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The Expression of Preferentially Expressed Antigen of Melanoma (PRAME) Gene in Acute Myeloid Leukemia

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ABSTRACT

Background: The preferentially expressed antigen of melanoma (*PRAME*) is a tumor associated antigen belonging to the group of cancer-testis antigens (CTA). CTAs expression is low or absent in almost all normal adult tissues. The *PRAME* transcript is highly expressed in acute and chronic leukemia patients and is usually associated with a favorable prognosis.

Aim: Our aim is to study the expression of *PRAME* gene in patients with acute myeloid leukemia (*AML*), and its association with clinical presentation and response to therapy.

Patients and Methods: Screening for *PRAME* gene expression was assessed using real-time reverse transcriptase polymerase chain reaction in bone marrow samples of 57 AML patients at diagnosis prior to therapy.

Results: *PRAME* positivity was found in 22/57 (38.6%) of patients. No significant correlation was found between PRAME gene positivity and any of the clinical or hematological variables except for percentage of BM promonocytes at diagnosis. *PRAME* positive patients showed good response to treatment compared to those who were PRAME negative. The rate of CR was 61.11% compared to 48.28% in *PRAME* positive and *PRAME* negative patients, respectively (p-value = 0.391). There was an increase in the overall survival among the *PRAME* positive compared to the *PRAME* negative group although the difference was not significant (p-value = 0.160).

Conclusions: Our results suggested that *PRAME* expression may be associated with better response to therapy and a trend towards improved survival. Study of a larger number of cases is needed for potential statistical significance.

Key Words: *PRAME – AML – qRT-PCR.*

INTRODUCTION

Acute myeloid leukemia (AML) is a genetically heterogeneous disease with accumulation of acquired genetic alterations in hematopoietic progenitor cells that disturb normal mechanisms of cell growth, proliferation and differentiation **[1]**. It is a disease with a high mortality rate. Therefore, analyzing the presence or absence of specific molecular abnormalities is not only useful for determining the overall prognosis more precisely, but can also guide treatment **[2]**.

The preferentially expressed antigen of melanoma (*PRAME*) was described as a tumor-associated antigen against a melanoma surface antigen recognized by autologous cytotoxic T cells and has been proposed as a promising candidate for tumor immunotherapy **[3]**. *PRAME* is only expressed at very low levels in normal tissues **[4]**. The physiologic role of *PRAME* remains controversial. *PRAME* was reported as a dominant repressor of retinoic acid receptor signaling, which induces proliferation arrest, differentiation and apoptosis **[5,6]**. Recently, *PRAME* has been shown to inhibit myeloid differentiation in normal hematopoietic and leukemic progenitor cells **[7]**.

PRAME is over expressed in a wide variety of malignancies **[8]**. The frequency of *PRAME* expression was reported to be about 30-64% in AML, 22-60% in chronic myeloid leukemia (CML), and 17-42% in acute lymphoblastic leukemia (ALL) **[9-11]**. High *PRAME* expression has been found to correlate with the stage of melanoma lesions **[12]**, with shorter overall survival of neuroblastoma **[13]**, and serous ovarian adenocarcinoma patients **[14]**. High *PRAME* mRNA levels were also shown to be an independent prognostic factor of poor clinical outcome in breast cancer **[8,15]**. However, the clinical relevance of *PRAME* expression in hematopoietic malignancies has been inconsistent. In acute myeloid leukemia, *PRAME* was

associated with favorable prognosis **[3,6,16,17]**. A high level of *PRAME* was also found to be associated with longer relapse free survival in cytogenetically normal AML **[18]**. It was further revealed that patients with acute promyelocytic leukemia with low *PRAME* expression had an unfavorable outcome **[19]**. However, in chronic myeloid leukemia, *PRAME* expression increased with disease progression and was associated with poorer therapeutic responses **[7]**.

Quantification of *PRAME* transcript in acute myeloid leukemia could be used to monitor minimal residual disease **[20]**.

In the current study, we investigated a group of adult with AML to assess the expression PRAME gene at diagnosis and to correlate its expression with the clinical response.

PATIENTS AND METHODS

The study was performed on 57 adult de novo AML patients including 27 males and 30 females; their ages ranged from 18 to 67 with a median of 37 years. They presented to the Medical Oncology clinics, National Cancer Institute (NCI), Cairo University, during the time period from October 2013 to October 2014. In addition after obtaining written consent, bone marrow samples were taken from 7 healthy donors and used as controls for gene expression, they presented as bone marrow transplantation donors to Nasser Institute for Research and Treatment, Ministry of Health, Cairo, Egypt. The study was approved by the Institutional Review Board (IRB) of the NCI and was conducted according to Helsinki declaration of studies performed on human subjects. A written informed consent was obtained from all patients and controls.

Patients were diagnosed according to standard methods including complete blood picture, bone marrow aspirate, cytochemistry as indicated, immunophenotyping, cytogenetics and molecular genetics. Patients' clinical characteristics are shown in Table (1). All patients received standard induction chemotherapy with 3+7 protocol (idarubicin as short infusion for 3 days with cytarabine 100mg/m^2 continuous infusion for 7 days). Patients, who achieved CR, according to their risk stratification, were offered consolidation with high dose cytarabine and HLA matching followed by allogeneic bone marrow transplantation. Refractory cases received re-induction with high dose cytarabine based regimen.

Clinical end points:

Complete remission (CR) is defined as bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 x 10^9/L; platelet count >100 x 10^9/L; independence of red cell transfusions, and treatment failure which includes either resistant disease or relapse. Resistant disease is defined as failure to achieve CR following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination. Relapse is defined as bone marrow blasts ≥5 percent; or reappearance of blasts in the blood; or development of extramedullary disease **[21]**.

Disease-free survival (DFS) was defined only for those patients achieving a CR. It was measured from the CR date until the date of relapse or death, regardless of cause, censoring for patients alive at last follow-up. Overall survival (OS) was measured from the protocol on study date until the date of death regardless of cause, censoring for patients alive at last follow-up **[21]**.

RNA extraction and cDNA synthesis:

RNA extraction was done using QIAamp RNA blood Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The amount of RNA was measured by nanodrop spectrophotometer. Subsequently, 1.0µg RNA was reverse transcribed into cDNA in 20µL reaction using random hexamer according to manufacturer's instructions (High capacity cDNA reverse transcription kit) (Applied Biosystems, USA).

Quantitative real-time PCR (qT-PCR):

The mRNA expression levels of *PRAME* and the endogenous housekeeping gene *GAPDH*, as a reference, were quantified by the TaqMan Probe Assay (QuantiFast Probe Assay Qiagen kits) according to the manufacturer's instructions. The assay IDs were (Hs_PRAME_FAM_1), Cat. no.: QF00326137 and (Hs_ GAPDH_ MAX_2), Cat. no.: QF00531132) using Applied Biosystems 7500 Instrument (Applied Biosystems, USA). The cycle number at which the reaction crossed an arbitrarily placed threshold (CT) was determined and the relative expression of PRAME regarding a housekeeping

gene (GAPDH), used as a control of RNA quality, was calculated using the equation $2^{-\Delta\Delta Ct}$ where ΔCT = CTPRAME – CTGAPDH and ΔΔCT= ΔCTsample –ΔCTHealthy BM (median) **[19,22]**. In order to carry out the ΔΔCT correction, we selected the median ΔCT value obtained in bone marrow samples from seven healthy donors. PRAME expression values were thus expressed as relative units (RU), where one RU is equivalent to the PRAME expression of the healthy donor bone marrow sample with the median ΔCT value **[19]**.

Statistical analysis:

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago A, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric *t*-test). Logistic regression was carried out using odds ratio. Cox-regression method was used to calculate the Hazard ratio (HR) with it 95% confidence interval (CI).

Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. A *p*-value <0.05 was considered significant.

RESULTS

In this study, the expression of *PRAME* mRNA was investigated in 57 adult AML patients by real-time PCR. *PRAME* expression was found in 22/57 (38.6%) of patients. For the $2^{-\Delta\Delta Ct}$ method we used bone marrow samples from seven healthy donors as a calibrator. The median ΔCT (CT_{PRAME}-CT_{GAPDH}) value in healthy samples was 8.17 (range 5.71 to 9.63). For AML samples, the median ΔCT value was 9.31 (range, 3.04 to 16.79) and the median *PRAME* expression was 0.566 RU (range, 0.003-35.017).

No significant correlation could be observed between *PRAME* expression and clinical characteristics such as age, sex, initial white blood cell count, hemoglobin level, platelet count, FAB-type, BM blasts and the percentage of blasts in the peripheral blood at the diagnosis. Statistical significance was encountered with stem cell marker CD34 expression on blast cells and with BM promonocytes in M4 and M5 at diagnosis (Table 1).

Table (1): Correlation of clinical and hematologic parameters with *PRAME* expression in AML patients.

Parameter		*PRAME* gene expression		*p*-value
		+ve (n=22/57)	–ve (n=35/57)	
Age (years)	Median (range)	41.5 (23-66)	35.0 (18-67)	0.112
Gender	Male (No - %) Female (No - %)	13-48.1% 9-30.0%	14-51.9% 21-70.0%	0.160
Organomegaly	Present (No - %)	9/17 (40.9%)	13/31 59.1%	0.464
Lymphadenopathy	Present (No - %)	4/17 (40.0%)	6/30 (60.0%)	0.776
TLC x 10^9/L*	Median (Range)	81.0 (3.6-175.0)	29.9 (0.8-207.0)	0.342
Hb (gm/dL)*	Mean ± S.D	7.1±2.2	7.2±2.1	0.920
Platelets x 10^9/L*	Median (Range)	32.0 (7.0-458.0)	25.0 (5.0-269.0)	0.734
PB blast %	No. Median (Range)	15 60.0 (4.0-97.0)	25 60.0 (30.0-98.0)	0.761
BM promonocytes	No. Median (Range)	9 29.0 (8.0-53.0)	14 11.5 (2.0-78.0)	0.016
BM blast %	No. Median (Range)	22 47.0 (12.0-90.0)	35 61.0 (10.0-90.0)	0.140
CD34 expression	CD34 +ve CD34 –ve	9 (42.9%) 12 (57.1%)	23 (69.7%) 10 (30.3%)	0.050
FAB classification	M0, M1 M2 M4, M5	5 (31.3%) 7 (43.8) 10 (40.0%)	11 (68.8%) 9 (56.3%) 15 (60.0%)	0.754

* Data available for 19 *PRAME* +ve and 31 *PRAME* –ve cases.

Out of the 57 studied patients, 47 were evaluable for response. Complete remission (CR) was achieved in 25 patients (53.19%), 5 (10.64%) failed to achieve CR, and 17 (36.17%) died during induction. In patients with positive *PRAME* expression, 61.11% responded to induction chemotherapy compared to 48.27% of patients with negative *PRAME* expression, Positive *PRAME* expressers had a statistically higher CR but this difference was not statistically significant (p=0.391).

Patients were followed-up for 0.03 to 15.76 with a median of 4.08 months; 39 (76.47%) patients died. The overall survival ranged from 1.021 to 7.137 with a median of 4.079 months. During the follow-up period 13/22 (59.09%) cases relapsed. The DFS ranged from 3.278 to 14.722 with a median of 9 months.

At the end of follow-up period: 13 patient (65.0%) in the positive PRAME group and 26 patients (83.87%) in the negative PRAME group died. Median OS was 6.4months (0.0-19.462) and 3.3months (0.35-6.163) respectively with no statistical significance difference between the two groups (p=0.160) (Fig. 1).

In PRAME positive group 6/10 (60.0%) patients relapsed with a median DFS of 9 months (2.8-15.19); the relapse rate in the PRAME negative group was 7/12 (58.33%), with a median DFS of 5 months (0.0-13.48) (p=0.627) (Fig. 2).

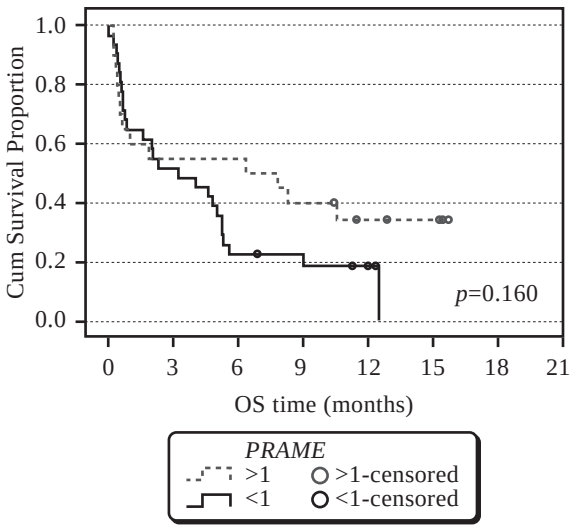

Fig. (1): Impact of *PRAME* expression on overall survival in AML patients.

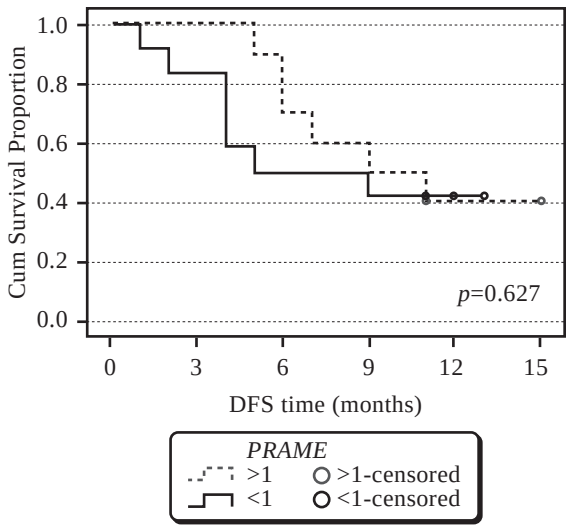

Fig. (2): Impact of *PRAME* expression on disease free survival in AML patients.

DISCUSSION

Preferentially expressed antigen of melanoma (PRAME) is expressed in a wide variety of tumors, but in contrast with most other tumor associated antigens, it is also expressed in leukemia. The physiologic role of PRAME has not yet been completely discovered **[23]**.

We have investigated the prevalence of *PRAME* expression in AML patients and seven normal bone marrow samples which served as controls. An increase of >1-log over the upper limit of normal bone marrow was defined as positive **[24]**.

In our study, the mean *PRAME* relative expression detected in the control group was 8.11±1.21 (range 5.71–9.63). Zhu et al. **[25]** found that the *PRAME* transcripts were 0-1.46% (median 0.18%) in 20 control cases using based real-time quantitative PCR with Eva Green dye; also Qin et al. **[17]** reported that all 22 normal bone marrow samples expressed *PRAME* mRNA and the upper limit was 0.28% using the TaqMan based real-time quantitative PCR methods. Steinbach et al., **[26]** were able to detect very low levels of *PRAME* in normal blood and bone marrow cells including CD34+ stem cells which might constitute a problem in using PRAME for tumor immunotherapy.

On the contrary, Khorshed et al. **[27]** found that *PRAME* mRNA transcript done by qualitative RT-PCR was not detected in normal bone marrow cells obtained from 10 healthy donors. Similar observation demonstrated highly tumor-restricted expression for *PRAME* gene **[10,28,29]**.

In the current study, we reported *PRAME* expression in 22/57 (38.6%) AML patients. This finding is comparable to that reported by Zhou et al., (42.9%) **[28]** and Tajeddine et al. (40%) **[30]**. Higher values were reported by Khorshedet al. (55%), Qin et al., (55.4%), Zhu et al.,(64%), Greiner et al., (64%) **[10,24,27,29]**. While lower values were reported by Paydas et al. (30%) **[11]**, Van Baren et al., (25%) **[3]**. In a study done by Matsushita et al. **[16]** on Japanese patients with leukemia and lymphoma, *PRAME* expression was found to be 42% using the semi quantitative RT-PCR. Mcelwaine et al. **[31]** reported that *PRAME* is highly expressed in Down syndrome with acute megakaryoblastic leukemia transformation but not in myeloproliferative disorder.

In the current study, no significant association was found between *PRAME* expression and age, gender, white blood cell count, hemoglobin level, platelet count, or percentage blasts in the peripheral blood at diagnosis; border line significant correlation was encountered with stem cell marker CD34 expression on blast cells. These results are in agreement with previous reports **[11,27,28,29]**. In our study out of 22 *PRAME* positive patients, 9 were M4, 7 M2, 4 M1, one M0 and one was M5, with *PRAME* expression more frequent in M4 FAB subtype but the numbers were too small to give statistical significance. However *PRAME* expression showed significant correlation with BM promonocytes at diagnosis. Previous studies demonstrated association of *PRAME* expression with M2 **[27]**, M1-M4 **[25,29]**, M2 with t(8;21) **[17,25]** and M3 with *PML-RAR* alpha fusion gene **[17,29]**.

As regards clinical data, no significant association was found between *PRAME* expression and organomegaly or lymphadenopathy. This is in agreement with Paydas et al. **[11]** and partial agreement with Khorshed et al. [27] who reported a significant association with hepatomegaly (p=0.036), but no association with splenomegaly or lymphadenopathy.

In the current study CR rate was higher in the *PRAME* +ve group though it did not achieve statistical significance. This is in line with Zhu et al. **[25,28]** who reported that high *PRAME* expression is a favorable prognostic marker. In contrast, some studies reported *PRAME* expression to be associated with lower CR remission rate **[27,32]** while others denied any impact of *PRAME* expression on response to therapy **[11,33]**.

Regarding overall survival, number of deaths was higher in low *PRAME* expressers though statistically insignificant (p-value 0.160). Overall survival was longer among the high *PRAME* compared to the low *PRAME* expressers though the difference was insignificant. Also, DFS was comparable for both groups (p-value 0.627). Previous reports on the association of *PRAME* expression with survival are controversial. Greiner et al. **[34]** found a significant correlation between high m-RNA levels of *PRAME* and longer overall survival. On the contrary, Khorshed et al. **[27]**, found an increase in the overall survival among the *PRAME* negative compared to the *PRAME* positive group although the difference was not significant (p-value = 0.06).

Also in a pediatric study done by Steinbach et al. **[26]**, overall and disease-free survival was associated with high *PRAME* expression. Accordingly, *PRAME* expression as an independent prognostic indicator could not be clearly highlighted. This is in agreement with most studies on acute leukemia cases that could not draw a similar conclusion **[11,26,33]**. On the contrary, high *PRAME* expression was found to be an independent prognostic marker of poor outcome in breast cancer and neuroblastoma **[13]**. Additionally, Radich et al. **[35]** suggested PRAME as a predictive factor to determine the blastic phase in CML cases.

In conclusion, PRAME is a tumor-associated antigen and its level of expression should be detected during the course of the disease. *PRAME* may be a suitable indicator to predict remission or relapse. However, more studies should aim at detailed understanding of the mechanisms of PRAME actions and its use in predicting progression and the remission of diseases.

REFERENCES

1- Meenaghan T, Dowling M, Kelly M. Acute leukaemia: making sense of a complex blood cancer. Br. J. Nurs. 2012; 21 (76): 78-83.

2- Moore JO, George SL, Dodge RK, Amrein PC, Powell BL, Kolitz JE, et al. Sequential multiagent chemotherapy is not superior to high-dose cytarabine alone aspostremission intensification therapy for acute myeloid leukemia in adults under 60 years of age: Cancer and Leukemia Group B Study 9222. Blood. 2005; 105: 3420-7.

3- Van Baren N, Chambost H, Ferrant A, Michaux L, Ikeda H, Millard I, et al. PRAME, a gene encoding an antigen recognized on a human melanoma by cytolytic T cells, is expressed in acute leukaemia cells. Br. J. Haematol. 1998; 102: 1376-9.

4- Wadelin F, Fulton J, McEwan PA, Spriggs KA, Emsley J, Heery DM. Leucine-rich repeat protein PRAME: expression, potential functions and clinical implications for leukaemia. Mol. Cancer. 2010; 9: 226.

5- Epping MT, Wang L, Edel MJ, Carlée L, Hernandez M, Bernards R. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. Cell. 2005; 122: 835-47.

6- Epping MT, Wang L, Plumb JA, Lieb M, Gronemeyer H, Brown R, Bernards R. A functional genetic screen identifies retinoic acid signaling as a target of histone deacetylase inhibitors. Proc. Natl. Acad. Sci. USA. 2007; 104: 17777- 17782.

7- Oehler VG, Guthrie KA, Cummings CL, Sabo K, Wood BL, Gooley T, et al. The preferentially expressed antigen in melanoma (PRAME) inhibits myeloid differentiation in normal hematopoietic and leukemic progenitor cells. Blood. 2009; 114: 3299-308.

8- Epping MT, Hart AA, Glas AM, Krijgsman O, Bernards R. PRAME expression and clinical outcome of breast cancer. Br. J. Cancer. 2008; 99: 398-403.

9- Paydas S, Tanriverdi K, Yavuz S, Seydaoglu G. PRAME mRNA levels in cases with chronic leukemia: Clinical importance and review of the literature. Leuk. Res. 2007; 31: 365-9.

10- Greiner J, Ringhoffer M, Taniguchi M, Li L, Schmitt A, Shiku H, et al. mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the development of specific immunotherapies. Int. J. Cancer. 2004; 108: 704-11.

11- Paydas S, Tanriverdi K, Yavuz S, Disel U, Baslamisli F, Burgut R. PRAME mRNA levels in cases with acute leukemia: Clinical importance and future prospects. Am. J. Hematol. 2005; 79: 257-61.

12- Haqq C, Nosrati M, Sudilovsky D, Crothers J, Khodabakhsh D, Pulliam BL, et al. The gene expression signatures of melanoma progression. Proc. Natl. Acad. Sci. USA. 2005; 102: 6092-7.

13- Oberthuer A, Hero B, Spitz R, Berthold F, Fischer M. The tumor-associated antigen PRAME is universally expressed in high-stage neuroblastoma and associated with poor outcome. Clin. Cancer Res. 2004; 10: 4307-13.

14- Partheen K, Levan K, Osterberg L, Claesson I, Fallenius G, Sundfeldt K, et al. Four potential biomarkers as prognostic factors in stage III serous ovarian adenocarcinomas. Int. J. Cancer. 2008; 123: 2130-7.

15- Doolan P, Clynes M, Kennedy S, Mehta JP, Crown J, O'Driscoll L. Prevalence and prognostic and predictive relevance of PRAME in breast cancer. Breast Cancer Res. Treat. 2008; 109: 359-65.

16- Matsushita M, Ikeda H, Kizaki M, Okamoto S, Oqasawara M, Ikeda Y, et al. Quantitative monitoring of the PRAME gene for the detection of minimal residual disease in leukemia. Br.J. Hematol. 2001; 112: 916-26.

17- Qin YZ, Li JL, Zhu HH, Li LD, Chang Y, Hao L, et al. PRAME Mrna expression in newly diagnosed acute myeloid leukemia patients and its application to monitoring minimal residual disease. Zhonghua Xue Ye Xue Za Zhi. 2008; 29 (7): 441-5.

18- Santamaría CM, Chillón MC, García-Sanz R, Perez C, Caballero MD, Ramos F, et al. Molecular stratification model for prognosis in cytogenetically normal acute myeloid leukemia. Blood. 2009; 114: 148-52.

19- Santamaría C, Chillón MC, García-Sanz R, Balanzategui A, Sarasquete ME, Alcoceba M, et al. The relevance of preferentially expressed antigen of melanoma (PRAME) as a marker of disease activity and prognosis in acute promyelocytic leukemia. Haematologica. 2008; 93: 1797-805.

20- Steinbach D, Bader P, Willasch A, Bartholomae S, Debatin KM, Zimmermann M, et al. Prospective validation of a new method of monitoring minimal residual disease in childhood acute myelogenous leukemia. Clin. Cancer Res. 2015 Mar 15; 21 (6): 1353-9.

21- Cheson BD, Bennett JM, Kopecky KJ, Büchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J. Clin. Oncol. 2003; 21: 4642.

22- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real- time quantitative PCR and the 2(–Delta DeltaC(T)) Method. Methods. 2001 Dec; 25 (4): 402–408.

23- Xu Y, Rong LJ, Meng SL, Hou FL, Zhang JH, Pan G. PRAME promotes in vitro leukemia cells death by regulating S100A4/p53 signaling. Eur. Rev. Med. Pharmacol. Sci. 2016; 20 (6): 1057-63.

24- Qin YZ, Zhu H, Li J, Lu X, Li L, Ruan G, et al. Expression patterns of WT1 and PRAME in acute myeloid leukemia patients and their usefulness for monitoring minimal residual disease. Leuk Res. 2009 Mar; 33 (3): 384-90.

25- Zhu ZH, Qian J, Lin J, Yao DM, Qian Z, Wang YL, et al. Quantification of the PRAME transcripts in

patients with acute myeloid leukemia. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2010; 27 (2): 149-52.

26- Steinbach D, Hermann J, Viehmann S, Zintl F, Gruhn B. Clinical implications of PRAME gene expression in childhood acute myeloid leukemia. Cancer Genet Cytogenet. 2002; 133: 118-23.

27- Amira M Khorshed, Ghada I Mossallam, Magda M Assem, Thoraya M Abdel Hamid, Gihane Abdel Basset, Roxane E Shafik. Expression of PRAME gene in Egyptian adult acute myeloid leukaemia and its correlation with clinical response. Life Science 2012; 9 (2): 1117-1121.

28- Zhou PY, Li WJ, Wei CX, Zhou Z. Expression of PRAME gene in adult acute leukemia and its significance in prognosis. Zhongguo Shi Zhonghua Xue Ye Xue Za Zhi. 2007; 15 (6): 1177-81.

29- Zhu YL, Liu J, Zhu P, DU JW, Zhang Y, GuJY. Expression of PRAME gene in acute leukemia and its clinical significance. Zhongguo Shi Zhonghua Xue Ye Xue Za Zhi. 2007; 15 (6): 1144-9.

30- Tajeddine N, Millard I, Gailly P Gala JL. Real-time RT-PCR quantification of PRAME gene expression for monitoring minimal residual disease in acute myeloblastic leukemia. Clin. Chem. Lab. Med. 2006; 44 (5): 548-55.

31- Mcelwaine S, Mulligan C, Groet J, et al. Microarry transcript profiling distinguishes the transient from the acute type of megakaryoblastic leukemia in Down syndrome, revealing PRAME as a discriminating marker. Br. J. Hematol. 2004; 125: 729.

32- Kawano R, Karube K, Kikuchi M, et al. Oncogene associated cDNA micro array analysis shows PRAME gene expression is a marker for response to anthracycline containing chemotherapy in patient with diffuse large B-cell lymphoma. J. Clin. Exp. Hematopathol. 2009; 49 (1): 1-7.

33- Spanaki A, Perdikogianni C, Linardakis E, Kalmanti M. Quantitative assessment of PRAME expression in diagnosis of childhood acute leukemia. Leuk. Res. 2007; 31: 639-42.

34- Greiner J, Schmitt M, Li L, et al. Expression of tumor-associated antigens in acute myeloid leukemia: Implications for specific immunotherapeutic approaches. Blood. 2006; 108 (13): 4109-17.

35- Radich JP, Dai H, Mao M, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc. Natl. Acad. Sci. USA. 2006; 103: 2794-9.