PVR (CD155) Expression as a Potential Prognostic Marker in Multiple Myeloma

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Abstract

Poliovirus receptor (PVR, CD155) is upregulated during tumor progression, and PVR expression is associated with poor prognosis in patients with cancer; however, prognostic implications for PVR in multiple myeloma (MM) have not been investigated. We assessed PVR expression in bone marrow samples of 125 patients via immunohistochemistry (IHC) and in those of 22 patients via enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction. Additionally, we evaluated TIGIT levels in bone marrow $CD8^+$ T cells and natural killer cells in 14 patients using flow cytometry. Soluble PVR and TIGIT levels in 22 patients were measured using ELISA. PVR clinical and prognostic significance were assessed using a histoscore via IHC. PVR was highly expressed in patients with MM, and membrane PVR expression showed significant correlation with soluble PVR levels. Moreover, PVR expression was significantly associated with Revised-International Staging System stage, presence of extramedullary plasmacyoma and bone lesions, percentage of bone marrow plasma cells, and β2-microglobulin levels, suggesting a possible role in advanced stage and metastasis. Furthermore, we found that TIGIT expression was significantly correlated with the percentage of bone marrow plasma cells. Patients with high PVR expression had significantly shorter overall and progression-free survival, and PVR expression was identified as an independent prognostic factor for poor survival in MM. These findings indicated that PVR expression is associated with MM stage and poor prognosis, suggesting PVR expression as a potential prognostic marker for MM.

Introduction

The poliovirus receptor (PVR, CD155), a member of the nectin-like protein family, has recently emerged as a promising target for immunotherapy to enhance antitumor responses [1]. PVR is upregulated during tumor progression and promotes tumor proliferation and migration [2]. Additionally, PVR present on the cancer cell surface reportedly promotes tumor invasiveness, and its upregulation in tumor-infiltrating myeloid cells impairs antitumor T lymphocyte and natural killer (NK) cell functions, thereby suppressing antitumor immunity [3]. Moreover, this upregulation is associated with an increased metastatic capacity of cancer cells [4]. Furthermore, PVR is overexpressed in several types of cancers, including serous ovarian cancer, colorectal carcinoma, hepatocellular carcinoma, lung adenocarcinoma, esophageal small cell carcinoma, and cholangiocarcinoma [5–10], and highly expressed in bone marrow cells in multiple myeloma (MM) [11]. Thus, the effects of PVR expression on progression and metastasis in patients with MM are of interest.

There is an increasing interest in the prognostic effects of PVR expression in cancer patients, as an elevated PVR expression is associated with poor survival in most cancer types [8–10, 12]. For instance, in a previous study conducting on lung cancer patients, PVR-positive patients had significantly shorter overall survival (OS) and progression-free survival (PFS) than PVR-negative patients [8]. In another study, esophageal cancer patients in the PVR-positive group showed unfavorable survival as compared to those in the PVR-negative group [9]. Additionally, PVR expression is an independent poor prognostic factor for OS in patients with cholangiocarcinoma [10], and high *PVR* mRNA levels are associated with shorter OS

and a recurrence-free interval in patients with breast cancer [12]. In a study of hematologic malignancies, high PVR expression was identified as an independent negative prognostic marker for survival in patients with acute myeloid leukemia (AML) [13]. However, the clinical and prognostic significance of PVR in patients with MM has not yet been reported, thereby requiring further studies to determine the prognostic value of PVR expression in MM.

T cell immunoreceptors with Ig and ITIM domains (TIGIT) is a class of transmembrane glycoprotein expressed on NK cells and T cell subsets [14]. TIGIT expression is upregulated in both T and NK cells, where it inhibits cytotoxic activity [15]. TIGIT has several ligands, including PVR, CD112, and CD113, with PVR interactions showing the highest affinity [14]. PVR plays an immunomodulatory role by interacting with TIGIT, CD226, and CD96, and TIGIT binds PVR with the highest affinity, followed by CD96 and CD226 [14]. TIGIT expression appears to be associated with an advanced disease status and poor clinical outcomes in several cancers [16–18]. A previous meta-analysis of cancers demonstrated that a high TIGIT expression is correlated with worse OS and PFS [19], and in hematological malignancies, high TIGIT expression is associated with poor clinical outcomes in patients with follicular lymphoma [20] and AML [21, 22]. In a previous study on MM, TIGIT expression was upregulated in $CD8^+$ T cells during myeloma progression and associated with impaired effector functions [23]. However, the prognostic implication of TIGIT expression in patients with MM is still unclear.

Therefore, in this study, we evaluate PVR expression and investigate whether its expression has a prognostic role in patients with MM. Additionally, we evaluated the relationship of TIGIT expression, as the primary PVR ligand, with clinical factors and patient prognosis. Furthermore, we assessed PVR as a potential immune biomarker for predicting the prognosis of patients with newly diagnosed MM.

Materials And Methods

Patients

We evaluated patients with newly diagnosed MM who underwent a bone marrow examination at the Korea University Anam Hospital (Seoul, Korea). This retrospective cohort included 171 patients diagnosed with MM between May 2010 and October 2019. Of these, 46 were excluded, because their bone marrow specimens were unavailable. Thus, this study examined 125 bone marrow specimens obtained at the time of diagnosis. Additionally, bone marrow-aspiration samples were collected from 22 patients with multiple myeloma for validation, four monoclonal gammopathy of undetermined significance (MGUS) patients, and three patients without cancer for comparative purposes. All patients included in this study were diagnosed with MM according to the International Myeloma Working Group criteria [24]. The study protocol was approved by the Institutional Review Board of the Korea University Medical Center (2018AN0150), and the patients provided written informed consent to participate in the study. All research was conducted in accordance with the principles of the Declaration of Helsinki.

Immunohistochemistry (IHC) staining and analysis

Formalin-fixed and paraffin-embedded bone marrow specimens were sectioned (4–5 μm thickness) and placed on glass slides that were baked in an incubator for 1 h at 60°C to soften the paraffin. The sections were then deparaffinized with xylene and rehydrated using an ethanol gradient. Antigen retrieval was performed by boiling in a pressure cooker for 10 min in sodium EDTA buffer (pH 9.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min. Primary antibodies for PVR (1:100; Elabscience, Houston, TX, USA) and CD138 (clone B-A38; 1:100; Cell Marque, Rocklin, CA, USA) were used, and the slides were incubated overnight at 4°C. Thereafter, the Polink DS-MR-Hu C1 kit (GBI Labs, Bothell, WA, USA) was used to double stain for PVR and CD138 according to manufacturer instructions. We calculated the proportion of PVR-expressing $CD138^+$ plasma cells with respect to all plasma cells and estimated the proportion score of positively-stained plasma cells (0, < 1/100; 1, 1/100 to 1/10; 2, 1/10 to 1/2; and 3, > 1/2). The intensity score was calculated based on the average intensity of the positively-stained plasma cells (0, none; 1, weak; 2, intermediate; and 3, strong). Proportion and intensity scores were added to obtain a histoscore (H-score), which ranged from 0 to 6. We defined the optimal cut-off value for high PVR expression based on the absolute value of the maximal log-rank statistic, as proposed by Contal and O'Quigley [25]. Two investigators, one pathologist and one hematologist examined each slide, and discrepancies were discussed until a consensus was reached. Optical microscopy (DS-Fi2; Nikon Metrology, Tokyo, Japan) was used for image acquisition and examination.

Gene-expression analysis

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was reverse transcribed from 1 μg of total RNA using the LaboPass cDNA synthesis kit (Cosmo Genetech, Seoul, Korea) according to manufacturer instructions. Real-time quantitative polymerase chain reaction (qPCR) was performed using Labopass SYBR Green Q master mix (Cosmo Genetech) in a CFX96 Touch real-time PCR detection system (Bio-Rad, Hercules, CA, USA), according to manufacturer instructions. The primers used were as follows: *PVR*, 5'-CTG GCT CCG AGT GCT TGC-3' and 5'-GAG GTT CAC AGT CAG CA-3'. *Glyceraldehyde 3-phosphate dehydrogenase* was used as the internal control. Relative *PVR* transcript levels were determined using the $2^{-\Delta\Delta Ct}$ method, with all samples tested in triplicate and mean values used for further analyses.

Enzyme-linked immunosorbent assay (ELISA)

Bone marrow cell lysates were prepared using the freeze (− 20°C)-thaw (room temperature) method. PVR protein in each cell lysate was quantified using a human PVR/CD155 ELISA kit (LS-F65983; LSBio, Seattle, WA, USA) according to manufacturer instructions, and soluble PVR and TIGIT proteins were measured in bone marrow plasma using a different human PVR/CD155 ELISA kit (MBS1752576; MyBioSource, San Diego, CA, USA) and a human TIGIT ELISA kit (MBS1607096; MyBioSource), respectively, according to manufacturer instructions. All samples were tested in duplicate, and the mean values were used for further analyses.

Flow cytometry

Bone marrow mononuclear cells were incubated with fluorescence-dye-conjugated antibodies for 15 min at 4°C. Antibodies used for staining were CD3 (clone SK7; BD Biosciences, Franklin Lakes, NJ, USA), CD8 (clone RPA-T8; BD Biosciences), CD56 (clone B159; BD Biosciences), and TIGIT (clone A15153G; BioLegend, San Diego, CA, USA). Stained cells were evaluated by flow cytometry using a FACSVerse instrument (BD Biosciences), and the obtained data were analyzed using the FlowJo software (Tree Star Inc., Ashland, OR, USA).

Statistical analysis

Categorical variables were evaluated using the chi-squared or Fisher's exact test, and continuous variables were evaluated using Student's *t*-test or Mann-Whitney *U* test. The Kruskal–Wallis test was used to compare three unmatched groups, and Dunn's multiple-comparison test was used for post-hoc analysis. Pearson's correlation coefficient was used to determine the relationship between the two variables. OS was calculated as the time from diagnosis to death from any cause, and PFS was calculated as the time from treatment initiation to disease progression or death from any cause. Survival curves were estimated using the Kaplan–Meier method, and differences in survival distributions were evaluated using the log-rank test. Cox proportional hazards models were used to analyze associations between survival outcomes and various prognostic factors, and we observed no major violation of the proportional hazards assumption when using the Schoenfeld method. All tests were two-sided, and a $P < 0.05$ was considered significant. Statistical analyses were performed using GraphPad Prism (v9.2.0; GraphPad Software, La Jolla, CA, USA) and SPSS (v25.0; IBM Corp, Armonk, NY, USA).

Results

PVR and TIGIT expression relative to clinical factors

We investigated 125 bone marrow biopsy samples for expression of PVR in plasma cells and clinical parameters, with PVR expression examined via double-staining IHC. Figure 1 shows the representative images based on the H-score, and Figure 2a shows the distribution of PVR expression in patients with MM. We then examined the 14 available bone marrow samples to assess TIGIT expression in $CD8^+$ T and NK cells using flow cytometric analysis (Fig. 2b), with the original flow cytometric data shown in Table S1.

We then compared PVR and TIGIT expression between categorical clinical parameters, including age, Eastern Cooperative Oncology Group (ECOG) performance status (< 2 vs. ≥ 2), serum free-light chain ratio [high (≤ 0.01 or ≥ 100) vs. low], cytogenetic abnormalities (high risk vs. others), Revised-International Staging System (R-ISS) stage (I vs. II vs. III), osteolytic bone lesion (presence vs. absence), and extramedullary plasmacytoma (EMP) (presence vs. absence). High-risk cytogenetics were defined as t(4;14), t(14;16), del(17/17p), *TP53* deletion, or chromosome 1 abnormalities, including gain(1q) and del(1p). Patients with R-ISS stage III showed significantly higher PVR-expression levels than those with stage I ($P = 0.004$) and II ($P = 0.001$) (Fig. 2c). Additionally, patients with bone lesions ($P = 0.034$) (Fig. 2d) and EMP ($P = 0.002$) (Fig. 2e) showed significantly higher PVR expression than those without bone

lesions and EMP. Analysis of continuous clinical parameters based on PVR- and TIGIT-expression status included age, serum M-protein levels, percentage of bone marrow plasma cells, β2-microglobulin levels, and lactate dehydrogenase (LDH) levels. Patients with positive PVR expression (H-score > 0) showed significantly higher bone marrow plasma cell (mean, 29.10% vs. 46.15%; $P < 0.001$) (Fig. 2f) and β2-microglobulin (mean, 5.064 mg/L vs. 7.642 mg/L; $P = 0.005$) (Fig. 2g) levels than patients with negative PVR expression (H-score = 0). Moreover, patients with high TIGIT expression in $CD8^+$ T cells (cut-off point, median) showed a significantly higher percentage of bone marrow plasma cells ($P = 0.037$) (Fig. 2h) than patients with low TIGIT expression. No significant differences were found among other factors.

Analysis of PVR expression using different detection methods

For validation of the results of PVR-expression analysis by the H-score, PVR protein levels were analyzed using ELISA and *PVR* mRNA levels using qPCR in the available 22 bone marrow-aspiration specimens (Table 1). The PVR protein levels measured via ELISA showed a significant correlation with the H-score from IHC analysis (r = 0.513, $P = 0.015$) (Fig. 3a). Moreover, the *PVR* mRNA levels evaluated using qPCR showed a significant correlation with PVR protein levels determined by the H-score from IHC analysis (r = 0.458, $P = 0.032$) (Fig. 3b); however, we observed no significant correlations between *PVR* mRNA levels and the protein levels determined by ELISA (r = 0.293, $P = 0.307$) (Fig. 3c).

Table 1. Expression of PVR using different detection methods

Patient	IHC (H-score)	ELISA (ng/mL)	qPCR (fold, *PVR/GAPDH*)
#1	2	0.251	0.520
#2	0	0.078*	0.348
#3	4	0.311	2.815
#4	3	0.181	1.643
#5	0	0.173	2.362
#6	0	0.078*	5.528
#7	2	0.243	0.143
#8	2	0.250	0.751
#9	2	0.276	1.170
#10	6	0.333	6.619
#11	6	0.166	1.248
#12	4	0.246	0.443
#13	2	0.167	0.475
#14	0	0.078*	0.227
#15	2	0.218	0.109
#16	2	0.188	0.373
#17	2	0.199	0.729
#18	0	0.182	0.223
#19	0	0.237	0.199
#20	0	0.180	0.285
#21	2	0.166	0.061
#22	5	0.242	6.176

* Values were below the detection limit range and replaced with half of the limit of detection values for statistical analysis.

ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IHC, immunohistochemistry; PVR, poliovirus receptor; qPCR, quantitative polymerase chain reaction.

Analysis of soluble PVR and TIGIT levels

Because PVR and TIGIT proteins can be found in soluble forms, we quantified soluble PVR protein levels in bone marrow plasma from patients with MM, MGUS, and patients without cancer. Although we found soluble PVR in both MGUS and patients with MM, we detected no significant differences in levels between the two patients subsets, although soluble PVR levels were significantly higher in patients with MM relative to those in patients without cancer ($P = 0.042$) (Fig. 4a). We identified soluble TIGIT in patients with MM but no significant correlations between soluble TIGIT and PVR protein levels (Fig. 4b).

We then examined the relationship between soluble PVR levels and surface PVR expression using various detection methods. Soluble PVR levels were significantly correlated with the surface PVR expression data obtained using ELISA (r = 0.525, $P = 0.012$) (Fig. 4c), IHC (r = 0.425, $P = 0.049$) (Fig. 4d) as well as mRNA levels obtained with qPCR (r = 0.565, $P = 0.006$) (Fig. 4e). We then examined the relationship between soluble TIGIT level and surface TIGIT-expression levels. Soluble TIGIT levels also positively correlated with surface TIGIT expression in $CD8^+$ T cells (r = 0.757, $P = 0.002$) (Fig. 4f) and NK cells (r = 0.887, $P < 0.001$) (Fig. 4g). ELISA data for soluble PVR and TIGIT protein levels are shown in Table S2.

Patient characteristics according to PVR-expression status

We determined the optimal cut-point value for high or low PVR expression based on the H-score of 3. Based on an H-score ≥ 3, we classified 27 patients as having high PVR expression and 98 as having low PVR expression. Table 2 summarizes patient characteristics according to PVR-expression status. We found no significant difference in median age between the high PVR expression [66.0; interquartile range (IQR): 58.0–72.0 years] and low PVR expression (66.0; IQR, 58.0–72.3 years) groups ($P = 0.865$). In total, 16 patients (59.3%) with high PVR expression and 37 (37.8%) with low PVR expression showed high LDH levels (greater than the upper limit of normal [ULN]; $P = 0.045$). Thirteen (48.1%) and 28 (28.6%) patients in the high and low PVR groups, respectively, had high-risk cytogenetic abnormalities ($P = 0.055$), and patients in the high PVR group showed a higher percentage of bone marrow plasma cells (46.5; IQR, 25.9–77.0) as compared with those in the low PVR group (30.4; IQR, 5.39–10.5) ($P = 0.018$). Additionally, patients in high PVR group had high β2-microglobulin levels (6.63; IQR, 25.9–77.0 mg/L) as compared with patients in low PVR group (4.44; IQR, 2.66–7.87 mg/L) ($P = 0.004$), and hemoglobin levels were lower in the high PVR group (8.30; IQR, 7.40–9.30 g/dL) than those observed in the low PVR group (9.65; IQR, 8.70–11.4 g/dL) ($P = 0.002$). Furthermore, most patients in the high PVR group were in stage III according to the R-ISS classification (n = 15; 55.6 %), whereas most patients in the low PVR group were in stage II (n = 66; 67.3 %) ($P < 0.001$).

Table 2. Comparison of clinical factors between patients with low and high PVR expression

	Total (n = 125)	Low (n = 98)	High (n = 27)	*P*
Age, y	66.0 (58.0–72.0)	66.0 (58.0–72.3)	66.0 (58.0–72.0)	0.865
Sex, female	56 (44.8)	46 (46.9)	10 (37.0)	0.360
ECOG PS, ≥ 2	8 (6.4)	6 (6.1)	2 (7.4)	0.682
BM plasma cells, %	32.6 (15.6–61.3)	30.4 (12.8–51.9)	46.5 (25.9–77.0)	0.018
Serum M-protein, g/dL	2.20 (0.45–4.75)	2.12 (0.39–4.63)	3.30 (0.50–5.00)	0.350
Albumin, g/dL	3.30 (2.75–3.90)	3.40 (2.80–3.90)	2.90 (2.50–3.70)	0.088
< 3.5 g/dL	71 (56.8)	54 (55.1)	17 (63.0)	0.465
β2-microglobulin, mg/L	4.87 (3.07–8.35)	4.44 (2.66–7.87)	6.63 (5.39–10.5)	0.004
≥ 5.5 mg/L	53 (42.4)	35 (35.7)	18 (66.7)	0.004
LDH, IU/L	390 (303–480)	363 (297–463)	434 (391–570)	0.070
> ULN	53 (42.4)	37 (37.8)	16 (59.3)	0.045
Calcium, mg/dL	9.10 (8.35–9.75)	9.20 (8.48–9.70)	8.50 (7.80–10.5)	0.886
> 11 mg/dL	12 (9.6)	4 (6.7)	8 (12.3)	0.285
Creatinine, mg/dL	1.07 (0.81–1.84)	1.03 (0.80–1.53)	1.20 (1.01–2.21)	0.123
> 2 mg/dL	24 (19.2)	10 (16.7)	14 (21.5)	0.490
Hb, g/dL	9.50 (8.30–11.3)	9.65 (8.70–11.4)	8.30 (7.40–9.30)	0.002
< 10 g/dL	75 (60.0)	28 (46.7)	47 (72.3)	0.003
Cytogenetic abnormalities				
High risk*	41 (32.8)	28 (28.6)	13 (48.1)	0.055
ISS				
Stage I	26 (20.8)	25 (25.5)	1 (3.7)	0.006
Stage II	46 (36.8)	38 (38.8)	8 (29.6)	
Stage III	53 (42.4)	35 (35.7)	18 (66.7)	
R-ISS				
Stage I	14 (11.2)	14 (14.3)	0 (0.0)	<0.001
Stage II	78 (62.4)	66 (67.3)	12 (44.4)	
Stage III	33 (26.4)	18 (18.4)	15 (55.6)	
Treatment regimen				

VTD	26 (20.8)	22 (22.4)	4 (14.8)	0.381
TD or RD	24 (19.2)	17 (17.3)	7 (25.9)	
VMP	57 (45.6)	45 (45.9)	12 (44.4)	
Others	7 (5.6)	4 (4.1)	3 (11.1)	
Supportive only	11 (8.8)	10 (10.2)	1 (3.7)	
Transplantation				
Auto-SCT	30 (24.0)	22 (22.4)	8 (29.6)	0.439
Allo-SCT	0	0	0	
None	95 (76.0)	76 (77.6)	19 (70.4)	

Data are shown as number (percentage) or median (interquartile range).

* High-risk cytogenetics were defined as t(4;14), t(14;16), del(17/17p), *TP53* deletion, or chromosome 1 abnormalities, including gain(1q) and del(1p).

BM, bone marrow; ECOG, Eastern Cooperative Oncology Group; ISS, International Staging System; Hb, hemoglobin; LDH, lactate dehydrogenase; PS, performance status; PVR, poliovirus receptor; RD, lenalidomide/dexamethasone; R-ISS, revised international staging system; SCT, stem cell transplantation; TD, thalidomide/dexamethasone; VMP, bortezomib/melphalan/prednisone; VTD, bortezomib/thalidomide/ prednisone; ULN, upper limit of normal.

Survival analysis

For investigating whether PVR expression is associated with the survival of patients with MM, we analyzed the OS and PFS of patients in relation to PVR-expression status. For PFS analysis, patients receiving only supportive treatment (n = 11) were excluded in accordance with the definition. Among all patients, those with high expression of PVR showed significantly inferior OS compared to those with low expression (median, 24 vs. 68 months; $P = 0.02$) (Fig. 5a), and those with high PVR expression also had significant inferior PFS compared to patients with low PVR expression (median, 15 vs. 29 months; $P = 0.04$) (Fig. 5b). In the subgroup of patients who received immunomodulatory drug (IMiD)-based therapy, we observed no significant differences in OS (Fig. 5c) or PFS (Fig. 5d) between the two groups. Analysis of patients who received non-IMiD therapy revealed that those with high PVR expression had significantly shorter OS (median, 24 vs. 54 months; $P = 0.02$) (Fig. 5e) and PFS (median, 8 vs. 24 months; $P = 0.03$) (Fig. 5f) relative to those with low PVR expression. We then assessed whether TIGIT expression was associated with survival outcomes of patients with MM; results showed that the median OS of the high TIGIT expression group (cut-off, median) was 14 months, whereas low expression group did not reach the median level ($P = 0.06$) (Fig. 5g). Moreover, patients with high TIGIT expression showed a shorter PFS than those with low PVR expression ($P = 0.03$) (Fig. 5h).

Prognostic implication of PVR expression

For evaluating the prognostic effects of PVR expression, we used the Cox proportional hazard model (Table 3). In univariate analysis, inferior prognosis was significantly correlated to an ECOG performance status ≥ 2, LDH levels > ULN, high-risk cytogenetics, and high PVR expression. Additionally, poor prognosis was associated with older age with borderline significance (P = 0.052). Multivariate analysis, using the backward stepwise elimination method that included all of the variables used in univariate analysis, showed that poor OS was independently predicted by high PVR expression [hazard ratio (HR), 2.029; 95% confidence interval (CI): 1.003–4.103; P = 0.048], ECOG performance status ≥ 2 (HR, 3.768; 95% CI: 1.185–11.983; P = 0.025), LDH levels > ULN (HR, 2.069; 95% CI: 1.040–4.113; P = 0.038), and high-risk cytogenetics (HR, 2.373; 95% CI: 1.165–4.843; P = 0.017). Collectively, these findings suggested PVR expression as a possible independent poor prognostic factor for survival in patients with MM.

Table 3
Univariate and multivariate analyses for OS

Prognostic factors	Univariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*
Age, y	1.032	1.000, 1.065	0.052	1.032	0.997, 1.068	0.075
ECOG performance status, ≥ 2	3.142	1.097, 8.994	0.033	3.768	1.185, 11.983	0.025
BM plasma cells, %	1.004	0.992, 1.016	0.525	0.887	0.765, 1.028	0.111
Serum M-protein, mg/dL	0.950	0.835, 1.080	0.434	2.069	1.040, 4.113	0.038
Albumin, g/dL	0.900	0.595, 1.359	0.615	2.373	1.165, 4.834	0.017
β2-microglobulin, mg/L	1.041	0.987, 1.099	0.139	2.029	1.003, 4.103	0.048
LDH, > ULN	2.912	1.545, 5.488	0.001			
Cytogenetics, high-risk*	2.072	1.086, 3.956	0.027			
PVR expression, high	2.127	1.114, 4.065	0.022			

* High-risk cytogenetics were defined as t(4;14), t(14;16), del(17/17p), *TP53* deletion, or chromosome 1 abnormalities, including gain(1q) and del(1p).

BM, bone marrow; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PVR, poliovirus receptor; ULN, upper limit of normal.

Discussion

This study determined the prognostic value of PVR expression in patients with MM. We found that high PVR expression predicted inferior prognosis. Moreover, PVR expression was correlated with the presence of EMP, which indicates the presence of metastatic lesions, bone marrow plasma cells, and β2-

microglobulin levels that are associated with tumor burden. Furthermore, multivariate analysis confirmed that high PVR expression was associated with poor OS in patients with MM.

The effect of PVR on the prognosis of cancer patients may be mediated by endogenous biological and immunological functions [1]. First, according to its endogenous function, overexpressed PVR supports proto-carcinogenic roles by promoting tumor cell invasion, migration, proliferation, and angiogenesis [1]. Moreover, PVR overexpression is associated with poor prognosis and enhanced tumor progression [1, 8–10, 12]. In this respect, we examined PVR expression in relation to R-ISS stage and EMP, both of which were positively associated with PVR expression according to the H-score. Additionally, patients with elevated PVR expression showed a higher tumor burden based on the percentage of bone marrow plasma cells and β2-microglobulin, the levels of which are reportedly correlated with tumor stage [26], than those with lower PVR expression. Second, several recent studies focused on the immune effects of PVR protein [1]. For example, PVR overexpression in tumor cells increases the activation of immune cells and tumor cell death through interaction with CD226 [27]. By contrast, TIGIT, and CD96 are inhibitory receptors and PVR upregulation in heterogeneous tumor cells results in tumor cell recognition and binding by activating receptor CD226 and the inhibitory receptors TIGIT and CD96 [27]. A previous study noted that PVR shows the highest binding affinity for TIGIT, followed by CD96 and CD226, with these effects acting as immune checkpoints [2]. Thus, we examined TIGIT expression in bone marrow $CD8^+$ T cells and NK cells and evaluated its association with PVR and clinical factors in patients with MM. In our study, we found that PVR expression did not correlate with TIGIT expression, although TIGIT expression in $CD8^+$ T cells was positively associated with the percentage of bone marrow plasma cells but not β2-microglobulin levels or other clinical factors. However, we examined TIGIT expression in the 14 available samples and did not investigate functional interactions between PVR and TIGIT; therefore, further studies are required to clarify these results.

Previous studies have reported the prognostic implications of PVR expression in various solid and hematological cancer patients. Overall, these studies showed consistent results, indicating that elevated PVR expression is associated with decreased survival in patients with solid cancer, including lung cancer [8, 28], esophageal cancer [9], cholangiocarcinoma [10], and breast cancer [12, 29], and hematologic cancers, including AML [13]. However, the prognostic potential of PVR in patients with MM is still unclear. In the present study, survival outcomes of patients with MM were associated with PVR expression. Interestingly, subgroup analyses showed that PVR expression did not affect survival outcomes in patients treated with IMiD therapy. These findings contradict the independent correlation of PVR expression with survival outcomes in patients treated with non-IMiD therapy. Presumably, these findings suggest that IMiD modulates the immune effects of PVR in MM. For example, when PVR is highly expressed, immune evasion is predominant, and IMiD might enhance the antitumor response by involving the PVR-related immune pathway. Although IMiD therapy or combination IMiD therapy with a PVR inhibitor could be a promising strategy depending on PVR expression status, confirmatory conclusions on the relationship between PVR and IMiD therapy cannot be drawn from the present results, as we only observed differences in OS and PFS related to high PVR expression in patients who received IMiD therapy.

Therefore, additional studies are required to identify the effects of IMiDs in relation to PVR expression in patients with MM.

PVR is produced as a transmembrane protein with two alternate splicing isoforms (α and δ) that produce variants lacking transmembrane regions (designated as β and γ) [1, 30], with PVRβ and PVRγ described as soluble and secreted isoforms, respectively [31]. Thus, we analyzed the soluble PVRβ isoform in the present study. A previous study demonstrated significantly higher levels of soluble PVR in patients with cancer relative to healthy donors, with these levels positively correlated with tumor stage [32]. Furthermore, high levels of soluble PVR are reportedly associated with poor prognosis in several cancer types, including hepatocellular carcinoma, breast cancer, and AML [33–35]; however, the role of soluble PVR in MM has not been studied. Therefore, this represents the first analysis of soluble PVR levels in patients with MM, as well as those with MGUS and without cancer. The results showed that patients with MM had higher soluble PVR levels than non-cancer patients, although no significant differences were observed between patients with MGUS and MM. We then analyzed the correlation between membrane and soluble PVR expression, revealing that soluble PVR levels correlated with membrane PVR levels detected by various methods. Although the potential differences between the two forms of PVR have not been studied in detail, measuring soluble PVR expression in bone marrow plasma cells may be a simpler and easier method for prognostication and treatment selection. Additionally, previous studies reported that TIGIT exists as a soluble protein [11, 36], and the present results showed that soluble TIGIT levels in plasma correlated with its expression in bone marrow $CD8^+$ T cells and NK cells. Although we observed no significant correlation between soluble PVR and TIGIT levels in patients with MM.

This study has several limitations. First, the most accurate methods for determining PVR expression in bone marrow specimens were not defined. Although double-staining IHC and H-score were used in this study, it is necessary to establish standardized methods. Second, an optimal cut-off value for high or low PVR expression measured with IHC has not been reported. Although we established a study-defined cut-off value based on our reported statistical method, a more standardized and reliable method is required. Third, we could not perform more in-depth molecular analyses; therefore, combined functional analysis of the effects of PVR overexpression would help clarify the mechanism related to the prognostic effects of PVR expression. Fourth, we did not investigate the expression of CD226, which is a co-stimulatory molecule opposed to TIGIT and influences TIGIT interactions with immune cells. This experiment will be conducted in a follow-up study. Furthermore, since we included patients with newly diagnosed MM, our findings may not be applicable to relapsed and refractory MM, making it necessary to conduct studies evaluating the prognostic value of PVR in these subsets of MM patients.

In conclusion, we found that PVR expression was associated with poor prognosis in patients with newly diagnosed MM, suggesting its role as a potential prognostic marker. Additionally, the findings suggested that IMiD therapy may present a clinical benefit for patients having high PVR expression in MM. Follow-up studies are required to investigate the prognostic effects of the combination of PVR and its ligands, including CD226, CD96, and TIGIT, in MM.

Abbreviations

AML, acute myeloid leukemia; ECOG, Eastern Cooperative Oncology Group; ELISA, enzyme-linked immunosorbent assay; EMP, extramedullary plasmacytoma; H-score, histoscore; IHC, immunohistochemistry; IMiD, immunomodulatory drug; IQR, interquartile range; LDH, lactate dehydrogenase; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; NK, natural killer; OS, overall survival; PFS, progression-free survival; PVR, poliovirus receptor; R-ISS, Revised-International Staging System; TIGIT, T cell immunoreceptor with Ig and ITIM domains; ULN, upper limit of normal

Declarations

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Competing Interests Statement

The authors declare that there are no competing interests.

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Authors' Contributions

Byung-Hyun Lee: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization

Ji-Hea Kim: Investigation, Resources, Data Curation

Ka-Won Kang: Investigation, Resources

Se Ryeon Lee: Validation, Resources

Yong Park: Writing - Review & Editing

Hwa Jung Sung: Supervision, Writing - Review & Editing

Byung Soo Kim: Conceptualization, Supervision, Writing - Review & Editing, Project administration

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

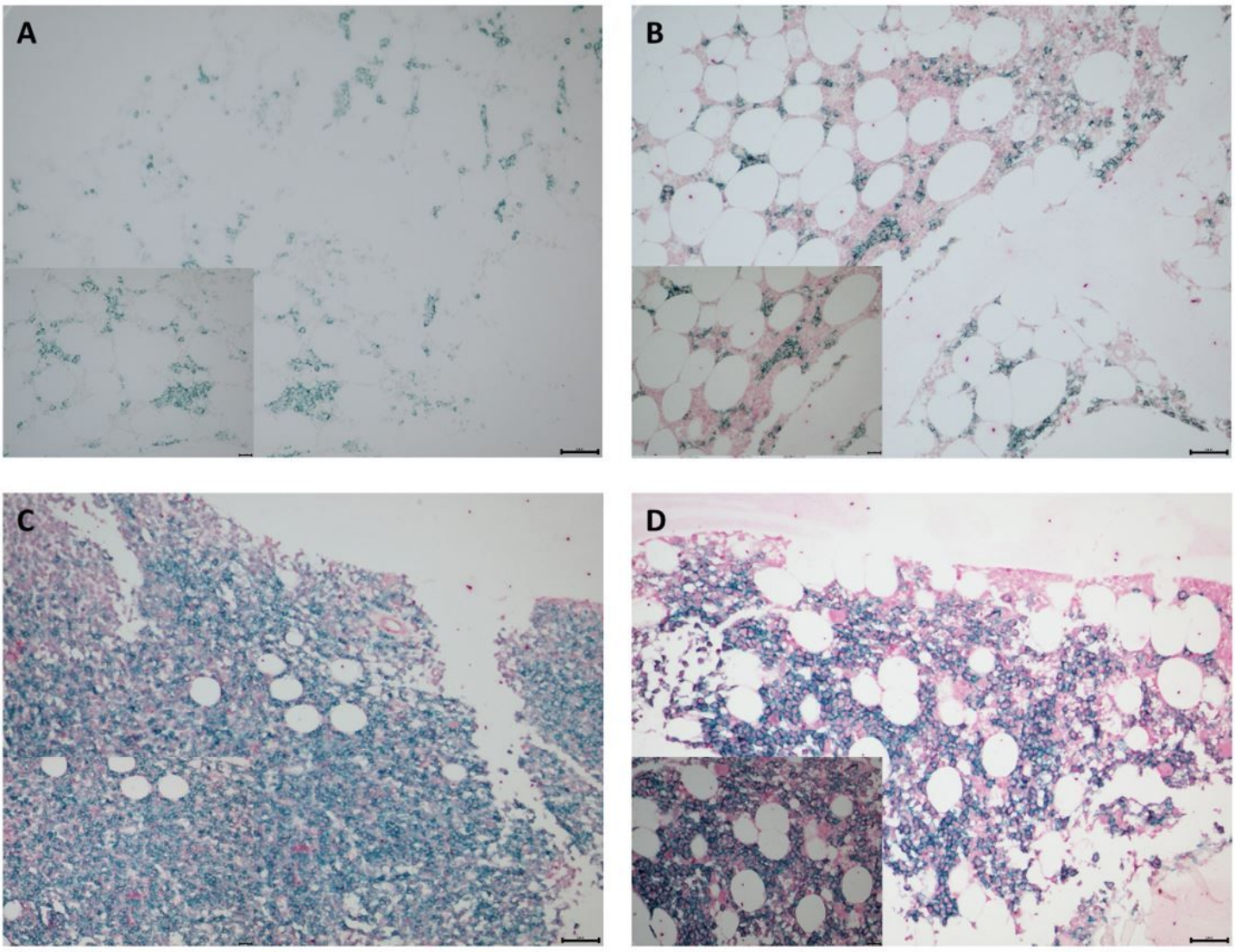

Figure 1

IHC staining to determine PVR expression in bone marrow plasma cells obtained from patients with MM. $CD138^{+}$ plasma cells were stained using Emerald chromogen (blue-green color) and PVR-expressing cells

were stained using Permanent Red chromogen (red color). Representative images depicting low and high PVR expression based on H-score. H-scores of **(a)** 0 and **(b)** 2 represented low PVR expression, and **(c)** 4 and **(d)** 6 were used to represent high PVR expression. Top row, original magnification: 200×; bottom row (inset), original magnification: 400×. H-score, histoscore; IHC, immunohistochemistry; MM, multiple myeloma; PVR, poliovirus receptor.

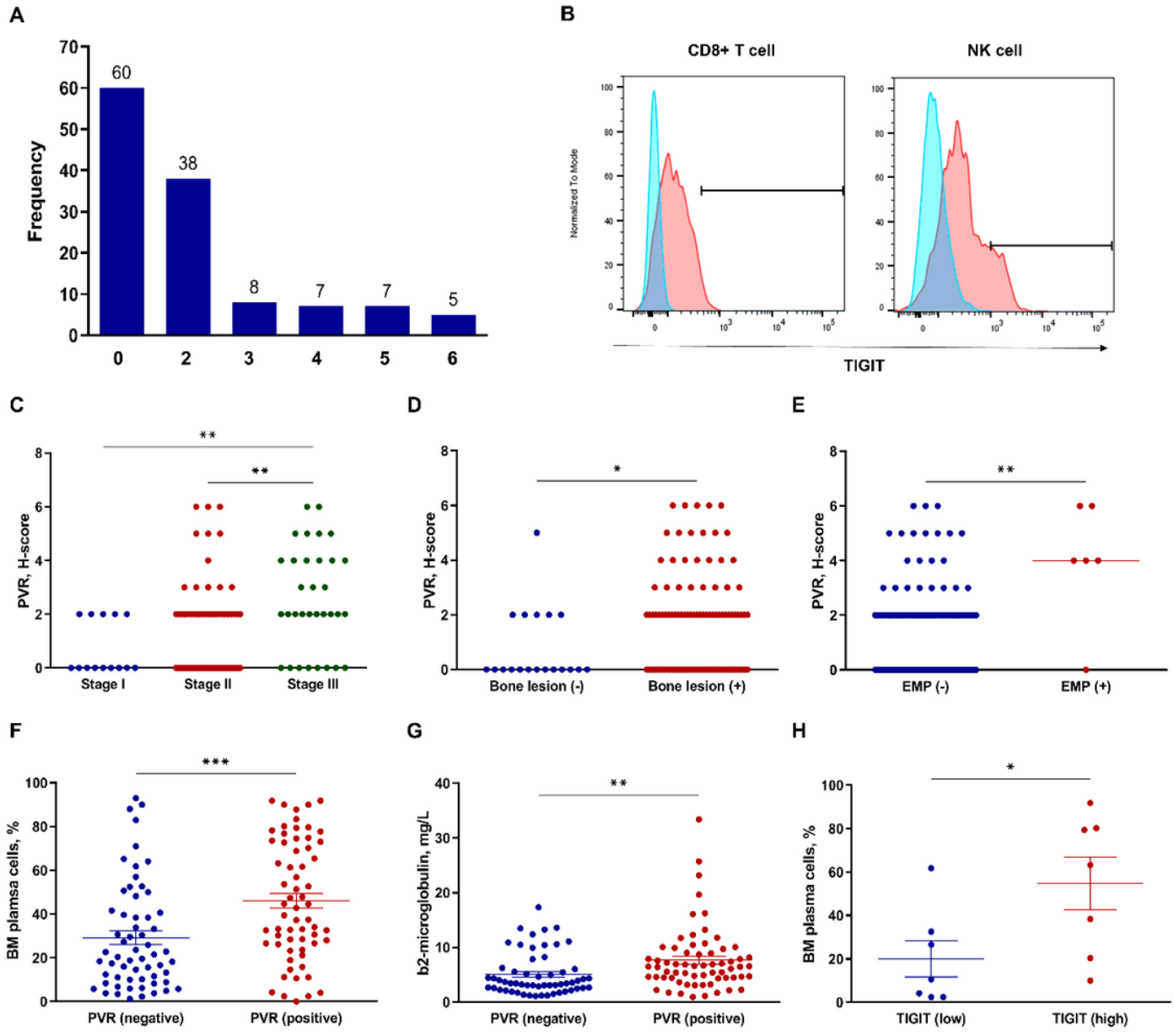

Figure 2

Assessment of PVR and TIGIT expression in relation to clinical factors in patients with MM. **(a)** Distribution of PVR expression (H-score) in bone marrow plasma cells in all patients. **(b)** Representative histogram of TIGIT expression in $CD8^+$ T cells and NK cells. Comparison of PVR expression based on **(c)**

R-ISS, presence of **(d)** bone lesion, and **(e)** EMP. *P < 0.05, **P < 0.01; Kruskal–Wallis and Mann–Whitney U tests. Comparison of clinical factors based on PVR- and TIGIT-expression status. **(f)** Percentage of bone marrow plasma cells and **(g)** concentration of β2-microglobulin in the negative (H-score: 0) and positive (H-score: > 0) PVR-expression groups (n = 125). Error bars indicate the mean ± standard error of the mean. *P < 0.05, **P < 0.01, ***P < 0.001; unpaired t test. **(h)** Percentage of bone marrow plasma cells in the low (< median) and high (≥ median) TIGIT-expression groups (n = 14). *P < 0.05; Mann–Whitney U test. BM, bone marrow; EMP, extramedullary plasmacytoma; H-score, histoscore; MM, multiple myeloma; NK, natural killer; PVR, poliovirus receptor; R-ISS, Revised-International Staging System; TIGIT, cell immunoreceptor with Ig and ITIM domains.

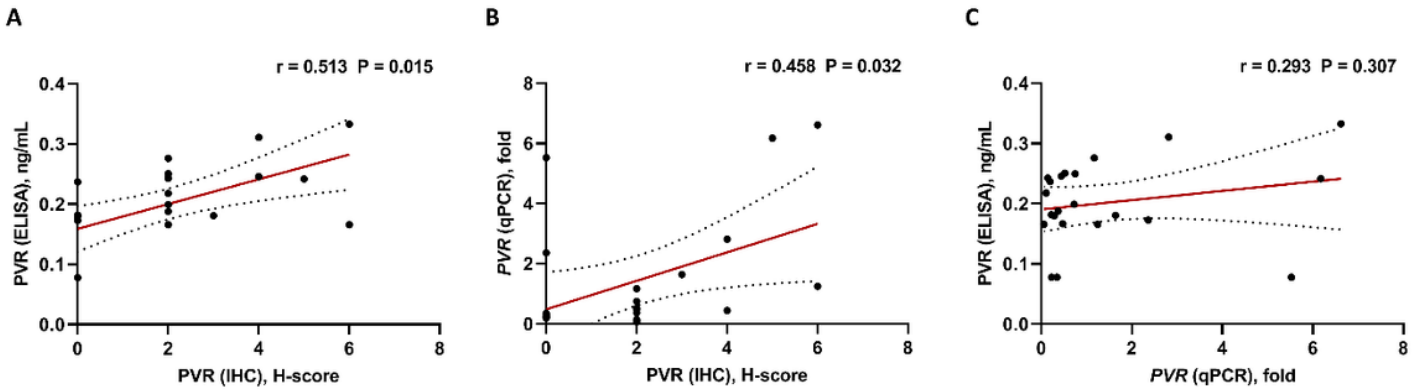

Figure 3

Correlations based on PVR expression using different detection methods (n = 22). **(a)** IHC vs. ELISA, **(b)** IHC vs. qPCR, and **(c)** qPCR vs. ELISA. Pearson's or Spearman's correlation coefficients (r) were used for analyses. ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; qPCR, quantitative polymerase chain reaction.

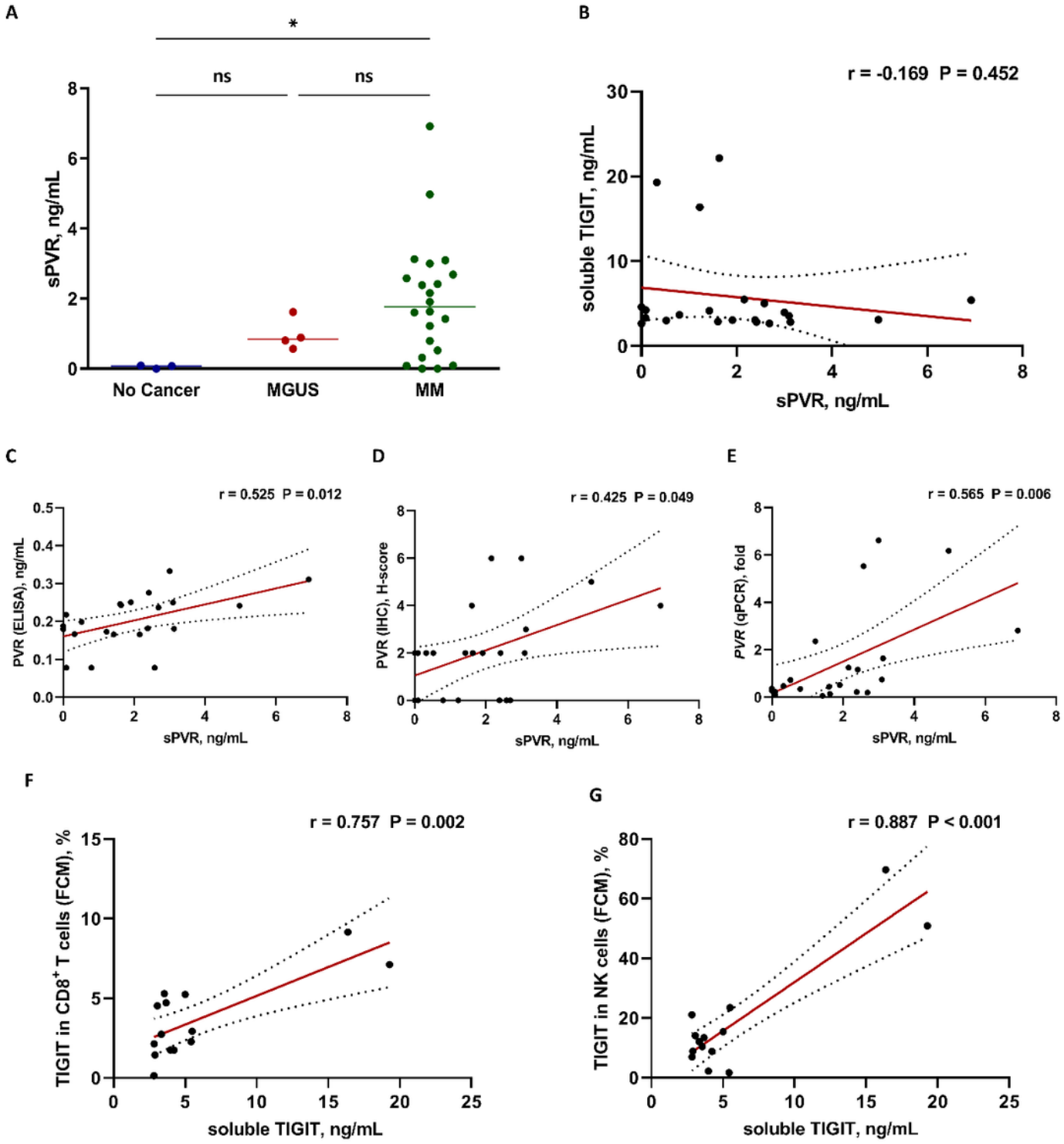

Figure 4

Correlation analysis based on soluble PVR (sPVR) and TIGIT levels in bone marrow plasma from patients with MM. **(a)** sPVR levels from patients without cancer (n = 3), patients with MGUS, (n = 4) and patients with MM (n = 22). *$P < 0.05$; Kruskal–Wallis test. **(b)** Relationship between sPVR and soluble TIGIT (n = 22). No significant correlations were observed. Positive correlation between sPVR and PVR expression in bone marrow plasma cells as measured by **(c)** ELISA, **(d)** IHC, and **(e)** qPCR (n = 22). Positive correlation

between soluble TIGIT and TIGIT expression in bone marrow **(f)** $CD8^+$ T cells and **(g)** NK cells (n = 14) measured by FCM. Pearson's or Spearman's correlation coefficients (r) were used for analyses. BM, bone marrow; ELISA, enzyme-linked immunosorbent assay; FCM, flow cytometry; IHC, immunohistochemistry; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; PVR, poliovirus receptor; qPCR, quantitative polymerase chain reaction; TIGIT, cell immunoreceptor with Ig and ITIM domains.

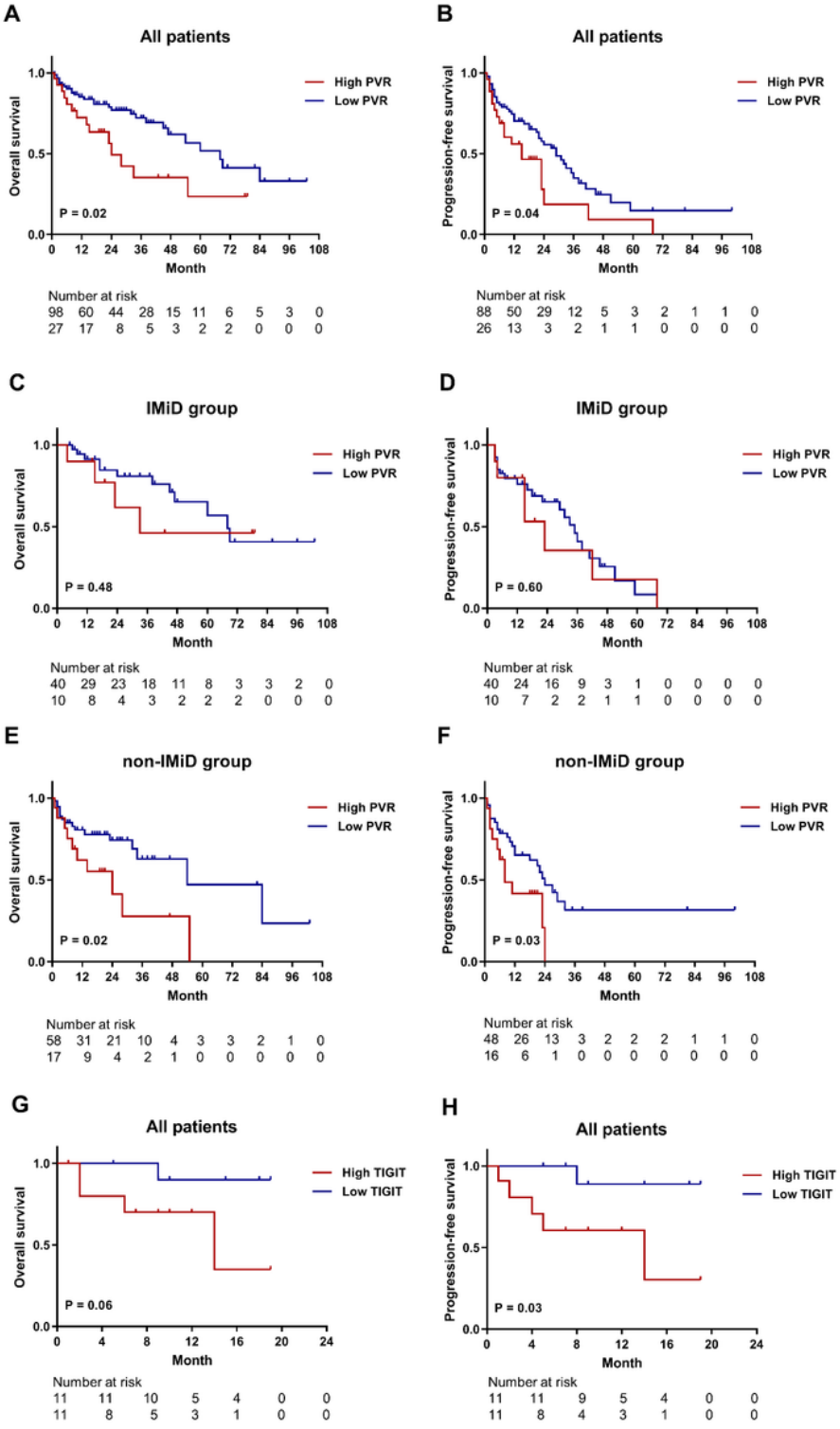

Figure 5

Kaplan–Meier survival curves for OS and PFS according to PVR expression. **(a, b)** OS and PFS curves for all patients. The median OS was 24 months in the high PVR-expression group and 68 months in the low PVR-expression group (n = 125). The median PFS was 15 and 29 months in the high and low PVR-expression groups, respectively (n = 114). **(c, d)** OS and PFS curves for the subgroups of patients (n = 50) who received IMiD therapy. The median OS (33 vs. 68 months) and PFS (23 vs. 35 months) showed no

significant differences between low and high PVR-expression groups. **(e, f)** OS (n = 75) and PFS (n = 64) curves for the subgroups of patients who did not receive IMiD therapy. The median OS (24 vs. 54 months) and PFS (8 vs. 24 months) were significantly shorter in the low PVR-expression group relative to the high PVR-expression group. **(g, h)** Kaplan–Meier survival curves for OS and PFS according to TIGIT expression (n = 22). IMiD, immunomodulatory drug; OS, overall survival; PFS, progression-free survival; PVR, poliovirus receptor; TIGIT, cell immunoreceptor with Ig and ITIM domains.

Supplementary Files

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