

E14a2 BCR-ABL1 transcript is associated with a higher rate of treatment-free remission in individuals with chronic myeloid leukemia after stopping tyrosine kinase inhibitor therapy

Treatment-free remission (TFR) is a new therapy goal for patients with chronic phase chronic myeloid leukemia (CML) receiving tyrosine kinase inhibitors (TKIs), with approximately 40% sustaining deep molecular responses after stopping treatment.¹⁻³ However, it is difficult to predict precisely who will achieve TFR and the subject remains controversial.³ We present data from 64 patients who stopped TKI therapy. Data show a significant association between the type of BCR-ABL1 transcript and age on the probability of TFR.

Subjects were in 1st chronic phase and had a deep molecular response (\geq MR4 on the International Scale) for one year or more before stopping TKI therapy, equivalent to the eligibility criteria of the Euro-Ski trial.⁴ Molecular response level was calculated by standard criteria⁵ and molecular relapse defined as loss of MR3. Time to molecular relapse was measured from the date of TKI discontinuation to the first of 2 or more consecutive quantitative real-time polymerase chain reaction (qRT-PCR) assessments confirming less than MR3.

Treatment-free remission was defined as the interval between the date of stopping TKI therapy and the date of molecular relapse or, if this did not happen, the date of last contact. Continuous variables were dichotomized to assess prognostic values for TFR using the median value. Sensitivity analysis was performed for these variables excluding outlier values. To explore the impact of age, we interrogated cut-off points at the median age (51 years) and at ages 40 and 60 years. $P < 0.05$ (two-tailed) was considered significant. Potential predictive variables for TFR were analyzed in univariate analysis using the Kaplan-Meier method. Only statistically significant variables were included in multivariate analysis using a Cox proportional hazard regression model.

Subject-, disease- and therapy-related variables before stopping TKI therapy are shown in Table 1. Median follow up from stopping TKI therapy was 26 months (range 6-121 months). Forty-one subjects [64%; 95% confidence interval (CI): 53, 75%] stopped TKI because of intolerance, 7 (11%; 95%CI: 5, 19%) in order to conceive, and 16 (25%; 95%CI: 14, 36%) were elected to stop treatment on achieving a sustained deep response. At the time of discontinuing TKI, 32 subjects (50%; 95%CI: 38, 63%) were receiving imatinib and 32 (50%; 95%CI: 38, 63%), dasatinib or nilotinib. The frequency of patients with e13a2 (42%) or e14a2 transcripts (58%) is similar to that reported within the European LeukemiaNet (ELN) registry, at 45% and 55%, respectively.⁶

Thirty-seven subjects (58%; 95%CI: 45, 70%) remain in molecular remission at a median of 26 months (range 7-64 months) after stopping TKI therapy. The 3-year actuarial probability of TFR is 53% (95%CI: 38, 66%). Twenty-seven subjects (42%; 95%CI: 30, 55%) had a molecular relapse at a median of four months (range 1-30 months) after stopping TKI therapy.

In multivariate analysis of factors found to be predictive of TFR in univariate analysis (i.e. transcript type, age \geq 40 years, duration of \geq MR4, depth of response, and percentage of TKI dose at the time of interruption), only e14a2 transcript type [Hazard Ratio (HR) = 0.38 (0.18, 0.84); $P = 0.016$] and age at diagnosis 40 years or over [HR

Table 1. Subject-, disease- and therapy-related variables (n=64).

Sex	
Males	22
Females	42
Age at diagnosis (y; median, range)	
	51 (19-87)
BCR-ABL1 transcript type	
e14a2	37
e13a2	27
Sokal score at diagnosis	
Low	23
Intermediate	15
High	14
Unknown	12
Prior interferon	
	11
Interval diagnosis to \geq MR3 (mo; median; range)	
	7 (2-87)
Interval diagnosis to \geq MR4 (mo; median; range)	
	24 (3-108)
\geq MR4 duration* (mo; median; range)	
	60 (12-156)
Duration of TKI therapy (y; median; range)	
	7 (2-15)
Reason for stopping TKI	
Adverse event	41
Pregnancy	7
Achievement of deep sustained response	16
Imatinib 1 st -line at stop	
After optimal response	28
After suboptimal response (BCR-ABL IS >10% at 3 months)	4
2G-TKI 1 st -line at stop	
	13
2G-TKI 2 nd -line at stop	
After prior imatinib intolerance	10
After prior imatinib failure	4
2G-TKI 3 rd -line at stop	
After prior TKI-intolerance	4
After prior TKI-resistance	1
2G-TKI at stop	
Nilotinib	17
Dasatinib	15
TKI-dose at stop (% of standard dose)	
100	21
75-50	35
<50	7
Missing	1

mo: months; y: years; IS: International Scale; TKI: tyrosine kinase inhibitor; 2G-TKI: 2nd-generation tyrosine kinase inhibitor. *Corresponding to the interval between the achievement of a sustained BCR-ABL < 0.01% (on the International Scale) and the date of TKI interruption.

= 0.3 (0.13, 0.66); $P = 0.003$] remained significantly-associated with TFR (Table 2). Figure 1 shows the cumulative incidence of losing MR3 for all subjects and those with e13a2 (64%; 95%CI: 50, 77%) and e14a2 transcripts (35%; 95%CI: 15, 56%).

Twenty-six of 27 subjects with molecular relapse returned to MR3 or over at a median of three months (range 1-9 months) after re-starting TKI therapy. At last follow up, all were alive and in MR3 (5 subjects at a median 6 months of follow up), MR4 (8 subjects at a median 8 months of follow up), or more than MR4 (13 subjects at a median 26 months of follow up after re-starting TKI therapy). One patient, who stopped TKI in

Cumulative incidence of molecular relapse

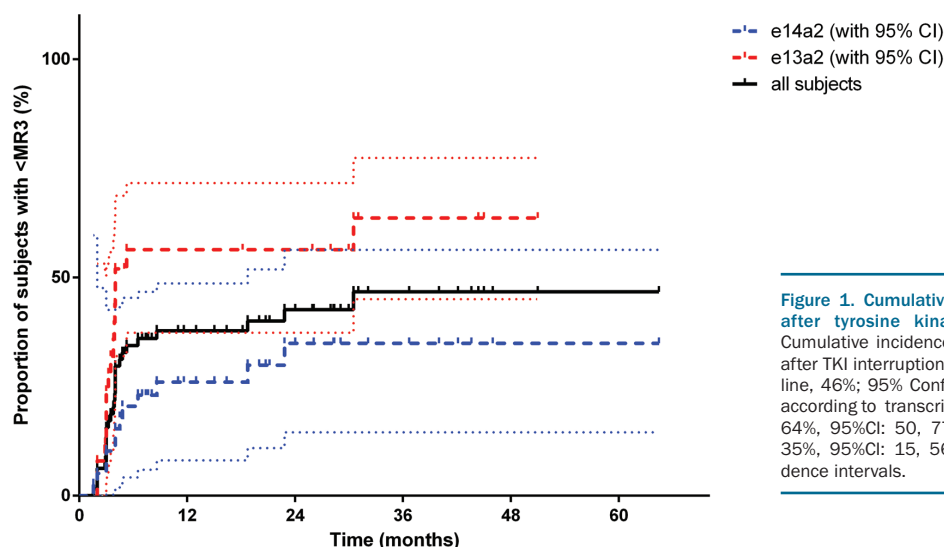


Figure 1. Cumulative incidence of molecular relapse after tyrosine kinase inhibitor (TKI) interruption. Cumulative incidence of molecular relapse (MR3 loss) after TKI interruption for the entire patient cohort [black line, 46%; 95% Confidence Interval (CI): 31, 60%] and according to transcript type (for e13a2, red dashed line, 64%, 95%CI: 50, 77%; for e14a2, blue dashed line, 35%, 95%CI: 15, 56%). Dotted lines represent confidence intervals.

Table 2. Univariate and multivariate analysis.

Variable	Cumulative incidence of MR3 loss over time (n=64)	
	Univariate analysis HR (95% CI); P	Multivariate analysis HR (95% CI); P
BCR-ABL1 transcript e14a2	0.4 (0.18, 0.85); P=0.019	0.38 (0.18, 0.84); P=0.016
Age at diagnosis ≥ 40 y	0.31 (0.14, 0.68); P=0.003	0.3 (0.13, 0.66); P=0.003
Sokal score low+intermediate	0.7 (0.28, 1.72); P=0.44	
Male	1.39 (0.63, 3.0); P=0.41	
Prior interferon	0.94 (0.32, 2.72); P=0.91	
TKI therapy >7 y	0.95 (0.45, 2); P=0.9	
Time to achieve MR3 <7 mo	0.71 (0.31, 1.55); P=0.39	
Time to achieve MR4 <24 mo	0.67 (0.38, 1.47); P=0.32	
\geq MR4 duration >60 mo	0.37 (0.16, 0.84); P=0.017	0.88 (0.31, 2.5); P=0.824
Depth of response at stop >MR4	0.38 (0.17, 0.86); P=0.021	0.75 (0.28, 1.97); P=0.56
2G-TKI at stop	0.61 (0.28, 1.33); P=0.21	
<100% of TKI standard dose at stop	0.45 (0.21, 0.96); P=0.043	0.58 (0.26, 1.32); P=0.19

HR: Hazard Ratio; CI: Confidence Interval; y: years; mo: months; 2G-TKI: 2nd-generation tyrosine kinase inhibitor.

order to conceive, lost MR3 at 24 weeks of pregnancy. She re-started TKI two months after a normal delivery but has not yet regained MR3 at one month from TKI resumption.

We found that the e14a2 *BCR-ABL1* transcript was significantly associated with a higher rate of TFR. Several studies have explored the correlation between *BCR-ABL1* transcript type and response to TKI therapy, with the e14a2 transcript reported to predict increased response to imatinib.⁷ One recent study showed higher rates of MR4.5, better event-free survival and better transformation to blast phase-free survival in subjects with an e14a2 transcript compared with those with e13a2, regardless of initial TKI therapy;⁸ lower response rates for e13a2 were also found by other authors.⁶ Another study in subjects receiving first-line imatinib came to the conclusion that different transcript types had no impact on overall sur-

vival or CML-related death.⁹

The association we report of *BCR-ABL1* transcript type and TFR, if confirmed, might reflect possible increased tyrosine kinase activity of the e13a2 transcript.⁷ Alternatively, increased immunogenicity of the e14a2 transcript eliciting a stronger host immune-mediated anti-CML effect is also described, although this seems an unlikely explanation.¹⁰⁻¹²

Age 40 years or over was also associated with a higher likelihood of TFR after stopping TKI therapy. Recent data suggest that CML patients aged 5-29 years have less frequent cytogenetic and molecular responses to TKI therapy compared with older individuals and an increased risk of transformation to blast phase.^{13,14} These data are consistent with our findings of an unfavorable impact of age on the probability of TFR. Others report a similar association.¹⁵

Our study has important limitations, including its retrospective nature, the small sample size and the fact that a substantial proportion of patients stopped TKI therapy because of intolerance rather than from a planned stopping strategy. Although our conclusions require validation, our data suggest that the presence of e14a2 BCR-ABL1 transcript type and age 40 years or over at diagnosis increase the probability of TFR.

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