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The Importance of CD319 Marker in Diagnosis and Prognosis of Plasma Cell Myeloma Patients

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Abstract

Background: The surface antigen CD319 (CS1,SLAMF7) is a marker of normal and malignant plasma cells in plasma cell myeloma. It is encoded by the SLAMF7 gene that located on chromsome 1 on long arm (1q23.3). It acts as a human NK cell receptor which its function activated through homophilic interactions.In contrast to CD138 (the traditional plasma cell marker), CD319/SLAMF7 is much more stable and allows proper isolation of malignant plasma cells from delayed or even cryopreserved samples. So SLAMF7 expression (CD319) play an important role in diagnosis of plasma cell myeloma. This study aimed to evaluate the use of CD319 as a diagnostic and prognostic marker of plasma cell myeloma. **Methods**: This is a Cohort study . It was conducted on newly diagnosed plasma cell myeloma patients at Internal Medicine Department-Hematology unit at Zagazig University Hospitals and Clinical Pathology Department .It included 18 cases (11 males and 7 females) with a male to female ratio of 1.5:1. Mean age of cases was 53 ± 9.8 years. Patients were followed up three months later after initiation of treatment. The study started at February 2019 to February 2021. **Results:** This study showed that CD319 (SLAMF7, CRACC, CS1) is a stable marker with high expression (MFI) on normal and malignant plasma cells. In this study , CD319 is found positive in all PCM patients . Low expression of CD319 (if MFI below 128.3) associated with a significant better response to treatment and good prognosis but high expression of CD319accompanied by bad response to treatment. It can be effectively used as a diagnostic and prognostic marker for PCM. **Conclusion:** CD319 is found positive in all PCM patients and shows strong positive correlation with CD138/CD38 and CD56 . It can be effectively used as a diagnostic marker for PCM. Low expression of CD319 associated with a significant better response to treatment. This indicates importance of CD319 as a prognostic marker of PCM .

keywords: CD319, Plasma cell myeloma , Bone marrow aspiration , Flow cytometry.

INTRODUCTION

Plasma cell myeloma is a malignant tumor of plasma cells that amass in bone marrow, causing bone loss and marrow failure. It is frequently coupled with a M protein in blood and indications of organ damage from the plasma cell neoplasm. Because of the accumulation of aberrant immunoglobulin chains in the tissues, the disease has a clinical range ranging from asymptomatic to aggressive. A combination of pathological, radiological, and clinical features is used to diagnose plasma cell myeloma(1).SLAMF7 (Signaling lymphocytic activation molecule) family member 7 is a protein discovered in humans that is encoded by the SLAMF7 gene. The surface antigen CD319 (SLAMF7) is a marker of normal and malignant plasma cells in plasma cell myeloma. It is found on

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chromosome 1 on the long arm (1q23.3). Given the potential therapeutic utility of SLAMF7targeting antibodies such as elotuzumab, detecting SLAMF7 expression levels on aberrant plasma cells should be useful in assessing therapy responses[(2)]**.** The aim of this study is to evaluate role of CD319 marker in diagnosis and prognosis of plasma cell myeloma patients

SUBJECTS AND METHODS

This study was carried out at Clinical Pathology Department and Internal Medicine Department-Hematology unit at Zagazig University Hospitals and aimed to evaluate role of CD319 marker in diagnosis and prognosis of PCM patients.Also to correlate between CD319 marker and response to therapy. This study included 18 patients of newly diagnosed PCM . They are 11 males and 7 females and mean age of cases was 53 ± 9.8 years old .

Inclusion criteria: Newly diagnosed patients with plasma cell myeloma. Patients who had any plasma cell myeloma patients under treatment and follow up. Other malignancy and autoimmune disease. The thesis was accepted by the Faculty of Medicine's ethical review committee at Zagazig University. In compliance with the World Medical Association's Code of Ethics.

All patients were subjected to full history taking, full clinical examination. Laboratory investigations; Complete blood count (CBC) using sysmex XP Japan, with examination of Leishman stained PB smears for differential leucocytes count.The samples were obtained during the course of routine analysis and collected in EDTA anticoagulant tubes. Liver, kidney function tests, serum calcium and LDH (lactic acid dehydrogenase) using automated analyzer "Cobas 8000 platform-702c module, China". Also ESR (erythrocyte sedimentation rate): using automated analyzer "VISION–B, China".

Myeloma specific tests; Serum protein Electrophoresis and Immunofixation.Serum B2 microglobulin.Bence Jones protein in urine. Bone marrow aspiration and examination of Leishman stained smears to detect the percentage of BM plasma cells. Also bone marrow biopsy film stained with H&E .Skeletal bone survey including plain x-ray. Multicolor flow cytometricimmunophenotyping by using a panel of monoclonal antibodies against CD56, CD19, CD20, CD138, CD38, and CD45 using FACS Calibur Flow Cytometry, Becton-Dickinson (BD), USA .

Detection of CD319 marker (CS1, SLAMF7, CRAAC) by using multicolor flow cytometry and its mean fluresnece intensity (MFI).

For MCF analysis 100µl of blood count_adjustedanticoagulatedsample . First add lyse then red blood cells were lysed and washed twice with PBS. The supernatant was discarded and the cell pellet was suspended in PSB. Bone marrow sample was incubated with monoclonal antibodies in the dark at room temperature for 15 min. After incubation , stained cells were quickly detected and analyzed using MCF (FACS Calibur Becton Dickinson, San Joe USA) Cell Quest software BD Bioscience , FACS Calibur 4 color using the following panel of cluster of differentiation (CD) antigens :CD138 PerCP , CD38 FITC, CD45 APC, CD54 PE, CD19 PerCP, CD20 FITC, CD319 PE, κ and λ light-chain Ig (PE & FITC respectively) to confirm clonality. For identification of PCs at least 30,000 cells were acquired , cells were considered positive for a marker when more than 20% of cells expressed that marke[**[3]**].

Identification of PCs by CD 38 , CD138 occcurs after setting a first PC gate in a CD38 vs. CD138 bivariate dot plot .It was used as the reference PC gating approach and considered as the 100% threshold for PC identification and for further comparison of the performance of CD319 gating in combination with CD38, CD138, or 56. In case of compromised /low expression of CD138, PCs were detected through the use of the other

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PC markers present in the tube. Expression levels of individual markers were reported as mean fluorescence intensity values (MFI).

CD319 expression level was assessed as the mean fluorescence intensity, defined as mean of the fluorescence intensity of CD319-PE-positive PCs, using the Infinicyt software (Cytognos, Salamanca, Spain)

To ensure importance of CD319 in prognosis of PCM, we follow up PCM patients three months later after initiation of treatment. Then assess response of PCM patients to treatment.

In this study all PCM patients receive three cycles of chemotherapy during course of treatment. They receive VRd , bortezomib in day 1,8,15,22 , lenalidomide in day 1-21 (one week off) and dexamethasone in day 1,2,8,9,15,16,22,23 for each cycle , then start another cycle . Almost patients receive biphosphate therapy (zometa) due to bone lesions .

After the third cycle re-evaluation of cases occur by CBC, serum B2 microglobulin, serum protein electrophoresis and immunofixation, BMA and BM biopsy. Then we assess response to treatment according to National Comprehensive Cancer Network **NCCN**[4] regarding revised IMWG response criteria[5]**.**

STATISTICAL ANALYSIS

Data from the history, basic clinical examination, laboratory tests, and outcome measures were coded, entered, and analyzed in Microsoft Excel software. The data was then imported into the Statistical Package for the Social Sciences (SPSS version 20.0) program for analysis.

RESULTS

This study included 18 PCM patients, the mean ± SD of age was 53±9.8 years and the majority of them were males (61.1%). The demographic and basic characteristics data of PCM patients are shown in figure (1) .

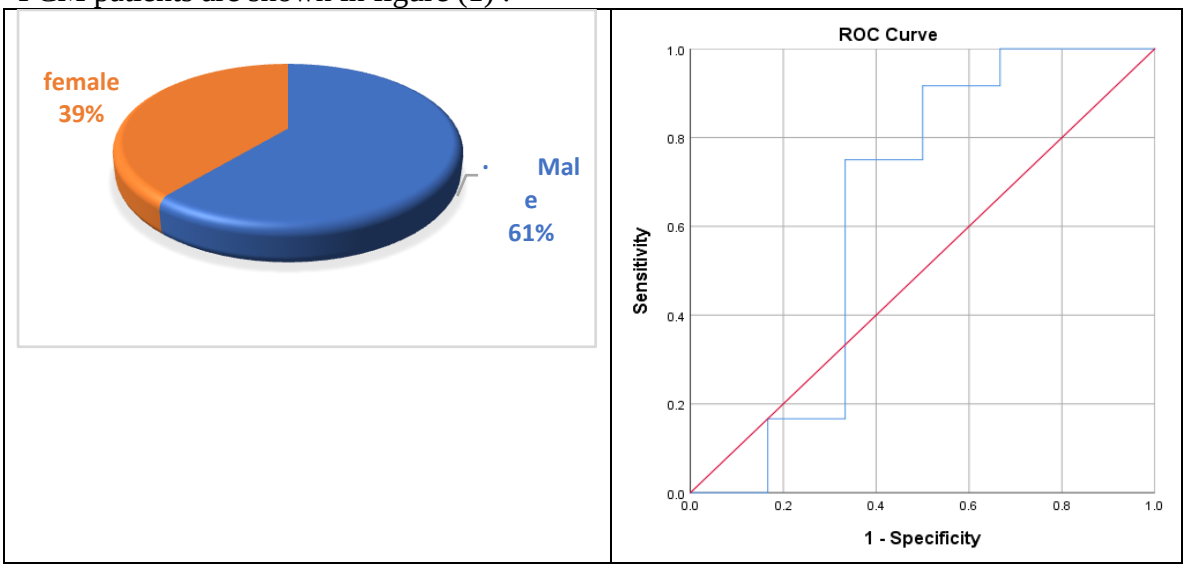

Fig 1: Sex among studied group.

Table 1 : ROC curve for CD319 MFI in plasma cell myeloma :

Variable	AUC	Cutoff point	Sensitivity	Specificity
MFI	0.64	128.3	90	66

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Correlation between expression of CD319 MFI and demographic & characteristic data of PCM patients

Table 2; This study showed that There was **statistically significant correlation** between high expression of CD319 and low Hb concentration of plasma cell myeloma patients ($p<0.05$) . In addition there was **highly statistically significant correlation** between high expression of CD319 and high expressions of CD138/38 and CD56 ($p< 0.001$).

Table 2: Correlation between expression of CD319 MFI, demographic and characteristic data of PCM patients :

Variable	Expression of CD319 MFI		t-test	P-value
	Low expression (N=11)	High expression (N=7)		
Age (yrs): ***Mean ± SD***	51.9 ± 11.5	54.7 ± 6.9	-0.58	0.57
Sex: ***Male*** ***Female***	7 (63.6) 4 (36.4)	4 (51.7) 3 (42.9)	Fisher	1
TLC: ***Mean ± SD***	7.1 ± 1.2	6.9 ± 0.9	2.6	0.679
Hb: ***Mean ± SD***	10.5 ± 2.3	7.8 ± 1.2	**3.9**	**0.013* (S)**
Platelets: ***Mean ± SD***	229.7 ± 68	167.3 ± 87.1	0.63	0.108
BM plasma cell (%): ***Mean ± SD***	30.5 ± 12.5	26 ± 7.5	3.5 (MW)	0.403
Ca: ***Mean ± SD***	10.5 ± 1.6	10.2 ± 0.78	0.371	0.715
Creatinine: **Mean ± SD**	2.2 ± 2.6	1.1 ± 0.95	2.9 (MW)	0.325
Albumin: ***Mean ± SD***	3.3 ± 0.8	2.2 ± 1.5	3.7 (MW)	0.057
B2microglobulin: ***Mean ± SD***	197.3 ± 51.4	187.7 ± 55.9	0.155	0.715
LDH: ***Mean ± SD***	162.6 ± 51.9	155 ± 27.1	1.6	0.726
M protein: ● ***IgG kappa*** ● ***IgG lambda*** ● ***IgA lambda***	4 (36.4) 4 (36.4) 3 (27.3)	5 (71.4) 2 (28.6) 0 (0)	2.8 **(χ2)**	0.093
Bone lesion: ● ***Multiple*** ● ***Solitary***	9 (81.8) 2(18.2)	7 (100) 0(0)	fisher	0.497
CD138/38 %: ***Mean ± SD***	29.5 ± 4.9	47 ± 11.9	**7.4**	**0.000* (HS)**
CD56 %:				**0.000***

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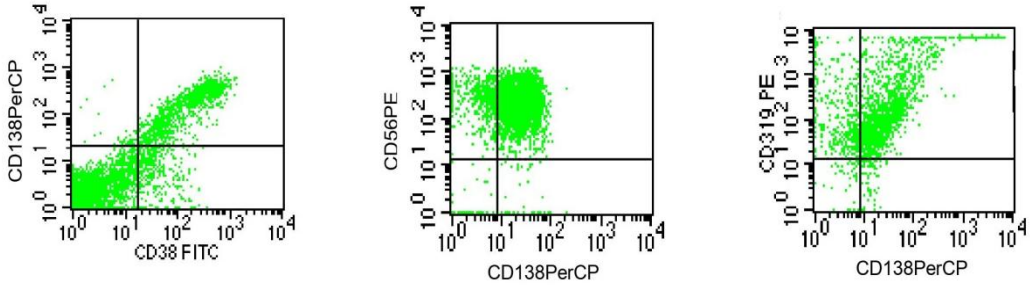
Mean* ± *SD	24.8 ± 3.1	34.3 ± 2.7	**0.412**	**(HS)**
CD 319 %: ***Mean* ± *SD***	58.9 ± 14.5	91.8 ± 5.7	**7.5**	**0.000* (HS)**

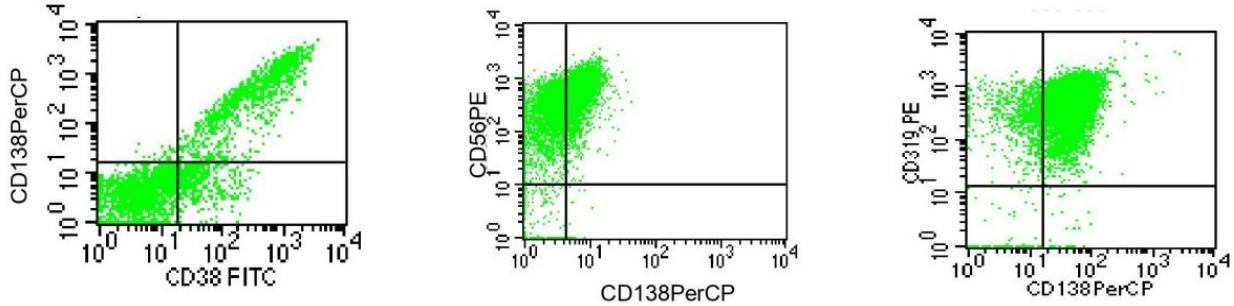
MW: MannWhittny P value <0.05 : is significant(S) P value<0.001 : is highly significant(HS)

Figure 2,3; showed positive CD138/38, positive CD56/CD138 and positive CD319/CD138 in the same sample of the PCM patient for each figure sepearte .



2 (A) 2 (B) 2 (C)

Figure 2: Immunophenotyping of plasma cells from BM aspirate of PCM patient revealed
A - positive CD138/38
B- positive CD56 /CD138
C- positive CD319/CD138



3 (A) 3 (B) 3 (C)

Figure 3: Another immunophenotyping of plasma cells from BM aspirate of PCM patient revealed :
A - positive CD138/CD38
B- positive CD56 /CD138
C- positive CD319/CD138

Figure 4;This study showed that there was highly statistically significant association between CD 319% and response to treatment (p< 0.001).

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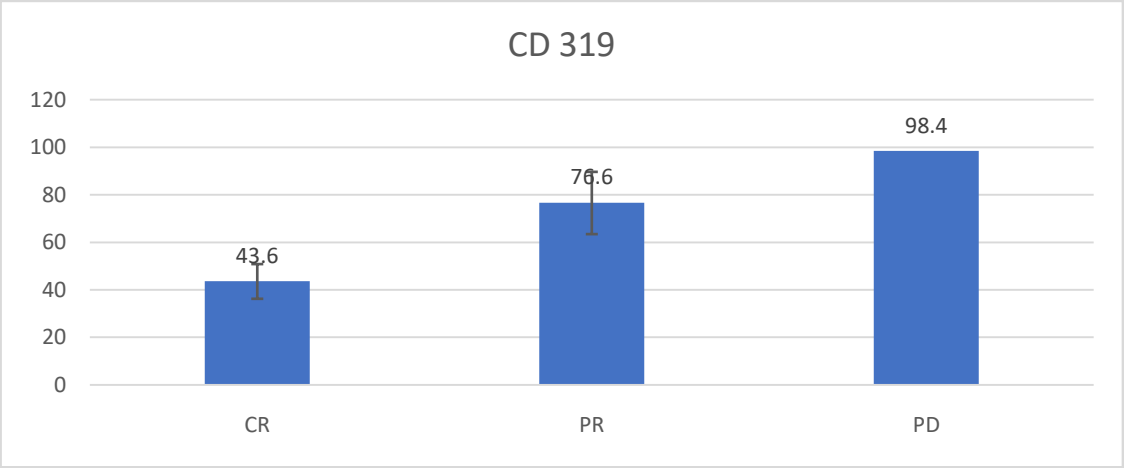

Fig 4: Association between between CD 319% and response to treatment.

Figure 5, 6 ; . There was **highly statistically significant correlation** between CD319 and CD138/38 , also CD319 and CD56 ($p < 0.001$).

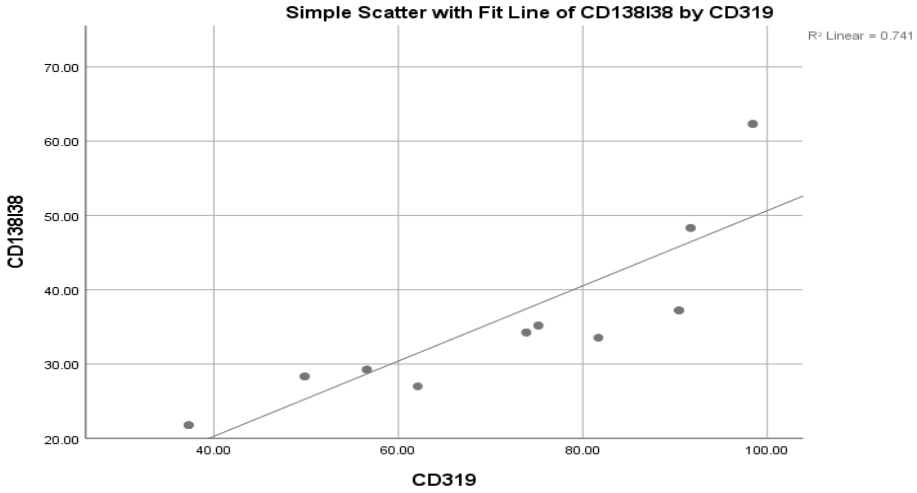

Fig 5 : Correlation between CD 319 and CD 138/ 38

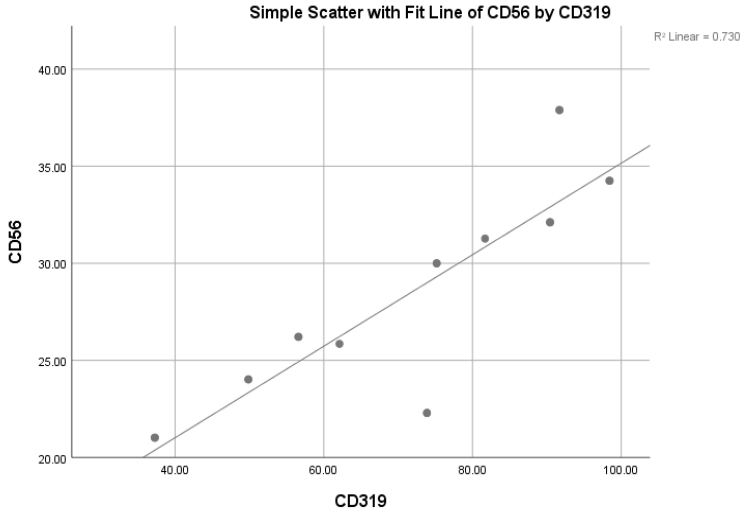

Fig 6 : Correlation between CD 319 and CD 56.

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There was **statistically significant** association between expression of CD319 and response to treatment ($p< 0.05$). As low expression of CD319 associated with a **significant** better response to treatment (Table 3) .

Table 3: Association between expression of CD319 MFI and response to treatment among the studied group:

Variable	Expression of CD319 MFI				$\chi 2$	P-value
	Low expression		high expression			
	N=11	%	N=7	%		
Response to treatment:						
● **CR**	**4**	**36.4**	**0**	**0**		**0.021***
● **PR**	**7**	**63.6**	**5**	**71.4**	**5.3**	**(S)**
● **PD**	**0**	**0**	**2**	**28.6**		

CR : complete response PR : partial response PD : progressive disease

DISCUSSION

According to this study, the average age of PCM patients is 53 years, and the majority of them (61.1 %) are males, with a male to female ratio of 1.5:1. In the study conducted by Bouatay A et al. [6], the average age of the cases was 67 years, with a male to female ratio of 1.7:1. Furthermore, Pojero et al. [7] discovered that the average age of PCM patients was 60 years, and the majority of them (56 %) were males, with a male to female ratio of 1.2:1.

In terms of M protein, half of PCM patients (50%) were IgG kappa, 33.3% were IgG lambda, and 16.7 percent were IgA lambda, according to the current study. These findings are consistent with recent studies conducted by Bouatay et al. [6] and Aita Marta Helena et al. [8], which show a preponderance of IgG kappa in MM patients.

Despite the presence of hypercalcemia, increased creatinine, and bone lesions, all of which are associated with myeloma, none of these clinical factors exhibited a significant correlation with positive CD319 and its expression in our study. These findings are consistent with those of Mariko Ishibashi et al[9], who discovered that CD319 was not associated with any clinical factors.

The percentages of BM plasma cells were not linked with CD319 (SLAMF7) levels in the current investigation. These findings are potentially comparable to those reported by Mariko Ishibashi et al. [9], who discovered that soluble SLAMF7 (sSLAMF7) levels were not linked with the percentages of BM plasma cells and SLAMF7 mRNA levels in PCM patients' BM plasma cells.

Except for Hb and multiple myeloma immunophenotypic indicators, laboratory variables Positive CD319 percent and MFI were not found to be statistically significantly linked with any of them. Hb had a statistically significant negative correlation because high expression of CD319 was associated with low Hb concentration in plasma cell myeloma patients, but there was a highly statistically significant strong positive correlation between CD 319 and its expression and CD138/38,CD 56 ($p<0.001$). This is consistent with the findings of Flores-Montero et al[10], who discovered a positive association between CD138/38, CD56, and CD319.

Aside from CD138 and CD38, a few other PC-associated markers have emerged as possible candidates for MCF detection of BM PCs in MM. CD54, CD229, and CD319 have been identified as PC-associated markers and possible candidates to replace CD38 and/or CD138 in the detection of BMPCs in PCM patients[11].

For the initial detection of both nPCs and aPCs in BM in PCM, CD319 plus CD38 combinations may be considered as robust alternatives to CD38 vs. CD138.

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This could be very useful in select PCM situations, particularly those with loss or downregulation of CD138 due to delayed sample arrival or frozen samples[11].

Overall, our findings indicate that CD319 (SLAMF7, CRACC, CS1) is a persistent marker with high expression (MFI) in both normal and malignant plasma cells. CD319 was found to be positive in all PCM patients in our investigation. It can be utilized efficiently as a PCM diagnostic marker. This is consistent with the findings of HarshiniSriram et al.[12], who discovered that CD319 is a stable immunophenotypic marker with strong, homogeneous expression in PCs and can be used effectively as a gating marker in addition to traditional markers for plasma cell quantitation, particularly after daratumumab therapy.

Our findings are also consistent with those of the Veillette A and Guo[13] studies. Their findings reveal that CD319 is expressed at high levels on myeloma PCs regardless of their cytogenetic/molecular background, prompting Frigyesi et al. [11] to argue that CD319 is more robust than CD138 for the isolation of BM PCs.

Concerning the results of CD319 and its high expression on plasma cells, these may be similar to those reported by Touzeau et al. [14], who discovered that high expression levels of SLAMF7 were observed in PCM patients with high-risk and low-risk molecular profiles, as well as those with and without cytogenetic abnormalities.

Recently, Soh KT et al. [15] demonstrated that CD319 is a viable alternative to CD38 for recognizing plasma cells. CD319 may be utilized alone or in combination with CD38 to detect PCs in patients receiving daratumumab or elotuzumab.

CD319 and its expression (MFI) were investigated in relation to treatment response to further understand the predictive importance and utility of CD319 in determining outcome. There was a strong statistically significant relationship between CD 319 percentage and expression (MFI) and treatment response ($p<0.001$).

In our study, high expression of CD319 (above cut off point 128.5), both PD and PR, was linked with a substantial poor response to treatment and a poor prognosis in PCM patients.

This is consistent with the findings of Mariko Ishibashi et al. [9], who discovered for the first time that PCM patients with high soluble SLAMF7 (sSLAMF7) levels had shorter progression free survival (PFS) times, and that sSLAMF7 in PCM patients' serum may be a useful indicator of disease progression.

Furthermore, low CD319 expression, including CR and/or PR, was associated with a much better response to treatment (positive prognosis), which agrees with the Mariko Ishibashi et al. [9] study. They discovered that soluble SLAMF7 (sSLAMF7) levels in MM patients' serum were either undetectable or decreased when they achieved CR or PR after antimyeloma therapy. In contrast, Lisenko et al. [16] discovered that SLAMF7 expression intensity on PCs decreases from MGUS cases to MM cases in relapsed/progressive stages of the disease using mean fluorescence intensity analysis.

Furthermore, Mariko Ishibashi et al. [9] discovered that serum sSLAMF7 may reflect MM disease progression and may be a valuable prognostic indication in newly diagnosed MM. This is consistent with our findings, which show the function of CD319 (SLAMF7) as a predictive marker in PCM patients.

Conclusion

CD319 is a stable immunophenotypic marker with high expression on PCs, and there was a highly statistically significant strong positive connection between CD319 percentage and expression (MFI) and CD138/38, CD56. This demonstrated its utility as a PCM diagnostic marker and demonstrated that it can be utilized as a gating sign in addition to standard indicators. Low CD319 expression was related with a significantly

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better response to treatment and a favorable prognosis, whereas high CD319 expression was associated with a poor response to treatment and a poor prognosis. This highlights the significance of CD319 as a PCM prognostic marker.

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