Review Article

Insights in Blood Disorders

BAALC-Expressing Earlier Leukemic Progenitors: Crucial Role in AML Relapses with Evaluation of Their Treatment and Prevention Efficacy

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ABSTRACT

A new concept of acute myeloid leukemia (AML) relapses is proposed which is linked with direct participation of BAALC-expressing earlier leukemic progenitors (ELP). The latter may be studied effectively by means of real-time quantitative polymerase chain reaction (RT-qPCR). Recent findings in support of this conception and ongoing prospective studies in the field are shortly discussed.

Introduction

Over two decades a lot of articles concerned the studies of Brain And Acute Leukemia, Cytopasmic (BAALC) gene overexpression in patients with acute myeloid leukemia (AML) and its poor prognostic role in origin of relapses [1-8]. Meanwhile, the convincing experiments with transplantation of human sorted leukemic cells into immunodeficient mice showed that earlier leukemic progenitors (ELP) with immune-phenotype CD34+CD38- are responsible for this transplanted success [9-12]. On the other hand, the data were obtained which presumed direct participation of ELP in selective expression of the BAALC gene [13,14]. On the basis of these data our hypothesis was drawn linking poor-risk BAALC overexpression directly with BAALC-expressing ELP [15-17] which can replace the older doubtful explanation of this phenomenon through BAALC-mRNA. If this explanation is true, then similar RT-qPCR might be used effectively in clinical setting for serial quantitative evaluation of BAALC-expressing ELP bulks.

In order to prove this concept, we have recently performed several closely tied projects. One of them [15] concerned of a crucial role of BAALC-expressing ELP in pathogenesis of relapses in both pediatric and adult AML, treated with hematopoietic stem cell transplantation (HSCT). The study enrolled 50 AML patients (37 adults and 13 children) who were treated by means of HSCT at Memorial Research Institute of Pediatric Oncology, Hematology and Transplantation. Simultaneous measurements of *BAALC*- and *WT1*- expression rates have been performed by means of standard real-time qPCR. Increased number of *BAALC*-expressing ELP were more common in patients with M1, M2, V4 and M5 FAB-variants of AML being at equal frequency in adults and children (Table 1). Notably, the increased ratio of *BAALC*-expressing ELP was rather common in combination with the increased *WT1*-expressing cells thus predicting poorer prognosis (Figure 1). A conclusion was made that this molecular approach offers insights into the crucial role of these cells in emergence and development of post-transplantation relapses, which is of both theoretical and practical importance.

The second project was devoted to the study of *BAALC*-expressing ELP in pediatric patients with *EVI1*-positive AML compared to those in adults [18]. As a result, there was found numbers of *BAALC*-expressing ELP were found to be lower in pediatric patients, thus being in good agreement with previously found differences in biology of these AML variants [19].

Table 1: *BAALC* and *WT1* gene expression levels at diagnosis, before allo-HSCT (D0) and at PTRs after hematopoietic stem cell transplantation in the patients with different AML FAB-variants.

Patients			Disease stage											
			Diagnosis			D0			PTR					
№	FAB	Gender age, years	*BAALC,%*	*WT1, copies*	Blasts,%	*BAALC,%*	*WT1, copies*	Blasts,%	*BAALC,%*	*WT1, copies*	Blasts,%	*PTR day*	Both BAALC-WT1 increase (clin stage)	OS, days
1	M1	M, 21	*n/d*	*n/d*	*n/d*	0.5	**379**	2.5	**329**	**7858**	70	146	PTR	306†
2	M1	F, 48	17	**386**	60	*n/d*	*n/d*	*n/d*	**117**	**281**	40	61	PTR	98†
3	M1	F, 54	*n/d*	*n/d*	*n/d*	**32**	**18439**	79.2	**77**	**32981**	35.4	24	D0, PTR	61†
4	M1	F,26	**130**	**10493**	84.2	*n/d*	*n/d*	*n/d*	**125**	**6500**	11.6	400	Diag, PTR	692†
5	M1	F,60	**2617**	**1728**	96.6	3	29	0.6	**378**	197	37	155	Diag	382†
6	M1	F, 18	**370**	**402**	32	*n/d*	*n/d*	*n/d*	-	-	-	-	Diag	95†
7	M1	M, 18	**242**	**13542**	93.2	*n/d*	*n/d*	*n/d*	-	-	-	-	Diag	730+
8	M1	F, 25	*n/d*	*n/d*	*n/d*	**40**	**1764**	13.2	-	-	-	-	D0	613†
9	M1	F, 44	*n/d*	*n/d*	*n/d*	29	**2355**	8.8	*n/d*	*n/d*	*n/d*	21	-	45†
10	M1	F, 32	*n/d*	*n/d*	*n/d*	21	40	2	4	41	7.8	98	-	672+
11	M1	M, 26	*n/d*	*n/d*	*n/d*	2	47	2.6	2	35	48	143	-	179†
12	M2	M, 30	4	**725**	26.8	*n/d*	*n/d*	*n/d*	**153**	**2363**	23	53	PTR	194†
13	M2	F,15	**89**	37	71.2	34	322	9	**67**	**2033**	45	67	D0, PTR	30†6
14	M2	M 39	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	83	**18872**	26	77	PTR	350†
15	M2	F, 38	*n/d*	*n/d*	*n/d*	**787**	**8756**	59.4	-	-	-	-	D0	407†
16	M2	M, 58	*n/d*	*n/d*	*n/d*	**366**	**26100**	45.8	*n/d*	*n/d*	*n/d*	763	D0	763†
17	M2	M, 35	*n/d*	*n/d*	*n/d*	**34**	**4238**	5	-	-	-	-	D0	20†
18	M2	M 8	**321**	**1236**	69.6	22	7	4.2	-	-	-	-	Diag	469+
19	M2	M, 9	**99**	**10789**	21.2	27	4	1.6	-	-	-	-	Diag	730+
20	M2	M, 28	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	23	5518	24	68	-	162†
21	M2	F, 49	*n/d*	*n/d*	*n/d*	14	4	6.5	*n/d*	*n/d*	*n/d*	188	-	214†
22	M2	M, 15	0,6	37	8.8	*n/d*	*n/d*	*n/d*	-	-	-	-	-	730+
23	M0	F, 42	*n/d*	*n/d*	*n/d*	**521**	**8296**	14	*n/d*	*n/d*	*n/d*	138	D0	166†
24	M0	M, 25	**124**	2.5	84.6	*n/d*	*n/d*	*n/d*	**82**	5	28.2	98	-	103†
25	M0	F, 12	0.01	**11168**	99	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	112	-	129†
26	M4	M, 6	**982**	**183**	54.6	*n/d*	*n/d*	*n/d*	**7118**	**296**	41.2	246	PTR	779†
27	M4	F, 34	*n/d*	*n/d*	*n/d*	**549**	**10819**	11.6	*n/d*	*n/d*	*n/d*	526	D0	560
28	M4	M, 39	*n/d*	*n/d*	*n/d*	**503**	**3548**	62	-	-	-	-	D0	614+
29	M4	F, 21	**107**	**867**	17	*n/d*	*n/d*	*n/d*	**389**	**529**	12	83	Diag, PTR	393†
30	M4	F, 25	**107**	**1790**	82	*n/d*	*n/d*	*n/d*	-	-	-	-	Diagn	730+
31	M4	M, 5	**95**	**999**	65.8	*n/d*	*n/d*	*n/d*	**35**	**411**	12.6	223	Diag, PTR	730+
32	M4	M, 21	*n/d*	*n/d*	*n/d*	**39**	41	5.4	-	-	-	-	-	518+
33	M4	F, 28	*n/d*	*n/d*	*n/d*	**34**	**7367**	7.6	*n/d*	*n/d*	*n/d*	195	D0	730+*
34	M4	F, 16	**34**	**1479**	22.5	*n/d*	*n/d*	*n/d*	-	-	-	-	Diag	128†
35	M4	M, 19	27	**14929**	95	9	**1319**	25.4	-	-	-	-	-	311†
36	M4	F, 19	*n/d*	*n/d*	*n/d*	25	96	10.6	-	-	-	-	-	730+*
37	M4	M, 27	*n/d*	*n/d*	*n/d*	20	**1696**	10	-	-	-	-	-	459†
38	M4	M, 18	*n/d*	*n/d*	*n/d*	10	**836**	7.2	-	-	-	-	-	61†
39	M4	M, 17	*n/d*	*n/d*	*n/d*	5	51	3	6	**362**	7	99	-	197†
40	M4	F, 55	4	**11686**	68	*n/d*	*n/d*	*n/d*	0,06	**1020**	20	57	-	101†
41	M4	M, 17	*n/d*	*n/d*	*n/d*	2	**2258**	12	-	-	-	-	-	730+*
42	M5	F, 32	*n/d*	*n/d*	*n/d*	**272**	**3779**	31.6	-	-	-	23	D0	142†
43	M5	M, 11	**45**	**11753**	88	*n/d*	*n/d*	*n/d*	-	-	-	-	Diag	730+*
44	M5	M, 37	2	177	8	2	77	3.8	24	**4542**	21	195	-	423†
45	M5	M, 22	17	25	94.4	0,6	6	1.4	10	25	9.6	42	-	730+*
46	M5	M, 55	1.3	**9631**	40	*n/d*	*n/d*	*n/d*	-	-	-	-	-	730+*
47	M3	F, 17	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	3	**32684**	24.4	136	-	160†
48	M3	M 18	0.05	**4375**	33.6	*n/d*	*n/d*	*n/d*	-	-	-	-	-	730*
49	M7	F, 3	0.25	**282**	12.8	3	**3693**	13.4	0,3	**1049**	20.8	60	-	258†
50	2ndMDS	M, 8	*n/d*	*n/d*	*n/d*	**51**	**2048**	7.2	**121**	**10239**	30.4	181	D0, PTR	317†

n/d – no data; **-** – no PTR; * – censored; 2ndMDS –secondary AML from MDS, numbers of *BAALC*- and *WT1*-expressing cells higher cut off levels (31% and 250 copies for BAALC and WT1, respectively) are shown by **bold**, +, patient alive, † - patient was dead.

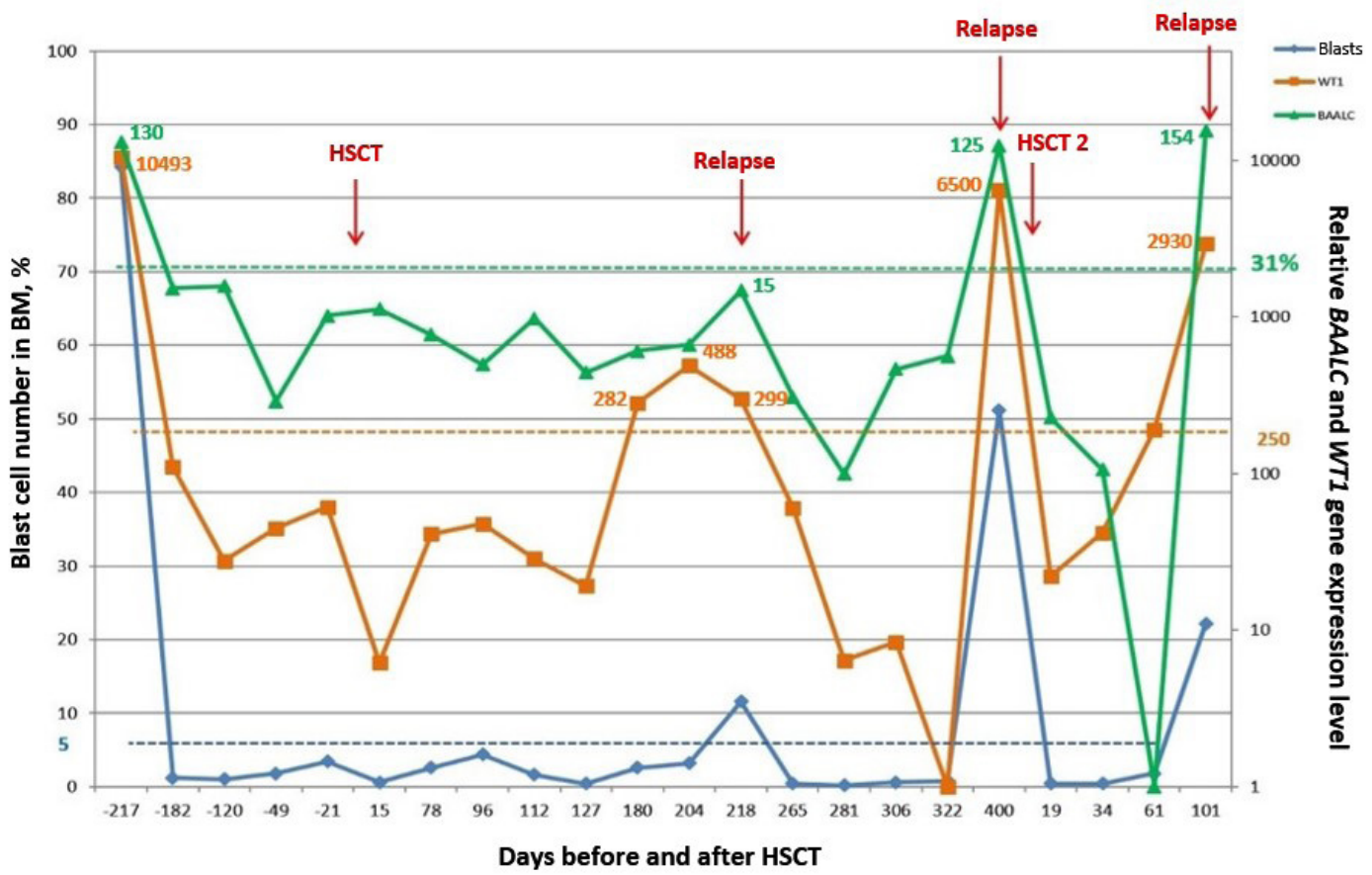

Figure 1: Association of higher numbers of *BAALC* expressing ELP combined with that of WT1 gene at post-transplant relapses of a female patient, aged 26 years, with M1 FAB variant of AML (#4) and complex 47,XX,der(11)add(q15),del(q23),+21 karyotype with EVI1 overexpression. In this patient simultaneous expression increase of both genes was observed at the time of primary AML diagnosis as well as at the 2nd and 3rd relapses, which caused death 692 days after the 1st allo-HSCT.

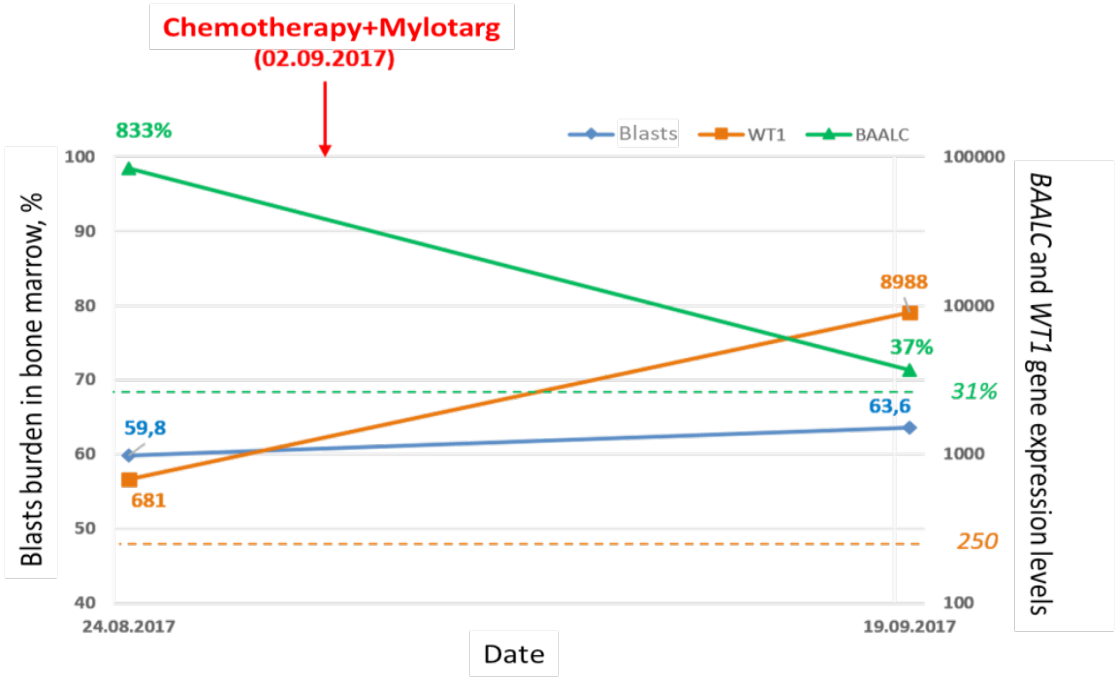

Figure 2: Evidence of direct inhibitory effect of Mylotarg onto *BAALC*-expressing ELP in adult patient with AML.

The third project revealed direct inhibitory effect onto *BAALC*-expressing ELP using such a targeted medicine as of Gemtuzumab ozogamicin (GO. Mylotarg, Figure 2). In this case measurements of *BAALC*- and *WT1*- expressing cells were performed in 14 of specially selected AML patients treated with combination of GO, various kinds of chemotherapy as well allogeneic HSCT. As a results, we have revealed the superior 3-year overall survival (OS) rate in general group of patients with normal karyotypes, and *FLT3* mutated AML variants as compared to those with more complex karyotypes and *EVI1* gene overexpression (85.7% vs 16.7%; p=0.032) [20]. Hence, utility of such approach to serial studies of leukemic hematopoiesis became evident.

The fourth project [21] showed: a) more often higher numbers of *BAALC*-expressing ELP in the patients with different cytogenetic variants of MDS, compared to those with AML; and b) presence of close link between these results and cytogenetic anomalies of the studied cells (Table 2).

Table 2: Numbers of *BAALC*-expressing ELP in 14 patients with MDS-EB1 and MDS-EB2 (n=2 and 12, respectively) associated with various cytogenetic variants.

Patients	Blasts, %	BAALC, %	Karyotypes
1.	3,6	30	del(5q) (CK)
2.	3,8	29	del(5q)
3.	5,4	55	del(5q)
4.	5,6	64	del(5q) (CK)
5.	7,4	58	del(5q) (CK)
6.	9,2	45	i(14),-Y
7.	11,8	76	+8
8.	13,4	154	inv(3q)
9.	15,8	39	-7
10.	15,8	52	+8
11.	17,4	262	del(5q) (CK)
12.	17	217	inv(3q)
13.	18,2	119	inv(3q)
14.	27,6	293	inv(3q)

Notes: CK-complex karyotype; Cut-off for distinguish higher and lower levels of BAALC-expressing ELP is 31%.

Conclusion

In whole, these findings show great prospects, utility and reality of this approach for the studying different cytogenetic and molecular variants of AML on the level of ELP, including their progression and relapses. On the other hand, it may be offered for testing in clinical settings some newly-developed medicines (e.g. retinoids) and therapeutic measures aiming to better preparation of AML patients for HSCT, as well as for detection and prevention of post-transplant relapses. Among nearest prospective studies, one may discuss: a) elucidation of ELP crucial role in pathogenesis of different specially selected cytological, cytogenetic or some molecular AML-variants under quantitative molecular assays of their burden, and b) search of newly-developed medicines and therapeutic approaches exerting direct inhibitory effects onto ELP followed by careful testing it at the oncohematological clinics.

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