The Prognostic Significance of the BMI-1 and BAALC Genes in Adult Patients with Acute Myeloid Leukemia

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Abstract The aim of this work is to investigate the different expression patterns of B cell-specifics moloney murine Leukemia virus integration site-1 (BMI-1) and brain and acute leukemia, cytoplasmic (BAALC) genes, their prognostic and clinical significance in newly diagnosed cytogenetically heterogenous adult acute myeloid leukemia patients. BMI-1 and BAALC expression was detected in the bone marrow of patients using quantitative real-time reverse transcription polymerase chain reaction with cut off value set at 50th percentile for both genes. BMI-1 and BAALC overexpression was detected in 50% of cases which suggest their potential as molecular markers. A statistical significant correlation was found between BMI-1 expression with hepatomegaly (P value = 0.007), hemoglobin level-grouped (P value = 0.047) and cytogenetic risk groups (P value = 0.036). There was a statistically significant correlation between BAALC and age (P value = 0.015), lymphadenopathy (P value = 0.043),

CD34 expression (P value = 0.003) and near statistical significance with FAB sub-groups (P value = 0.054). No statistical significance was noted for other hematological findings and response to treatment except for BAALC gene and treatment response (P value = 0.014). No statistical significance in overall survival and disease free survival for both genes was found. Their prospective screening in combination with other molecular markers can help refine myeloid leukemia staging and prognosis toward optimizing therapeutic interventions.

Keywords AML · Acute myeloid leukemia · BMI-1 · B-cell-specifics moloney murine Leukemia virus integration site-1 · BAALC · Brain and acute leukemia cytoplasmic · Prognosis · Overall survival

Introduction

Clonal karyotypic abnormalities were one of the first imperative factors predicting clinical outcome in AML, and were used to guide risk-adapted treatment strategies; nonetheless, nearly 45% of all patients lack any karyotypic abnormality, thus the majority of patients fall into an intermediate risk category, making cytogenetics alone insufficient to accurately assess prognosis for all AML patients [1].

The B-cell specifics moloney murine Leukemia virus integration site-1 (BMI-1) gene maps on the short arm of chromosome 10 (10p11.23). Through repression of target genes, BMI-1 is implicated in the uncontrolled proliferation of hematopoietic cells, self-renewal of hematopoietic stem cell and cancer stem cell, leading to malignant transformation [2].

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The brain and acute leukemia, cytoplasmic (BAALC) maps on the long arm of chromosome 8 at 8q22.3. It encodes for a protein physiologically implicated in neuroectodermal and hematopoietic development [3]. Though the site and cellular mechanisms of its effects are still unclear, the BAALC gene has a prognostic significance in cytogenetically normal-AML (CN-AML). It has been associated with significantly higher refractoriness to induction treatment, lower rates of complete remission (CR), poor overall survival (OS) and disease-free survival (DFS) for patients with high BAALC expression independent of other prognostic molecular markers with a gene expression signature consistent with less differentiated AML blasts [4–6].

Both BMI-1 and BAALC are new targets for therapy in malignancies characterized by their overexpression.

This study was designed to investigate the impact of BAALC and BMI-1 expression on the prognosis of different cytogenetic categories AML patients and if they would serve as biomarkers to predict disease aggressiveness and the possible selection of candidates for target therapy.

Patients and Methods

This study was carried out on 95 adult newly diagnosed adult AML patients (47 male and 48 female); age ranged from 18 to 70 years with a median of 40 years. They presented to the medical oncology clinics, National Cancer Institute, Cairo University, during the period from July 2014 to July 2015. Written informed consent was obtained from all patients.

Also after obtaining written consent, bone marrow samples were taken from 20 healthy age and sex-matched donors and used as controls for gene expression. The controls were bone marrow transplantation donors.

The study was approved by the International Review Board (IRB) ethical committee, review board of NCI, Cairo University in accordance with Helsinki guidelines for the protection of human subjects.

Patients were diagnosed according to standard methods in compliance with the French–American–British (FAB) and the World Health Organization (WHO) criteria. Patients' clinical characteristics are shown in Table 1.

All patients received standard induction chemotherapy with 3 + 7 protocol (idarubicin as a short infusion for 3 days with cytarabine 100 mg/m² 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.

Table 1 Clinical and hematological characteristics of adult AML patients

Parameter	Frequency/total (%)
Male	47/95 (49.5%)
Female	48/95 (50.5%)
< 50	67/95 (71%)
≥ 50	28/95 (29%)
Hepatomegaly	38/95 (40%)
Splenomegaly	33/95 (34.7%)
Lymphadenopathy	20/95 (21.1%)
White blood cells count ($\times 10^9/L$)	
Mean $\pm$ SD	52.7 $\pm$ 69.2
Median (range)	24.1 (1–345)
< 30	51/95 (53.7%)
30–100	28/95 (29.5%)
> 100	16/95 (16.8%)
Haemoglobin level (gm/dL)	
Mean $\pm$ SD	7.9 $\pm$ 1.8
Median (range)	7.7 (3.1–12.3)
< 8	53/95 (55.8%)
≥ 8	42/95 (44.2%)
Platelets count ($\times 10^9/L$)	
Mean $\pm$ SD	58.7 $\pm$ 71.9
Median (range)	38 (1–436)
< 100	81 (85.3%)
≥ 100	14 (14.7%)
Peripheral blood blasts	
Mean $\pm$ SD	51.4% $\pm$ 26.6
Median (range)	51% (0–98)
Bone marrow blasts	
Mean $\pm$ SD	62.2% $\pm$ 20.3%
Median (range)	67% (20–97)
< 50	70 (73.6%)
≥ 50	25 (26.3%)
Bone marrow cellularity	
Normocellular marrow	15/95 (15.8%)
Hypercellular marrow	80/95 (84.2%)
Phenotype	
Myeloid (M1, M2, M3, M7)	68/95 ^a (71.6%)
Myeloid with monocytic (M4, M5)	27/95 (28.4)
CD34	
Positive	47/90 ^b (52.2%)
Negative	43/90 (47.8%)
FAB classification	
M1	20/95 (21%)
M2	37/95 (38.9%)
M3	9/95 (9.4%)
M4	22/95 (23.2%)
M5	5/95 (5.2%)
M7	2/95 (2.1%)
Karyotyping	

Table 1 continued

Parameter	Frequency/total (%)
Normal karyotype	19/62 ^c (30.6%)
t(15,17)	9/62 (14.5%)
t(8,21)	8/62 (12.9%)
Inv(16)	1/62 (1.6%)
t(9,22)	10/62 (16.1%)
Complex karyotyping ^c	6/62 (9.7%)
Other abnormalities	9/62 (14.5%)
FLT3	
Mutant	14/95 (14.7%)
Wild	81/95 (85.3%)
Cytogenetic risk groups	
Favorable	18/62 (29%)
Intermediate	21/62 (33.9%)
Unfavorable	23/62 (37.1%)
Early response treatment	
Achievement of CR	56/85 ^d (65.9%)
Early death before day 28	29/85 (34.12%)

^aTotal number of AML cases^bTotal number of AML cases with valid CD34 results^cNumber of cases with valid cytogenetic results^dTotal number of AML cases with available response to treatment data^eComplex karyotyping: show 3 or more cytogenetic aberrations; other abnormalities include – 2, – 3, – 7, – 11, – 13, – 21, – 22, + 8, + 13, + 17 and + 21

Clinical Endpoints

Complete remission (CR) was defined as bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count > $1.0 \times 10^9/L$; platelet count > $100 \times 10^9/L$; and independence of red cell transfusions.

Treatment failure included either resistant disease or relapse. The resistant disease was defined as the failure to achieve CR following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination. Relapse was defined as bone marrow blasts $\geq 5\%$; or reappearance of blasts in the blood; or development of extramedullary disease [7].

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 [7].

Quantitative Real-Time Reverse-Transcription-Polymerase Chain Reaction (qRT-PCR) Amplification of BMI-1, BAALC

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

BMI-1 gene expression and the endogenous house-keeping gene $\beta 2$ M, as a reference gene, were quantified by *QuantiTect SYBR Green PCR kit* (QIAGEN) (catalog number: 204141) and BAALC gene expression and the endogenous housekeeping gene GPI, as a reference gene, were quantified by the Taqman Probe Assay (Applied Biosystems, Life Technologies, USA) (catalog number: 4440043) according to the manufacturer's instructions.

Available sequences and cycling conditions are given in Table 2.

The cycle number at which the reaction crossed an arbitrarily placed threshold (CT) was determined and the

Table 2 Primers and probes sequences and cycling conditions

BMI-1 primers	Forward: 5'-TAA GCA TTG GGC CAT AGT-3' Reverse: 5'-ATT CTT TCC GTT GGT TGA-3'	Amplification was performed after initial incubation at 95 °C for 10 min in a 3-step cycle procedure (95 °C, 15 s; 60 °C, 60 s and 72 °C, 30 s) for 45 cycles
$\beta 2$ M primers	Forward: 5'-TAC ACT GAA TTC ACC CCCAC-3' Reverse: 5'-CAT CCA ATC CAA ATG CGGCA-3'	
BAALC gene	Forward: 5'GCCCTCTGACCCAGAAACAG Reverse: 5'CTTTTGCAGGCATTCTCTTAGCA	Amplification was performed after initial incubation at 95 °C for 10 min in a 2-step cycle procedure (95 °C, 15 s and 60 °C, 60 s) for 45 cycles
GPI gene	Forward: 5'TCTTCGATGCCAACAAGGAC Reverse: 5'GCATCACGTCTCCGTCAC	
Taqman Probe BAALC	5'-FAM-CTCTTTTAGCCTCTGTGGTCTGAAGGCCAT-TAMRA	
Taqman Probe GPI	5'-HEX-TTCAGCTTGACCCTCAACACCAAC-TAMRA	

relative expression of BMI-1 and BAALC genes regarding the housekeeping gene ($\beta 2M$ and GPI respectively), used as a control of RNA quality, among the leukemic and normal samples, was calculated using the equation $2^{-\Delta\Delta CT}$.

Statistical Analysis

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) versus 23. Numerical data were summarized using means and standard deviations or medians and ranges, as appropriate. Categorical data were summarized as numbers and percentages. Numerical data were explored for normality using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. The exploration of data revealed that the collected values were not normally distributed. Comparisons between the groups were done by the Mann-Whitney test. Chi square or Fisher's tests were used to compare the groups with respect to categorical data, as appropriate. To measure the strength of the association between numeric variables, Spearman's correlation coefficients were calculated. Survival was estimated using the Kaplan and Meier method. Differences between the survival curves were assessed for statistical significance with the log-rank test. All P values are two-sided. P values < 0.05 were considered significant.

Results

Determination of Cut-Off Values for High and Low Expressers

BMI-1 and BAALC expression were studied in the 95 AML patients and 20 normal healthy controls. An increase of > 1 -log over the upper limit of the normal bone marrow was defined as positive for BMI-1 or BAALC transcript, thus, the RQ of the studied controls < 1 for both gene expression.

The cohort was subdivided into quartiles of expression of each gene. The 50th percentile (median) was taken as a cut-off value (234 for BMI-1 and 4.83 for BAALC) below which the patient is considered as low expresser and above it, the patient is considered as high expresser.

Associations of BMI-1 and BAALC Expression with Clinical and Molecular Characteristics

No significant correlation could be observed between high and low expressions for both genes (BAALC and BMI-1) regarding sex, splenomegaly, initial white blood cell count, platelet count, BM cellularity, BM blasts and FLT3 mutational status in AML patients.

Low BMI-1 expressers had statistically significant hepatomegaly ($P = 0.007$) while high BMI-1 expressers were in the favorable cytogenetic risk group ($P < 0.036$). High BMI-1 expressers tended to have a lower Hb level (< 8) ($P = 0.081$) as shown in Table 3.

High BAALC patients presented with statistically significant lymphadenopathy ($P = 0.043$) and CD34 positivity ($P = 0.003$). Also, high BAALC expression was associated with the more immature FAB subtypes (M1/M2) compared to the monocytic differentiated FAB (M4 and M5) ($P = 0.054$).

Prognostic Value of BMI-1 and BAALC Expression

There was no statistically significant difference in BMI-1 levels concerning early treatment response (CR and early death), DFS and OS. However, high BAALC expressers achieved CR more often than low BAALC expressers (77.8% vs 52.5%; $P \leq 0.014$) as shown in Table 4.

The 12 months survival was 44.3% with a median observation time of 7.1 months. The OS of low BMI-1 and low BAALC expressers was worse than high expressers of both genes but not reaching statistical significance ($P = 0.360$ and 0.246 respectively). Similarly, the 12 months DFS was 37.8% with a median observation time of 7 months. No significant statistical differences in BMI-1 and BAALC levels with respect to DFS ($P = 0.398$ and 0.483 respectively) as shown in Fig. 1.

Discussion

Many studies have shown that BMI-1 expression is frequently upregulated in various types of human cancers, including lung cancer, ovarian cancer, acute myeloid leukemia, nasopharyngeal carcinoma, breast cancer, and neuroblastoma, which indicates that BMI-1 might play important roles in cancer initiation and progression [8–13].

It was proposed that BAALC promotes leukemia cell proliferation by activating the ERK pathway while blocking the differentiation of leukemia cells by preventing ERK-pathway mediated KLF4 accumulation in the nucleus. This unique dual function of BAALC might contribute to the formation of more aggressive leukemia. The inhibition of the ERK pathway together with the induction of KLF4 is a new promising strategy for refractory AML with high BAALC expression [14].

In our study, we analyzed the level of expression of BMI-1 and BAALC by quantitative real-time RT-PCR to gain insight into its correlated characteristics in AML. An increase of > 1 -log over the upper limit of normal bone marrow was defined as positive expression [15].

Table 3 Pretreatment clinical characteristics and molecular features at diagnosis according to BMI-1 and BAALC expression status in AML cases

Parameter	BMI-1 expression			BAALC expression			Total
	< 234 N (%)	≥ 234 N (%)	<i>P</i>	< 4.83 N (%)	≥ 4.83 N (%)	<i>P</i>	
Sex							
Male	24 (51.1%)	23 (48.9%)	0.759	22 (46.8%)	25 (53.2%)	0.607	47
Female	23 (47.9%)	25 (52.1%)		25 (52.1%)	23 (47.9%)		48
Hepatomegaly							
No	22 (38.6%)	35 (61.4%)	0.007	29 (50.9%)	28 (49%)	0.672	57
Yes	25 (65.8%)	13 (34.2%)		18 (47.4%)	20 (52.6%)		38
Splenomegaly							
No	27 (43.5%)	35 (56.5%)	0.093	32 (51.6%)	30 (48.4%)	0.514	62
Yes	20 (60.6%)	13 (39.4%)		15 (45.5%)	18 (54.5%)		33
Lymphadenopathy							
No	35 (46.7%)	40 (53.3%)	0.262	41 (54.6%)	34 (45.3%)	0.043	75
Yes	12 (60%)	8 (40.0%)		6 (30%)	14 (70%)		20
TLC-grouped							
< 30	24 (47.1%)	27 (52.9%)	0.812	27 (52.9%)	24 (47.1%)	0.692	51
30–100	14 (50%)	14 (50%)		12 (42.9%)	16 (57.1%)		28
> 100	9 (56.3%)	7 (43.8%)		8 (50%)	8 (50%)		16
Hemoglobin							
< 8	22 (41.5%)	31 (58.5%)	0.081	26 (49.1%)	27 (50.9%)	0.927	53
≥ 8	25 (59.5%)	17 (40.5%)		21 (50%)	21 (50%)		42
PLT—grouped							
< 100	39 (48.1%)	42 (51.9%)	0.534	41 (50.6%)	40 (49.4%)	0.592	81
≥ 100	8 (57.1%)	6 (42.9%)		6 (42.9%)	8 (57.1%)		14
BM cellularity							
Hyper	39 (48.8%)	41 (51.3%)	0.745	41 (51.3%)	39 (48.8%)	0.424	80
NC	8 (53.3%)	7 (46.7%)		6 (40%)	9 (60%)		15
BM blasts-grouped							
< 67% ^b	24 (51.1%)	23 (48.9%)	0.759	27 (57.4%)	20 (42.6%)	0.124	47
≥ 67%	23 (47.9%)	25 (52.1%)		20 (41.7%)	28 (58.3%)		48
FAB grouped							
Myeloid	33 (48.6%)	35 (51.5%)	0.618	29 (42.6%)	39 (57.4%)	0.054	68
Myeloid with monocytic	14 (51.9%)	13 (48.1%)		18 (66.7%)	9 (33.3%)		27
CD34 expression							
Negative	25 (58.1%)	18 (41.9%)	0.140	29 (67.4%)	14 (32.6%)	0.003	43
Positive	20 (42.6%)	27 (57.4%)		17 (36.2%)	30 (63.8%)		47
Cytogenetic risk groups							
Favorable	5 (27.8%)	13 (72.2%)	0.036	7 (38.9%)	11 (61.1%)	0.271	18
Intermediate	13 (61.9%)	8 (38.1%)		13 (61.9%)	8 (38.1%)		21
Poor	15 (65.2%)	8 (34.8%)		14 (60.9%)	9 (39.1%)		23
FLT3+							
Mutant	8 (57.1%)	6 (42.9%)	0.534	9 (64.3%)	5 (35.7%)	0.230	14
Wild	39 (48.1%)	42 (51.9%)		38 (46.9%)	43 (53.1%)		81
Response to treatment ^a							
CR	26 (61.9%)	30 (69.8%)	0.445	21 (52.5%)	35 (77.8%)	0.014	56
Early death	16 (38.1%)	13 (30.2%)		19 (47.5%)	10 (22.2%)		29

P ≤ 0.05 is considered significant^aOut of 85 AML cases with available response to treatment data^bBone marrow blasts median

Table 4 Clinical outcomes in patient subgroups defined according to expression levels of BMI-1 and BAALC

Outcome	Total	BMI-1			BAALC		
		< 234 (N = 44)	≥ 234 (N = 43)	<i>P</i>	< 4.83 (N = 42)	≥ 4.83 (N = 45)	<i>P</i>
CR				0.445			0.014
No.	56	26	30		21	35	
%		61.9	69.8		52.5	77.8	
DFS				0.398			0.483
Median, months	7.1	4	9.5		2.9	8	
12 months DFS (%)	37.8	36.7	39.4		40	37.3	
OS				0.360			0.246
Median, months	7.1	4.1	13.3		2.9	9.3	
12 month survival (%)	44.3	38.9	50		38.9	48.8	

According to the median level of expression, the patients were dichotomized into 2 groups: high (≥ 234 RQ) and low (< 234 RQ) BMI-1 expressers. Same cutoff value (median) was adopted by previous studies [16–18]. Similarly for BAALC, according to its median level of expression, the patients were divided into 2 groups: high (≥ 4.83) and low (< 4.83) BAALC expressers. Same cut-off value (median of BAALC expression) was adopted by the majority of the studies [3, 19, 20]. In contrast, Brand et al. [21] adopted the 30th percentile as a cut off while Haferlach et al. [22] adopted the 75th percentile as a cutoff.

There were no statistically significant differences in BMI-1 or BAALC gene expression levels concerning gender, initial TLC, platelet count, BM cellularity or BM or PB blasts at presentation. These results were in agreement with previous studies [17, 18, 23, 24]. However, Metzeler et al. [25] and Soliman et al. [26] found a statistically significant difference between high BAALC expressers and high TLC count. Also, Saudy et al. [17] found that BM blasts were significantly higher in high BMI-1 versus low BMI-1 gene expression groups.

To the best of our knowledge, no published studies had correlated between BMI-1 expression and hepatomegaly or splenomegaly or lymphadenopathy. BMI-1 expression tended to be lower in most cases of hepatomegaly and splenomegaly (P value = 0.007, 0.093 respectively).

BAALC gene expression tended to be higher in most cases of lymphadenopathy (P value = 0.043). This is consistent with the results previously encountered [26].

Our study revealed that high BMI-1 expressers were more likely to be in the favorable cytogenetic risk group (P value = 0.036). Grubach et al. [27] support this result as they, interestingly, reported frequent overexpression of BMI-1 in t(8,21) as well as inv(16) positive patients concluding that different leukemogenic pathways are involved in the *CBF*-mutated AMLs. This is contrary to the previous reports [2, 16, 18]. In agreement with Chowdhury et al. [16] and Saudy et al. [17], we found no significant

correlation between FAB classification and BMI-1 gene expression. Contrary to these findings, Sawa et al. [10] and Nishida et al. [18] reported that M0 was highest among AML subtypes in cases of BMI-1 gene overexpression raising the possibility that BMI-1 expression is negatively correlated with differentiation and contribute to leukemogenesis. Also in our cases, the BMI-1 levels were not significantly affected by the mutational status of FLT3 or CD34 expression status. These results were similar to that reported by Grubach et al. [27] and Nishida et al. [18] which point to actual deregulation within the leukemic cells.

Regarding BAALC, in agreement with Damiani et al. [3], there were no significant differences in BAALC levels concerning cytogenetic risk groups.

Our study failed to identify a correlation between high BAALC expression and the *FLT3-ITD* mutation which is consistent with results previously reported [3, 24, 25]. But Contrary to the finding of other studies [19, 28, 29].

Regarding CD34 expression, we found that high BAALC levels were frequently detected in CD34 positive cases (P value = 0.003). This was consistent with the hypothesis of its leukemogenic role in immature BM stem cell progenitors and may confirm partially the physiological expression of BAALC in CD34+ cells. This is in agreement with that encountered by [3, 30, 31].

There was a trend of high BAALC expression to be associated with the more immature FAB subtypes such as M1/M2 whereas the monocytic differentiated FAB correlated with low BAALC expression (P value ≤ 0.054). These data are in agreement with other studies [3, 19, 24, 29]. In contrast to these results, Eid et al. [32] and Soliman et al. [26] reported that there is no correlation between BAALC expression and different FAB subtypes.

In the present study, there was no significant difference in BMI-1 expression levels with respect to the early treatment response (achievement of CR and early death before day 28) as well as OS and DFS. This was in

(A) Kaplan-Meier curve of the effects of BMI-1 gene expression on overall survival of AML patients (*p* value 0.360)

(B) Kaplan-Meier curve of the effects of BAALC gene expression on overall survival of AML patients (*p* value 0.246)

(C) Kaplan-Meier curve of the effects of BMI-1 gene expression on disease free survival (DFS) of AML patients (*p* value 0.398)

(D) Kaplan-Meier curve of the effects of BAALC gene expression on disease free survival (DFS) of AML patients (*p* value 0.483)

Fig. 1 Kaplan–Meier curves showing the effects of BMI-1 and BAALC genes on overall survival and disease free survival of AML patients

agreement with Grubach et al. [27]. Saudy et al. [17] failed to demonstrate a correlation between high and low BMI-1 expression groups with the clinical outcome as single variate but by using multivariate analysis, they reported that higher BMI-1 expression group had a significantly shorter OS and DFS than low BMI-1 expression group. These results were contrary to Chowdhury et al. [16] who

reported that BMI-1 expression was significant by both univariate and multivariate analyses for OS, DFS and CR, also that patients with higher BMI-1 expression had less chance of achieving CR, higher chance of relapse and reduced duration of survival but the majority of cases had normal karyotype (intermediate cytogenetic risk group), so

they recommended further investigations for the role of BMI-1 in other cytogenetic groups.

Nishida et al. [18] and Sahasrabudhe [2] revealed that higher BMI-1 expression in patients lead to significantly shorter OS and confirmed its contribution to leukemogenesis and unfavorable prognosis in AML respectively.

Discrepancies in the outcome might be explained by the heterogeneity in PcG expression between patients that reflect the diversity in the AML with respect to their molecular background as well as the different treatment regimens used in different studies.

There was a statistically significant difference between BAALC gene expression and early response to treatment (CR and early death before day 28). A trend of higher death rate among low BAALC expressers and a higher incidence of CR among high BAALC expressers (P value ≤ 0.014). These results were contrary to other studies [19, 25, 26, 28, 31, 33].

Our results showed that there was no impact of BAALC expression on OS or DFS. This was in agreement with Metzeler et al. [25] and Haferlach et al. [22]. But contrary to previous findings [19, 26, 28, 31, 33].

Discrepancies in the outcome might be due to the fact that the majority of these studies have investigated the prognostic role of BAALC overexpression in the subset of AML with normal cytogenetics and most of them were concordant in finding negative impact of high BAALC expression on CR and survival while few studies investigated the impact of high BAALC in AML cases displaying different cytogenetic abnormalities. Besides, the different types of methodologies used in the studies are believed to be a major source of heterogeneity.

Conclusion

1. Our results suggest the potential of BMI-1 and BAALC as molecular markers hence their high expression in 50% of AML cases, but further studies are needed.
2. The prospective screening for BMI-1 and BAALC expression in combination with other molecular markers can help refine myeloid leukemia staging and prognosis toward optimizing therapeutic interventions, including perhaps BMI-1 and BAALC targeted inhibitors, in order to improve the currently disappointing cure rate of patients with AML.
3. Targeting BMI-1 activity might offer more curative success for the hematologic malignancies associated with its aberrant activity.

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Compliance with Ethical Standards

Conflict of interest Authors declare that they have no conflict of interest.

Ethical Approval The study was approved by the International Review Board (IRB) ethical committee, review board of National Cancer Institute, Cairo University in accordance with Helsinki guidelines for the protection of human subjects.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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