Brief report

EVI-1 oncogene expression predicts survival in chronic-phase CML patients resistant to imatinib treated with second-generation tyrosine kinase inhibitors

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Activation of the *EVI-1* oncogene has been reported in acute myeloid leukemia, chronic myeloid leukemia (CML) in blast crisis, and less commonly, in chronic-phase CML patients. We screened an unselected cohort of 75 chronic-phase CML patients who had failed imatinib for expression of *EVI-1* and sought a correlation with subsequent outcome on the second-generation tyrosine kinase inhibitors dasatinib (n = 61) or nilotinib (n = 14). The 8 patients (10.7%) who expressed *EVI-1* transcripts detectable by real-time polymerase chain reaction had significantly lower event-free survival, progression-free survival, and overall survival than patients with undetectable transcript. The predictive value of *EVI-1* expression was validated in an independent cohort. In a multivariate analysis, *EVI-1* expression status and the best cytogenetic response obtained on imatinib were the only independent predictors for overall survival, progression-free survival, and event-free survival. Our data suggest that screening for *EVI-1* expression at the time of imatinib failure may predict for response to second-line TKI therapy and consequently aid clinical management. (*Blood*. 2010;116(26): 6014-6017)

Introduction

Second-generation tyrosine kinase inhibitors (2G-TKIs), such as dasatinib or nilotinib, are efficacious therapies for chronic myeloid leukemia (CML) patients in chronic phase (CP) who have failed imatinib.[1,2] However, more than 50% of patients fail to achieve major cytogenetic response (MCyR) on 2G-TKI, and a significant proportion of these eventually experience disease progression.[1-3] As both 2G-TKI and allogeneic stem cell transplantation are possible therapies for patients failing imatinib, there is an urgent need for additional factors present at the point of imatinib resistance that predict for responses (or lack of response) to 2G-TKI and would therefore help in management decisions.

The *EVI-1* gene at chromosome band 3q26 exhibits several properties consistent with its role as an oncogene and is activated in a subset of most myeloid leukemias, either via rearrangement of chromosome band 3q26 or by other as yet undetermined mechanisms.[4] In acute myeloid leukemia, *EVI-1* expression has been associated with a poor prognosis, particularly in younger patients.[5,6] The mechanistic contribution of *EVI-1* expression to a more aggressive disease phenotype remains speculative but may be related to its interaction with several epigenetic regulators, including methyltransferases.[7] *EVI-1* activation has also been described in CML blast crisis, and less commonly in CP, but the value of this expression in predicting patient outcome has not been investigated.[8,9] We therefore sought to investigate the frequency and prognostic value of detectable *EVI-1* expression in imatinib-resistant CP CML.

Methods

Patients

Between April 2005 and July 2008, we studied 75 consecutive patients with CML in CP resistant to imatinib who were treated with dasatinib (n = 61) or nilotinib (n = 14). Written informed consent was obtained from all patients before enrollment. Patient characteristics were typical of those with imatinib-treated "late" CP (Table 1). No patient harbored a 3q26 rearrangement in the Ph-positive clone, as assessed by conventional cytogenetics and fluorescent in situ hybridization using an *EVI-1* gene–specific probe (Kreatech Diagnostics). The median follow-up from starting 2G-TKI was 30 months (range, 6-53 months); 95% of the patients were followed for at least one year. Dasatinib and nilotinib were administered as previously described.[1-3]

Bone marrow morphology and cytogenetics were assessed before 2G-TKI therapy and then every 3 months. CP, complete hematologic response, minor cytogenetic response (MiCyR), MCyR, complete cytogenetic response (CCyR), and major molecular response were defined by conventional criteria.[3,10] The 30-month cumulative incidences of MCyR, CCyR, and major molecular response were 59.1%, 55.1%, and 25.7%, respectively. The 30-month probabilities of overall survival (OS), progression-free survival (PFS), and event-free survival (EFS) were 90.7%, 88.9%, and 86%, respectively.

EVI-1 expression

Peripheral blood was collected from the 75 patients before starting treatment with the chosen 2G-TKI. This was approved by the Institutional

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Table 1. Patient characteristics at the time of starting 2G-TKI and 2.5-year probabilities of EFS, PFS, and OS*

Variable	n	EFS, %	PFS, %	Survival, %
Age, y		*P* = .84	*P* = .86	*P* = .77
> 55	40	84.5	87.6	91.8
≤ 55	35	87.2	89.9	89.2
Sex		*P* = .13	*P* = .11	*P* = .37
Male	35	80.6	82.7	88.4
Female	40	91.5	94.8	92.6
Sokal risk group		*P* = .65	*P* = .77	*P* = .28
Low	15	92.3	92.3	100
Intermediate	29	82.2	85.9	83.2
High	31	86.4	89.8	93.5
Status at the onset of imatinib therapy		*P* = .8	*P* = .94	*P* = .48
Newly diagnosed CP patients	28	85.1	89.1	86.1
Late CP	47	86.7	89.0	93.2
Additional cytogenetic abnormalities at start of 2G-TKI		*P* = .17	*P* = .44	*P* = .25
No	63	88.3	90.0	92.5
Yes	12	74.1	83.3	81.5
Percentage of Ph-positive marrow metaphases at start of 2G-TKI		*P* = .064	*P* = .14	*P* = .29
< 95%	28	96.4	95.2	96.4
≥ 95%	47	79.9	84.5	87.9
Time from imatinib failure to starting 2G-TKI		*P* = .42	*P* = .60	*P* = .84
≤ 6 months	15	93.3	93.3	90.9
> 6 months	60	84.2	87.8	90.5
Best cytogenetic response on imatinib†		*P* = .002	*P* = .043	*P* = .017
0%-94% Ph^+	42	63.7	76.5	75.3
≥ 95% Ph^+	33	92.6	92.6	96.4
Hematologic resistance to imatinib		*P* = .29	*P* = .44	*P* = .78
Yes	55	79.7	90.3	89.5
No	20	88.3	85.0	91.4
Maximal dose of imatinib		*P* = .55	*P* = .68	*P* = .82
400 mg/day	16	93.8	93.8	93.8
600 mg/day	29	81.5	85.4	87.9
800 mg/day	30	86.2	89.8	92.0
KD mutation at start of 2G-TKI		*P* = .51	*P* = .31	*P* = .11
No	55	84.9	86.9	100
Yes	20	90.0	95.0	86.7
Transcript type		*P* = .57	*P* = .62	*P* = .72
e14a2	26	83.9	83.9	86.3
e13a2	34	91.1	91.1	93.2
e14a2 and e13a2	15	78.3	93.3	93.3
***EVI-1* at start of 2G-TKI**		*P* = .0001	*P* < .0001	*P* = .0003
Negative	67	90.6	93.8	95.2
Positive	8	43.7	43.7	47.5
Percentage of blasts in bone marrow at start of 2G-TKI		*P* = .02	*P* = .1	*P* = .3
≤ 5%	54	90.4	88.4	90.4
> 5%	21	66.7	77.1	83.8
Hemoglobin level at start of 2G-TKI†		*P* = .3	*P* = .5	*P* = .5
Normal	41	92.8	92.9	96.4
Low	34	82.3	86.7	89.6

P values were calculated using the log-rank method. Variables with a *P* value < .1 were included in the multivariate analysis for each outcome.

*Other peripheral blood and bone marrow parameters are not included in the table, but they did not significantly predict for any of the outcomes.

†Normal values are > 12.5 for males and > 11.5 for females.

Review Board of Imperial College London, Hammersmith Hospital. Total RNA was extracted from cells using the automated QIAcube and the RNeasy kit (QIAGEN) and was reverse transcribed to generate cDNA. *EVI-1* expression was measured using a multiplex TaqMan assay using *EVI-1* primers and probes described by Vinatzer et al.[4] A VIC-MGB-labeled G6PD probe, designed in house, was used as an endogenous control. Six standards of known concentration were used, prepared by serial dilution of 2 plasmids containing part of the *EVI-1* and G6PD genes, respectively (supplemental data, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Standards were tested in triplicate, and all patient samples were tested in duplicate. The intercept of the standard curve slope (theoretically one transcript) was used as a threshold to indicate *EVI-1* positivity. As expected, cDNA extracted from the peripheral blood of 20 healthy volunteers with no history of hematologic malignancy showed no evidence of *EVI-1* expression by this method.

Statistical methods

Probabilities of OS, PFS, and EFS were calculated using the Kaplan-Meier method. Univariate and multivariate analyses for the different outcomes were carried out as previously described.[3] The associations between *EVI-1* expression and other pretherapy characteristics were studied using the Fisher exact test. *P* values were 2-sided and 95% confidence intervals (CI) computed.

Results and discussion

EVI-1 expression was detected by real-time polymerase chain reaction in 8 (10.7%) of the 75 patients enrolled in this study. The median level of *EVI-1* expression relative to *G6PD* was 2×10^{-4} (range, 4.43×10^{-6} to 5.46×10^{-2}). This was significantly lower than that observed in 3 CML blast crisis (BC) patients with 3q26 rearrangements (median, 3.46×10^{-2}; range, 2.65×10^{-2} to 0.13; $P = .036$). We could not find any significant association between expression of *EVI-1* and any of the patient characteristics shown in Table 1 (supplemental Table 1). Interestingly, there was no significant association between detectable *EVI-1* expression at the start of 2G-TKI therapy and characteristics commonly associated with progression to advanced phase, such as clonal cytogenetic evolution ($P = 1.0$), presence of KD mutations ($P = .67$), Sokal score at diagnosis ($P = .4$), and hematologic resistance to imatinib ($P = .43$). The CD34 count in the bone trephine and the percentage of blast in the bone marrow were also comparable between patients with (0.8% and 3.9%) and without (1.4% and 3.5%) *EVI-1* expression ($P = .9$ and $P = .7$, respectively).

Patients with positive *EVI-1* expression at the onset of 2G-TKI therapy had a significantly lower 30-month EFS (43.7% vs 90.6%, $P = .0001$), PFS (43.7% vs 93.8%, $P < .0001$), OS (47.5% vs 95.2%, $P = .0003$), and cumulative incidence of CCyR (12.0% vs 59.7%, $P = .05$) (Table 1; Figure 1A) than patients without *EVI-1* expression. We performed multivariate analysis for OS, PFS, and EFS. Expression of *EVI-1* (present or absent) and the achievement of at least a MiCyR during the prior imatinib therapy were the only independent predictors for OS (relative risk [RR] = 0.11, CI, 0.02-0.58, $P = .009$; and RR = 5.2, CI, 1.03-24.2, $P = .05$), PFS (RR = 0.07, CI, 0.02-0.3, $P = .0003$; and RR = 4.9, CI, 1.2-20.4, $P = .03$), and EFS (RR = 0.08, CI, 0.02-0.3, $P = .0004$; and RR = 8.1, CI, 2.1-30.1 $P = .002$).

We validated the prognostic value of *EVI-1* using an independent cohort of 28 patients who were treated in the Liverpool Royal University Hospital with nilotinib (n = 13) or dasatinib (n = 15) after imatinib failure while still in CP. Patients with positive *EVI-1* expression at the onset of 2G-TKI therapy had a significantly lower 30-month EFS (0% vs 84.2%, $P = .0001$), PFS (0% vs 88.6%, $P < .0001$), and OS (50% vs 95.7%, $P = .007$) than patients without *EVI-1* expression.

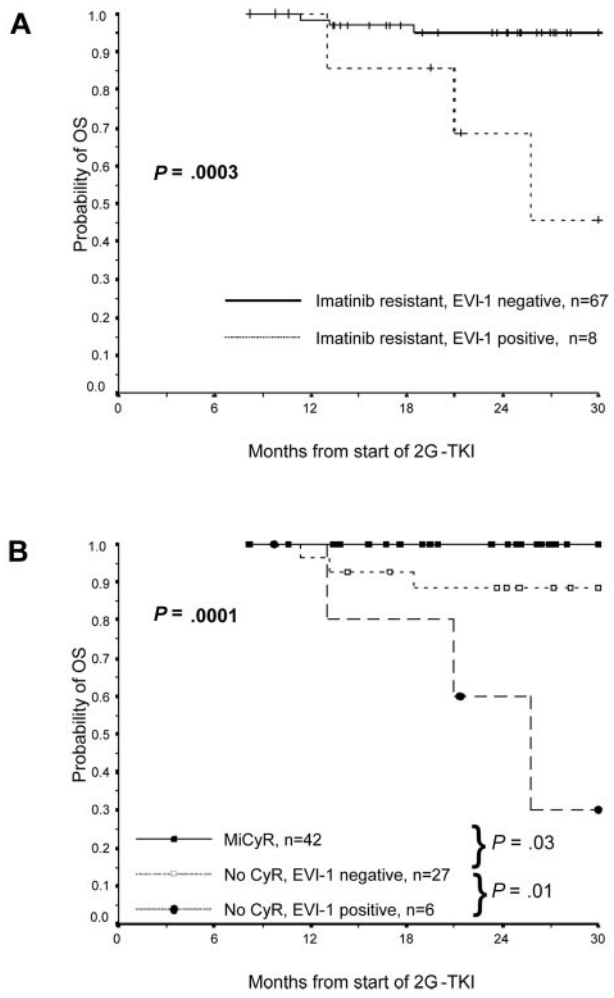

Figure 1. Survival analysis of imatinib-resistant CML patients treated with 2G-TKI according to EVI-1 expression status. (A) OS for imatinib-resistant patients with and without detectable *EVI-1* expression at the onset of 2G-TKI therapy. The probability of survival at 30 months was 95.2% for *EVI-1* nonexpressors (median survival time, not reached; blue line) versus 47.5% for expressors (median survival time, 26 months; red line) ($P = .0003$). (B) Survival of CML patients receiving 2G-TKI therapy according to the presence of at least a MiCyR (< 95% Ph-positive metaphase) at 3 months (green line), absence of both MiCyR and *EVI-1* expression at 3 months (blue line), or absence of MiCyR with presence of *EVI-1* expression (red line). The graph demonstrates that, in patients who fail to achieve a MiCyR on 2G-TKI, positive *EVI-1* expression is a strong predictor of poor outcome. The *P* values were calculated using the log-rank method.

At 3 months, 42 patients had achieved at least a MiCyR. These patients had a significantly better OS, PFS, and EFS than the 33 nonresponders, namely, 100% versus 79.9% ($P = .005$), 100% ver-sus 75.1% ($P = .001$), and 97.4 versus 71.9 ($P = .002$), respectively. We repeated the multivariate analysis, including the level of cytogenetic response achieved at 3 months. Expression of *EVI-1* and the achievement of MiCyR were the only independent predictors for OS (RR = 0.09, CI, 0.02-0.5, $P = .004$; and RR = 8.3 CI, 1.4-41.5, $P = .02$), PFS (RR = 0.09, CI, 0.02-0.4, $P = .001$; and RR = 10, CI, 2.5-38.6, $P = .01$), and EFS (RR = 0.16, CI, 0.04-0.6, $P = .007$; and RR = 11.1, CI, 1.4-90.9, $P = .025$; Figure 1B).

Significantly, none of the patients in our cohort in whom *EVI-1* expression was detected harbored a *BCR-ABL1* KD mutation known to confer resistance to 2G-TKIs; this observation should exclude this mechanism as an alternative explanation for their inferior outcome. Retrospective analysis of archived material revealed that *EVI-1* expression was not present in diagnostic samples of patients who were later found to express the transcript but emerged at a median of 31 months after diagnosis (range, 15-103 months). We also screened diagnostic samples from a further 23 CML patients, 10 of whom responded to imatinib and 13 did not. None of these samples had detectable *EVI-1* expression, suggesting that *EVI-1* expression is not commonly present in patients at diagnosis, regardless of subsequent response to imatinib.

We have shown that detection of expression of the *EVI-1* oncogene in imatinib-resistant CP CML patients at the start of 2G-TKI therapy is a strong predictor for disease progression and shorter survival, and validated this result using an independent cohort. Measurement of *EVI-1* expression at the point of imatinib failure may therefore identify patients who would fare badly on a 2G-TKI regimen and might be better served by early referral for transplantation. Our findings also suggest that, in patients who fail to achieve MiCyR after 3 months of 2G-TKI therapy, *EVI-1* expression status may help to distinguish those patients with poor disease outcome from those who have a significantly higher chance of long-term PFS.

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Authorship

Contribution: A.G.R., D. Marin, and J.S.K. conceived and designed the study; D. Marin, D. Milojkovic, J.F.A., L.F., and R.E.C. provided study materials or patients; M.D., J.S.K., G.G., C.P., D. Marin, D. Milojkovic, P.C.M., V.A.D.M., and L.W. collected and assembled the data; A.G.R., D. Marin, M.D., J.S.K., and G.G. analyzed and interpreted data; and A.G.R., J.S.K., D. Marin, J.M.G., J.F.A., and L.F. wrote the manuscript.

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