EUROPEAN HEMATOLOGY ASSOCIATION
haematologica
Journal of the European Hematology Association

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

by Simone Claudiani, Jane Apperley, Robert Peter Gale, Richard Clark, Richard Szydlo, Simona Deplano, Renuka Palanicawandar, Jamshid Khorashad, Letizia Foroni, and Dragana Milojkovic

Haematologica 2017 [Epub ahead of print]

Citation: Claudiani S, Apperley J, Gale RP, Clark R, Szydlo R, Deplano S, Palanicawandar R, Khorashad J, Foroni L, and Milojkovic D. E14a2 BCR-ABL1 transcript is associated with a higher rate of treatment-free remission in persons with chronic myeloid leukemia after stopping tyrosine kinase-inhibitor therapy. Haematologica. 2017; 102:xxx
doi:10.3324/haematol.2017.168740

Title:

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

Running title: E14a2 transcript is associated with TFR in CML

Authors: Simone Claudiani[1], Jane Apperley[1], Robert Peter Gale[1], Richard Clark[2], Richard Szydlo[1], Simona Deplano[1], Renuka Palanicawandar[1], Jamshid Khorashad[1], Letizia Foroni[1], Dragana Milojkovic[1]

Affiliations:

[1] Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

[2] Royal Liverpool University Hospital, Liverpool, United Kingdom

Contact information for correspondence:

Simone Claudiani; email address: Simone.Claudiani@imperial.nhs.uk; mailing address: Haematology Department, Hammersmith Hospital, Du Cane Road, **London** W12 0HS.
Tel number: +440208-383-1627. Fax number: +440208-383-3965.

Word count: 883.

Acknowledgments:

The authors would like to thank the BRC for their support. SC acknowledges Ariodante Mattucci for the informatics support. RG acknowledges support from the National Institute of Health Research (NIHR) Biomedical Research Centre funding scheme.

Treatment-free remission (TFR) is a new therapy-goal for persons 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, predicting who will achieve TFR is imprecise and controversial (3). We report data from 64 persons who stopped TKI-therapy and showed 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 (≥MR4 on International Scale) for ≥1 year before stopping TKI-therapy, equivalent to the eligibility criteria of the Euro-Ski trial (4). Molecular response level was calculated by standard criteria (5) 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 consecutive quantitative real-time polymerase chain reaction (qRT-PCR) assessments confirming <MR3.

TFR was defined as the interval between the date of stopping TKI-therapy and 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 done for these variables excluding outlier values. For the age we interrogated cut-points at the median (51 years) and at ages 40 and 60 years. P-values <0.05 (two-tailed) were considered significant. Potential predictive variables for TFR were analysed in univariate analyses by the Kaplan-Meier method. Only the statistically significant variables were included in multivariate analyses using a Cox proportional hazard regression model.

Subject, disease and therapy related variables before stopping TKI-therapy are displayed 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%; [5, 19%]) in order to conceive and 16 (25%; [14, 36%]) were elected to stop treatment because of the achievement of sustained deep response. At the time of discontinuing TKI 32 subjects (50% [38, 63%]) were receiving imatinib and 32 (50% [38, 63%), dasatinib or nilotinib. The

frequency of patients with e13a2 (42%) or e14a2 transcripts (58%) is similar to that reported within the ELN registry at 45% and 55% respectively (8).

Thirty-seven subjects (58% [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% (38%, 66%). Twenty-seven subjects (42% [30, 55%]) had a molecular relapse at a median of 4 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 ≥40, duration of ≥MR4, depth of response and percent TKI dose at the time of interruption) only e14a2 transcript type (HR=0.38 [0.18, 0.84], p=0.016) and age at diagnosis ≥40 years (HR=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% [50, 77%]) and e14a2 transcripts (35% [15, 56%]).

Twenty-six of 27 subjects with molecular relapse returned to ≥MR3 at a median of 3 months (range, 1-9 months) after re-starting TKI-therapy. At last follow-up, all were alive and in MR3 (5 subjects at 6 months median of follow-up), MR4 (8 subjects at 8 months median follow-up), or >MR4 (13 subjects at 26 months median follow-up after re-starting TKI-therapy). One patient, who stopped TKI in order to conceive, lost MR3 at 24 weeks of pregnancy. She restarted TKI 2 months after normal delivery and has not yet regained MR3 at 1 month from TKI resumption.

We found that the e14a2 *BCR-ABL1* transcript was significantly associated with a higher rate of TFR. Several studies interrogated the correlation between *BCR-ABL1* transcript type and response to TKI-therapy, with the e14a2 transcript reported to predict increased response to imatinib (6). One recent study showed higher rates of MR4.5, better event free- (EFS) and better transformation to blast phase-free survival in subjects with an e14a2 transcript compared with those with e13a2, regardless of initial TKI-therapy (7); lower response rates for e13a2 were also found by other authors (8). Another study in subjects receiving first-line imatinib concluded

for absence of impact of different transcript types on overall survival and 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 (6). Alternatively, increased immunogenicity of the e14a2 transcript eliciting a stronger host immune-mediated anti-CML effect is also described but seems an unlikely explanation (10-12).

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

Our study has important limitations including the retrospective nature, the small sample size and a substantial proportion of subjects stopping 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 at diagnosis improve the probability of TFR.

References:

1. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029-1035.
2. Rousselot P, Charbonnier A, Cony-Makhoul P, et al. Loss of major molecular response as a trigger for restarting tyrosine kinase inhibitor therapy in patients with chronic-phase chronic myelogenous leukemia who have stopped imatinib after durable undetectable disease. J Clin Oncol. 2014;32(5):424-430.
3. Dulucq S, Mahon FX. Deep molecular responses for treatment-free remission in chronic myeloid leukemia. Cancer Med. 2016;5(9):2398-2411.
4. Mahon FX, Richter J, Guilhot J, et al. Cessation of Tyrosine Kinase Inhibitors Treatment in Chronic Myeloid Leukemia Patients with Deep Molecular Response: Results of the Euro-Ski Trial. Blood. 2016;128(22):787.
5. Cross NC, White HE, Colomer D, et al. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia. 2015;29(5):999-1003.
6. Lucas CM, Harris RJ, Giannoudis A, et al. Chronic myeloid leukemia patients with the e13a2 BCR-ABL fusion transcript have inferior responses to imatinib compared to patients with the e14a2 transcript. Haematologica. 2009;94(10):1362-1367.
7. Jain P, Kantarjian H, Patel KP, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. Blood. 2016;127(10):1269-1275.
8. Hanfstein B, Lauseker M, Hehlmann R, et al. Distinct characteristics of e13a2 versus e14a2 BCR-ABL1 driven chronic myeloid leukemia under first-line therapy with imatinib. Haematologica. 2014;99(9):1441-1447.
9. Pfirrmann M, Evtimova D, Saussele S, et al. No influence of BCR-ABL1 transcript types e13a2 and e14a2 on long-term survival: results in 1494 patients with chronic myeloid leukemia treated with imatinib. J Cancer Res Clin Oncol. 2017;143(5):843-850.
10. Clark RE, Dodi IA, Hill SC, et al. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. Blood. 2001;98(10):2887-2893.
11. Rea D, Dulphy N, Henry G, et al. Low natural killer (NK) cell counts and functionality are associated with molecular relapse after imatinib discontinuation in patients (pts) with chronic phase (CP)-chronic myeloid leukemia (CML) with undetectable BCR-ABL transcripts for at least 2 years: preliminary results from immunostim, On Behalf Of STIM Investigators. Blood. 2013;122(21):856.
12. Gale RP, Opelz G. Is there immune surveillance against chronic myeloid leukemia? Possibly, but not much. Leuk Res. 2017;59:109-111.
13. Castagnetti F, Gugliotta G, Baccarani M, et al. Differences among young adults, adults and elderly chronic myeloid leukemia patients. Ann Oncol. 2015;26(1):185-192.
14. Pemmaraju N, Kantarjian H, Shan J, et al. Analysis of outcomes in adolescents and young adults with chronic myelogenous leukemia treated with upfront tyrosine kinase inhibitor therapy. Haematologica. 2012;97(7):1029-1035.
15. Mori S, Vagge E, le Coutre P, et al. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. Am J Hematol. 2015;90(10):910-914.

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 ≥MR3 (mo; median; range)	7 (2-87)
Interval diagnosis to ≥MR4 (mo; median; range)	24 (3-108)
≥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 1st-line at stop	32
After optimal response	28
After suboptimal response (BCR-ABL IS >10% at 3 months)	4
2G-TKI 1st-line at stop	13
2G-TKI 2nd-line at stop	14
After prior imatinib intolerance	10
After prior imatinib failure	4
2G-TKI 3rd-line at stop	5
After prior TKI-intolerance	4
After prior TKI-resistance	1
2G-TKI at stop	32
Nilotinib	17
Dasatinib	15
TKI-dose at stop (% of standard dose)	
100%	21
75-50	35
<50%	7
Missing	1

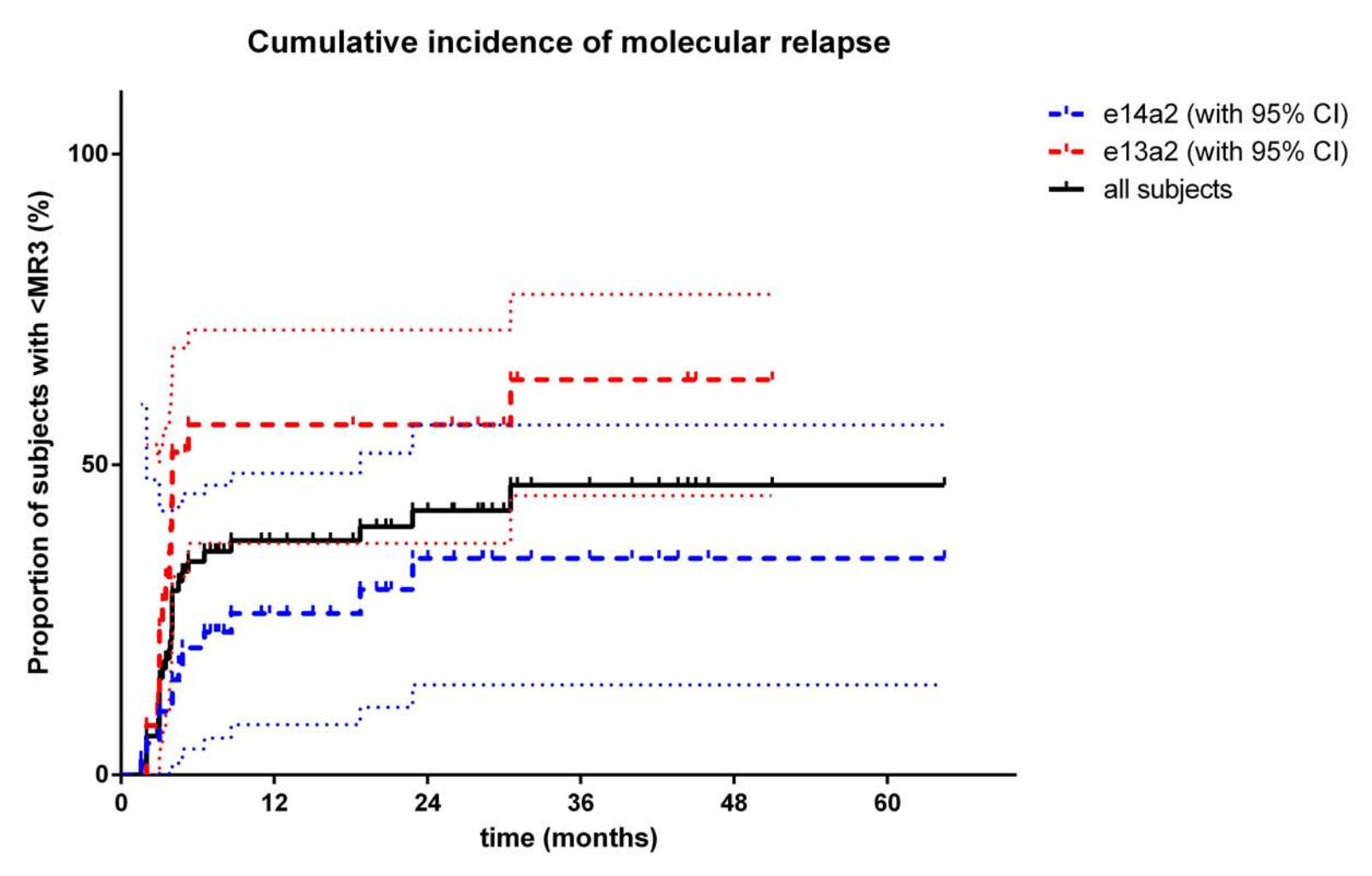
Legend: mo=months; IS=International Scale; TKI= Tyrosine Kinase Inhibitor; 2G-TKI, 2nd generation TKI.
*corresponding to the interval between the achievement of a sustained BCR-ABL < 0.01% (on International Scale) and the date of TKI interruption.

Table 2. Univariate and Multivariate Analysis.

	Cumulative incidence of MR3 loss over time (n=64)	
Variable	Univariate analysis HR (95% CI); p value	Multivariate analysis HR (95% CI); p value
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 sex	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	
≥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

Legend: y=years; mo=months; 2G-TKI= 2nd generation Tyrosine Kinase Inhibitor.

Figure 1. Cumulative incidence of molecular relapse after TKI interruption.
The figure shows the cumulative incidence of molecular relapse (MR3 loss) after TKI interruption for the entire patient cohort (black line, 46% [CI = confidence interval, 31, 60%] and according to the transcript type (for e13a2, red dashed line, 64% [CI 50, 77%]; for e14a2, blue dashed line, 35% [CI 15, 56%]). The dotted lines represent confidence intervals.


Cumulative incidence of molecular relapse
e14a2 (with 95% CI)
e13a2 (with 95% CI)
all subjects
Proportion of subjects with <MR3 (%)
100
50
0
0
12
24
36
48
60
time (months)