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PRAME Expression and Its Clinical Relevance in Hodgkin's Lymphoma

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Key Words

Disease-free survival · Hodgkin's lymphoma · Immunohistochemistry · Overall survival · Preferentially expressed antigen of melanoma · Prognosis · Real-time polymerase chain reaction

Abstract

Objectives: Although Hodgkin's lymphoma (HL) is one of the most curable cancers in adult patients, new targets have to be defined in cases resistant to traditional chemotherapy. The preferentially expressed antigen of melanoma (PRAME) is a cancer testis antigen and its expression is very scarce or absent in normal tissues. For this reason PRAME is a promising candidate for tumor immunotherapy. The aim of this study is to understand the correlation of PRAME expression with prognostic factors in HL, to determine the utility of PRAME as a targeted molecule for immunotherapy and to compare real-time polymerase chain reaction (real-time PCR) and immunohistochemistry (IHC) for the detection of PRAME. ***Methods:*** In 82 patients, PRAME was studied using real-time PCR and IHC. Data analyses were performed using statistical methods such as t test, Mann-Whitney U test, χ^2 test, Kaplan-Meier method, log-rank test and Cox regression analysis. ***Results:*** PRAME was detected in 15 (18.3%) patients using IHC and in 8 (9.8%) patients using real-time PCR. A correlation was found between PRAME positivity and higher International Prognostic Score ($p = 0.039$). PRAME positivity detected using real-time PCR was found to be correlated with shorter disease-free survival (DFS) and overall survival (OS, $p = 0.0005$). ***Discussion:*** The demonstration of PRAME especially in histiocytes and Reed-Sternberg cells may provide guidance for immunotherapy. Although PRAME positivity increases the risk for death (3.56), independent risk factors that affected DFS and OS occurred in advanced age and high-risk groups. ***Conclusion:*** Although real-time PCR is sensitive in the detection of PRAME, IHC can be another useful method. Despite the need for studies conducted on larger patient samples, PRAME expression is considered as a poor prognostic parameter in HL.

Introduction

Hodgkin's lymphoma (HL) is a heterogeneous tumor that develops from germinal center and postgerminal center B cells and that has a cell composition including Reed-Sternberg cells and some of its variant neoplastic cells on an inflammatory basis [1]. Although HL is one of

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the tumors for which cure is most achieved, 20–30% of the cases do not respond to conventional chemotherapeutic regimens. For this reason it is important to determine novel prognostic and predictive factors and also immunotherapy approaches.

Preferentially expressed antigen of melanoma (PRAME) has been described in malignant melanoma as a tumor-related antigen recognized with autologous cytotoxic T cells against surface antigen [2]. It is not expressed or only slightly expressed in normal tissues, except testicles, ovaries, endometrium and adrenal glands [3, 4]. PRAME has been shown to be expressed in many tumors as a tumor antigen and PRAME expression has been detected in 88–95% of malignant melanoma, in 39% of head and neck carcinoma, in 46–78% of non-small cell lung cancer, in 41% of renal-cell cancer, in 39% of malignant mesenchymal tumors, in 27% of breast cancer and in 33% of acute leukemia [5]. In most of these tumors PRAME has been found to be a poor prognostic factor [6–15]. Information about PRAME expression and its prognostic value in HL is very limited. In a study performed by Staege et al. [16] it was demonstrated that PRAME has been found to be expressed only in cell lines belonging to patients with resistant HL [17]. In the study performed by Willenbrock et al. [18], when patients with HL were compared with those with anaplastic large cell lymphomas and B cell non-Hodgkin lymphoma, PRAME expression was found to be higher in HL.

The aim of this study is to investigate the PRAME expression in HL and to determine the correlation of PRAME with very well-known prognostic factors in HL and also to compare immunohistochemistry (IHC) and real-time polymerase chain reaction (real-time PCR) in the detection of PRAME.

Materials and Methods

Prior to the beginning of the study, the study protocol was reviewed and approved by the local ethics committee, in accordance with the ethical principles for human investigations, as outlined by the Second Declaration of Helsinki. We included 82 HL patients diagnosed and treated between 1998 and 2012 in the study. The risk of the patients was determined according to the International Prognostic Score (IPS). Staging was performed according to the Ann Arbor staging system (Cotswolds modification).

The patients were divided into three risk groups: early-stage favorable (without risk factor) and unfavorable (with risk factor) groups and advanced-stage group (stage IIB with risk factors and stage III–IV). Risk factors were high erythrocyte sedimentation rate (≥50 mm/h), any B symptoms, mediastinal mass ratio greater than 0.33, number of nodal sites >3 and the presence of a mass >10 cm.

Immunohistochemistry

Immunohistochemical evaluation was done by avidin-biotin-peroxidase method and graded as (–), (+) and (++) by PRAME staining characteristics. As the number of PRAME (+) subjects was relatively small, the subjects were divided into two groups according to their PRAME staining characteristics.

Real-Time PCR

Total RNA was isolated from formalin-fixed and paraffin-embedded tissue sections by using the High Pure miRNA Isolation Kit (Roche Diagnostics, 05080576001). QuantiTect PRAME and ACTB (beta-actin as housekeeping gene) primers (Qiagen) were used for real-time PCR reactions. cDNA reactions performed by using the miScript RT Kit (Qiagen) with manufacturers' recommendations. cDNA samples were preamplified by using PRAME and ACTB primer pools with TaqMan PreAmp Master Mix (Life Technologies) for 14 cycles. Unbound primers were removed by using exonuclease (New England Biolabs, MO293L). Real-time PCR analyses were performed with Fast EvaGreen qPCR Master Mix (Biotium, 31003-1) on a PikoReal 96 Real-Time PCR System (Thermo Scientific).

Statistical Method

The consistence of the data with normal distribution was tested; continuous variables that showed normal distribution were analyzed using t test in independent groups, and continuous variables that did not show normal distribution were analyzed using Mann-Whitney U test. Categorical variables were analyzed using χ^2 test. For survival analyses, the Kaplan-Meier method and log-rank test were used. For multiple comparisons, Cox regression analysis was used. The results were expressed as mean ± standard deviation, median (lower limit and upper limit), number and percentage; $p < 0.05$ was considered as statistically significant. Statistical analysis of the data was performed using SPSS 16.0 software.

Results

The duration of mean follow-up was found to be 51.5 ± 39.0 months and median follow-up was 45.6 months (min. 1, max. 152) for all patients. Forty-three (52.4%) of 82 patients were male and 61 (74.4%) were under the age 45. Fifty-six of the patients (68.3%) had B symptoms. Seven of the patients (8.5%) had stage I, 40 (48.8%) had stage II, 24 (29.3%) had stage III, and 11 (13.4%) patients had stage IV disease. Forty-four (53.6%) of the patients had mediastinal involvement, 15 (18.3%) had extranodal involvement. Twenty-nine (35.4%) patients were in the favorable early-stage group, 23 (28%) patients in the early-stage unfavorable group and 30 (36.6%) patients had advanced-stage disease. IPS was low (1–3) in 73 (89%) patients and high (≥4) in 9 (11%). Complete response has been achieved in 58 patients (70.7%), recurrent disease has been observed for 28 (34.1%).

As a first-line therapy, the ABVD protocol (doxorubicin, bleomycin, vinblastine, and dacarbazine) was administered to 77 (93.9%) of 82 patients. In 28 patients, disease recurrence occurred. In patients with first disease recurrence, the DHAP protocol (dexamethasone, cytarabine, cisplatin) was the most frequently used treatment regimen, as salvage therapy. The ICE protocol (ifosfamide, carboplatin, etoposide), gemcitabine plus either vinorelbine or oxaliplatin or cisplatin, and brentuximab alone were the other salvage protocols used with less frequency than DHAP.

Immunohistochemistry

PRAME expression was detected in 15 patients using IHC staining (18.3%). Among these patients, 4 had mixed cellular type, 9 had nodular sclerosis (NS), 1 had nodular lymphocyte predominant, and 1 had lymphocyte-depleted type HL.

Real-Time PCR

PRAME was detected in 8 patients (9.8%) by real-time PCR. PRAME expression by this method was highly variable and changed from very slight expression to an expression higher than 244-fold (1.52- to 244.51-fold). Of the patients in whom PRAME positivity was detected using real-time PCR, 3 had mixed cellular, 4 had NS, 1 had lymphocyte-depleted HL whereas 1 had HL that could not be classified.

There was no difference for age, basal biochemical tests, involved area and IPS in PRAME-positive and negative cases both in IHC and real-time PCR.

PRAME detected by IHC was more commonly found in men as compared with women. The incidence of PRAME expression detected using real-time PCR was higher in patients aged 45 years and above. The presence of B symptoms was more commonly seen in PRAME-positive patients detected both by IHC and real-time PCR, but the difference was statistically nonsignificant. When compared by stages, PRAME expression was detected mostly in advanced-stage disease (stage III–IV disease) and also in patients who were found to have involvement of 4 or more lymph node sites. Mediastinal involvement was found to be more common in subjects with PRAME expression. However, none of these comparisons showed statistically significant differences. The incidence of PRAME expression detected by IHC was similar among the low- and high-risk groups. However, PRAME expression detected by real-time PCR was found to be higher in the high-risk group of patients. Again the differences were not statistically significant. Higher IPS was found in cases with PRAME expression detected by real-time PCR and the difference was significant ($p = 0.039$). Table 1 shows the PRAME expression by IHC and real-time PCR.

Survival Analyses

We found shorter disease-free survival (DFS) in cases with PRAME expression detected by real-time PCR ($p = 0.0005$, table 2). Although PRAME positivity was associated with longer DFS as compared with PRAME-negative cases (118 vs. 61 months), the difference was not statistically significant. DFS was found to be shorter in older age (age above 45 years, $p = 0.0001$), in advanced stage ($p = 0.007$), in advanced risk group ($p = 0.05$), in patients with high IPS ($p = 0.0001$), in the presence of recurrence ($p = 0.005$) and relapse duration less than 12 months ($p = 0.035$).

In overall survival (OS) analysis, the patients with PRAME expression by real-time PCR had shorter survival time as compared to negative patients ($p = 0.005$, table 3). Although the patients in whom PRAME expression was detected using IHC had an OS advantage (149 vs. 79 months), no statistically significant difference was found. Older age ($p = 0.0001$), advanced stage ($p = 0.007$), and high IPS ($p = 0.0001$) were found to be associated with shortened OS and these differences were found to be statistically significant (table 3).

Multiple regression (Cox regression) analysis was used to determine independent risk factors for DFS and OS and results are shown in table 4. Age and risk groups were found to be independent risk factors affecting survival. Although PRAME positivity was found to be an increased risk for death (OR 1.63, 95% CI 0.31–8.61), this was not a significant factor.

Discussion

In this study, PRAME expression was studied on paraffin-embedded tissue using both real-time PCR and IHC methods. Nearly in all studies designed to detect the PRAME expression to date fresh tissue and the real-time PCR method have been used. In this study, in addition to the real-time PCR, the IHC method was applied to the same samples and it was aimed to investigate the comparability of real-time PCR and IHC methods. Our primary aim was to see whether PRAME can be analyzed using a practical, easy and cost-effective method. In this study PRAME positivity was detected in 15 patients (18.3%) with IHC analysis and in 8 patients (9.8%) by real-time

Table 1. Comparison of patient characteristics with PRAME IHC and real-time PCR data

	PRAME IHC		p	PRAME real-time PCR		p
	negative, n	positive, n (%)		negative, n	positive, n (%)	
Gender						
Female	34	5 (12.8)	0.175	35	4 (10.3)	0.587
Male	33	10 (23.3)		39	4 (9.3)	
Age, years						
<45	50	11 (18)	0.575	57	4 (6.6)	0.111
≥45	17	4 (19)		17	4 (19)	
Localization (N = 76)						
Below diaphragm	5	1 (16.7)	0.525	5	1 (16.7)	0.790
Above diaphragm	37	7 (15.9)		40	4 (9.1)	
Below and above diaphragm	19	7 (26.9)		24	2 (7.7)	
B symptoms						
Yes	45	11 (19.6)	0.765	49	7 (12.5)	0.425
No	22	4 (15.4)		25	1 (3.8)	
Leukocytes, $\times 10^9$/l						
<15	63	14 (18.2)	0.646	70	7 (9.1)	0.410
≥15	4	1 (20)		4	1 (20)	
Hemoglobin, g/dl						
≥10.5	49	13 (21)	0.226	56	6 (9.7)	0.629
<10.5	18	2 (10)		18	2 (10)	
Lymphocytes, $\times 10^9$/l						
≥0.6	56	15 (21.1)	0.092	64	7 (9.9)	0.709
<0.6	11	0 (0)		10	1 (9.1)	
Albumin, g/dl						
≥4	40	9 (18.4)	0.983	45	4 (8.2)	0.409
<4	27	6 (18.2)		29	4 (12.1)	
Sedimentation/h						
<50	37	10 (21.3)	0.304	44	3 (6.4)	0.206
≥50	30	5 (14.3)		30	5 (14.3)	
β_2-Microglobulin (N = 54)						
>N	17	6 (26.1)	0.766	21	2 (8.7)	0.488
N	24	7 (22.6)		27	4 (12.9)	
Stage						
I	6	1 (14.3)	0.697	7	0 (0)	0.637
II	33	7 (17.5)		36	4 (10)	
III	18	6 (25)		22	2 (8.3)	
IV	10	1 (9.1)		9	2 (18.2)	
Number of sites involved[a] (N = 76)						
1–3	39	9 (18.8)	0.777	44	4 (8.3)	0.513
≥4	22	6 (21.4)		25	3 (10.7)	
Mediastinal involvement						
No	33	5 (13.2)	0.204	35	3 (7.9)	0.442
Yes	34	10 (22.7)		39	5 (11.4)	
Extranodal involvement						
No	54	13 (19.4)	0.449	62	5 (7.5)	0.157
Yes	13	2 (13.3)		12	3 (20)	
Risk group						
Stage I–II good	23	6 (20.7)	0.743	28	1 (3.4)	0.227
Stage I–II poor	20	3 (13)		21	2 (8.7)	
Stage III–IV	24	6 (20)		25	5 (16.7)	
IPS						
1–3	59	14 (19.2)	0.480	68	5 (6.8)	0.039

Table 1. (continued)

	PRAME IHC		p	PRAME real-time PCR		p
	negative, n	positive, n (%)		negative, n	positive, n (%)	
≥4	8	1 (11.1)		6	3 (33.3)	
Recurrence						
No	43	11 (20.4)	0.361	49	5 (9.3)	0.558
Yes	24	4 (14.3)		25	3 (10.7)	
Type of response						
Complete	47	11 (19)	0.539	53	5 (8.6)	0.430
Partial	20	4 (16.7)		21	3 (12.5)	
Relapse duration (N = 28)						
≤12 months	6	0 (0)	0.470	6	0 (0)	0.611
>12 months	19	3 (13.6)		20	2 (9.1)	

n = Number of patients; N = number of evaluated patients.
[a] Sites involved were positive in 14 patients for IHC and in 7 patients for PCR.

Table 2. DFS data in the patients in whom PRAME expression was analyzed

	Total, n	Events, n[a]	Mean	Median	p[b]
PCR					
(–)	74	10	124	155	0.0005
(+)	8	4	40	20	
IHC					
(–)	67	12	118	155	0.800
(+)	15	2	61	–	
Gender					
Male	43	9	100	120	0.132
Female	39	5	128	155	
Age, years					
<45	61	5	135	155	0.0001
≥45	21	9	37	47	
B symptoms					
Yes	56	10	106	120	0.467
No	26	4	131	155	
Sedimentation/h					
<50	47	6	115	120	0.608
≥50	35	8	115	155	
β_2-Microglobulin (N = 54)					
>N	23	3	109	–	0.426
N	31	4	119	–	
Stage					
I	7	1	127	–	0.007
II	40	2	136	155	
III	24	5	107	–	
IV	11	6	72	47	
Number of sites involved (N = 76)					
1–3	48	6	125	120	0.080
≥4	28	7	92	97	
Mediastinal involvement					
Yes	44	9	113	155	0.500
No	38	5	118	120	
Extranodal involvement					
Yes	15	5	88	–	0.066
No	67	9	124	155	
Risk group					
Stage I–II good	29	2	135	–	0.050
Stage I–II poor	23	2	114	120	
Stage III–IV	30	10	96	97	
IPS					
0	13	2	136	155	0.0001
1	29	2	132	–	
2	23	2	114	120	
3	8	1	50	–	
4	3	2	18	18	
5	6	5	41	20	
IPS					
1–3	73	7	129	155	0.0001
≥4	9	7	36	20	
Recurrence					
No	54	5	141	155	0.005
Yes	28	9	86	97	
Type of response					
Complete	58	8	86	97	0.905
Partial	24	6	119	155	
Relapse duration (N = 28)					
≤12 months	6	1	7	–	0.035
>12 months	22	8	102	120	
Overall	82	14			

n = Number of patients; N = number of evaluated patients.
[a] Number of events n = 7 for β_2-microglobulin, n = 13 for the number of sites involved, n = 9 for relapse duration. [b] log-rank test.

Table 3. OS data in the patients in whom PRAME expression was analyzed

	Total, n	Events, n[a]	Mean	Median	p[b]
PRAME real-time PCR					
(–)	74	10	156	198	0.005
(+)	8	4	68	80	
PRAME IHC					
(–)	67	12	149	198	0.707
(+)	15	2	79	–	
Gender					
Male	43	9	107	124	0.145
Female	39	5	160	198	
Age					
<45	61	5	169	198	0.0001
≥45	21	9	71	66	
B symptoms					
Yes	55	10	116	–	0.800
No	27	4	154	198	
Sedimentation/h					
<50	47	6	119	124	0.832
≥50	35	8	151	198	
β_2-Microglobulin (N = 54)					
>N	23	3	117	–	0.485
N	31	4	121	–	
Stage					
I	7	1	131	–	0.007
II	40	2	178	198	
III	24	5	117	–	
IV	11	6	80	66	
Number of sites involved (N = 76)					
1–3	48	6	139	124	0.124
≥4	28	7	103	–	
Mediastinal involvement					
Yes	44	9	148	198	0.413
No	38	5	125	–	
Extranodal involvement					
Yes	15	5	97	–	0.122
No	67	9	156	198	
Risk group					
Stage I–II good	29	2	139	–	
Stage I–II poor	23	2	120	124	0.057
Stage III–IV	30	10	123	100	
IPS					
0	13	2	173	198	0.0001
1	29	2	138	–	
2	23	2	118	124	
3	8	1	112	–	
4	3	2	53	25	
5	6	5	52	25	
IPS					
1–3	73	7	165	198	
≥4	9	7	53	49	0.0001
Recurrence					
No	54	5	179	198	
Yes	28	9	108	124	0.083
Type of response					
Complete	58	8	104	–	0.991
Partial	24	6	151	198	
Total survival	82	14			

n = Number of patients; N = number of evaluated patients.
[a] Number of events n = 7 for β_2-microglobulin, n = 13 for the number of sites involved. [b] log-rank test.

Table 4. The results of multiple regression (Cox regression) analysis used to determine independent factors that determine total survival and DFS

	Overall survival				DFS			
	b	OR	95% CI	p	b	OR	95% CI	p
PRAME (IHC)	0.49	1.63	0.31–8.61	0.568	–0.30	0.74	0.15–3.70	0.716
Risk group								
No		ref.				ref.		
Low (F)	–0.07	0.93	0.12–7.06	0.947	0.47	1.61	0.22–11.98	0.644
High (III–IV)	1.62	5.04	1.03–24.62	0.046	1.87	6.51	1.36–31.13	0.019
Age	0.09	0.000	1.05–1.14	0.000	0.10	1.11	1.05–1.16	0.000
PRAME (PCR)	1.28	3.58	0.89–14.48	0.074	0.90	2.47	0.67–9.06	0.174
Risk group								
No		ref.				ref.		
Low (F)	0.23	1.26	0.16–9.77	0.826	–0.25	0.78	0.10–5.85	0.810
High (III–IV)	1.62	5.05	0.99–25.66	0.051	1.29	3.64	0.70–18.80	0.123
Age	0.10	1.11	1.05–1.16	0.000	0.09	1.10	1.05–1.14	0.000

OR = Odds ratio; CI = confidence interval; b = coefficient; F = favorable.

PCR. The detection of PRAME in fewer subjects using real-time PCR may be attributed to RNA loss that occurred during the fixation or waxing steps. For this reason, fresh tissue is more appropriate for PRAME analysis with real-time PCR. We found that the rate of PRAME in HL is between 10 and 20%. We could not compare these results with other studies due to the lack of larger studies of PRAME expression in HL.

In 4 of the patients, PRAME was detected both by IHC and real-time PCR. On the other hand, PRAME was detected in 4 patients using real-time PCR, but in these patients PRAME could not be detected using IHC. Of the patients in whom PRAME was detected using real-time PCR, 3 had mixed cellular HL, 4 had NS, and 1 had lymphocyte-depleted HL, whereas 1 had unclassified classical HL. Briefly, while there was no marked difference in subtypes of HL in whom PRAME was detected using real-time PCR, 60% of the subjects in whom PRAME was detected by IHC had NS type. The clinical relevance of this finding is not clear, but the higher rate of PRAME expression in patients with NS type may suggest that PRAME may be a target for immunotherapy in this subtype.

In our study patients with PRAME detected by real-time PCR had older age and had higher IPS risk score ($p = 0.077$ and $p = 0.039$, respectively). Indeed, despite the absence of a statistical difference, mean IPS was 2.5 in the patients in whom PRAME was detected using real-time PCR and 1 in PRAME-negative patients. In other words, PRAME expression was correlated with poor IPS in both methods. This finding is important due to the correlation between PRAME expression and two strong prognostic parameters. This finding suggests that the PRAME expression detected by real-time PCR may be a poor prognostic indicator in patients with HL.

In survival analyses, although both progression-free survival and OS analysis were found to be shorter in PRAME expression detected by IHC and/or real-time PCR, only the PCR method was found to be statistically significant (tables 2, 3). Based on multiple regression analysis PRAME positivity was found to be associated with risk of death. In the high-risk group, DFS was one of the independent risk factors affecting DFS; the risk was found to be increased in cases expressing PRAME.

In various tumors, PRAME expression has been found to be correlated with poor prognosis [6–15]. In hematopoietic neoplasias, PRAME has been found to be a poor prognostic indicator. However, this is not a rule and PRAME has been found to be correlated with good prognosis in some cases of acute myelocytic leukemia and in some cases with acute promyelocytic leukemia [19–29]. However, the situation is different in chronic leukemias. The detection of PRAME in the progression of chronic myelocytic leukemia is quite exciting [5, 25, 30]. Studies exploring PRAME expression in HL are limited. Van Baren [24] detected PRAME in only 1 of 7 patients. On the other hand, Staege et al. [16] detected PRAME expression only in patients with resistant HL [31, 32]. Willenbrock et al. [18] found very high PRAME expression in cases with HL as compared with patients with anaplastic large cell lymphoma and B-cell non-Hodgkin lymphoma. These findings suggest that PRAME is a poor prognostic indicator in HL. In our series, 9 of 15 patients in whom PRAME was detected by IHC had NS subtype and 4 of 8 patients in whom PRAME was detected by real-time PCR had NS subtype. In a study performed using monoclonal antibody that recognizes MAGE-A4, 11 of 53 patients with HL (21%) showed expression. Interestingly, strong expression was detected especially in Reed-Sternberg cells and it was not detected in other cells of the environment [33]. In our IHC analysis, the demonstration of expression in Reed-Sternberg cells may suggest that these cells can be a good target for immunotherapy. With these results we can suggest that especially when a PRAME study is planned in fresh tissues in HL, it would be useful to perform the PRAME study by separating these cells by microdissection.

PRAME may be a potential target for therapeutic approaches in the future if it is detected in larger series where PRAME is expressed at high rates in resistant HL [16]. On the other hand, high PRAME expression has been found to be associated with increased resistance to chemotherapy in diffuse large B cell lymphoma and in HL. Kawano et al. [34] examined the gene expression pattern in patients who are susceptible and those who are refractory to anthracycline-containing therapy. They found that the expression of 9 genes was increased in the refractory group and greater increase was seen in PRAME among these genes. In this study, DFS was found to be shorter in PRAME-positive patients with diffuse large B cell lymphoma. Additionally PRAME was found in 50% of the patients in whom progression was detected while in 18% of patients achieving complete response [34].

As seen in many malignant tumors, immunotherapy seems to be an attractive option in HL. The basic principle of immunotherapy is the determination of the ideal target and this target should be expressed in tumor cells only but not in normal tissues. From this point of view, PRAME has become a promising candidate for tumor immunotherapy since it is recognized by autologous cytotoxic T lymphocytes [3, 16].

Conclusion

We found a correlation between PRAME expression and IPS ≥4, age >45 years, advanced stage, high-risk group, recurrent disease, shorter relapse time, shorter DFS and OS and increased risk for death. These findings suggest that PRAME expression may predict poor prognosis. In these analyses, as the results obtained using real-time PCR were more significant, real-time PCR was found to be a more reliable method for the detection of PRAME expression.

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Disclosure Statement

None.

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