVL effect after alloSCT.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2019.01.013.

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