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Influence of bcr-3 PML-RARα transcript on outcome in Acute Promyelocytic Leukemia patients of Kashmir treated with all-trans retinoic acid and/or arsenic tri-oxide

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Abstract

Aims: Distinct types of PML-RARα hybrid transcripts viz bcr-1, bcr-2 and bcr-3 result from translocation between chromosomes 15 and 17 *t*(15;17) in Acute Promyelocytic Leukemia patients. We aimed to determine the frequencies of the PML-RARα transcripts and FLT3-ITD mutations in APL patients to evaluate their prognostic implications and also to analyze their impact on disease outcome.

Main method: RT-PCR and Rq-PCR were adopted for transcript typing and quantitation of PML-RARα transcripts while FLT3-ITD was detected by PCR in APL patients.

Key findings: PML-RARα bcr-1, bcr-2 and bcr-3 transcripts were found in 26, 3 and 16 cases respectively. 64.4% patients achieved complete remission, 22.2% expired early wherein majority of the cases expressed bcr-3 transcript ($p=0.03$). 50% relapse rate was observed in patients with bcr-3 transcripts. Multivariate analysis showed expression of bcr-3 transcript associated with early death ($p=0.027$) and increased relapse risk ($P=0.046$). Patients expressing bcr-3 hybrid transcript showed lowest OS of 28.0 months (± 5.26) ($p=0.027$). FLT3-ITD mutation was detected in 5 (11.1%) patients and presence of these mutations was not associated either with PML-RARα transcripts or with disease outcome.

Significance: bcr-3 transcript has a more lethal outcome and is also associated with frequent relapse risk in APL patients of our region.

Keywords Acute Promyelocytic Leukemia, ATRA, Arsenic Tri-Oxide, PML-RARα, Relapse, Remission, Kashmir.

Introduction

Acute promyelocytic leukemia (APL), a subtype of AML characterized by emergence of fusion gene PML-RARα that results from the balanced reciprocal translocation among two chromosomes 15 and 17 denoted as *t*(15;17) [1]. The chimeric product consists of the retinoic acid receptor alpha gene (RARα) on chromosome 17 and the Promyelocytic leukemia gene (PML) on chromosome 15 in more than 98% of the cases. The breakpoint location on RARα gene lies within the second intron [2,3]. Variation in expression of fusion transcripts results from multiple breakpoint sites also known as breakpoint cluster regions (bcr) within the PML gene and due to post transcriptional alternative splicing of the PML sequence [4–6].

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Approximately 40–45% of *t*(15;17) positive cases express bcr-3 fusion transcript whose breakpoint is located inside intron 3 of the PML gene and is created by the fusion of PML exon 3 with RARα exon 3. The bcr-1 hybrid transcript detected in approximately 45–55% of *t*(15;17) positive cases has breakpoint within intron 6 of the PML gene and is formed by the joining of PML exon 6 and RARα exon 3. The least common fusion transcript bcr-2 detectable in only 8–10% of *t*(15;17) positive cases contain breakpoint at inconsistent sites within exon 6 of the PML [7,8].

The bcr-2 hybrid transcript results from the combination of a variable portion of PML exon 6 with third exon of the RARα gene [9]. The variation in position of breakpoints within the PML gene produces PML-RARα transcripts of different sizes namely long (bcr-1), variant (bcr-2) and short (bcr-3), respectively.

The heterogeneity of PML-RARα hybrid transcripts in APL patients have clinical relevance and their detection by molecular techniques (RT and Rq-PCR) is a specifically sensitive test for the diagnosis of APL. It is also used to measure minimal residual disease after chemotherapy, differentiation therapy and bone marrow transplantation for their optimal management [7,10–13]. Reappearance of PML-RARα hybrid transcripts post treatment in the bone marrow is indicative of leukemic relapse [14,15].

Although arsenic trioxide (ATO) and ATRA in combination with chemotherapy has revolutionized the therapy of acute promyelocytic leukemia by achieving complete remission rates more than 90% and long-term remission rates above 80%, high incidence of early death still remains associated with this leukemia more frequently in high-risk APL patients. High risk group generally exhibit WBC count $> 10 \times 10^9$/L at presentation, expression of CD34, CD56 and CD2 cell antigens, short PML-RARα isoform and FLT3-internal tandem duplication (ITD) mutations [16]. Among these FLT3 gene is an important member of tyrosine kinase class III receptor family that induces signals for cell proliferation. Internal tandem duplication mutation in exons 14 and 15 of FLT3 causes incessant activation of the FLT3 tyrosine kinase receptor without ligand stimulation [17–19] that leads to a continual proliferation of leukemic promyelocytes and an unfavorable prognosis [20,21]. In AML patients, the most frequent abnormality is the over expression of the FLT3 gene [22]. Although in AML, internal tandem duplication mutation in FLT3 gene is associated with poor outcome but its prognostic significance in APLs still remains debatable.

In view of this, we conducted a first of its kind study from our region (Kashmir, North India) to investigate the frequencies of the PML-RARα transcripts in a series of 45 APL patients at different stages of treatment. We also aimed to evaluate the association of these subtypes with various prognostic factors in particular FLT3-ITD status with respect to disease outcome.

Material and methods

In this study, 45 newly diagnosed and relapsed patients were studied from May 2013 to September 2017. Diagnosis of APL was made according to FAB classification and *t*(15;17) was confirmed by the cytogenetic analysis. Reverse transcription polymerase chain reaction (RT-PCR) detected the type of fusion gene formed from PML-RARα rearrangement which was then quantified by Rq-PCR. Further, FLT3-ITD mutation status was detected by conventional PCR. Patients were stratified into low, intermediate and high risk groups according to their baseline white cell count and platelet counts and treated with (IC-APL 2006 and ATO) protocols. 36 patients were treated with ATRA (45 mg/m2/d) along with Daunorubicin (DNR) 60 mg/m2/d and Dexamethasone 2.5 mg/m2/12 h $\times$15 days (if WBC $> 5 \times 10^9$/L) for remission induction followed by consolidation with 3 cycles of ATRA for 15 days, Daunorubicin (DNR) 25–35 mg/m2/d (d 1, 2, 3, 4), Methotrexate (MTZ) 10 mg/m2/d (d 3–5), Cytosine Arabinoside (Ara-C) 1–150 gm/m2/d (d 1, 2, 3, 4) in case of high risk patients. After the consolidation, patients who achieved clinical remission were subjected to maintenance chemotherapy which includes ATRA 45 mg/m2/d $\times$ 15 (every 3 months) and Methotrexate 15 mg/m2/d (Weekly) for two years.

Arsenic Trioxide (ATO) protocol was given to 8 patients which consists of an initial course of 10 mg ATO injection until complete remission or 60 days (Max), followed by second course of ATO injection for 28 days (if patient continues to be in remission in the interval). This is followed by a maintenance course of ATO injection for 10 days/ month (6 months).

The study was approved by the 'Institute Ethics Committee' (IEC) of SKIMS. The patients were evaluated at baseline, after consolidation and during as well as after maintenance stage of treatment for their response to conventional chemotherapy (IC-APL 2006 and ATO) by RT-PCR and Rq-PCR at the Department of Immunology and Molecular Medicine, SKIMS. Peripheral blood samples were taken in heparinised and EDTA vials after informed consent from the patients.

RNA extraction

Total RNA was extracted from 1.5–2.0 ml of peripheral blood using RNA extraction kit (Qiagen, Germany). 260/280 nm ratio was used to measured the concentration and purity of extracted RNA spectrophotometrically (Eppendorf, Germany). A ratio of 1.90 to 2.00 was accepted as good quality RNA. RNA integrity was checked by DEPC-treated Gel electrophoresis.

cDNA synthesis

Reverse Transcription of the extracted RNA to cDNA was done using the Maxima cDNA synthesis kit (Thermo Scientific, USA) that contained Enzyme Mix (M-MuLV RT Enzyme and Ribolock Rnase Inhibitor), 5$\times$ Reaction Mix (reaction buffer, dNTPs, oligo(dt)18 and random hexamer primers and Nuclease free water). 2 µg of mRNA was reverse transcribed to cDNA by an incubation of 10 minutes at 25 °C, 15 min at 50 °C, and 5 min at 85 °C in a total volume of 20 µl that contained 4 µl of 5X Reaction Mix, 2 µl of Maxima Enzyme Mix and 12 µl of nuclease free water.

Reverse transcriptase polymerase chain reaction (RT-PCR: qualitative)

Multiplex PCR reaction was performed in a 25 µl reaction volume consisting of a 55 mM $MgCl_2$, a 0.2-µMol

concentration of dNTPs, 400 nm concentrations of all PML-RARα primers **PML-A1- 5′-*CAGTGTACGCCTTCTCCATCA*-3′, PML-A2- 5′-*CTGCTGGAGGCTGTGGAC*-3′, RARA-B- 5′-*GCTTGTAGATGCGGGGTAGA*-3′, PML-RP- 5′-*CTGACTGTACCACAGCCATAGG*-3′** as described by Van Dongen et al. [23]. Thermal conditions included an initial denaturation of 94 °C for 5 min, followed by 35 cycles (30 s at 94 °C, 30 s at 54 °C, and 30 s at 72 °C) with a final extension of 7 min at 72 °C. 6–8 µl of the PCR product was size-fractionated by 2% agarose gel electrophoresis and visualized under a UV transilluminator (Flourchem, HD2-Cell Biosciences) at 365 nm.

In case of PML-RARα, PCR products of different sizes ranging from 345 to 381 bp were found with RARA-B primer which is a common reverse primer for bcr-1, bcr-2 and bcr-3 transcripts. A single product of either 381 or 345 bp was observed with PML-A1 primer that corresponded to the translocation breakpoint in intron 6 (bcr-1) or exon 6 (bcr-2) and 376 bp product was observed with PML-A2 primer corresponding to (bcr-3) whose breakpoint is located in intron 3. Primers PML-A2 and PML-RP (self designed) displayed an additional PCR product of 450 bp which served as an internal control and ensured good quality of cDNA used [Supplementary Fig. 1].

Real-time polymerase chain reaction (Rq-PCR: quantitative)

Rq-PCR was assessed at baseline, at the end of consolidation and thereafter every three months during maintenance phase of treatment. After maintenance it was assessed every 6 months. Quantitation was carried on the Rotor Gene Q series platform using Ipsogen PML-RARα kits from Qiagen (Germany) [Supplementary Fig. 2].

Analysis of FLT3-ITDs

Genomic DNA from the peripheral blood/bone marrow leukocytes of APL patients was extracted using QIAamp DNA Blood Mini Kit (Cat No. 51104), Qiagen (Germany). The concentration and purity of the extracted DNA was measured using Biospectrophotometer (Eppendorf AG; Serial No: 6137EQ102539; Germany).

FLT3 ITDs is located in exons 14 and 15 and amplification of the respective exons along with its detection was carried out in 45 APL patients at baseline using primer set ***11F (5′-GCAATTTAGGTATGAAAGCCAGC-3′)*** and ***12R(5′-CTTTCAGCATTTTGACGGCAA CC-3′)*** as described by Fröhling et al. [24]. In FLT3-ITD^{-} cases, the primer set amplified a single 328 bp normal *FLT3* gene product whereas two bands of 328 bp and 350 bp were amplified in FLT3-ITD^{+} patients [Supplementary Fig. 3].

Statistical analysis

Overall survival (OS) was measured from the start of treatment until to the last follow-up or to the date of death from any cause. Disease free survival (DFS) was measured from the start of treatment until loss of molecular and hematological remission (relapse). The difference between variables was analysed by the χ^2 and Fischer exact test as applicable. The Cox hazard model was used to identify prognostic variables influencing the OS and DFS in multivariate analysis. All statistical tests were two sided with the significance level set as ≤ 0.05 using the IBM SPSS statistics.

Results

A total of 45 APL patients included in the study group comprised 28 (62.2%) males and 17 (37.8%) females. Median age was 31 years (range 06 to 92 years). 41 (91.1%) had bone marrow leukemic promyelocyte count $\geq$60%. Clinical and laboratory features at diagnosis are shown in [Table 1]. No differences were observed in age, sex, leukocyte count and bone marrow infiltration by leukemic promyelocytes except for platelet count ($p < 0.05$) in patients expressing different types of PML-RARα fusion gene transcripts [Table 1]. RT-PCR analysis of the 45 APL cases at baseline showed amplification of the bcr-1 transcript in 26(57.8%) cases, bcr-2 in 3 (6.7%) cases and bcr-3 in 16 (35.5%) cases.

36 patients (80.0%) were treated according to the standard ICAPL-2006 chemotherapy protocol and 09 patients (20.0%) with Arsenic Trioxide (ATO). The median follow-up was 33 months (range 0.5–52). 08(17.7%) patients (02 expressing bcr-1 and 06 expressing bcr-3) died early after diagnosis before completion of induction chemotherapy ($p = 0.02$). Complete remission after induction therapy was obtained in 37 patients (82.2%).

Among patients that achieved CR, relapse rate was observed more in cases that expressed bcr-3 transcripts as compared to either patient with bcr-1 and bcr-2 transcripts. Out of total 08 patients who relapsed, 50% of the patients belonged to bcr-3 group, compared to 37.5% of patients with bcr-1 and 12.5% harboring bcr-2 transcript ($p = 0.18$). Among 8 relapsed patients, 2 expired (each expressing bcr-1 and bcr-3 transcript) due to cerebral hemorrhage ($p = 0.02$) [Table 2].

In this study, 5 (11.1%) of the 45 patients were positive for FLT3 ITD mutation. Of the FLT3 ITD positive cases 04(8.9%) were males and 01(2.2%) female. 04(8.9%) of the FLT3 ITD were $<$30 years of age and 01(2.2%) $>$30 years. 3 (6.7%) FLT3 ITD positive patients had bcr-1 and 2 (4.4%) patients had bcr-3 PML-RARα transcript [Table 3].

Survival analysis

At multivariate analysis, expression of bcr-3 fusion transcript was confirmed as an independent poor prognosis factor for overall survival (OS) (HR, 2.2; 95% CI, 1.02–4.62; $p = 0.027$) and increased relapse risk (HR, 2.07; 95% CI, 0.98–4.38, $P = 0.046$). Other tested variables like age, sex, WBC and platelet counts, FLT3 ITD status (OS hazard ratio: 2.1; 95% CI: 0.44–9.99; $p = 0.33$); (DFS hazard ratio: 1.5; 95% CI: 0.19–12.8; $p = 0.67$) did not influence the survival outcome of the patients [Table 4].

Kaplan–Meir analysis was used to determine overall survival (OS) and disease free survival (DFS) of patients expressing these fusion transcripts. A significant difference in OS [log

Table 1 Clinico-pathological parameters of 45 acute promyelocytic leukemia patients at diagnosis.

Particulars	No's	Baseline			p Value
	($n=45$)	bcr-1 26(57.8%)	bcr-2 3(6.7%)	bcr-3 16(35.5%)	
Gender					
Male	28(62.2%)	16(57.1%)	01(3.6%)	11(39.3%)	0.507
Female	17(37.8%)	10(58.8%)	02(11.8%)	05(29.4%)	
Age					
<30	28(62.2%)	16(57.1%)	03(10.7%)	09(32.1%)	0.355
≥30	17(37.8%)	10(58.8%)	00(0.0%)	07(41.2%)	
Leukemic Promyelocytes					
<60%	04(8.9%)	03(75.0%)	00(0.0%)	01(25.0%)	0.72
≥60%	41(91.1%)	23(56.1%)	03(7.3%)	15(36.6%)	
TLC					
$<10\times10^9$/L	35(77.8%)	22(62.9%)	02(5.7%)	11(31.4%)	0.433
$>10\times10^9$/L	10(22.2%)	04(40.0%)	01(10.0%)	05(50.0%)	
Platelets					
$<40\times10^9$/L	39(86.7%)	24(61.5%)	00(0.0%)	15(38.5%)	0.00
$\geq40\times10^9$/L	06(13.3%)	02(33.3%)	03(50.0%)	01(16.7%)	

Table 2 Treatment and clinical outcome of 45 APL patients according to the PML-RARα transcript type.

	Total cases	bcr-1	bcr-2	bcr-3	P-Value
Treatment					
ICAPL-2006	36 (80.0%)	20(55.6%)	03(8.3%)	13(36.1%)	0.631
Arsenic	09 (20.0%)	06(66.7%)	00(0%)	03(33.3%)	
Survival	35 (77.8%)	23 (65.7%)	3 (8.6%)	9 (25.7%)	**0.03**
Death	10 (22.2%)	03(30.0%)	0 (0%)	7(70.0%)	
Remission	29 (64.4%)	21(72.4%)	2 (6.9%)	6(20.7%)	0.18
Relapse	8 (17.7%)	3 (37.5%)	1(12.5%)	4 (50.0%)	

Table 3 Association of different PML-RARα transcript with FLT3-ITD.

T Type	Total ($n=45$)	FLT3-ITD status		p Value
		Positive ($n=05$)	Negative ($n=40$)	
bcr-1	26(57.8%)	03(60.0%)	23(57.5%)	0.91
bcr-2	03(6.7%)	0(0.0%)	03(7.5%)	—
bcr-3	16(35.5%)	02(40.0%)	14(35.0%)	0.82

Table 4 Multivariate analysis of APL patients according to the PML-RARα transcript type and FLT3-ITD status.

Hazard ratio						
Overall Survival (OS)				Disease Free Survival (DFS)		
Transcript type	Hazard ratio	95% CI	p Value	Hazard ratio	95% CI	p Value
bcr-1	0.297	0.077–1.150	0.079	0.272	0.065–1.146	0.076
bcr-2	0.044	0.000–1.652	0.561	1.292	0.159–10.521	0.561
bcr-3	2.2	1.02–4.62	**0.027**	2.07	0.98–4.38	**0.046**
FLT3-ITD	2.1	0.44–9.99;	0.33	1.5	0.19–12.8	0.67

rank value ($p=0.027$)] (Fig. 1(A)) was observed in patients with bcr-1(88.5%), bcr-2 (100%) and bcr-3 (56.3%) fusion transcript with the lowest mean OS of 28.0 months (±5.26) for patients expressing bcr-3 fusion transcripts. DFS was analyzed in 37 patients who achieved Clinical Remission. The estimated DFS of 86.9%, 66.7% and 60.0% was calculated for patients expressing bcr-1, bcr-2 and bcr-3 fusion transcript respectively (log-rank test: P = 0.116) (Fig. 1(B)) with a lower mean DFS of 34.3 months (±3.46) for patients with bcr-3 [Table 5].

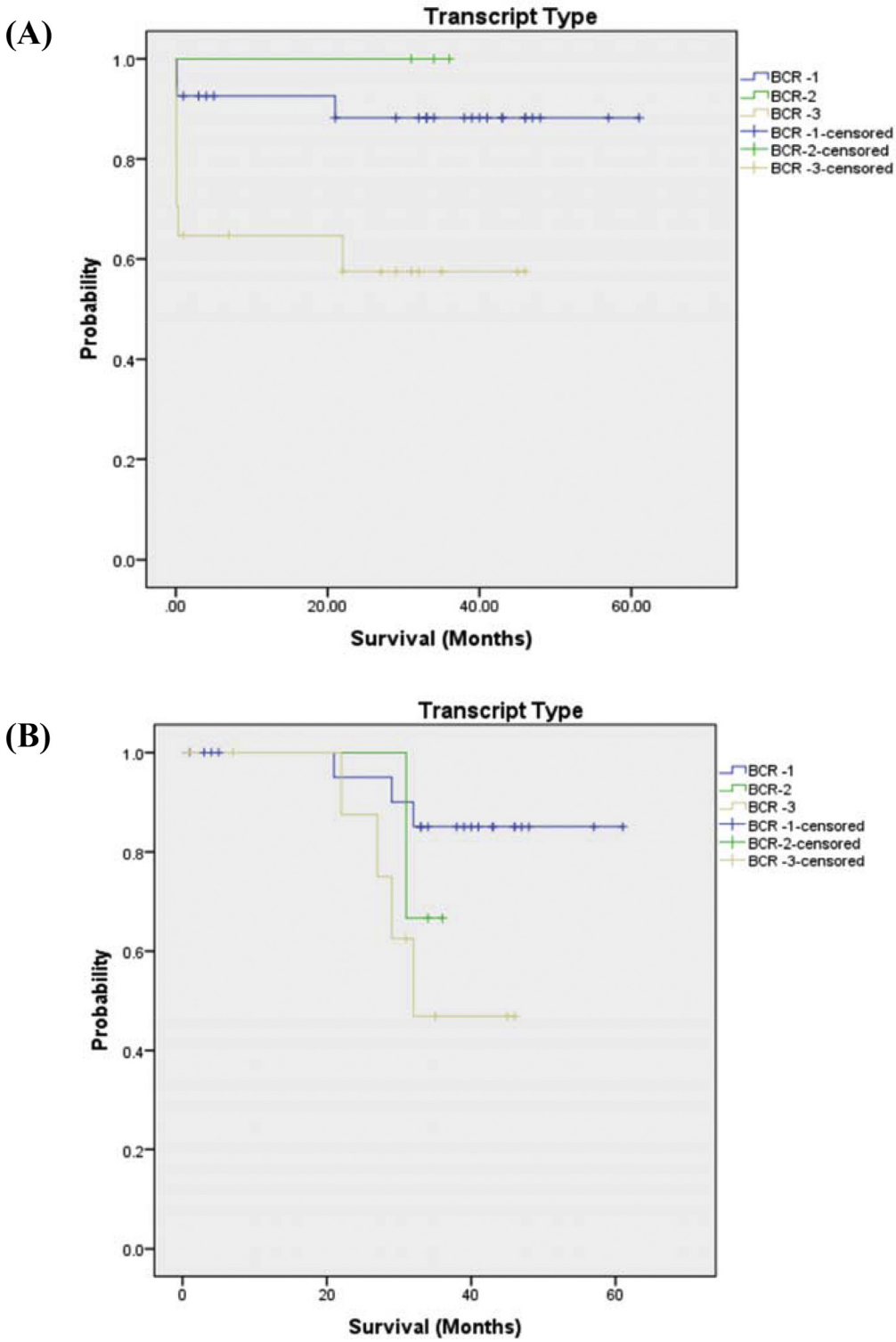

Fig. 1 Kaplan Meier (A) overall survival and (B) disease-free survival plots of acute promyelocytic leukemia patients according to the PML-RARα fusion gene transcripts.

Discussion

Three distinct transcript types of PML-RARα hybrid transcripts viz long (bcr-1), short (bcr-3), and variant (bcr-2) have been identified in different APL patients as a result of the translocation *t*(15;17). The prominent heterogeneity of these fusion transcripts may have clinical relevance and have an impact on the treatment and management of the APL. The reports that have featured so far have produced controversial reports on the influence of breakpoint site on

Table 5 Kaplan Meier Overall (OS) and Disease free survival (DFS) estimate of APL patients according to the PML-RARα transcript type.

Survival analysis								
Overall Survival (OS)				p Value	Disease Free Survival (DFS)			p Value
Transcript type	Estimate (Months)	SD Error	95% CI		Estimate (Months)	SD Error	95% CI	
bcr-1	54.72	3.43	47.99–61.45		55.95	2.71	50.62–61.27	
bcr-2	34.33	1.36	31.66–37.00	**0.027**	36.31	3.46	29.53–43.09	0.116
bcr-3	28.07	5.27	17.75–54.64		34.33	1.36	31.66–37.00	

patient outcome but all have claimed their definite role in the clinical outcome of the disease. For this reason we chose to type the fusion transcripts to observe the pattern and distribution of bcr-1, bcr-2 and bcr-3 in APL patients in addition to analyze their prognostic significance and impact on patient survival. To further strengthen the prognostic implications, we specifically emphasized on other prognostic factors which have impact on disease outcome either independently or with respect to fusion transcripts which in particular include evaluation of FLT3-ITD mutations. Therefore, we conducted a study to understand the clinical implications of these transcripts in the outcome of the APL patients through qualitative RT-PCR and quantitative Rq-PCR analysis in addition to FLT3-ITD mutations.

We evaluated the transcript types of all the 45 APL patients at different treatment stages where bcr-1 frequency was found to be highest in 26 (57.8%) followed by bcr-3 transcript in 16 (35.5%) and 03 (6.7%) patients were found with bcr-2 fusion transcript. There has been a slight variation in the distribution of these isoforms as per the studies across the globe but overall frequency of the respective isoforms remains more or less same. The frequency of bcr-1 is highest amongst the three followed by bcr-3 and bcr-2. The aggregate frequency of brc-1 and bcr-2 in our study is in complete agreement with a study conducted by Melo et al. [25] (68.8% versus our study 64.4%) whereas the frequency of bcr-3 also showed concordance (31.4% versus 35.5% our study). The distribution of bcr-1 among our series of patients agrees with Douer et al. [26] who also reported a significantly higher frequency of bcr-1 isoform. Further, the frequency of the three hybrid transcripts in our series of patients is in accordance with several studies from Europe and the USA which reported an incidence of approximately 50–55% for PML(L) RARα (bcr-1), 8–20% for PML(V) RARα (bcr-2) and 27–49% for PML(S) RARα (bcr-3) [26].

Given an established role of transcript types in the outcome of APL disease, many authors have tried to correlate them with different clinical parameters at diagnosis [11,27,28] and also their impact on the patient survival [29–31]. A higher frequency of relapse and shorter survival have been reported in patients who express the bcr-3 hybrid transcript compared to patients with the long isoform (bcr-1) [29,30]. Many reports across the globe found no difference in disease-free survival between patients either with long or short isoforms [11]. Our study found that 50% APL patients relapsed with a short transcript type (bcr-3) and had unfavorable prognosis as compared to 37.5% patients with a long transcript type (bcr-1) ($p = 0.02$). Further, relapse was seen in 33.3% (1 out of 3) patients who presented with bcr-2 transcripts [Table 2]. In this study, we observed that patients with the long isoform bcr-1 had remission in majority (86.9%) of cases as compared to 66.7% and 60% with bcr-2 and bcr-3, respectively. The difference in rémission between the three groups showed favorable outcome for bcr-1($p = 0.01$). These results were in conformity with a study conducted by Joseph et al. [32] that also showed patients with the short isoform than long transcript to have a shorter remission duration and overall survival.

Our study detected a frequency of 11.1% (5 of 45) FLT3 -ITD mutations in our series of APL patients. Yoo et al. [33] reported nearly a similar frequency of 12% (9 of 75) FLT3 -ITD mutations in APL, but a frequency as high as 38% have been reported by Kuchenbauer et al. [34]. Of the 5 FLT3 –ITD$^+$ positive cases detected, 2 (40%) patients had bcr-3 fusion gene transcripts but no association was found with different PML-RARA fusion transcripts ($p > 0.05$) [Table 3]. Further, our findings were consistent with few of the investigations conducted across the globe (Yoo et al. [33],Callens et al. [35] and Mathews et al. [36]) who reported nearly similar frequency of bcr-3 transcripts but did not find any association with FLT3 -ITD mutation status.

Multivariate analysis showed that the patient group expressing bcr-3 transcripts had an increased number of early death with a hazard ratio of 2.2 ($p = 0.027$) as against patients with bcr-1 and bcr-2 transcripts. Further, presence of bcr-3 transcript conferred an increased risk of relapse with hazard ratio 2.07 ($P < 0.05$) [Table 4]. These findings revealed expression of bcr-3 fusion transcript as an independent poor prognostic factor. The higher rate of deaths in patients expressing bcr-3 has also been documented earlier by Greco et al., whereby a more fatal APL was characterized by presence of bcr-3 including some other factors [37]. In yet another report by Huang et al. [29], bcr-3 has been shown to confer more fatal and frequent relapse risk in APL patients which further support our findings.

Again multivariate analysis revealed that the presence of FLT3-ITD has no significant impact on overall survival and disease free survival outcomes (OS, $P = 0.33$; DFS, $P = 0.67$) [Table 4]. Consistent with our results, there are similar studies that have also substantiated no significant impact of FLT3-ITD on survival outcomes and relapse risk in patients with APL [38–40].

Kaplan–Meier analysis showed a significant difference in overall survival for APL patients with respect to expression of different fusion transcripts ($P = 0.027$). Patients expressing bcr-1 and bcr-2 transcript were found to have better OS (88.5% and 100%, respectively) as against those harboring bcr-3 with relatively short OS (56.3%). However, no significant difference was observed in DFS between patient groups expressing these transcript types ($p = 0.116$) but patients expressing bcr-3 transcript showed lower DFS (60.0%) of 34.3 months [Table 5]. Similar scenario was depicted by Huang et al. [29], who has reported unfavorable prognostic outcome for

patients with a bcr-3 fusion transcript than patients with bcr-1 and bcr-2. One more study by Vahdat et al. [30] has depicted the similar picture regarding the prognosis of the APL patients on the basis of presence of the fusion gene transcripts.

Conclusion and study limitations

Despite clear indication from this report that different fusion transcripts play an important role in the outcome of APL patients independent of FLT3-ITD mutations. Further investigations in larger series are needed to establish whether different PML breakpoints are associated with variable prognosis and/or aid in the management of the disease.

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Conflicts of interest

There is no conflict of interest between the authors.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2018.12.003.

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