Review Article

The Impact of *BCR-ABL1* Transcript Type on Tyrosine Kinase Inhibitor Responses and Outcomes in Patients With Chronic Myeloid Leukemia

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Although the majority of patients with chronic myeloid leukemia do well with treatment with tyrosine kinase inhibitors (TKIs), some patients still have inferior outcomes. There are many factors that might play a part, including the different *BCR-ABL1* transcript types at baseline. The current study was performed to determine the possible impact of different transcripts on the treatment responses and outcomes of patients with chronic myeloid leukemia who are receiving TKI therapy. The authors performed a systematic literature search by using the terms "b2a2/b3a2," "e13a2/e14a2," or "transcript type." e14a2 was the more common transcript type. The majority of the studies demonstrated no significant difference regarding age, sex, leukocyte counts, and hemoglobin levels between patients with the e13a2 and e14a2 transcripts. However, in approximately one-half of the studies, the e14a2 transcript was associated with higher platelet counts. Almost no studies demonstrated a significant association between disease risk scores and transcript types. In the majority of studies, having the e14a2 transcript was associated with earlier, deeper, and higher molecular response rates. Although better event-free survival was observed in patients with the e14a2 transcript in some of the studies, the majority demonstrated that transcript type did not have an impact on progression-free and overall survival. Treatment-free remission currently is a topic of much interest, and to the authors' knowledge there are limited data with conflicting results regarding the possible effects of transcript types on the outcomes of patients after discontinuation of TKIs. Because having the e14a2 transcript appears to be related to a favorable outcome, choosing second-generation TKIs for frontline therapy might be a convenient approach in patients with chronic myeloid leukemia with the e13a2 transcript. The authors believe this finding warrants further investigation. ***Cancer* 2018;124:3806-3818**. *© 2018 American Cancer Society.*

KEYWORDS: *BCR-ABL1*, chronic myeloid leukemia (CML), e13a2, e14a2, response, transcript, tyrosine kinase inhibitor (TKI).

INTRODUCTION

In 1959, Hungerford and Nowell defined a critical chromosomal abnormality in patients with chronic myeloid leukemia (CML).[1] This abnormality was the juxtaposition of the *ABL1* gene of the 9th chromosome onto the *BCR* gene of the 22nd chromosome. Today, we know that approximately 95% of patients with CML are positive for the Philadelphia chromosome (Ph+), and this translocation serves as a target for the tyrosine kinase inhibitors (TKIs).

The *BCR* gene contains 4 breakpoint cluster regions termed major (*M-BCR*), minor (*m-BCR*), micro (μ*-BCR*), and nano (*v-BCR*). *M-BCR* has 5 exons termed e12 to e16 (formerly b1-b5), and in 95% of patients with Ph+ CML, a break occurs at this point.[2] After the translocation of the *ABL1* gene, a chimeric messenger RNA (mRNA) transcripted from this fusion gene is translated into the p210 protein. Usually, the e13 to e14 exons of the *M-BCR* gene and the a2 to a3 exons of the *ABL1* gene are the origin of this protein whereas fusions such as e1a2 (in which the break occurs at *m-BCR* and encodes the p190 protein) and e19a2 (in which the break occurs at μ*-BCR* and encodes the p230 protein) also are possible.[3,4] These rare forms of *BCR-ABL1* transcript types are for the most part observed in patients with Ph+ acute lymphoblastic leukemia. The frequency of p190 among patients with CML is reported to be approximately 1% and these patients usually are identified as being at high risk due to their inferior responses to treatment with imatinib.[5] In patients with Ph+ CML, the e13a2 ("b2a2") and e14a2 ("b3a2") fusion genes have the highest frequency and differ from one another by 75 base pairs. To our knowledge, the clinical impact of these extra 25 amino acids in the e14a2 transcript remains unclear. However, it is known that there is a structural difference occurring in the Src homology 1 (SH1)-, SH2-, SH3-, and DNA-binding domains of the p210 protein, which can cause an alteration in kinase activity.[6]

Chimeric fusion proteins are expressed only in CML cells and presented by major histocompatibility complex (MCH) class I and II molecules on the cell surface, and antitumor responses of cytotoxic T cells are based on these human leukocyte

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antigen (HLA) proteins in patients with CML. It previously has been shown that peptide-specific CD8-positive (CD8+) or CD4+ cytotoxic T cells proliferate as a response to HLA class I or II molecules presenting proteins encoded from e13a2 and e14a2.[7-10] Bocchia et al[11] assessed the affinity of e13a2 and e14a2 transfusion proteins to HLA class I molecules and found that 4 peptides derived from the e14a2 breakpoint had high or intermediate affinity for HLA A3, A11, and B8 molecules, but this was not true for e13a2, which did not demonstrate any affinity for these HLA class I molecules. The authors suggested that this might play a role in the impaired CD8+ cytotoxic T-cell response observed in patients with the e13a2 transcript.[11]

These studies have provided essential information for the development of vaccines targeting these CML-specific peptides. A peptide-specific vaccine derived from e14a2 (CMLVAX100, Originator: Memorial Sloan-Kettering Cancer Center, Developer : Breakthrough Therapeutics) was used in 16 patients with CML in the chronic phase (CML-CP) who had the e14a2 transcript and who were resistant to previous treatments (imatinib in 10 cases and interferon-α [IFN-α] in 6 cases), and 5 patients receiving imatinib achieved a complete cytogenetic response (CCyR) after 6 vaccinations, 3 of whom had an undetectable transcript amount. In addition, 2 of the 6 patients receiving CMLVAX100 plus IFN-α achieved a CCyR.[12] Jain et al[13] also assessed the efficacy of a vaccine derived from a mixture of peptides from both the e13a2 and e14a2 sequences in 10 patients with CML-CP, all with e13a2 transcripts (including 2 patients with coexpression of e13a2 and e14a2), who were in CCyR while receiving imatinib. Vaccination in combination with imatinib therapy induced improved molecular responses, a finding that then was supported by another study demonstrating promising results with the vaccine, which was derived from the e13a2 transcript.[14] Recently, Vinhas et al[15] used a gold nanoconjugate (a single-stranded DNA and gold nanoparticles for protection against Rnase activity) specific to the e14a2 fusion point and triggered apoptosis in *BCR-ABL1*-expressing cells, which might overwhelm the imatinib resistance. Although it was shown that the addition of these vaccines to imatinib therapy may result in improved outcomes, second-generation TKIs (2GTKIs) generally are used in the management of imatinib-resistant cases of CML in daily clinical practice.

The studies demonstrating the impact of *BCR-ABL1* transcript types on the outcomes of patients with CML receiving TKIs have had conflicting results. Hematologic parameters such as platelet and leukocyte counts, spleen size, or risk scores (including the Sokal and European Treatment and Outcome Study [EUTOS] scores but not the Euro-Hasford score) have provided opposing results by transcription type. This also makes it difficult to predict TKI response and long-term outcomes in patients with CML with regard to different transcript types. Many studies have demonstrated favorable outcomes with TKI therapy in patients with the e14a2 transcript type, but there are some that demonstrate just the opposite. In this review, we evaluated the current available literature for the differences in the short-term and long-term outcomes of patients with CML with different *BCR-ABL1* transcript types (e13a2/e14a2 or both) while receiving TKI therapy.

Methodology

We used the PubMed database and Google Scholar for a systematic literature search by using the terms "b2a2/b3a2," "e13a2/e14a2," or "transcript type." We accessed 420 articles in English through December 2017. Studies unrelated to this article, reviews, case reports, letters, and duplicates were excluded. Articles reporting other rare transcript types also were excluded. Although selected abstracts presented in the American Society of Hematology meetings relevant to this topic were included, for the most part 53 full-text articles were evaluated in detail for this review.

Molecular response classification was based on *BCR-ABL1* to control gene transcript ratios, expressed on the international reporting scale (IS), in which a major molecular response (MMR) or MR^3 is defined as $BCR\text{-}ABL1^{IS} \leq 0.1\%$, MR^4 is defined as $BCR\text{-}ABL1^{IS} \leq 0.01\%$, and $MR^{4.5}$ is defined as $BCR\text{-}ABL1^{IS} \leq 0.0032\%$. Early molecular response (EMR) is defined as a $BCR\text{-}ABL1^{IS} \leq 10\%$ at 3 or 6 months, and deep molecular response (DMR) generally is referred to as MR^4 or $MR^{4.5}$; some patients may achieve responses beyond the limit of detection of the assays used, which usually is termed a complete molecular response (CMR).[16]

Results and Discussion

The p190 (encoded by the e1a2 transcript) and p230 (encoded by the e19a2 transcript) proteins are relatively rare in patients with CML. In what to our knowledge is the largest study to date evaluating e1a2 transcript frequency and its clinical impact among patients with CML, only 14 were found in 1292 cases (approximately 1%). Nine of these patients had CML-CP, whereas 1 had CML in accelerated phase and 4 had CML in blast crisis. These patients had inferior TKI responses compared with patients with p210, and consequently, the authors declared these cases as high-risk patients.[3] In addition to

TABLE 1. Incidence of Transcript Types According to Different Studies From Different Countries

Country	Sample Size (No.)	e13a2, %	e14a2, %	Coexpression, %
Tunisia (Bennour 2013[2])	44	36.36	63.63	0
Argentina (Sastre 2007[18])	53	41.7	37.5	8.3
Ecuador (Paz-y-Mino 2002[19])	40	94.6	5.4	0
Sudan (Osman 2010[20])	43	53.5	41.9	2.3
United States (Jain 2016[21])	481	42	41	18
Syria (Al-Achkar 2016[22])	45	46.7	51.1	NR
Germany (Hanfstein 2014[23])	1105	41	45	14
Brazil (Pagnano 2017[24])	170	33	55	12
India (Sharma 2010[25])	87	38	54	8
India (Polampalli 2008[26])	202	32	68	0
ELN cohort (Pfirrmann 2017[27])[a]	1494	38	49	13
Italy (Castagnetti 2017[28])	559	36	52	11
India (Mir 2015[29])	200	24	68	8
Canada (Lin 2016[30])	166	36.7	50	13.3
Poland (Prejzner 2002[31])	27	29	62.3	8.2
India (Deb 2014[32])	80	41.25	56.25	NR
Korea (Lee 2018[33])	120	35	60	2.5
England (Claudiani 2017[34])	64	42	58	0
United States (Verschraegen 1995[35])	362	30.2	67.9	NR
England (Khorashad 2008[36])	319	41	46	12
Serbia (Todoric-Zivanovic 2011[37])	136	25	73.5	NR
Iran (Yaghmaie 2008[38])	75	21	62	5
India (Arun 2017[39])	1260	34.3	60	NR
Thailand (Udomsakdi-Auewarakul 2000[40])	99	31	61	NR
Korea (Goh 2006[41])	548	32.34	66.74	NR
Japan (Ito 2004[42])	226	30.2	67.5	NR

Abbreviations: Coexpression, coexpression of e13a2 and e14a2 transcripts; ELN, European LeukemiaNet; NR, not reported.
[a] The ELN cohort was an international cohort from Germany, Italy, Spain, and the Netherlands.

e1a2, there are only case reports reporting the e19a2 transcript in patients with CML.[4,17]

Because p210, which is encoded by the e13a2 and e14a2 transcripts, is the most frequent *BCR-ABL1* noted in patients with CML, this review focused mainly on the impact of these transcript types on outcomes.

Relationship between transcript types and patient characteristics

The incidence rates of the *BCR-ABL1* transcript types gathered from the full-text articles are given in Table 1.[2,18-42] Of 26 articles, 22 (85%) showed a higher incidence of the e14a2 transcript whereas only 4 articles demonstrated a higher incidence of the e13a2 transcript. The studies conducted among patients with CML from Argentina, Ecuador, and Sudan showed a higher frequency of the e13a2 transcript but had small sample sizes (53 patients, 40 patients, and 43 patients, respectively).[18-20] It is interesting to note that in the study by Paz-y-Mino et al,[19] the incidence of the e13a2 transcript was 94.6%, whereas that of e14a2 was only 5.4%. Ethnicity might play a role in these results, as well as the small sample size of the study. However, a larger cohort from the United States of 481 patients also demonstrated higher incidence rates for the e13a2 transcript type.[21]

In nearly all the studies, sex did not appear to have an impact on the transcript types, including the European LeukemiaNet (ELN) cohort, which to our knowledge has the largest sample size.[2,22-27,43-48] However, a small study consisting of 43 patients from Sudan with CML demonstrated a possible relationship between the e13a2 transcript and male sex,[20] which then was confirmed by other 2 large-scale studies from Italy (559 patients)[28] and India (200 patients)[29] ($P = .03$ and $P = .05$, respectively). In addition, Lin et al[30] demonstrated that female sex was more frequent among patients coexpressing both the e13a2 and e14a2 transcripts ($P < .05$), although no significant difference with regard to sex was shown in the e13a2 or e14a2 transcript groups.

Age did not appear to have an impact on transcript types in the majority of the studies. Bennour et al[2] found that patients with the e14a2 transcript type were significantly older than patients in the e13a2 group (mean age of 56 years vs 26 years; $P = .001$). Other studies did not find a correlation between age and transcript types.[21-24,26-29,43-47]

Relationship between transcript types and hematologic parameters and risk scores at diagnosis

Structural differences in different p210 proteins (ie, e14a2 and e13a2) may result in different tyrosine kinase activities and thus, theoretically, hematologic parameters

among patients with CML with the e13a2 and e14a2 transcript types may differ.

With regard to the association between transcript types and platelet counts at the time of diagnosis, the literature search demonstrated some contradictory results. In some studies, significantly higher platelet counts were found in patients with the e14a2 transcript compared with those with the e13a2 transcript,[2,21,44,46-48,50-52] whereas others demonstrated no difference (Table 2).[2,21-25,27-30,43-47,49,53,54] In addition, in the study by Jain et al,[21] significantly higher platelet levels were observed in the patients coexpressing the transcripts compared with patients with the e13a2 transcript alone. A study from Italy with 559 patients was unable to demonstrate any difference between transcript types ($P = .251$).[28] However, a German study with 1105 patients found that patients with the e13a2 transcript had significantly lower platelet levels compared with those with the e14a2 transcript and those coexpressing transcripts (e13a2 plus e14a2) ($P < .001$); however, after dissecting patients according to Euro-Hasford risk scores, this statistically significant difference disappeared.[23]

Many studies found no association between leukocyte counts and hemoglobin levels at the time of diagnosis and the transcript types.[2,22,24-26,28,43-45,47,49] However, in their study, the German investigators demonstrated that patients with the e13a2 transcript had significantly higher baseline median leukocyte counts compared with patients with the e14a2 transcript (88×10^9/L vs 65×10^9/L; $P < .001$), but no such relationship was shown for hemoglobin levels.[23] A recently published study from Brazil also reported similar findings and patients with the e13a2 transcript had a significantly higher median leukocyte count at the time of diagnosis compared with patients with the e14a2 transcript (147×10^9/L vs 129×10^9/L; $P = .049$) (Table 2).[2,21-25,27-30,43-47,49]

A German study demonstrated that there was no significant difference between patients having the e13a2, e14a2, and the coexpressing e13a2 plus e14a2 transcript types in terms of spleen size at the time of diagnosis.[23] Other studies from Poland, India, and Romania with smaller sample sizes also supported this finding ($P = .941$, $P = .868$, and $P = .680$, respectively).[25,31,47] Although having a larger spleen at the time of diagnosis was found to have a negative impact on achieving a MMR[33] and on event-free survival (EFS),[21] to our knowledge neither study investigated the difference in spleen size among different transcript groups.

The Sokal, Euro-Hasford, EUTOS, and EUTOS Long-Term Survival (ELTS) risk scores have important predictive value, especially for long-term outcomes. These scoring systems are based on different features, including platelet and blast counts, spleen size, and patient age. The Sokal and Euro-Hasford risk classification systems were developed before the introduction of TKIs, whereas the EUTOS and then ELTS systems were developed within the era of TKIs.[55,56] The EUTOS score used the percentage of basophils and spleen size to divide patients into 2 groups as high risk and low risk, and 5-year progression-free survival (PFS) was found to be significantly better in the low-risk compared with the high-risk group (90% vs 82%; $P = .006$).[55] Older patient age, more peripheral blasts, a larger spleen size, and low platelet counts were found to be significantly associated with increased probabilities of dying of CML and resulted in a new long-term survival score, the so-called "ELTS score," with 3 prognostic groups. Compared with the low-risk group, patients in the intermediate-risk and high-risk groups had significantly higher probabilities of dying of CML.[56] This new score differentiated the probabilities of dying of CML better than the Sokal, Euro-Hasford, and EUTOS scores.

Lucas et al[57] found no significant difference in the distribution of Sokal risk scores between the e13a2, e14a2, and e13a2 and e14a2 coexpression groups. This also was supported by the studies by Jain et al[21] (481 patients) and Prejzner et al[31] (61 patients), in which there were no differences noted in terms of Sokal risk groups among patients with different transcript types ($P = .53$ and $P = .734$, respectively). Studies from India and Thailand demonstrated similar results according to the Sokal and Euro-Hasford scores[25,54]; however, Deb et al[32] demonstrated that patients with the e13a2 transcript had higher baseline Sokal and EUTOS risk scores compared with those without this transcript type ($P < .05$).

In the study by Castagnetti et al,[28] which included 559 patients, there were no differences noted in terms of the distribution of the Sokal, Euro-Hasford, and EUTOS risk scores among patients with the e13a2 and e14a2 transcript types ($P = .525$, $P = .322$, and $P = .662$, respectively). Similarly, in the study by Pagnano et al,[24] the distribution of EUTOS and Sokal risk scores was similar in patients with the e13a2, e14a2, and e13a2 plus e14a2 transcript types (Table 3).[21,24,25,28,32,57] Pfirrmann et al[27] did not find any significant difference in terms of the distribution of ELTS scores between the different transcript types.

Distribution of responses to first-line imatinib according to different transcript types

In a study consisting of 481 patients with CML-CP, the distribution of the e13a2, e14a2, and e13a2 plus e14a2

TABLE 2. Distribution of Hematologic Parameters According to Transcript Types

Reference	Median/Mean Age, Years				Male Sex, %				Median Platelet Count, $\times 10^9$/L				Median WBC, $\times 10^9$/L				Median Hgb, g/dL			
	e13a2	e14a2	Co expression	P^a	e13a2	e14a2	Co expression	P^a	e13a2	e14a2	Co expression	P^a	e13a2	e14a2	Co expression	P^a	e13a2	e14a2	Co expression	P^a
Bennour 2013[2]	26.56	56.61	NR	**.001**	62.5	57.1	NR	.49	207	681.19	NR	**.001**	264.7	181.5	NR	.153	9.31	10.83	NR	.078
Jain 2016[21]	47	49	52	.11	NR	NR	NR	NR	288	405	358	.001	31	28	30	.94	12	12	13	.32
Al-Achkar 2016[22]	39	36	NR	.66	57.1	56.5	NR	1	293	257	NR	.44	143.1	115	NR	.64	11.2	10.2	NR	.28
Hanfstein 2014[23]	52	53	NR	NS	59.9	59.5	NR	NS	296	430	NR	NS	87.7	65.3	NR	**<.001**	12.2	12.5	NR	NS
Pagnano 2017[24]	43	52	48	.06	66	54	60	.36	343.3	431	378	.09	110	112	97.9	.7	11.7	12.1	11.6	.67
Sharma 2010[25]	36.58	37.54		.706	70.5	66.03		.196	340		360	.712	77		83	.923	11.8		12	.69
Kiani 2016[43]	46.38	48.91	NR	NS	55.5	37.83	NR	NS	364.5	392.9	NR	NS	131.8	126.8	NR	NS	11.12	10.84	NR	NS
Balatzenko 2011[44]	49.9	51	NR	NS	65	47.45	NR	NS	440.4	791.3	NR	**.007**	132.4	119.5	NR	NS	10.79	11.6	NR	NS
Rostami 2017[45]	52	45	NR	.73	56	51.4	NR	.93	379	386	NR	.77	140	120	NR	.07	NR	NR	NR	NR
Vasconcelos 2017[46]	NR	NR	NR	.51	63.2	55.3	NR	.28	287	429	NR	**.005**	147	129	NR	**.049**	10.5	10.5	NR	.93
Szanto 2014[47]	51.4	50.06	NR	.9	66.6	50	NR	.47	261.7	412.8	NR	**.003**	105.9	176	NR	.247	11.8	11.2	NR	.089
Castagnetti 2017[28]	52	52	NR	.374	66	57	NR	**.05**	293	401	NR	.251	61.6	52.2	NR	.174	12	12.3	NR	.413
Mir 2015[29]	NR	NR	NR	NR	68.75	62.5	62.5	**.03**	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Lin 2016[30]	54	60	62.5	NS	52.4	54.2	59	NS	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mondal 2006[49]	30.5	40	NR	NS	NR	NR	NR	NR	373	250	NR	NS	162	120	NR	NS	NR	NR	NR	NR
Pfirrmann 2017[27]	52	51	51	NS	59	58	62	NS	295	420	407	NR	NR	NR	NR	NR	NR	NR	NR	NR

Abbreviations: Coexpression, coexpression of e13a2 and e14a2 transcripts; Hgb, hemoglobin; NR, not reported; NS, not significant; WBC, white blood cell count.
[a] Bold type indicates statistical significance.

TABLE 3. Sokal, Euro-Hasford, and EUTOS Risk Scores in Different Transcript Types

Reference	Risk	e13a2, No.	e14a2, No.	Coexpression, No.	P[a]
Sokal					
Jain 2016[21]	High	11	11	9	.53
	Intermediate	47	48	21	
	Low	142	137	55	
Pagnano 2017[24]	High	18	16	10	.06
	Intermediate	15	31	4	
	Low	13	25	4	
Sharma 2010[25]	High	4	6	NR	.824
	Intermediate	7	14	NR	
	Low	23	33	NR	
Castagnetti 2017[28]	High	43	72	NR	.525
	Intermediate	74	110	NR	
	Low	86	108	NR	
Lucas 2009[57]	High	21	9	12	NS
	Intermediate	14	5	9	
	Low	15	6	9	
Deb 2014[32]	High	5	7	NR	**.03**
	Intermediate	17	15	NR	
	Low	11	25	NR	
Euro-Hasford					
Sharma 2010[25]	High	4	5	NR	.849
	Intermediate	8	10	NR	
	Low	22	38	NR	
Castagnetti 2017[28]	High	12	24	NR	.322
	Intermediate	101	142	NR	
	Low	90	124	NR	
Deb 2014[32]	High	4	1	NR	.24
	Intermediate	15	22	NR	
	Low	14	24	NR	
EUTOS					
Pagnano 2017[24]	High	7	9	4	.45
	Low	47	81	16	
Castagnetti 2017[28]	High	15	23	NR	.662
	Low	188	267	NR	
Deb 2014[32]	High	17	20	NR	**.027**
	Low	16	27	NR	

Abbreviations: Coexpression, coexpression of e13a2 and e14a2 transcripts; EUTOS, European Treatment and Outcome Study; NR, not reported; NS, not significant.
[a] Bold type indicates statistical significance.

transcript types were 200 patients (42%), 196 patients (41%), and 85 patients (18%), respectively. Patients were divided into 4 groups according to the frontline TKI therapy received as 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. Cumulative MMR and $MR^{4.5}$ rates were significantly inferior in the e13a2 group compared with the e14a2 and coexpression groups in all treatment arms ($P = .0001$ and $P = .00001$, respectively) (Table 4).[21,23,24,26,28-30] With regard to CCyR, patients with the e13a2 transcript who received imatinib at a dose of 400 mg/day had inferior response rates (77%) compared with other TKIs (90%-95%), but this trend was not observed in patients with the e14a2 or coexpression of the e13a2 plus e14a2 transcripts, in whom the CCyR rate with imatinib at a dose of 400 mg/day was similar (93%) to that of other treatment modalities (93%-96%).[21] In addition, time to achieve MMR and $MR^{4.5}$ was longer for e13a2 cohort, but it was similar for CCyR in all transcript groups. The transcript levels declined more slowly after 3 months and 6 months of TKI therapy in the e13a2 group compared with the e14a2 and coexpression groups. When treatment responses according to different transcript types were evaluated for each different TKI treatment modality, patients with the e13a2 transcript receiving imatinib at a dose of 400 mg/day had significantly inferior CCyR, MMR, and $MR^{4.5}$ rates compared with those observed among both patients with the e13a2 transcript who were receiving other TKI modalities and patients with other transcript types who were receiving imatinib at a dose of 400 mg/day.[21] Lower response rates for CCyR, MMR, and $MR^{4.5}$ in patients with the e13a2 transcript persisted, even at 60 months. The $MR^{4.5}$ response sustainability was lower in patients with the e13a2 transcript compared with patients with the e14a2 transcript and transcript coexpression ($P = .0021$). In addition, as expected, patients treated with standard-dose imatinib (400 mg/day) were found to have the longest time to achieve MMR and MR,[4.5] irrespective of the type of transcripts, with patients with the e13a2 transcript generally having longer times to MMR and $MR^{4.5}$ across all treatment types.[21]

In the study by Castagnetti et al,[28] patients receiving frontline imatinib were evaluated, and CCyR rates at 12 months for patients with the e13a2 and e14a2 transcripts were 75% and 79%, respectively ($P = .274$) (Table 5).[21,23-25,28,57] The median time to achieve CCyR was 6 months for both groups, but patients having the e13a2 transcript type had significantly inferior MMR rates at 18 months and MR^4 rates at 36 months compared with patients with the e14a2 transcript (52% vs 67% [$P = .001$] and 20% vs 30% [$P = .013$], respectively). In addition, the median times to achieve MMR and MR^4 were significantly longer for patients with the e13a2 transcript compared with those with the e14a2 transcript type (12 months vs 6 months [$P = .001$] and 61 months vs 41 months [$P = .001$], respectively).[28]

Among a patient cohort consisting of 1105 patients from Germany with CML, patients were divided into treatment arms of imatinib at a dose of 400 mg (300 patients), imatinib at a dose of 400 mg plus IFN-α (331 patients), imatinib at a dose of 400 mg plus cytarabine (150 patients), and imatinib at a dose of 800 mg (324 patients).[23] At the time of total sample analysis, a cumulative MMR incidence for the e14a2 group was better than that of patients with the e13a2 transcript ($P = .002$).

TABLE 4. Distribution of MMR, MR[4], and MR[4.5] Rates According to Different Transcript Types

	MMR																
	3 Months				6 Months				12 Months				Cumulative				
Reference	e13a2, %	e14a2, %	Coexpression, %	*P*[a]	e13a2, %	e14a2, %	Coexpression, %	*P*[a]	e13a2, %	e14a2, %	Coexpression, %	*P*[a]	e13a2, %	e14a2, %	Coexpression, %	*P*[a]	Duration
Jain 2016[21]	27	49	50	**<.05**	42	67	70	**<.05**	55	83	76	**<.05**	79	91	95	**.0001**	5 y
Hanfstein 2014[23]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	81	85	NR	**.002**	5 y
Pagnano 2017[24]	60	84	75	**.02**	51	72	53	.06	NR	NR	NR	NR	NR	NR	NR	NR	NR
Polampalli 2008[26]	NR	NR	NR	NR	41	48	NR	NS	76	55	NR	NS	NR	NR	NR	NR	NR
Castagnetti 2017[28]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	83	88	NR	**<.01**	80 mo
Mir 2015[29]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	64	72.1	NR	**.04**	NR
Lin 2016[30]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	60.7	77.1	81.8	**<.05**	2 y

	Cumulative MR[4] to MR[4.5b]				
Reference	e13a2, %	e14a2, %	Coexpression, %	*P*[a]	Duration
Jain 2016[21]	57	79	80	**<.001**	5 y
Hanfstein 2014[23]	58	76	NR	**<.001**	5 y
Castagnetti 2017[28]	52	67	NR	**.001**	80 mo

Abbreviations: Coexpression, coexpression of e13a2 and e14a2 transcripts; IS, international reporting scale; MMR, major molecular response; MR[4], *BCR-ABL1*^{IS}/*ABL1* $\leq$ 0.01%; MR[4.5], *BCR-ABL1*^{IS}/*ABL1* $\leq$ 0.0032%; NR, not reported; NS, not significant.

[a] Bold type indicates statistical significance.

[b] MR[4.5] for Jain et al[21]; MR[4] for others.

TABLE 5. CCyR Rates in Different Transcript Types Shown in the Literature

Reference	CCyR																	
	3 Months				6 Months				12 Months				Cumulative					
	e13a2, %	e14a2, %	Coexpression, %	P^a	e13a2, %	e14a2, %	Coexpression, %	P^a	e13a2, %	e14a2, %	Coexpression, %	P^a	e13a2, %	e14a2, %	Coexpression, %	P^a	Duration
Jain 2016[21]	59	67	63	NR	73	81	82	NR	NR	NR	NR	NR	89	94	94	NS	60 mo
Hanfstein 2014[23]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	94.6	93.3	93.6	NS	5 y
Pagnano 2017[24]	NR	NR	NR	NR	43	70	64	**.02**	62	78	79	.16	NR	NR	NR	NR	NR
Sharma 2010[25]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	61	35	NR	.396	2 y
Castagnetti 2017[28]	NR	NR	NR	NR	NR	NR	NR	NR	75	79	NR	.274	89	88	NR	.916	80 mo
Lucas 2009[57]	NR	NR	NR	NR	NR	NR	NR	NR	25	53.8	NR	**.01**	NR	NR	NR	NR	NR

Abbreviations: CCyR, complete cytogenetic response; Coexpression, coexpression of e13a2 and e14a2 transcripts; NR, not reported; NS, not significant.
[a] Bold type indicates statistical significance.

DMR (MR[4] or deeper) rates also were found to be superior in patients with the e14a2 transcript compared with patients with the e13a2 transcript (76% vs 58%; $P<.001$). Patients coexpressing both transcript types demonstrated no difference in MMR rates when compared with patients with the e13a2 or e14a2 transcripts, but this group had significantly lower MR[4] rates compared with patients with the e14a2 transcript ($P=.004$) (Table 4).[21,23,24,26,28-30] When evaluating the treatment arms separately, in the patients treated with imatinib at a dose of 400 mg plus IFN-α, those patients with the e14a2 transcript type achieved significantly higher MMR rates when compared with the e13a2 group ($P=.004$). In addition, MR[4] rates also were superior among patients with the e14a2 transcript compared with those with the e13a2 transcript among the patients treated with imatinib at a dose of 400 mg plus IFN-α, imatinib at a dose of 400 mg plus cytarabine, and imatinib at a dose of 800 mg ($P<.001$, $P=.004$, and $P=.028$, respectively). However, there were no significant differences in the cumulative incidence of major cytogenetic response, CCyR, PFS, and overall survival (OS) noted between different transcript types in all treatment arms, and the authors concluded that the transcript types did not serve as a useful prognostic tool with which to predict long-term outcomes, at least in this cohort of patients.[23]

A retrospective study from Canada with 166 patients with CML-CP demonstrated that MMR rates in the e14a2 and coexpression groups were both significantly higher than that of patients with the e13a2 transcript (77.1% and 81.8% vs 60.7%, respectively; P values not given) (Table 4).[21,23,24,26,28-30] The median time to achieve MMR did not differ between the groups, and similarly the disease progression rates and median time to disease progression were comparable. The authors concluded that having an e14a2 transcript type was associated with better molecular responses.[30] Another study from India with 200 patients supported the findings of the Canadian study[29] by demonstrating that the MMR rates of patients with the e14a2, e13a2, and coexpression transcripts were 72.1%, 24.7%, and 3%, respectively ($P=.04$). Complete hematologic response and MMR rates also were similar to what was observed in the study by Lin et al,[30] and also were superior in patients with the e14a2 transcript compared with those with the e13a2 transcript ($P=.05$ for complete hematologic response and $P=.04$ for MMR).[29] However, in this study, approximately 42% of patients were in accelerated phase or blast crisis, which was not the case in the Canadian study.[29]

A retrospective study evaluated 170 treatment-naive patients who were treated with imatinib at a dose of 400 or 800 mg/day as frontline therapy and compared responses according to transcript type within the entire cohort regardless of the daily imatinib dose. CCyR rates at 6 months were 43% for the e13a2 group, 70% for patients with the e14a2 transcript, and 64% for those demonstrating coexpression ($P = .02$). However, no such difference was observed at 12 months of TKI therapy ($P = .16$). EMR assessment at 3 months also favored patients with the e14a2 transcript ($P = .02$).[24] In the study by Lucas et al,[57] patients having the e14a2 transcript type had superior CCyR rates and achieved this response faster when compared with the e13a2 group ($P = .01$ and $P = .006$, respectively) in a cohort of 78 patients with CML receiving first-line imatinib (Table 5).[21,23-25,28,57]

Lee et al[33] divided a total of 120 patients who did not achieve EMR at 3 months into 3 groups as patients with the e13a2 transcript (group 1), those with the e14a2 transcript with a spleen measuring >9 cm (group 2), and those with the e14a2 transcript with a spleen measuring ≤9 cm (group 3). While receiving frontline imatinib treatment, overall MMR rates in these 3 patient groups were 19%, 20.8%, and 56.1%, respectively, and the authors concluded that having the e13a2 transcript type and splenomegaly >9 cm were negative predictors of achieving MMR.[33]

The study by Vega-Ruiz et al[58] reported similar results, in which the authors evaluated imatinib response in 251 newly diagnosed patients and 229 patients after IFN-α failure. Among the treatment-naive group, MMR and CMR rates (defined as undetectable transcript levels) were superior in patients with the e14a2 transcript compared with those with the e13a2 transcript (59% vs 77% [$P = .008$] and 25% vs 47% [$P = .002$], respectively). Improved MMR and CMR rates also were observed in the IFN-α failure group for patients having the e14a2 transcript compared with patients with the e13a2 transcript (34% vs 63% [$P = 0.001$] and 16% vs 42% [$P = 0.001$], respectively).[58]

Similarly, among patients with CML-CP receiving imatinib both in the upfront setting or after first-line IFN-α, Bonifacio et al[59] showed that having the e14a2 transcript was associated with durable MR^4 when compared with those with the e13a2 transcript ($P = .003$).[59]

Although in the majority of the publications it was stated that the patients with the e14a2 transcript usually achieved better responses and had favorable outcomes, there also are some studies with contradictory results. A study from India with 87 patients with CML demonstrated that those with the e13a2 transcript had better responses while receiving imatinib compared with patients having the e14a2 transcript. CCyR rates were 59% versus 28%, respectively, in favor of the e13a2 transcript type, and the difference was statistically significant ($P = .04$). However among this cohort, there were patients with a prior treatment other than imatinib (hydroxyurea or IFN-α), and there were only 30 treatment-naive patients. After performing the analysis within these treatment-naive patients, the CCyR rates among the different transcript groups were similar ($P = .396$).[25] Polampalli et al[26] found no difference in terms of MMR and CCyR rates at 6 months and 12 months between patients with the e13a2 and e14a2 transcript types, but there were more patients with the e13a2 transcript who progressed to myeloid blast crisis (Table 4).[21,23,24,26,28-30]

Distribution of responses under 2GTKIs according to different transcript types in the frontline setting

To the best of our knowledge, there are limited data evaluating the responses and outcomes under first-line 2GTKIs according to *BCR-ABL1* transcript types.

In a study from The University of Texas MD Anderson Cancer Center (MDACC), there were 105 patients receiving upfront dasatinib 50 mg twice daily or 100 mg daily and 108 patients receiving frontline nilotinib 400 mg twice daily.[21] In patients with the e13a2 transcript, overall CCyR rates for both 2GTKIs were superior to that of imatinib at a dose of 400 mg/day but comparable to that of imatinib at a dose of 800 mg/day (77% for imatinib at a dose of 400 mg/day vs 90%-95% for other treatment arms; *P* value not given). Overall MMR and $MR^{4.5}$ rates among patients with the e13a2 transcript receiving treatment with 2GTKIs also were higher than those for patients receiving imatinib at doses of 400 mg/day and 800 mg/day (*P* value not given). For MR^3 and $MR^{4.5}$, patients with the e13a2 transcript who were treated with imatinib at a dose of 400 mg/day had a trend toward an inferior response rate compared with those treated with other TKI modalities. In addition, MR^3 and $MR^{4.5}$ rates were comparable between all TKI groups for patients with the e14a2 transcript, with the exception of patients treated with nilotinib 800 mg daily, who demonstrated an inferior $MR^{4.5}$ rate in both the e13a2 and e14a2 cohorts compared with patients treated with imatinib at a dose of 800 mg/day or dasatinib 2×50 mg/day or 100 mg daily.[21] The $MR^{4.5}$ rate of nilotinib 400 mg twice daily in those patients with the e14a2 transcript was found to be inferior to that of imatinib at a dose of 800 mg/day and dasatinib 50 mg twice daily or 100 mg daily (64% for imatinib at a

dose of 400 mg/day, 85% for imatinib at a dose of 800 mg/day, 89% for dasatinib 50 mg twice daily or 100 mg/day, and 68% for nilotinib 800 mg/day). The CCyR and MMR rates of patients with the e14a2 transcript receiving first-line nilotinib 400 mg twice daily treatment were comparable to those of other treatment arms. Based on these results, the authors proposed a possible frontline therapy approach of 2GTKIs for patients with the e13a2 transcript and imatinib at a dose of 400 mg/day for patients with the e14a2 transcript.[21] They also stated that having the e14a2 transcript type (compared with patients with the e13a2 transcript, but not the coexpressing patients), receiving first-line treatment with imatinib at a dose of 800 mg/day or 2GTKIs, and having a spleen measurement of <10 cm at the time of the initial presentation have prognostic value for EFS. Positive predictors for treatment-free remission (TFR) were defined as having the e14a2 transcript or coexpressing the e13a2 plus e14a2 transcripts, and frontline treatments with imatinib at a dose of 800 mg daily or dasatinib 50 mg twice daily or 100 mg/day. However, the only positive predictor found for OS was having both the e13a2 and e14a2 transcripts.[21] The authors also evaluated patients according to ELN optimal response criteria and found that having the e14a2 transcript or coexpressing the e13a2 plus e14a2 transcripts were positive predictors for achieving MMR at 6 months and 12 months of TKI treatment. In addition, the e14a2 transcript had a prognostic value for superior major cytogenetic response at 3 months and improved CCyR at 6 months of TKI treatment.[21]

Castagnetti et al[48] demonstrated that the cumulative MMR (82% vs 88%; $P = .135$), MR^4 (60% vs 69%; $P = .101$), estimated PFS (88% vs 93%; $P = .547$), and estimated OS (89% vs 94%; $P = .436$) rates were comparable between patients having the e13a2 and e14a2 transcripts, respectively, among 328 patients with CML-CP receiving upfront nilotinib. Although there was a trend toward better responses and outcomes in patients with the e14a2 transcript, none reached statistical significance.[48]

In another study from MDACC, the authors evaluated the distribution of responses and outcomes among 85 patients (47 with recurrent/refractory disease and 38 newly diagnosed individuals) receiving ponatinib (a third-generation TKI) according to transcript type. In the patients with recurrent/refractory disease, the overall CCyR and MMR rates of patients with the e13a2, e14a2, and coexpression transcripts were 50%, 61%, and 50%, respectively, and 29%, 52%, and 30%, respectively.[60] While receiving frontline ponatinib, the median *BCR-ABL1*^{IS} levels at 3 months were at least MR^3 for all transcript groups, and at 6 months all groups showed an MR^4 or deeper response with no significant differences noted between different transcript groups. The 3-year probability of failure-free survival (FFS) was comparable for all groups, but patients with the e13a2 transcript demonstrated inferior results with regard to the 3-year probability of OS when compared with patients with the e14a2 and e13a2 plus e14a2 transcripts ($P = .08$ and $P = .03$, respectively).[60]

TABLE 6. Long-Term Outcomes and Survival Data According to Transcript Types

Reference	e13a2, %	e14a2, %	Coexpression, %	P[a]	Duration
OS					
Jain 2016[21]	88	95	98	.34	5 y
Pagnano 2017[24]	96	88	NR	NS	5 y
Pagnano 2017[24]	94	76	67	**.03**	10 y
Pfirrmann 2017[27]	89	93	93	.106	5 y
Castagnetti 2017[28]	83	90	NR	**.017**	7 y
EFS					
Jain 2016[21]	79	89	87	.09	5 y
Pagnano 2017[24]	82	71	71	.41	10 y
PFS					
Pagnano 2017[24]	94	89	75	.13	10 y
Castagnetti 2017[28]	81	89	NR	**.005**	7 y
FFS					
Castagnetti 2017[28]	54	71	NR	**<.001**	7 y
TFS					
Jain 2016[21]	91	97	99	**0.01**	5 y

Abbreviations: Coexpression, coexpression of e13a2 and e14a2 transcripts; EFS, event-free survival; FFS, failure-free survival; NR, not reported; NS, not significant; OS, overall survival; PFS, progression-free survival; TFS, transformation-free survival.
[a] Bold type indicates statistical significance.

Long-term outcomes and survival according to different transcript types

Long-term outcomes and survival data of the studies are summarized in Table 6.[21,24,27,28] In a recently published study in which 1494 patients who were treated with first-line imatinib-based regimens were included, there was no significant difference in 5-year OS noted between patients with the e13a2, e14a2, and coexpressing transcripts when patients were stratified according to ELTS risk scores (89%, 93%, and 93%, respectively; $P = .106$). In addition, the probability of dying of CML-related causes was similar in patients with the e13a2, e14a2, and e13a2 plus e14a2 transcripts (5%, 3%, and 2%, respectively; $P = .256$).[27]

In contrast to the previous study, Castagnetti et al[28] demonstrated that in patients with the e13a2 and e14a2 transcript types, the 7-year OS rates while receiving imatinib at a dose of 400 mg/day were 83% versus 91%,

whereas in the high-dose (800 mg/day) imatinib group, the 7-year OS rates were 82% versus 87% ($P = .038$ and $P = .232$, respectively), demonstrating that patients with the e14a2 transcript had a significantly higher 7-year OS compared with patients with the e13a2 transcript receiving standard-dose imatinib. In addition, patients with the e14a2 transcript had significantly better PFS for both daily imatinib doses ($P = .027$ for imatinib at a dose of 400 mg/day and $P = .039$ for imatinib at a dose of 800 mg/day), and the FFS rates were significantly superior in patients with the e14a2 transcript type for both the standard-dose and high-dose groups ($P = .004$ for imatinib at a dose of 400 mg/day and $P = .011$ for imatinib at a dose of 800 mg/day).[28] That said, in the same study, the authors suggested that transcript type may be able to predict survival regardless of the daily imatinib dose administered and that patients with the e14a2 transcript had significantly superior OS, PFS, and FFS when compared with patients with the e13a2 transcript ($P = .017$, $P = .005$, and $P < .001$, respectively).[28]

There were no differences in terms of 5-year EFS and OS noted between patients with the e13a2, e14a2, and coexpressing transcripts in the MDACC cohort.[21] However, patients with the e14a2 or coexpressed e13a2 plus e14a2 transcripts and who achieved optimal responses at 3 months of TKI therapy according to the 2013 ELN response criteria had better transformation-free survival rates than patients with the e13a2 transcript (95%, 99%, and 89%, respectively; $P = .033$).

As stated earlier, in the Korean study,[33] the cohort was divided into 3 groups, and patients in group 3 were found to have significantly higher MMR rates and better 5-year FFS compared with the other groups, but there was no significant difference with regard to OS and PFS noted between all groups. Another study with a relatively shorter follow-up period also demonstrated similar results, with no significant difference noted in the 2-year OS despite a better CCyR in patients with the e14a2 transcript.[57]

In the study by Pagnano et al,[24] there were no significant differences noted in terms of 5-year EFS, PFS, and OS between the transcript groups. Despite significantly better EMR (*BCR-ABL1*^{IS} $\leq$%10 at 3 months) and CCyR rates being observed in patients with the e14a2 transcript, the 10-year OS in patients with the e13a2 transcript was superior to that of patients with the e14a2 transcript and the coexpression group ($P = .03$).[24] Although there was no significant difference between the patient groups with regard to median age, patients in the e13a2 cohort were younger, and the authors concluded that the favorable OS detected in these individuals most likely was due to this age difference.[24]

Impact of *BCR-ABL1* transcript types on TKI discontinuation and TFR

Discontinuation of TKI therapy and TFR currently are topics of much interest for physicians who treat patients with CML, and to our knowledge the impact of transcript types on the outcome of TFR is not yet fully understood. Lee et al[61] assessed the effect of transcript types on sustained MMR rates and CMR at 12 months of imatinib cessation in the Korean Imatinib Discontinuation Study (KIDS). They demonstrated that sustained MMR rates and CMR at 12 months were comparable for both patients with the e13a2 and e14a2 transcript types ($P = .977$ and $P = .859$, respectively).[61]

In a recent study, Claudiani et al[34] analyzed 37 patients with the e14a2 transcript and 27 patients with the e13a2 transcript who achieved and maintained MR^4 or MR$^{4.5}$ for at least 12 months and then discontinued TKI therapy. Thirty-two patients received imatinib, 17 patients were treated with nilotinib, and the remaining 15 patients were receiving dasatinib at the time of TKI cessation. Thirteen patients received upfront 2GTKI treatment. After TKI discontinuation, 37 patients (58%) sustained molecular remission at a median of 26 months, and patients with the e13a2 transcript demonstrated inferior results compared with the e14a2 group (45% vs 70%).[34] The 3-year probability of TFR was 53% for the entire cohort, and this percentage was higher for patients with the e14a2 transcript compared with those with the e13a2 transcript (66% vs 38%, respectively). Having the e14a2 transcript and being aged $\geq$40 years at the time of diagnosis were marked as positive predictors of TFR ($P = .016$ for the e14a2 vs e13a2 transcript and $P = .003$ for age $\geq$40 years vs age $<$40 years).[34]

Conclusions

TKI therapy has revolutionized the management of patients with CML-CP, but some patients still have inferior responses and worse long-term outcomes. There are many factors that might play a role, including the different *BCR-ABL1* transcript types at baseline. In this review, we evaluated the current literature regarding the impact of different transcripts (e13a2, e14a2, or coexpression of e13a2 plus e14a2) on the short-term and long-term outcomes as well as the correlation of these transcript types with the disease characteristics at the time of the initial diagnosis. In approximately one-half of the studies, the e14a2 transcript was associated with higher platelet counts, whereas other studies did not demonstrate such an association. Almost no studies demonstrated a significant association between disease risk score and *BCR-ABL1*

transcript type. In the majority of the studies, having the e14a2 transcript at baseline was associated with higher molecular response rates (including EMR and DMR), whereas some studies demonstrated just the opposite. For the long-term outcomes, although some of the studies demonstrated better EFS in patients with the e14a2 transcript, the majority of studies demonstrated that transcript type does not have an impact on PFS and OS. TFR is a novel topic for discussion in the management of patients with CML, and to our knowledge there are limited data with conflicting results regarding the possible effects of transcript type on the outcomes of patients after TKI discontinuation.

Because having the e14a2 transcript appears to be related to favorable outcomes, choosing alternative therapies such as 2GTKIs in the frontline setting might be a convenient approach in patients with the e13a2 transcript, which generally is associated with an inferior outcome, and we believe this warrants further investigation. Prospective and randomized controlled trials with larger sample sizes still are needed to determine the impact of transcript type on the short-term and long-term outcomes in patients with CML who are receiving TKI therapy.

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