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REVIEW ARTICLE

Chronic myelogenous leukemia

Chronic myeloid leukemia: the concepts of resistance and persistence and the relationship with the BCR-ABL1 transcript type

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Abstract

Chronic myeloid leukemia is driven by a hybrid gene, *BCR-ABL1*, that codes for a leukemogenic tyrosine kinase (TK) protein of 210 KDa ($p210^{BCR\text{-}ABL1}$). Resistance to TK inhibitor (TKI) therapy occurs in relatively few patients, no more than 10%, while persistence of minimal residual disease during TKI therapy occurs in the great majority of patients. Resistance is a cause of death, persistence is compatible with a fairly normal length and quality of life, but may require lifelong treatment. The causes of resistance are heterogeneous, including the development of other genomic abnormalities or the altered expression of other genes, requiring different treatments. The causes of persistence may not be the same as those of resistance. We hypothesize that the variability in breakpoint position within the Major-breakpoint cluster region (M-*bcr*), resulting in two different messenger RNAs that may or may not include exon 14 of *BCR* (e13a2 and e14a2, respectively), and, as a consequence, in two $p210^{BCR\text{-}ABL1}$ proteins that differ by 25 amino acids, may be a cause of persistence. The hypothesis is based on a critical review of the relationships between the *BCR-ABL1* transcript types, the response to TKIs, the outcome of treatment, and the immune response, suggesting that the e14a2 transcript is associated with more and deeper molecular responses, hence with a higher probability of achieving treatment-free remission (TFR). Investigating this putative cause of persistence may help bringing more patients into stable TFR.

For more than one century, chronic myeloid leukemia (CML) has been an almost fatal disease. Today, with the availability of several targeted agents, the tyrosine kinase inhibitors (TKIs), the survival of CML patients approaches the survival of non-leukemic people, and only about 50% of deaths are due to leukemia progression, that is to say to true resistance to TKIs [1–5]. The cases and the causes of resistance have been investigated in depth, particularly as far as the characteristics and the biologic properties of the so-called leukemic stem cells are concerned [reviewed in [6]]. A new and challenging problem is how to achieve a durable treatment-free remission (TFR), since even in patients who do not develop resistance, Philadelphia chromosome-positive, *BCR-ABL1*-positive cells persist, being sometimes hardly detectable or even undetectable [1, 5–8]. Although these cells are sensitive and remain sensitive to TKIs, only a minority of such patients can discontinue treatment without molecular recurrence of leukemia [1–7]. Therefore, we must face two conditions "resistance" and "persistence". Are they two faces of the same problem, do they share the same molecular bases, and do they require the same therapeutic policies? The issue is clinically relevant: persistence is not a problem of living or dying, yet it is a problem that concerns many patients, involving the quality of life, the side effects, the complications and the cost of chronic treatment, as well as the very concept of "cure".

In a minority of cases, the cause of resistance to a TKI can be easily identified, namely a point mutation in the *BCR-ABL1* kinase domain [1, 5, 9]. A mutation can be relatively easy to overcome using another TKI. However, the identification of other molecular mechanisms of resistance has been hampered by the fact that true resistance is a relatively rare event, and is likely to be multifactorial. Many studies have tried to investigate the effects of $p210^{BCR\text{-}ABL1}$ on several downstream molecular pathways, or to identify other genetic or genomic abnormalities, or to show an altered expression of other genes [1, 5, 6, 10]. Some studies have led to the identification of alternative candidate targets

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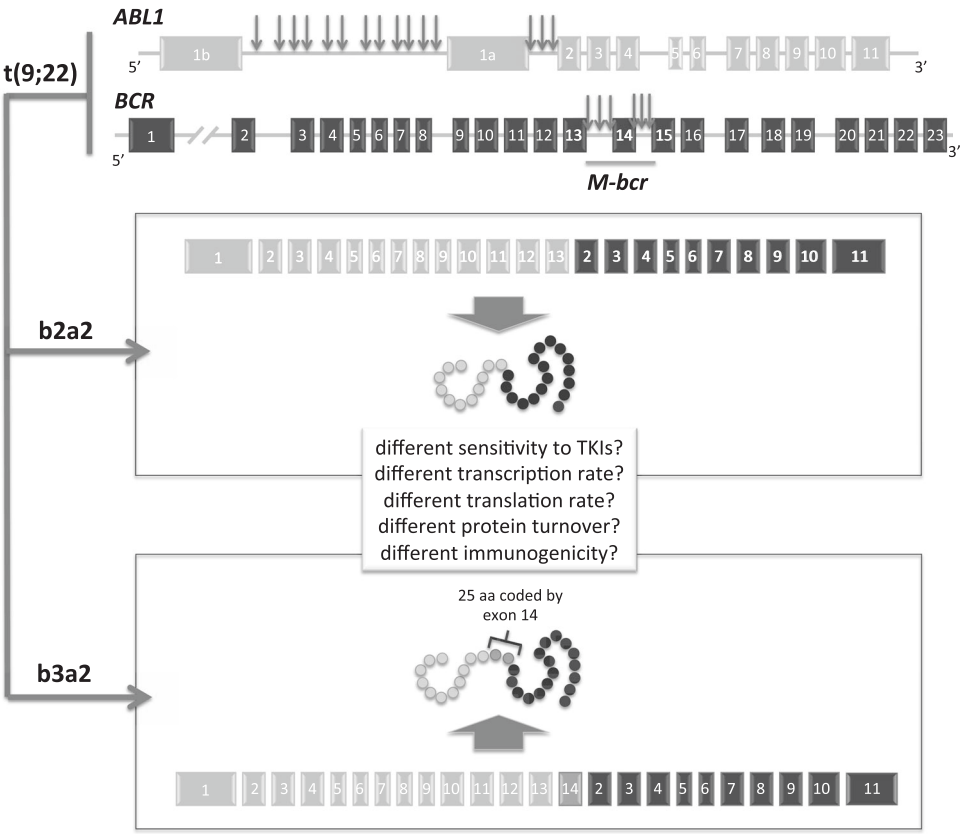

Fig. 1 Schematic representation of breakpoint positions in the *BCR* and *ABL1* genes and structure of the resulting e13a2 and e14a2 fusion transcripts. In CML, almost 98% of the genomic breakpoints in *BCR* occur within the so-called Major-breakpoint cluster region (M-*bcr*) between intron 13 and intron 14. Breakpoints falling within intron 13 or exon 14 will give rise to a mature transcript where, after splicing, exon 13 (e13) of the *BCR* gene is juxtaposed to exon 2 (a2) of *ABL1*, called e13a2. Breakpoints falling more 3', within intron 14, will give rise to a mature transcript including also exon 14 (e14) of *BCR*, called e14a2 Therefore, the e14a2 fusion transcript codes for a protein that is slightly different from that coded by e13a2, being 25 amino acid (aa) longer

for therapy, but until now none has been translated into novel, effective treatment options. After the introduction of imatinib, all progresses in therapy have been due to the development of other TKIs, the so-called second- and third-generation TKIs, that are more potent than imatinib in the inhibition of unmutated as well as mutant $p210^{BCR\text{-}ABL1}$.

The case of persistence is likely to be different. Persistence is the case of a patient who achieves a major molecular response (MMR, *BCR-ABL1* ≤ 0.1%^{IS}) slowly, or who does not achieve a deep molecular response (DMR, *BCR-ABL1* ≤ 0.01%^{IS}), or, if he does, who relapses after treatment discontinuation, but continues to be alive and well on TKI therapy. We hypothesize that a cause of persistence may be found in qualitative or quantitative differences in $p210^{BCR\text{-}ABL1}$. A qualitative difference is defined as a difference that makes the protein a more or less suitable target for TKIs, resulting in different levels of inhibition, or a more or less powerful trigger of an immune response. A quantitative difference is defined as a difference that concerns the balance between the amount of the target ($p210^{BCR\text{-}ABL1}$) and the amount of the inhibitor. In both cases, the degree of inhibition may be sufficient to keep leukemic cells quiescent, avoiding proliferation and progression, but it may not be sufficient to eradicate leukemia. The current concept of CML as a monolytic disease—one gene, one mRNA, and one protein—does not explain why patients with the "same" disease, driven by the same "target", respond differently to targeted therapy, and does not take into account the possibility that response to treatment and outcome may differ, slightly but importantly, depending on differences in the "target" itself, that is $p210^{BCR\text{-}ABL1}$. The genomic breakpoints that leads to the formation of the *BCR-ABL1* hybrid gene can occur at different sites in *BCR* (and sometimes also in *ABL1*) [11, 12] (Fig. 1). In CML, almost 98% of the genomic breakpoints in *BCR* fall within the so-called Major-breakpoint cluster region (M-*bcr*), a region of ~3 kb between intron 13 and intron 14. In *ABL1*, breakpoints cluster in intronic regions over an area of 200 kb between exon 1b and exon 2 (more rarely exon 3). When the breakpoint falls in intron 13 or in exon 14 of *BCR*, the resulting mature fusion transcript will be e13a2, where exon 13 (e13) of *BCR* is juxtaposed to exon 2 (a2) of *ABL1* (if the breakpoint is within exon 14, the partial exon is eliminated during the splicing process) [11, 12]. When the breakpoint falls in intron 14, the resulting mature fusion transcript will be e14a2, where exon 14 (e14) of *BCR* is juxtaposed to exon 2 (a2) of *ABL1*. Both transcripts are translated into a protein of 210 kDa ($p210^{BCR\text{-}ABL1}$) but the protein resulting from e14a2 features 25 amino acids more than the protein resulting from the e13a2 transcript (Fig. 1).

Unanswered questions are (a) are the two p210$^{BCR\text{-}ABL1}$ proteins equally sensitive to TKIs? and (b) are the transcription and translation rates of the two fusion genes and transcripts, respectively, and the turnover of the two proteins, different, resulting in different intracellular levels of the two proteins? Some studies favor indirectly the simple hypothesis that what counts is the amount of the protein, because altering the balance between the amount of the target (the protein) and the amount of the inhibitor (the TKI), results in a modulation of the resistance to TKIs. When a leukemic cell doubles the Philadelphia chromosome, as it happens sometimes when leukemia progresses from chronic to blastic phase and becomes resistant to TKIs, the presence of two *BCR-ABL1* gene copies results into an increase of p210$^{BCR\text{-}ABL1}$ [13]. When the genes coding for the multidrug-resistant proteins are overexpressed, the intracellular level of the inhibitor is reduced, and cells are less sensitive to TKIs [14]. Several studies by the Adelaide group [15, 16] have shown that the expression of the hOCT1 transporter regulates the cellular intake of TKIs, mainly of imatinib, so that when hOCT1 is underexpressed less TKI is transported into the cells, cells are less responsive to treatment, and the clinical response to treatment is poorer. Regrettably, the cellular levels of the two different mRNAs and of the two different p210$^{BCR\text{-}ABL1}$ proteins have not been studied extensively and compared so far, but recently it has been reported that when the expression of the BCR-ABL1 mRNA is normalized to cell number to generate an expression ratio, the expression of e13a2 mRNA is lower than that of e14a2 [17]. It has also been reported that a high baseline level of the transcript (before any treatment) predicts an inferior response to imatinib, and a high transcript level may reflect a larger leukemic cell burden but also a higher level of transcript per cell [18].

Are e13a2 and e14a2 CMLs identical? The hematologic phenotype and the baseline risk of CML patients according to the M-*bcr* transcript type do not differ, apart from a higher platelet count in e14a2 patients [19–21]. The distribution by gender and by age differs, as e13a2 is less frequent in females than in males (36.2% vs 39.2%, $P < 0.0001$) and its prevalence decreases with age, from 38.6% in children and adolescents to 31.6% in the elderly ($P < 0.0001$) [21]. These differences are small, but are consistent with the findings that the prognosis of females is better and that the disease is more aggressive in children and adolescents [1]. A relationship between the M-*bcr* transcript type and survival was reported but was not confirmed in the pre-TKI era [20, 22]. In patients treated first line with TKIs, this relationship has been analyzed in few studies (Table 1). The complete cytogenetic response (CCyR) rate was reported to be the same in four studies [23–26], and to be significantly lower in e13a2 patients in three studies [27–29]. The MMR rate was reported to be the same in one study [25], and to be lower in e13a2 patients in seven studies (with the difference achieving statistical significance in three) [23, 24, 26, 28–31]. The DMR rate was never found to be the same, since it was reported to be lower in e13a2 patients in all nine studies (statistically significantly in seven) [23–26, 28, 31–34]. Progression-free survival (PFS) or transformation-free survival(TFS) was reported to be the same in three studies [23, 29, 31], and to be significantly worse in two [28, 35]. Overall Survival (OS) was reported to be the same in three studies [23, 27, 31] and to be significantly worse in two [26, 36]. In a study of second-line treatment, e14a2 was found to be the strongest predictor of MMR ($P = 0.009$) and of failure-free survival ($P = 0.064$) in 120 patients who were switched to a second-generation TKI after failing imatinib at three months [37]. Overall, all studies have reported one or more significant differences in response and outcome favoring e14a2 patients, while no studies reported a better response or outcome in e13a2 patients. Moreover, in a review of 283 cases of blast crisis, the proportion of e13a2 patients was reported to be higher than expected at diagnosis (53% vs 39%) [21, 38], suggesting that a progression to blast crisis was more frequent in e13a2 patients. It should be noticed that in most studies, first-line treatment was imatinib or imatinib-based, while in three studies it included also second-generation TKIs, and in one study it was not specified. It is not surprising that more differences, and deeper differences, were found in molecular response than in survival, because the type of the transcript is expected to modulate the depth of the response, hence the probability of achieving TFR, not the survival. A relationship, though indirect, between the M-*bcr* transcript type and TFR can be found in few papers [25, 34, 35, 39–41] (Table 2). In these studies, the proportion of e13a2 patients who were enrolled or reported in TFR trials, having achieved a stable DMR, ranged between 34% and 18%, always lower than expected from the proportion of e13a2 patients at diagnosis, that is 40% [21]. In one study [34] the reported proportion of patients with molecular recurrence was higher in e13a2 patients that in e14a2 patients (65% vs 34%, $P = 0.008$). It is also worth remembering that female sex and older age, that are both associated with a higher proportion of e14a2, were reported to predict for a stable DMR and a higher TFR rate, respectively [42]. However, no differences between males and females were reported in the STIM1 and in the ENESTfreedom studies [43, 44].

It should not be overlooked that all comparisons between patients harboring e14a2 and e13a2 have so far been based on quantitation of the mRNA. Therefore, it may be possible that some of the differences that have been reported depend, at least to some extent, on technical issues. Polymerase chain reaction (PCR)-based assays may generate artifacts and may display differences in performance, resulting in differences in the accuracy of e13a2 vs e14a2 quantitation.

Table 1 Data on response to TKIs and outcome according to transcript type from 13 reports published between 2009 and 2019

Ref.	TKI	No. of pts	F-Up	CCyR rate, e13a2 vs e14a2	MMR rate, e13a2 vs e14a2	DMR rate, e13a2 vs e14a2	PFS/TFS rate, e13a2 vs e14a2	OS rate, e13a2 vs e14a2
[27]	I	71	2 y	At 1 y, 25% vs 54% ($P = 0.01$) At 2 y, 39% vs 58%	NR	NR	NR	=
[23]	I	1105	5 y	=	At 5 y, 78% vs 86% ($P = 0.002$)	$MR^{4.0}$, 55% vs 75% ($P < 0.001$)	=	=
[32]	I	320	6 y	NR	NR	$sMR^{4.0}$, 53% vs 61% ($P = 0.07$)	NR	NR
[30]	I	166	NR	NR	61% vs 71% ($P = 0.04$)	NR	NR	NR
[26]	I	559	6 y	=	83% vs 88% ($P < 0.01$)	$MR^{4.0}$, 52% vs 67% ($P = 0.001$)	At 7 y, 81% vs 89% ($P = 0.005$)	At 7 y, 83% vs 90% ($P = 0.017$)
[29]	I	170	6 y	At 6 m, 43% vs 70% ($P = 0.02$) At 12 m, 62% vs 78% ($P = 0.16$)	At 18 m, 54% vs 69% ($P = 0.46$)	NR	=	=
[36]	I	1494	6.5 y	NR	NR	NR	NR	At 5 y, 89% vs 93% ($P = 0.02$)[a]
[33]	I	172	5 y	NR	NR	($P = 0.001$ in favor of e14a2)	NR	NR
[28]	I, N, D	481	8 y	At 6 m, 73% vs 81%	At 1 y, 55% vs 83%	$MR^{4.5}$ 55% vs 80% ($P = 0.0001$)	At 5 y, 91% vs 97% ($P = 0.04$)	=
[24]	I, N, D	603	8.5 y	=	At 1 y, 36% vs 46%	$sMR^{4.5}$, 34% vs 45%	NR	NR
[25]	I,N,D	173	5 y	=	=	sDMR, 27% vs 48% ($P = 0.004$)	NR	NR
[31]	N	345	5 y	NR	At 1 y, 66% vs 72%, ($P = 0.24$) 82% vs 88% ($P = 0.13$)	At 3 y, $MR^{4.0}$, 56% vs 66% ($P = 0.06$) $MR^{4.0}$, 60% vs 65% ($P = 0.10$)	=	=
[34]	NR	280	NR	NR	NR	At 6 y, $MR^{4.5}$, 52% vs 70%	NR	NR

In all studies, the cytogenetic and the molecular responses were either equal or better for e14a2 patients, and in many cases the difference with e13a2 was reported to be significant ($P < 0.05$). PFS/TFS was reported in five studies, with a small but significant advantage for e14a2 patients in two studies. OS was reported in seven studies, with a small but significant advantage for e14a2 patients in two studies. When the time points are not indicated, the percentage represents the cumulative incidence. *P* values are reported only if and as they were calculated in the original papers

The symbol "=" indicates no difference between e14a2 and e13a2

D Dasatinib, *I* Imatinib, *N* Nilotinib, *y* years, *m* months, *F-up* follow up, *CCyR* complete cytogenetic response (absence of Ph+ metaphases), *MMR* major molecular response (BCR-ABL1 ≤ 0.1%^{IS}), *DMR* deep molecular response ($MR^{4.0}$, BCR-ABL1 ≤ 0.01%^{IS}; $MR^{4.5}$, BCR-ABL1 ≤ 0.0032%^{IS}), *sDMR* stable DMR, *$sMR^{4.0}$* stable $MR^{4.0}$, *$sMR^{4.5}$* stable $MR^{4.5}$, *PFS* progression-free survival, *TFS* transformation-free survival, *OS* Overall Survival (all deaths), *NR* not reported

[a]In this study, the OS was reported to be the same when e14a2 was compared with e13a2, but to be different when e14a2 + e13a2 were compared with e13a2, in favor of e14a2 + e13a2

Table 2 Distribution by transcript type of the patients who met the criteria for TKI discontinuation and who experienced molecular recurrence after discontinuation, as reported in five studies

Ref.	TKI	No. of pts	Percentage of pts who discontinued, e13a2 vs e14a2	Percentage of pts with molecular recurrence after discontinuation, e13a2 vs e14a2
[39]	I	85	22% vs 78%	NR
[35]	I	111	31% vs 70%	15% vs 7%
[34]	NR	96	34% vs 66%	66% vs 35% ($P = 0.008$)
[40]	I, N, D	111	22% vs 78%	NR
[41]	I, N	252	25% vs 75%	NR
[25]	I, N, D	51	18% vs 82%	68% vs 41%

In all studies, the proportion of e14a2 patients was higher than the proportion found at diagnosis (60%) and expected if the type of transcript were irrelevant. In one study, the proportion of e14a2 patients with molecular recurrence after TKI discontinuation was significantly lower in e14a2 patients, in spite of the fact that the number of e14a2 patients (66%) was higher than the number of e13a2 pts (34%). Rates and *P* values are as reported in the original papers. Abbreviations as in Table 1

Usually, a single assay is used that may amplify both e13a2 and e14a2 BCR-ABL1 transcript variants. A polymorphism in exon 13 of *BCR* has been described [45]. Polymorphisms may reduce the binding efficiency of the primer used to detect e13a2, resulting in inferior PCR yield. In addition, evidences have been brought suggesting that the shorter e13a2 amplicon may be amplified more efficiently than the e14a2 by real-time PCR assays [46]. However, since it is the protein that matters, future studies should focus more on the comparison between the $p210^{BCR\text{-}ABL1}$ proteins that are encoded by the two mRNAs, as it has been already done for p210 $^{BCR\text{-}ABL1}$ vs $p190^{BCR\text{-}ABL1}$ [47, 48].

Another important cause of persistence may be related to the immune response, i.e., the capacity of the immune system to control or to eradicate the BCR-ABL1-positive stem cells that survive the TKIs. The importance of the immune response in the setting of CML was already shown with allogeneic stem cell transplantation [1, 5, 49], and is supported, although indirectly, by the therapeutic activity of interferon-α [1]. Since the proteins encoded by the two M-*bcr* transcripts differ in 25 amino acids at the fusion junction of *BCR* and *ABL1*, the two proteins may display different immunogenicity and may trigger different responses [50–53]. Few studies have reported on a relationship between immunogenicity and transcript type and it is of interest that they all focused on the e14a2 transcript, as already highlighted [21]. Patients with e14a2, but not e13a2, were shown to produce interferon γ in response to stimulation with Ph+ monocyte-derived dendritic cells [50]. An immune response and a clinical benefit were reported in 11 of 16 e14a2 patients who were vaccinated with a peptidic vaccine derived from the sequence p210–e14a2 [51]. Nineteen e14a2 imatinib-treated patients were vaccinated with peptides spanning the BCR-ABL1 e14a2 fusion junction, and 14 of 19 developed T cell responses to those peptides [52]. In a pilot study of vaccination with autologous non-irradiated dendritic cells, T-cells recognizing leukemia-associated antigens became detectable in three of ten patients, and all three had the e14a2 transcript [53].

In conclusion, CML is no longer a fatal disease, and relatively few patients become resistant to treatment and die of leukemia. The causes of resistance to TKIs are heterogeneous, and at least in part must be searched in the development of other, additional, genomic abnormalities that are neither easy to identify nor easy to manage [6]. Up to 90% of all patients remain alive and relatively well, but only a minority of them can achieve the ultimate goal of TFR, so that many continue to be TKI-dependent lifelong. These are cases of persistence. The causes of persistence of Ph-positive BCR-ABL1-positive cells might be heterogeneous as well, but are likely to be different from the causes of resistance. We hypothesize that one cause of persistence may depend on the gene and we propose that more investigations should be designed and performed to evaluate the rate of transcription of the gene, the rate of translation of the mRNA into protein, and particularly the cellular amount, the characteristics, and the immunogenicity, of $p210^{BCR\text{-}ABL1}$. We do not predict to find major differences, because exploring persistence is not like exploring a "black or white" difference. In the setting of TFR in CML, any difference is likely to be subtle, but it is not as subtle for the patients, considering the difference between living on treatment and living off treatment. In any case, identifying a difference will be relevant, as it will provide useful data for a better understanding of CML response to TKIs, and for improving the TFR rate.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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