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LETTER TO EDITOR

Impact of the Major BCR-ABL1 Transcript Type on Clinical and Biological Parameters and Molecular Response in Patients With Chronic Myeloid Leukemia

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Abstract

Aim: **In chronic myeloid leukemia (CML), the impact of MBCR-ABL1 major transcript type on disease phenotype and response to treatment still controversial to date. This work aims to study the influence of Mb3a2 and Mb2a2 transcripts on clinico-biological parameters and the molecular response in patients with chronic phase chronic myeloid leukemia (CP-CML) treated with Imatinib as frontline therapy.**

Methods: **This is six years prospective study started in March 1 st, 2013. 67 patients with newly CP-CML were treated by Imatinib as frontline therapy. Clinical and biological characteristics disease were collected for all patients. Molecular typing was performed by multiplex RT-PCR and quantification of transcripts by real-time quantitative PCR (qRT-PCR). The cumulative incidence of deep molecular response (DMR) was estimated by the Kaplan-Meier method. The comparison was made using the parametric Log-Rank test. A value of $P \leq 0.05$ is considered significant.**

Results: **61% of patients expressed b3a2, 35.82% b2a2 and 2.98% expressed a rare transcript of type e19a2. At diagnosis, the b2a2 type had a higher level of expression than that of b3a2 (67.92 vs 53.79%; $P = 0.03$). This insignificant difference between the two transcript subgroups was also observed for rates below 1% at 6 months (54 vs 39; $P = 0.26$) and below 0.1% (54 vs 44 %; $P = 0.50$), (77 vs 50%; $P = 0.09$) and (81 vs 78 %; $P = 0.52$) at 12, 18 and 24 months respectively. The two types of transcript had almost the same kinetics. Nevertheless, the absolute value of the BCR-ABL1/ABL ratio decrease was faster in the group of patients expressing b3a2, than in those expressing b2a2. At 18 months post IM therapy, patients with a b3a2 transcript have a trend of better MMR that those with b2a2 (77 vs 50%; $P = 0.09$). The DMR was not significantly different between two groups at 24 months (50 vs 32%; $P = 0.20$) and 36 months (75 vs 70%; $P = 0.54$) respectively. The cumulative probability of achieving MRD at 5 years was higher in patients with b3a2 type but not statistically significant; (85 vs. 68%; $P = 0.17$).**

Conclusion: **Patients with b3a2 transcript may be associated with a better response to Imatinib therapy.**

Keywords: BCR-ABL1 transcript, Chronic myeloid leukemia, Imatinib therapy, Major molecular response, qRT-PCR

1. Introduction

In the context of chronic myeloid leukemia (CML), b3a2 (e14a2) or b2a2 (e13a2) transcripts are found in more than 95% of cases. Rarely, the break points interrupting the BCR and ABL1 genes can be in alternative regions leading to very rare and atypical transcripts: e8a2, e6a2, e19a2, e1a3, b2a3, b3a3, and

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e1a2 [1]. The $p210^{BCR\text{-}ABL1}$ chimeric protein encoded by the BCR-ABL1 fusion gene became the target of tyrosine kinase inhibitors, with imatinib (IM) as the first-line therapy. This improved the overall survival of patients with CML which reached that of the general population [2]. Many studies investigated the influence of major transcripts types on the phenotype of disease and the response to treatment. However, the results of these studies remain controversial. The aim of this paper is to examine the influence of Mb3a2 and Mb2a2 transcripts on clinical and biological parameters and the molecular response in patients with chronic phase CML (CP-CML) treated by IM as frontline therapy.

This is a 6-year (2013–2019) prospective, monocentric study, involving 67 patients with newly diagnosed CP-CML and treated by IM as frontline therapy. The qualitative research of the BCR-ABL1 transcript was performed by reverse transcriptase polymerase chain reaction (PCR) multiplex technique using the Seeplex Leukemia BCR/ABL kit (Seegene, Seoul, Korea). The BCR-ABL1 transcript levels were measured by real-time quantitative PCR in peripheral blood samples using a Rotor-Gene analyzer (Qiagen) with a standardized kit (Ipsogen kit (Qiagen) BCR-ABL1 Mbcr IS-MMR; Réf 670823) according to the European Leukemia Net (ELN) recommendations [3]. The major molecular response (MMR) was defined by a reduction of more than $3\log_{10}$ (ratio $\leq$ 0.1%) of the expression level of BCR-ABL1. Crosstabs and Student t test were performed for the comparison of two averages. Cumulative incidence of deep molecular response (DMR) was estimated by the Kaplan–Meier method and log rank test using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). A value of $p \leq 0.05$ was considered significant.

Among the 67 patients, 41 (61%) patients expressed b3a2, 24 (35.82%) patients expressed b2a2, and two (2.98%) patients expressed a rare transcript type e19a2. The comparative groups involved 65 patients with major transcripts b3a2 and b2a2, including 32 (49%) men and 33 (51%) women with a sex ratio F/M of 1.04. Mean age was 46 ± 13.85 (19–78) years. Epidemiological and clinical characteristics according to the two transcript types of our series are summarized in Table 1. The median follow-up duration was 36 (6–72) months. At diagnosis, the median BCR-ABL1/ABL1 ratio was significantly higher in the b2a2 transcript than in b3a2 transcript (67.92% vs. 53.79%; $p = .03$) (Table 2). Furthermore, 64% of patients expressing b3a2 have a 3-month rate less than 10% compared with 54% of patients expressing b2a2 ($p = .43$). This insignificant difference between the two transcript subgroups

Table 1. Baseline Characteristics of Patients with Chronic Myeloid Leukemia by Type of Major Transcript.

Characteristic			*P*
Type of transcript	b3a2	b2a2	
Patients, *n* (%)	41(63)	24 (37)	0.076
Age, yr (range)	51 (19–78)	46 (20–77)	0.49
Sex, n (%)			
Men	17 (53)	24 (73)	0.98
Women	15 (47)	09 (27)	0.044
Sokal risk score, n (%)			
Low	10 (24.5)	7 (29)	0.73
Intermediate	21(51)	8(33.5)	0.65
High	10 (24.5)	8 (37.5)	0.90
EUTOS score, n (%)			
Low	36 (88)	17 (71)	0.99
High	5 (12)	7 (29)	0.99
Blood cell count, Median			
WBC, $\times 10^9$/L	171	351	0.086
Hemoglobin, g/L	10.5	11.6	0.78
Platelets, ×109/L	458	507	0.61

Note. EUTOS = European Treatment and Outcome Study; *n* = number; WBC = white blood cell count.

was also observed for rates below 1% at 6 months (54% vs. 39%; $p = .26$) and below 0.1% (54% vs. 44%; $p = .50$), (77% vs. 50%; $p = .09$), and (81% vs. 78%; $p = .52$) at 12, 18, and 24 months, respectively. The two types of transcript had almost the same kinetics. Nevertheless, the absolute value of the BCR-ABL1/ABL ratio decrease was faster in patients expressing b3a2 than in those expressing b2a2 (Fig. 1).

The frequency of patients who achieved MMR increased to 51%, 68%, and 80%, at 12, 18, and 24 months, respectively, with median time to obtain of 12 months. The frequency of DMR decreased from 43% to 73% between 24 and 36 months. At 18 months post IM therapy, patients with a b3a2 transcript had a trend of better MMR that those with a b2a2 transcript (77% vs. 50%; $p = .09$). DMR was not significantly different between two groups at 24 months (50% vs. 32%; $p = .20$) and 36 months (75% vs. 70%; $p = .54$; Fig. 2). The cumulative probability of achieving DMR at 5 years was higher in patients with b3a2 but not statistically significant (85% vs. 68%; $p = .17$; Fig. 3).

In this paper, we studied the clinical and biological characteristics, the MMR and MRD obtained in each type of major transcript in patients with newly diagnosed CP-CML and treated frontline with IM. The major transcript was the most common with an incidence of molecular isoform b3a2 higher than that of b2a2. Several study groups reported different frequencies of major transcripts b3a2 and b2a2. In a meta-analysis study, 22 (85%) out of 26 reviewed articles had a high incidence of the b3a2 transcript,

Table 2. BCR-ABL1 ratio at diagnosis.

	Effective	Mean	Median	Standard deviation	Range	*p*
Ratio BCR-ABL1	65	59	60.00	25.62	14.00–98.00	NA
Ratio Mb3a2	41	53.79	51.00	26.16	14.00–97.00	**0.03**
Ratio Mb2a2	24	67.92	77.00	24.37	21.00–98.00	

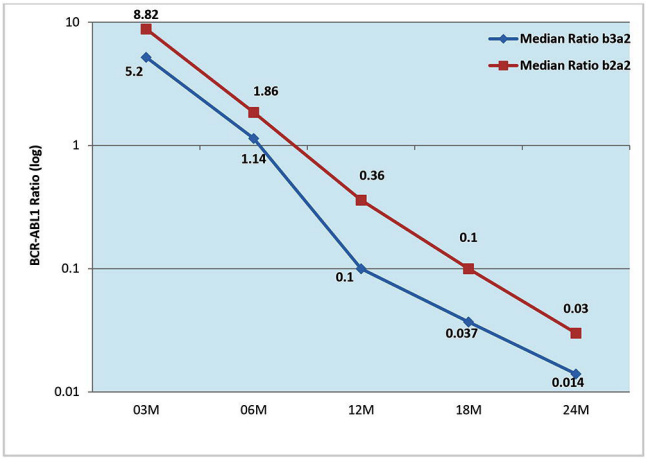

Fig. 1. Decrease in the BCR-ABL1/ABL1 ratio depending on the type of rearrangement.

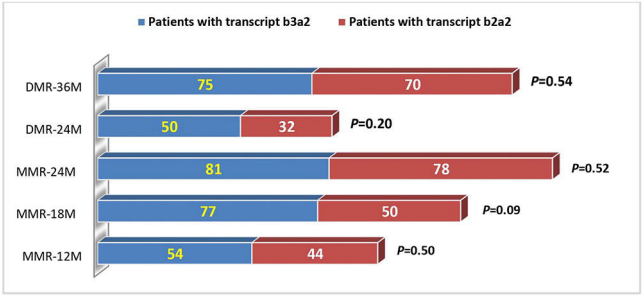

Fig. 2. Proportion of patients with MMR and DMR depending on the type of major transcript. Note. DMR = deep molecular response; MMR = major molecular; M = months.

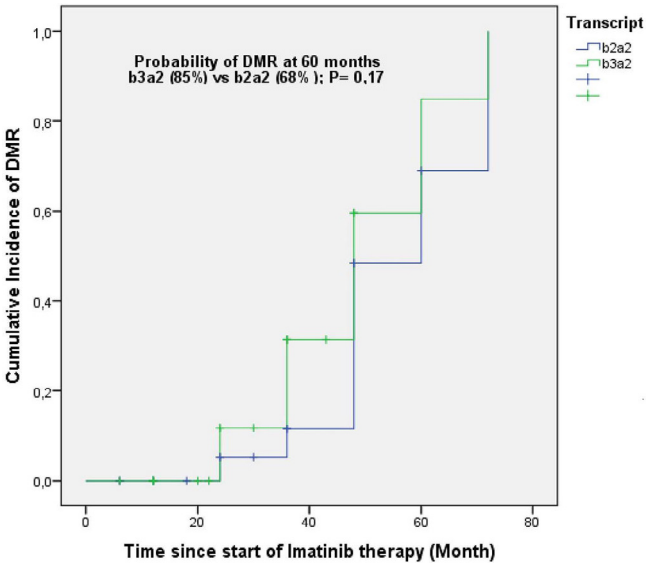

Fig. 3. Cumulative incidence of DMR stratified according to the type of major transcript. Note. DMR = deep molecular response.

whereas the other articles had a high incidence of the b2a2 transcript [4]. In a series of 45,503 patients with newly diagnosed CML reported from 45 countries, the proportion of b2a2 and b3a2, including the cases coexpressing b3a2 and b2a2, was 37.9% and 62.1%, respectively [5].

In the era of tyrosine kinase inhibitors, the impact of the type of transcript on clinical and biological parameters and molecular response remains controversial. Moreover, 420 articles concerning this topic reported that the majority of studies found no significant differences in terms of age, sex, leukocyte count, and hemoglobin (Hb) level. However, in about half of the studies, the b3a2 transcript was associated with higher platelet counts and only a few studies found a significant association between the disease risk scores and transcript types [4]. In our series, the b3a2 transcript was significantly more frequent than the b2a2 transcript in women. Our results agree with those of the large study of the ELN registry [6]. However, in the investigation study by Baccarani et al. [5] having concerned 180 centers in Africa, Asia, Australia, Europe, North America, and South America, the proportion of these two transcripts was correlated with sex, b2a2 being more frequent in men (39.2%) than in women (36.2%; $p < .0001$).

No differences were found in prognostic scores. Similar results have been found in other studies [7–11]. By contrast, Deb et al. [12] demonstrated that patients expressing b2a2 had higher Sokal and EUTOS scores ($p < .05$). In our study, patients expressing b2a2 appeared to have higher mean leukocyte count indicating greater tumor charge than those expressing b3a2. No difference was observed for average number of platelets or Hb concentration. Our results were similar to other reports, which do not show a correlation between the type of transcript and the hematological parameters, especially the platelets count [13,14]. However, other studies have revealed a statistically significant

difference. A larger study from the German group [15] evaluated 1105 patients treated with IM and showed that patients expressing b3a2 were characterized by a low number of white blood cell count (65 vs. 88 $\times$ 10^9 g/L, $p < 10^{-3}$) and a higher platelet count (430 vs. 296 $\times$ 10^9 g/L, $p < .001$). These results, drawn from a large cohort of patients, strongly confirm the existence of a distinct disease phenotype between b2a2 and b3a2; this may reflect a different response to treatment.

Serial assays performed during the molecular follow-up allowed us to evaluate the decrease in the kinetics of the BCR-ABL1/ABL1 ratio and showed that the median diagnosis rate was significantly higher in patients expressing b2a2 than those expressing b3a2.

Our results conform to those obtained in the study by Kagita et al. [11] (99.52 $\pm$ 97.25 vs. 71.32 $\pm$ 66.69; $p = 0.0351$).

We also found that the kinetics of reduction is different according to the type of transcript at different times of follow-up (12, 18, and 24 months); however, these results were not statistical significant.

In our study, the most clinically relevant finding is the association of the b3a2 fusion transcript with a better molecular response and a shorter median time to obtain MMR. Our results are correlated with several published studies that suggested that in patients expressing the b3a2 transcript, the response to treatment is faster and deeper [4,8,10,15]. Hanfstein et al. [15] found that the cumulative probability of achieving MMR and $MR^{4.0}$ at 5 years was significantly higher in patients with b3a2 transcript (85% vs. 81%; $p = .002$) than in patients with b2a2 transcript (76% vs. 58%; $p < .001$). They also reported that the median time to obtain MMR and $MR^{4.0}$ was also shorter in patients expressing b3a2 (14.2 vs. 18.4 months) than in those expressing b2a2 (32.4 vs. 55.2 months) [15]. Lucas et al. [8] suggested that this may be due to patients with b2a2 having a higher BCR-ABL tyrosine kinase activity.

In conclusion, our results suggest that the b3a2 and b2a2 transcripts may have different phenotypes, which can have an impact on the outcome of the disease. The b2a2 transcript appears to have a higher tumor load and a higher tyrosine kinase activity than the b3a2 transcript. Our study findings are in agreement with the literature data. Indeed, we found that frontline IM-treated patients expressing the b3a2 transcript of BCR-ABL1 presented higher rates of MMR and DMR than those expressing the b2a2 transcript. However, these observations warrant further confirmation from a larger series. The biological mechanism responsible for this difference should also be investigated in larger trials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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