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Review Article

Prognostic Significance of Transcript-Type *BCR-ABL1* in Chronic Myeloid Leukemia

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Abstract. Chronic myeloid leukemia (CML) is characterized by the presence of the *BCR-ABL1* fusion gene. In more than 95% of CML patients, the typical *BCR-ABL1* transcript subtypes are e13a2 (b2a2), e14a2 (b3a2), or the simultaneous expression of both. Other less frequent transcript subtypes, such as e1a2, e2a2, e6a2, e19a2, e1a3, e13a3, and e14a3, have been sporadically reported. The main purpose of this review is to assess the possible impact of different transcripts on the response rate to tyrosine kinase inhibitors (TKIs), the achievement of stable deep molecular responses (s-DMR), the potential maintenance of treatment-free remission (TFR), and long-term outcome of CML patients treated with TKIs. According to the majority of published studies, patients with e13a2 transcript treated with imatinib have lower and slower cytogenetic and molecular responses than those with e14a2 transcript. They should be considered a high-risk group that would most benefit from frontline treatment with second-generation TKIs (2GTIKIs). Although few studies have been published, similar significant differences in response rates to 2GTKIs have been not reported. The e14a2 transcript seems to be a favorable prognostic factor for obtaining s-DMR, irrespective of the TKI received, and is also associated with a very high rate of TFR maintenance. Indeed, patients with e13a2 transcript achieve a lower rate of s-DMR and experience a higher probability of TFR failure. According to most reported data in the literature, the type of transcript does not seem to affect long-term outcomes of CML patients treated with TKIs. In TFR, the e14a2 transcript appears to be related to favorable responses. 2GTKIs as frontline therapy might be a convenient approach in patients with e13a2 transcript to achieve optimal long-term outcomes.

Keywords: Chronic myeloid leukemia; Transcript type; e13a2; e14a2; Treatment-free remission.

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Introduction. Chronic myeloid leukemia (CML) is a hematological malignancy that has an estimated incidence of 1-2 cases per 100,000 adults and accounts for nearly 15% of new leukemia diagnoses in adults. The prevalence of CML in the US was estimated at approximately 80,000-100,000+ in 2017.[1] Pathognomonic CML is a cytogenetic aberration well-known as the Philadelphia (Ph) chromosome, which represents the first chromosome alteration associated with a specific human malignancy.[2] The Philadelphia chromosome derives from a reciprocal translocation involving the 3′ region of the proto-oncogene *c-ABL* (9q34) and the 5′ region of the breakpoint cluster region (*BCR*) gene on chromosome 22q11 (t(9;22)(q34;11).

This balanced translocation determines the production of the *BCR-ABL1* oncogene, which encodes a protein with constitutive tyrosine kinase activity that promotes leukemogenesis.[3,4] The major breakpoint cluster region (*M-BCR*) consists of 5 exons called e12 to e16 (formerly b1-b5) located within the central region of the *BCR* gene, and more than 95% of CML patients have a breakpoint in this specific region. Two major breakpoints are identified, one after the 13th exon producing b2a2 (e13a2) fusion and the other after the 14th exon consisting of b3a2 (e14a2) fusion.[5,6] These fusion mRNAs encode two 210-kDa tyrosine kinase proteins ($p210^{BCR-ABL}$). Approximately 5-10% of CML patients may co-express both e13a2 and e14a2 transcripts. These proteins act as tyrosine kinases, have masses of 210-kDa, and differ by 25 amino acids coded by the b3 exon and an amino acid substitution (Glu903Asp).[7] The difference between the two proteins was found in the secondary structural elements, specifically, in five α-helices and nine β-strands related to differences in the SH1, SH2, SH3, and DNA-binding domains. These variations may explain the distinct activities exerted by the two isoforms in mediating signal transduction during the evolution of the disease.[8,9] The p210 protein is also detected in nearly 40% of adults and 10% of children with t(9;22)-positive precursor B-lymphoblastic leukemia (B-ALL).[10] In approximately 60-80% of patients with Ph-positive acute lymphoblastic leukemia (ALL) and rare cases of CML, the breakpoint occurs in the minor-*BCR* (*m-BCR*) region, thereby resulting in the shorter isotype p190 *BCR-ABL1* encoded from the e1a2 type mRNA.[11] In CML, the e1a2 transcripts may be co-expressed with e13a2 (b2a2)/e14a2 (b3a2) and CML presenting only the e1a2 transcript is uncommon (approximately 1% of all CML) and shows an inferior outcome to treatment with TKIs.[12,13] An extreme 3' breakpoint seldom arises after exon 19 (e19) of *BCR* in the designated *μ-BCR* region and produces the larger (p230) fusion protein. $P230^{BCR-ABL}$ has been associated with a rare disease known as CML with neutrophil's maturation.[14] Several atypical *BCR-ABL1* transcripts (e1a3, e13a3, e14a3, e19a3, e6a2, and e8a2, which account for less than 1% of CML cases) deriving from chromosomal breakpoints outside the *ABL* intron 1 or *BCR* intron 1, 13, or 14 have been described.[15] The most frequent breakpoint regions in the *c-ABL* gene are 5′ of the second exon resulting in a2 junctions. Other breakpoint regions detected between the second and the third exon have been observed, determining a3 junctions.[16]

The *BCR-ABL1* gene can be studied by several molecular techniques (fluorescence in situ hybridization [FISH], Southern blotting, and reverse-transcription polymerase chain reaction [RT-PCR]). RT-PCR is the most common method used for detecting *BCR/ABL1* transcript type due to its simplicity, rapidity, and sensitivity. However, recent new molecular techniques have been developed to detect all kinds of transcripts using a more rapid and appropriate approach. The multiplex PCR technique applying primers coupled to distinct fluorochromes and the optical system of a sequencer can simultaneously detect either the transcript (fluorescence) or the class of junction that it holds (size).[17] This technique accurately identifies the transcript at diagnosis and allows follow-up at the molecular level.[17,18] RT-qualitative/quantitative PCR is useful to identify the typical transcripts (e13a2 or e14a2) at baseline and to monitor their quantitative fluctuations during the treatment. Atypical transcripts may yield a false negative PCR using routine primer/probe sets in qualitative or quantitative reverse transcriptase PCR protocols. If not tested at diagnosis, a false impression may be given that a patient is in complete molecular response after TKI treatment. Therefore, cytogenetics should be done in patients with atypical *BCR-ABL1* transcripts that cannot be measured by RT-quantitative PCR. FISH monitoring may also need in patients with atypical transcripts.

Several studies investigated the impact of *BCR-ABL1* transcript types on CML patients receiving TKIs; the patients' characteristics at baseline, TKI response, and long-term outcomes provided different results by transcript type. In treatment-free remission (TFR), it is essential to evaluate whether the transcript type may identify a group of patients who more likely may achieve an s-DMR and may have a high probability of maintaining DMRs during drug discontinuation. In this review, we evaluated the impact of different *BCR-ABL1* transcripts on responses, long-term outcomes, and TFR rates in CML patients in TKIs.

Relationship Between Transcript Type and Outcome in pre-TKIs. In conventional chemotherapy and interferon (IFNα), different studies have evaluated whether the type of transcript identified at baseline may affect the outcome of CML patients;[19,20,21,76,77] overall, none of these studies found a significant and robust influence of transcript type on response and clinical outcome in this setting. However, in pre-TKI, the breakpoint in the 3' portion of the *BCR* region was associated with more aggressive disease and faster transformation in blast crisis.

In 1989, Mills et al. mapped the breakpoint within the *BCR* in peripheral blood leukocyte-derived DNA from 22 CML patients and first studied whether there was a correlation between the site of breakpoint and outcomes. No associations between the breakpoint site and the disease's clinical phase emerged. Still, a notable relationship between the breakpoint site, length of time elapsed from the presentation, and occurrence of acute phase was reported. Indeed, the median time of chronic phase duration in patients harboring a 3' breakpoint was 52 weeks, while that in patients with a 5' breakpoint was 203 weeks and the rate of progression to blast crisis was

significantly different between the two groups (p<0.02).[19] The authors concluded that patients with a 3' breakpoint had worse outcomes, showing a four-fold more rapid transition to blast crisis then patients with a 5' breakpoint.

Later, an English group analyzed the correlation between mRNA transcripts and clinical characteristics, cytogenetic response, duration of chronic phase, and outcomes in a large cohort of 216 CML patients treated with IFNα. No differences were found between clinical characteristics (hemoglobin concentration, white cell and platelet count, basophil numbers, blast cell numbers, and spleen and liver size) of patients with e13a2 and e14a2 breakpoints except for a Sokal risk group, which was inferior among those with e13a2 transcripts (p=0.04). No significant differences were also observed in terms of the duration of the chronic phase and outcomes in patients with the e13a2 and e14a2 transcripts. Five-year survival was 52% and 54% (p=0.95) for e13a2 and e14a2, respectively.[21]

An Italian group reported the transcript type impact on outcomes in 146 CML patients who were enrolled in a prospective study that provided IFNα treatment for at least one year. A trend in favor of e14a2 cases was observed or in cytogenetic response after 1 year of IFNα treatment (39% in the e14a2 group vs 24% in the e13a2 group) in 5-year survival rates (71% in e14a2 patients vs 57% in e13a2 patients) (**Table 1**).[22]

In IFNα, although e13a2 transcript was associated with an unfavorable trend in outcomes and treatment responses, no data were sufficient to define the type of transcript as an independent prognostic factor.

Type of Transcript and Response to Imatinib. In the currently available literature, several studies[23,24,25,26,27,28,29,30,79] analyzed whether the two transcripts (e14a2 and e13a2) have different or similar responses to imatinib treatment.

A German CML study group analyzed a large cohort of 1,105 newly diagnosed imatinib-treated patients by transcript type at baseline (e13a2, n=451; e14a2, n=496; and e13a2+e14a2, n=158). Patients expressing e14a2 transcript showed a better cumulative incidence (CI) of major molecular response (MMR) (p=0.002) than those with e13a2 transcripts, while patients co-expressing e13a2 and e14a2 transcripts did not differ from the other two groups (p=ns). There was also a significant difference in median time to MMR comparing patients harboring e13a2 and e14a2 transcripts, respectively (18.4 vs. 14.2 months). The CI of MR4.0 and the median time to MR4 (55.2 vs. 32.4 months) were significantly better in the e14a2 group (p<0.001). Patients co-expressing e13a2 and e14a2 transcript differed from e14a2 (p=0.004) but not from e13a2 in terms of MR4 achievement. Patients were also evaluated separately in accordance with the treatment arms. MR4.0 rates were higher in the group expressing the e14a2 transcript than the e13a2 transcript group in the three treatment arms, which included imatinib at a dose of 400 mg plus IFNα, imatinib at a dose of 400 mg plus cytarabine, and imatinib at a dose of 800 mg (p<0.001, p=0.004, and p=0.028, respectively).[23]

In the MDACC study that included 481 patients with chronic phase CML (CML-CP), the authors assessed the prognostic significance of transcripts in four groups of patients treated frontline with different TKI therapies (imatinib at a dose of 400 mg, imatinib at a dose of 800 mg, dasatinib 50 mg twice daily or 100 mg daily, and nilotinib 800 mg/day). Complete cytogenetic response (CCyR) rates were inferior in the e13a2 group treated with imatinib 400 mg daily (77%) compared with other TKIs (90%-95%). Regarding molecular responses, the CI of MMR and MR4.5 were significantly superior in the e14a2 and co-expression groups than the e13a2 group in all of the treatment arms (p<0.001 and p<0.001, respectively). When treatment responses were assessed for each specific TKI treatment option, patients with the e13a2 transcript who had received imatinib 400 mg daily showed a significantly lower rate of CCyR, MMR, and MR4.5 than those reported among both patients expressing the e13a2 transcript receiving other TKI treatments and patients harboring different transcript

Table 1. Responses to treatment according to transcript types in pre-TKIs era.

Reference	Total number of patients	E13a2 %	E14a2 %	E13a2+e14a2 %	Treatment	Comment
Lee et al. (68)	134	38	52	10	Interferon-α	MCyR[1]: 55% vs 24% in e13a2 and e14a2, respectively; p<0.0001.
Prejzner-Rego et al. (21)	62	29.5	62.3	8.2	Interferon-α	No significant differences in terms of response.
Shepherd et al. (20)	219	40	55	5	Interferon-α	No significant differences in terms of response and outcome.
Italian co-operative study group (22)	146	43	57	0	Interferon-α	E14a2: trend for higher MCyR[1] and 5-year OS (p=ns[2]).
Mondal et al. (67)	122	27	56.5	5	Hdroxyurea, interferon-α	E13a2: trend for younger age, and higher WBC (p = ns[2]).

MCyR: major molecular response; Ns= not significant.

types receiving imatinib 400 mg daily. After a long-term follow-up of 60 months, higher CCyR, MMR, and MR4.5 responses persisted in the group of patients with the e14a2 transcript. In contrast, the MR4.5 response sustainability was lower in patients with the e13a2 transcript than those with the e14a2 transcript and transcript co-expression (p<0.001).[24]

An Italian study conducted by the GIMEMA group including a large cohort of 559 patients treated with imatinib frontline observed that MMR rates at 18 months and MR 4.0 rates at 36 months were significantly inferior in patients expressing the e13a2 transcript (52% vs. 67%, p=0.001, and 20% vs. 30%, p=0 .013, respectively). The median time to MMR in the e14a2 and e13a2 groups was 12 and 6 months, with 83% and 88% estimated probability of achieving MMR (p<0.001), respectively. The median time of attaining MR4.0 was 61 and 41 months, and the estimated rate of MR4.0 was 52% and 67% in the two classes of patients (p=0.001), respectively.[25]

Lin at al. retrospectively analyzed a cohort of 166 patients (36.7% of patients had e13a2 transcripts, 50% had e14a2, and 13.3% co-expressed e13a2 and e14a2 at baseline) treated for up to 10 years, focusing on the correlation between *BCR-ABL1* transcript type and molecular responses to imatinib after a long-term follow-up. Patients with e14a2 or both e14a2 and e13a2 transcripts had higher MMR rates than those with e13a2 (81.8% vs 60.7% [p=0.023] for e14a2 vs e13a2, respectively, and 77.1% vs 60.7% (p=0.043) for both transcripts vs e13a2, respectively). The median time to achieve MMR, disease progression rates and the median time to disease progression did not differ between the three groups.[27]

A Korean study evaluated outcomes in patients who received imatinib frontline with EMR failure at three months to individualize potential predictive factors for an overall MMR. In this specific subset of patients, multivariate analyses showed that a transcript type of e13a2 compared with e14a2 and larger spleen size (>9 cm spleen size) represented independent risk factors for failure of overall MMR. According to these results, the authors identified a high-risk group of patients with the previously cited features who would benefit from early decision-making regarding treatment change.[29] Another study by the MDACC group assessed responses to imatinib according to the transcript not only in 251 patients who received imatinib frontline but also in 229 patients treated with imatinib after IFN-α failure. The CCyR rates were similar for patients with e14a2 and e13a2 in both the newly diagnosed (91% and 82%) and post-IFN failure (72% and 78%) groups. The rates of MMR and complete molecular response (CMR) (defined as undetectable transcript levels) were significantly higher in patients who harbored the e14a2 transcript than those with the e13a2 transcript (59% vs. 77%; p=0.008 and 25% vs. 47%; p=0.002, respectively) in the treatment-naive group. Similar results were also found among the group of patients who had failed IFNα long-term treatment; the rates of MMR and CMR were superior in patients with the e14a2 transcript than those with the e13a2 transcript (34% vs. 63%; p=0.001 and 16% vs. 42%; p=0.001, respectively).[30]

Among studies that investigated the impact of transcripts on imatinib response, only one conducted by an Indian group showed better responses in patients with e13a2 than those with e14a2. In this study, the CCyR rates were significantly higher in patients with e13a2 transcripts (59% vs. 28%; p=0.04). However, in the cohort of 70 patients analyzed, some patients (n=40) had received previous treatment with hydroxyurea or IFNα. Therefore, the authors analyzed only the cohort of treatment-naive patients and reported similar CCyR rates among different transcript groups (p=0.396)[31] (**Table 2** and **Table 3**). According to these data, patients with e13a2 transcripts treated with imatinib had a lower and more slow achievement of CCyR, MMR, and DMR than those with e14a2 transcripts. They probably should be considered a high-risk group who would most benefit from treatment with 2GTKIs. To date, the main endpoint of TKIs has become the achievement of s-DMR, allowing discontinuation of therapy. Therefore patients with e13a2 would probably require a more potent frontline therapy able to induce more profound and faster molecular responses. Data on responses to imatinib in patients with co-expression of both transcripts remain controversial. Still, most of the studies documented that this group of patients seems to have better responses and prognoses compared to the e13a2 group.

Type of Transcript and Response to 2GTKIs. The 2GTKIs dasatinib and nilotinib have increased the cytogenetic and molecular response rates in CML patients when used as a frontline approach or as second-line therapy after imatinib failure for resistance or intolerance.[32,33,34] To the best of our knowledge, few previously published studies have systematically and retrospectively analyzed response rates in patients who received 2GTKIs frontline according to the type of *BCR-ABL1* transcript detected at diagnosis.

The MDACC study included 105 patients who had received dasatinib (50 mg twice daily or 100 mg daily) and 108 patients who had received nilotinib (400 mg twice daily) as frontline treatment. Patients with the e13a2 transcript in both 2GTKI groups achieved overall CCyR rates superior to that of imatinib at a dose of 400 mg/day (95% vs. 77%), but similar to that of imatinib at a dose of 800 mg/day (95% vs. 90%). Similarly, for MMR and MR4.5, the e13a2 group who received imatinib 400 mg daily showed a trend of a lower response rate compared with the groups of patients treated with other TKI approaches. According to the MMR and MR4.5 response rates, they were

Table 2. Incidence and cumulative incidence of complete cytogenetic reponses by transcript types in patients treated with imatinib.

Reference	Total number of patients	Incidence of transcript			CI[4]CCyR[6]				
		E13a2 %	E14a2 %	E13a2+ e14a2 %	E13a2 %	E14a2 %	E13a2+e14a2 %	p-value	Follow-up
Hanfstein et al. (23)	1105	41	45	14	94.6	93.3	93.6	NS[5]	5 y[3]
Sharma et al. (31)	87	38	54	8	35	61	NR[1]	.396	2 y[3]
Jain et al.[24]	481	42	41	18	89	94	94	NS[5]	60 mo[2]
Castagnetti et al. (25)	559	36	52	11	89	88	NR[1]	.916	80 mo[2]
Pagnano et al. (28)	170	33	55	12	NR[1]	NR[10]	NR[1]	NR[1]	NR[1]
Vega‐Ruiz et al. (30)	480	39	49	11	91	89	NR[1]	NS[5]	62 mo[2]
Polampalli et al. (69)	202	32	68	0	NR[1]	NR[1]	NR[10]	NS[5]	1 y[3]
Mir et al. (26)	200	24	68	8	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]
Lin et al. (27)	166	36.7	50	13.3	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]

NR= not reported: mo= months; y=years; CI= cumulative incidence; NS= not significant; CCyR= complete cytogenetic response.

Table 3. Cumulative incidence of molecular responses by transcript types in patients treated with imatinib.

Reference	CI[4]MMR[5]					CI[4]DMR[6]				
	E13a2 %	E14a2 %	E13a2+e14a2 %	p-value	Follow-up	E13a2 %	E14a2 %	E13a2+e14a2 %	p-value	Follow-up
Hanfstein et al. (23)	81	85	NR[1]	0.002	5 y[3]	58	76	NR[1]	<0.001	5 y[3]
Sharma et al. (31)	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]
Jain et al. (24)	79	91	95	0.0001	5 y[3]	57	79	80	<0.001	5 y[3]
Castagnetti et al. (25)	83	88	NR[1]	<0.01	80 mo[2]	52	67	NR[1]	0.001	80 mo[2]
Pagnano et al. (28)	NR[1]	NR[1]	NR[1]	NR[10]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]
Vega‐Ruiz et al. (30)	59	77	NR[1]	0.008	62mo[2]	25	47	NR[1]	0.002	62mo[2]
Polampalli et al. (69)	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]
Mir et al. (26)	64	72.1	NR[1]	0.04	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]
Lin et al. (27)	60.7	77.1	81.8	<0.05	2 y[3]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]

NR= not reported; mo= months; y=years; CI= cumulative incidence; MMR= major molecular response; DMR= deep molecular response.

substantially comparable in all TKI modalities for patients expressing e14a2 transcripts except for those who received nilotinib, who had a lower rate of MR4.5 in both the e13a2 and e14a2 cohorts compared with patients treated with imatinib 800 mg daily or dasatinib 50 or 100 mg daily. In fact, the MR4.5 rate in patients with e14a2 transcripts treated with nilotinib 400 mg twice daily was inferior compared to the patients with e14a2 who received imatinib 800 mg/day and dasatinib 50 mg twice daily or 100 mg/day (64% in the group of patients treated with imatinib 400 mg/day, 85% in the group who received imatinib 800 mg/day, 89% in the group who received dasatinib 50 mg twice daily or 100 mg/day, and 68% in the group treated with nilotinib 800 mg/day).

In contrast, the CCyR and MMR response rates in patients with the e14a2 transcript treated with frontline nilotinib were comparable to those of other treatment arms. The authors also concluded that patients receiving 2GTKIs who expressed e14a2 had a trend in favor of achieving more rapid and deeper cytogenetic and molecular responses than those with e13a2 transcripts and were able to maintain these responses for a long time. Furthermore, they also added that expressing the e14a2

transcript (compared with patients harboring e13a2 transcripts but not the co-expressing patients), receiving frontline imatinib at a dose of 800 mg/day or 2GTKIs, and presenting a spleen size <10 cm at diagnosis represented prognostic factors for EFS. They also reported that expressing the e14a2 transcript or co-expressing the e13a2 plus e14a2 transcripts significantly increased the probabilities of achieving MMR at six months and 12 months of TKIs treatment. In a multivariate analysis, positive predictors for TFR were first-line treatments with imatinib 800 mg daily or dasatinib 50 mg twice daily or 100 mg/day and having the e14a2 transcript or co-expressing the e13a2 plus e14a2 transcripts.[24]

The Italian GIMEMA group assessed whether the *BCR-ABL1* transcript type (e14a2 vs. e13a2) affected responses and clinical outcomes in 345 newly diagnosed adult patients treated frontline with nilotinib. The response and outcome rates were uniformly lower in the group of patients with e13a2 transcripts (N=124) than the group with e14a2 transcripts (N=174), but these differences were not statistically significant: MMR by 12 months, 66% vs 72%, p=0.244; MR4.0 by 36 months, 56% vs 66%, p=0.067; estimated CI of MMR, 82% vs 88%, p=0.135; estimated CI of MR4.0, 60% vs 69%, p=0.101; estimated PFS, 88% vs 93%, p=0.547; and estimated OS, 89% vs 94%, p=0.436. The responses of patients who co-expressed the e13a2 and the e14a2 transcripts (N=30) were comparable to those of e14a2 patients.[35]

In another MDACC study, the authors assessed whether the transcript type might affect responses to ponatinib. The analysis included 85 patients (47 with recurrent/refractory CML and 38 newly diagnosed patients) treated with ponatinib. Among recurrent/refractory patients, responses to e13a2 and e14a2 and both transcripts were CCyR 50% vs 61% vs 50% and MMR 29% vs 52% vs 30%, respectively. Among patients in the frontline setting, the median levels of transcripts at three months were 0.098, 0.091, and 0.042 in patients with e13a2, e14a2a2, and both transcripts, respectively, and therefore, no differences in terms of responses were documented.[36]

In patients treated with second- and third-generation tyrosine kinase inhibitors, the type of *BCR/ABL1* transcript did not seem to affect cytogenetic and molecular responses, and the presence of e13a2 transcript was not considered an unfavorable factor for response achievement and time to response. However, further studies in larger patient cohorts are required to clarify these findings.

Impact of *BCR-ABL1* Transcript Type on the Achievement of Stable Deep Molecular Responses. A stable deep molecular response (s-DMR) in CML patients is a prerequisite for possible discontinuation. Patients reaching a transcript level of ≤0.01% achieved a 4-log reduction (MR4), whereas a *BCR-ABL1/ABL* ratio of ≤0.0032% identified a 4.5-log reduction (MR4.5); both identified a DMR. Several studies identified biologic features associated with the probability of achieving a DMR as a stable response (s-DMR for at least two years and treatment duration with TKIs ≥3 years).[24,37,38,39,40]

The MDACC group reported that patients with e14a2 and co-expressed transcripts had a significantly higher probability of achieving a stable MR4.5 than those with e13a2 (8-year probability, 43% vs. 24%; p=0.0021).[24]

An Italian cooperative group correlated the presence of e14a2 transcript types at baseline with a higher frequency of s-DMR (63% vs. 53%; p=0.07) in 320 patients who had received imatinib, but a group of patients included in the study had previously been treated with IFN and the analysis considered only MR4 responses and not MR4.5 response rates.[37]

Our Italian group reported that in univariate analysis (43% vs. 31%, p=0.02) and multivariate regression analysis (e14a2 vs. e13a2 type, HR 1.6, 95% CI: 1.3-2.9; p=0.03), the e14a2 type of transcript was associated with higher achievement of a stable MR4.5 compared to the e13a2 transcript in a series of 208 patients treated with imatinib frontline.[38] An Australian group showed that in a series of 298 patients, 48% of patients with e14a2 transcripts were candidates for a TFR attempt compared with only 32% of e13a2 transcripts after an 8-year follow-up.[39]

In another recent Italian study comparing patients who had achieved s-DMR and patients who did not achieve it, the authors did not find any significant difference according to sex, age, Sokal score distribution, frontline TKI treatment (imatinib vs. 2GTKIs), and duration of TKI treatment. Still, the type of *BCR-ABL1* transcript was the only baseline characteristic that significantly predicted the potential achievement of s-DMR. Indeed, the e14a2 transcript was detected at diagnosis in 56/75 (75%) s-DMR-positive patients and in 29/59 (49%) s-DMR-negative patients (p =0.0023)[40] (**Table 4**).

The results of these studies conducted in real-life settings indicated that the identification of the type of transcript at baseline might help to identify better those patients who are more likely to benefit from therapy discontinuation strategies.

Impact of *BCR-ABL1* Transcript Type on TFR- Treatment-free remission (TFR) is defined as the interval between the date of discontinuing TKI treatment and that of documented molecular relapse or if this did not happen, the date of the last follow-up. The TFR is a new endpoint for CML patients receiving TKIs, with approximately 40% s-DMR after discontinuing treatment.[41,42] However, precisely predicting who will achieve TFR and the subjects remains a difficult challenge.

Table 4. Stable deep molecular response rates by transcript types.

Reference	Total number of patients	Follow-up	s-DMR[1] (%)		
			E14a2	E13a2	p-value
Jain et al. (24)	481	8 years	43	24	0.021
Bonifacio et al. (37)	320	74 months	63	53	0.07
Shanmuganathan et al. (39)	298	8 years	48	32	NR[2]
Breccia et al. (39)	208	7 years	43	31	0.02
D'Adda et al. (40)	134	5 years	47.2	26.9	NR[2]

s-DMR= sustained deep molecular response; NR= not reported.

The Hammersmith group investigated the correlation between the type of *BCR-ABL1* transcript and the probability of TFR in 64 CML patients (37 patients with the e14a2 transcript and 27 patients with the e13a2 transcript) who stopped TKI therapy maintaining MR4.0 or MR4.5 for at least 12 months. At the time of stopping TKI, 32 patients were receiving imatinib and 32 nilotinib or dasatinib. Thirty-seven patients (58%) remained in molecular remission at a median time of 26 months (range 7-64 months) after discontinuing TKI treatment, and presenting the e14a2 *BCR-ABL1* transcript was significantly associated with superior probabilities of remaining in TFR compared with the e13a2 transcript (70% vs. 45%). The 3-year chance of TFR was 53% for the entire cohort, but patients with e14a2 transcripts had a higher 3-year probability of TFR than those with e13a2 transcripts (66% vs. 38%). The authors also found that the e14a2 transcript type (p=0.016) and age at diagnosis of 40 years or over (p=0.003) were the only factors significantly associated with TFR.[43]

In an Australian study including 82 patients, the most relevant finding was that patients eligible for TFR expressing e14a2 *BCR-ABL1* transcripts were more likely to maintain TFR at 12 months than those with e13a2 transcripts (65% vs. 34%; p=0.008) and that patients with either e14a2 or both transcript types were 2.24 times more likely to remain in TFR at 12 months than those with e13a2 transcripts. The authors hypothesized that the higher rate of TFR in the e14a2 transcript group might have been associated with a longer time in MR4.5 before discontinuation (4.1 years in the e14a2 cohort vs. 3.01 years in the e13a2 group).[39]

A recent Italian study showed that the type of *BCR-ABL1* transcript had a significant impact not only on the achievement of s-DMR but also on the maintenance of TFR. In fact, analyzing the DMR loss rate after 12 months from TKI discontinuation, the authors found that patients with e14a2 transcripts had a higher probability of maintaining TFR than those with e13a2 transcripts (79% vs. 40%; p=0.012)[40] (**Table 5**).

According to these data, having an e13a2 type of *BCR-ABL1* transcript is an adverse prognostic factor for achieving s-DMR and maintaining TFR, while presenting the e14a2 transcript is a favorable predictive factor for achieving s-DMR, regardless of the TKI type received and is associated with a consistent rate of TFR maintenance.

Type of Transcript and Long-Term Outcome. Several studies[24,25,28,44] analyzed long-term outcomes and survival data according to different transcript types in CML patients.

The Italian group observed that the 7-year OS (90% vs. 83%, p=0.017), PFS (89% vs. 81%, p=0.005), and failure-free survival (71% vs. 54%, p<0.001) rates were significantly higher in patients with e14a2 transcripts than those with e13a2 transcripts and that the transcript type might be a predictive factor of survival regardless of the daily imatinib dose.[25] Indeed, in the MDACC studies, there were no significant differences in 5-year EFS and OS comparing patients with the e13a2, e14a2, and co-expressing transcripts. However, patients with the e13a2 transcript had a worse transformation-free survival rate than those with the e14a2 transcript or co-expressed e13a2 plus e14a2 transcripts (89%, 95%, and 99%, respectively; p=0.033).[24]

The German group assessed the prognostic correlation between transcript type and long-term survival in 1,494 CML patients who received imatinib. The 5-year incidence of death for CML was 3%, 5%, and 2% (p=0.190) in patients with e14a2 transcripts, e13a2 transcripts, and both transcripts, respectively. There was also no significant difference in terms of 5-year OS comparing patients with the e13a2, e14a2, and co-expressing transcripts when patients were analyzed according to their ELTS risk scores at baseline (89%, 93%, and 93%, respectively; p=0.106).[44] Pagnano et al. observed a higher 10-year OS in patients with e13a2 transcripts than those with e14a2 transcripts (p=0.03) (**Table 6**), but the authors correlated this significance to the younger age of the patients in the e13a2 cohort.[28] According to most of the data reported in the literature, the type of transcript does not seem to affect long-term outcomes of CML patients treated with TKIs and appears to be a negative prognostic factor when OS, PFS, and EFS are considered.

Clinical and Prognostic Significance of Atypical *BCR-ABL1* Transcript Subtypes in CML. The typical

Table 5. Treatment free remission rates by transcript types.

Reference	Total number of patients	Follow-up	TFR[1] (%)		
			E14a2	E13a2	p-value
Claudiani et al. (43)	64	26 months	70	45	NR[2]
Claudiani et al. (43)	64	3 years	66	38	NR[2]
Shanmuganathan et al. (39)	82	12 months	65	34	0.008
D'Adda et al. (40)	75	12 months	75	49	0.0023
Lee et al. (70)	48	12 months	79.9	82.5	0.977

TFR= treatment free remission; NR= not reported.

Table 6. Long-term outcomes and survival data by transcript types.

Reference	Follow-up	E14a2 %	E13a2 %	E13a2+E14a2 %	p-value
OS[1]					
Pagnano et al. (28)	5 years	88	96	NR[4]	NS[5]
Pagnano et al. (28)	10 years	76	94	67	0.03
Castagnetti et al. (34)	7 years	90	83	NR[8]	0.017
Jain et al. (25)	5 years	95	88	98	0.34
Pfirmann et al. (43)	5 years	93	89	93	0.106
PFS[2]					
Pagnano et al. (28)	10 years	89	94	75	0.13
Castagnetti et al. (24)	7 years	89	81	NR[4]	0.005
EFS[3]					
Pagnano et al. (28)	7 years	71	82	71	0.09
Jain et al. (25)	5 years	89	79	87	0.41

OS=Overall Survival; PFS=Progression Free Survival; EFS=Event Free Survival; NR= not reported; NS= not significant.

BCR-ABL1 transcript subtypes are e13a2, e14a2, or expression of both simultaneously, but other less frequently detected transcript subtypes such as e1a2, e2a2, e6a2, e19a2, e1a3, e13a3, and e14a3 have also been studied.[45,46] Although there are several published studies on typical *BCR-ABL1* fusion transcripts in CML patients from different populations, studies on the influence of rare transcript subtypes on the disease course and patient outcomes remain controversial.

The MDACC group assessed the impact of the e1a2 transcript subtype in a large cohort of 2,322 CML patients treated with TKIs and observed that the incidence of e1a2 transcripts was extremely rare in CML patients (41 patients, 1.8%). According to the baseline characteristics, CML with e1a2 transcripts was diagnosed prevalently in older patients ($p<0.001$) and more likely presented a blast phase (BP) at diagnosis ($p<0.001$) compared to patients with typical transcripts. Furthermore, patients who expressed e1a2 transcripts showed a higher frequency of additional chromosomal abnormalities (ACAs) than those with typical transcripts (46.3% vs. 25.2%, $p=0.002$). According to treatment responses, patients with e1a2 transcripts responded more slowly and less likely achieved CCyR (median time to CCyR 53.1 vs. 18.8 months, $p=0.003$; overall CCyR rate 33.3% vs. 66.5%) and MMR (median time to MMR unreached vs. 31.7 months, $p=0.001$; overall MMR rate 18.5% vs. 63.7%) than those with typical transcripts. In addition, regarding outcomes, patients with e1a2 transcripts showed a significantly shorter OS than patients with typical transcripts, with a median OS of 69.5 vs. 206.8 months ($p<0.001$), respectively.[47]

Small series of patients co-expressing e1a2 and e13a2/e14a2 at diagnosis were described regarding responses to TKIs.[48,49] Our Italian group reported treatment responses and outcomes of 29 CML patients co-expressing p190 and p210 proteins. In our cohort, after a median follow-up of 7 years, median OS was 69 months, and EFS was 69%; 28.5% of patients developed resistance to imatinib, and 14.2% experienced a BP. Among eight patients who started frontline on nilotinib, 6 achieved MMR after a median time of 18.8 (range 4-36) months, and two obtained MR4.5 after three months of therapy. In our experience, co-expression of e1a2 and a13a2/e14a2 transcripts was associated with superior rates of resistance and disease progression in patients who received imatinib, whereas, even in a small cohort of patients, treatment with 2GTKIs frontline was associated with better outcomes.[49] Patients with e1a2 transcripts at diagnosis are rare and associated with a minor issue to therapy with imatinib. These patients need to be identified as high-risk patients and receive 2GTKIs as frontline treatment.

The e19a2 rearrangement was initially observed in

neutrophilic CML with a benign clinical evolution.[14,50] Still, it was later reported mainly in patients with typical CML, and some of these patients exhibited an aggressive clinical course. According to the published literature, approximately 50 patients with e19a2 *BCR-ABL1* have been reported in CML, and among them, 16 patients received TKIs.[51,52,53,54,55,56] Of the 16 patients, 13 received imatinib as frontline treatment; among them, six patients achieved CCyR, and 2 had the first MMR with imatinib and second MMR with dasatinib. Three out of 13 patients did not respond to imatinib. One out of 13 patients achieved first MCyR with imatinib and second with nilotinib, while one patient did not respond to imatinib but reached MCyR with dasatinib. Therefore, patients with e19a2 seem to have better responses to 2TKIs.

The e1a3 CML-related atypical translocation is associated with an indolent clinical course, low leukocyte count, long duration of chronic phase even without treatment, and a good rate of responses to TKIs.[57,58] However, Martinez-Serra et al. reported a case of an e1a3-positive patient who, after an initial response to imatinib, experienced a lymphoid blast crisis.[59]

In the literature, fewer than 20 CML cases were reported in which e6a2 fusion was usually associated with a clinically aggressive disease frequently presenting in accelerated or blast crisis phases.[60,61,62] Although responses to imatinib have been reported,[63,64] several cases of *ABL1* kinase domain mutation-associated imatinib-resistant e6a2 *BCR-ABL1* CML have been documented[65,66] with limited information on the efficacy of frontline 2GTKIs in this genotype.

The prognostic significance of atypical transcripts remains controversial (except for e1a2) due to different disease genotypes correlated with each transcript and the small number of patients treated with TKIs.

Conclusions and Future Directions. In this review, we reported on the influence of the transcript type on molecular and cytogenetic responses achieved after different TKI regimens in newly diagnosed CML patients and compared different transcripts according to survival outcomes and TFR rates.

Several studies reported that imatinib-treated patients with e14a2 transcript (and to some extent those with co-expression of e14a2 and e13a2) obtained more rapid and deeper cytogenetic and molecular responses than those with only e13a2 transcripts and maintained these responses longer.[24,25,28] However, in the majority of studies, e14a2 transcripts did not seem to be associated with better outcomes in terms of long-term OS, EFS, and PFS[24,28,44] in patients who received imatinib frontline. Therefore, the e13a2 *BCR-ABL1* transcript negatively affects the rate, depth, and speed of responses to imatinib, and including the transcript type in the calculation of the baseline risk scores may improve prognostic stratification and assist with choosing the best treatment policy.

Scant data on the prognostic influence of the *BCR-ABL1* transcript type in CML patients treated frontline with second- and third-generation TKIs are available. In this setting, although a trend in lower response rates and inferior outcomes in patients with e13a2 transcripts has been reported, the observed differences were predominantly not significant between e13a2 and e14a2 groups.[35,36] Further studies of larger patient cohorts are required to clarify whether 2GTKIs are able to overcome the adverse prognostic impact of transcript type, potentially improving the probability of achieving s-DMR and TFR rates and patient outcomes. To date, patients with e13a2 transcripts, if possible (no cardiovascular comorbidities or previous respiratory diseases), could be considered for treatment with 2GTKIs or, if patients are not eligible for 2GTKIs due to baseline comorbidities, the molecular monitoring should be conducted more strictly and carefully. However, the new version of ELN guidelines[71] does not recommend any specific treatment choice according to the type of transcript at baseline. The type of transcript has not yet been included in the prognostic scores generally used for patients with CML and age, comorbidities, and EUTOS Long Term Survival (ELTS) risk-score at diagnosis remain the main factors that guide the therapeutic strategy.

For CP-CML patients, the TFR is increasingly becoming a goal of therapy; however, the ability to predict success following attempted TFR remains limited. The new ELN guidelines[71] require the presence of typical e13a2 or e14a2 *BCR–ABL1* transcripts for potentially attempting TFR. Recent studies[38,39,40,43] found that the e14a2 *BCR-ABL1* transcript was significantly associated with a higher rate of TFR regardless of the TKI used. Furthermore, it was also observed that patients expressing e14a2 transcripts have a considerably higher incidence of stable MR4.5 response than those with e13a2 transcripts.[25,38,39,40] Therefore, the type of transcript may also increase the probability of reaching the endpoint required for treatment discontinuation.

Among atypical transcripts potentially associated with CML, the e1a2 transcript deserves particular attention. Patients with e1a2 transcripts are diagnosed at an older median age and are more likely to present in BP initially; those who do not present in BP at baseline have an increased risk of subsequent progression to BP, lower cytogenetic and molecular responses to TKI treatments, and dismal OS.[47,48] This transcript is a high-risk factor for disease progression, and patients should always be considered for frontline 2GTKI treatment.

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