Predictive value of early molecular response in patients with chronic myeloid leukemia treated with first line dasatinib

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Running title: dasatinib 3 months transcript level

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ABSTRACT
Dasatinib is effective therapy for newly diagnosed patients with chronic myeloid leukaemia, but not all patients respond well. We analysed the outcome of patients treated with dasatinib as first line therapy in order to identify patients who are more likely to fare poorly. The 8.6% of patients who at three months had a BCR-ABL/ABL ratio >10% had a significantly worse 2-year cumulative incidence of complete cytogenetic response (CCyR, 58.8% vs 96.6%, p<0.001) and molecular responses than the remaining patients with a lower transcript levels. The predictive value of the 3-month transcript level could be improved by using the dasatinib-specific transcript level cut-offs, namely 2.2%, 0.92% and 0.57% for CCyR, 3 log and 4.5 log reductions in the transcript level respectively. The study is registered at www.clinicaltrials.gov as NCT01460693.
INTRODUCTION

Second generation tyrosine kinase inhibitors (TKIs) such as dasatinib or nilotinib have proved to be very successful in treating chronic myeloid leukaemia (CML) patients who have failed imatinib.\textsuperscript{1,2} This observation led investigators to explore the efficacy of these drugs as first line therapy in CML. Indeed early reports with both of these second generation TKIs suggest a very high response rate,\textsuperscript{3,4} but despite these encouraging results, some patients still fail to respond to front line dasatinib or nilotinib and may benefit from an alternative treatment. In this study we define molecular milestones at 3 months that may identify patients treated with dasatinib as first line therapy who have a low probability of achieving an adequate response and are thus candidates for alternative treatment.

METHODS

Patients and therapy

Between August 2008 and September 2011, 142 consecutive adult patients with CML in chronic phase (CP) received dasatinib 100 mg daily as first line therapy as described elsewhere\textsuperscript{4} in the United Kingdom (UK) SPIRIT 2 study. Briefly, the UK SPIRIT 2 trial is a phase III study in which newly diagnosed CML patients in CP are randomly allocated to receive either imatinib 400mg daily or dasatinib 100mg daily (ClinicalTrials.gov Identifier: NCT01460693). Patient characteristics are shown in Table 1. The median follow up was 18.2 months (range 12-35). Bone marrow morphology and cytogenetics were assessed at diagnosis and every 12 months thereafter. CP was defined using conventional criteria.\textsuperscript{5} Complete cytogenetic response (CCyR) was defined either by the failure to detect any Philadelphia chromosome-positive metaphases in bone marrow examinations with a minimum of 20 metaphases examined or by the reduction of the BCR-ABL1 transcript numbers in peripheral blood to a level usually regarded as consistent with CCyR, namely 1\%.\textsuperscript{6,7}

Detection of BCR-ABL1 transcripts

BCR-ABL1 transcripts were measured in the blood at 3 month intervals using RQ-PCR as described previously.\textsuperscript{8,9} The tests were performed in molecular biology laboratory at Hammersmith Hospital. Results were converted to the international scale.\textsuperscript{10} Molecular responses (MR) were expressed according to the log reduction in
the transcript level below the conventionally defined starting point of 100%. MR3 (equivalent to major molecular response) was defined as a transcript level \( \leq 0.1\% \) on the international scale. MR4.5 was defined as BCR-ABL1 ratio of 0.0032\% on the international scale provided copies numbers of the control was at least 45,000, consistent with a 4.5 log reduction in the transcript level. Complete molecular response (CMR) was defined by the finding of two consecutive samples with no detectable transcripts with an ABL1 control >40,000 copies. Samples with an ABL1 control <10,000 were discarded. For samples included in this study the median copy number of the ABL1 control was 62,000.

**Statistical Methods**

The probabilities of cytogenetic and molecular responses were calculated using the cumulative incidence (CI) procedure, whereby cytogenetic or molecular responses were the events of interest and therapy discontinuation or death was the competitor. Probabilities of CI were examined using Fine-Gray regression.11-13 Univariate, and multivariate analyses were performed in accordance with standard methods; variables found to be significant at the \( p<0.10 \) level were entered in the multivariate analysis. Receiver operating characteristic (ROC) curve were plotted using the SPSS-19 statistical package.

**RESULTS AND DISCUSSION**

**Responses to dasatinib first line therapy**

The 2-year cumulative incidence of CCyR, MR3, MR4.5 and CMR were 89.8\%, 70.0\% 39.1\% and 6.5\% respectively. We examined the influence of the patient characteristics shown in Table 1 on the probability of achieving cytogenetic and molecular responses. The only independent predictors for the achievement of CCyR were Sokal risk group (RR= 0.713 and RR= 0.582, \( p= 0.027 \) for intermediate and high Sokal respectively) and the haemoglobin level at diagnosis (RR=1.236, \( p<0.001 \)). Similarly Sokal risk group (RR= 0.590 and RR= 0.412, \( p= 0.029 \) for intermediate and high Sokal respectively) and the haemoglobin level (RR=1.360, \( p<0.001 \)) were the only independent predictors for the achievement of MR3. Haemoglobin level at diagnosis was the only independent predictor for MR4.5.
The BCR-ABL1 transcript levels after 3 months on dasatinib therapy strongly predicts for cytogenetic and molecular responses

One hundred and twenty eight patients had a valid sample for RQ-PCR for BCR-ABL1 at 3 months. In the remaining 14 patients the sample was missing or had an ABL control <10000 copies. We examined the predictive value of the transcript level measured at 3 months on the probabilities of achieving cytogenetic and molecular responses. The 117 (91.4%) patients who after 3 months on dasatinib had a BCR-ABL1/ABL1 ratio of ≤10% had a significantly superior 2-year cumulative incidence of CCyR (91.4% vs 58.8%, p<0.001), MR3 (79.8% vs 14.3%, p<0.001), and MR4,5 (45.7% vs 0%, p<0.001), compared to the 11 (8.6%) patients with values greater than 10% (Figure 1), but it was not predictive for CMR (6.1% vs 0%, p=0.45). The 3 month transcript level (>10%) and haemoglobin level at diagnosis were the only independent predictors for CCyR (RR=0.239, p<0.001 and RR=1.248, p=0.001 respectively) and MR4,5 (RR=0.015, p<0.001 and RR=1.315, p=0.01 respectively)

The predictive power for cytogenetic and molecular responses of the BCR-ABL1 transcript level at 3 months can be greatly improved when therapy-specific transcript level cut-offs are used

As previously described we identified cut-offs in the 3-month transcript levels that predicted for achievement of cytogenetic and molecular responses with the maximal sensitivity and specificity using a ROC curve. The optimal transcript level cut-offs identified for CCyR were 2.26% (2-year CI of CCyR 100% vs 78.5%, p<0.001), for MR3 0.92% (2-year CI of MR3 95.8% vs 46.2%, p<0.001) for MR4,5 was 0.57% (2-year CI of MR4,5 70.9% vs 14.5%, p<0.001) and for CMR was 0.24% (2-years CI of CMR 14.1% vs 1.8%, p=0.004). When the multivariate analysis was repeated using these cut-offs, the BCR-ABL1 transcript level was the only independent predictor for CCyR, MR3, MR4,5 and CMR.

We have previously reported that patients treated with imatinib first line therapy who have a 3-month transcript level >9.84% have significantly worse survival and that the
measurement of the transcript level at three months is the most accurate way to identify patients who will fare poorly.\textsuperscript{14} Similar results have been reported by others.\textsuperscript{15-18} The transcript level at 3 months (lower than 10\%) can also be used to identify patients treated with second line dasatinib or nilotinib who have a lower probability of survival.\textsuperscript{19} In this analysis we show that the measurement of the transcript level at 3 months in patients treated with first line dasatinib is also predictive for outcome, allowing the identification of around 10\% of dasatinib-treated patients who have a low probability of achieving CCyR and deep molecular responses and for whom other forms of treatment might be considered. The predictive power of the 3-month RQ-PCR assessment can be greatly improved by identifying and then applying the optimal transcript level cut-offs for each specific outcome (CCyR, MR\textsubscript{3} and MR\textsubscript{4.5}). These cut-off values are significantly lower that the ones reported for imatinib (i.e. 2\% vs 10\%), highlighting the fact that patients treated with second generation TKIs have response kinetics that differ from patients treated with imatinib and need specifically tailored therapeutic milestones.\textsuperscript{20}

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AUTHORS DISCLOSURES:

David Marin, Dragana Milojkovic, Jane Apperley, Stephen O’Brian and John Goldman received research support from Novartis and Bristol Myers-Squibb.

AUTHOR CONTRIBUTIONS:

Corinne Hedgley assembled the patients data and co-ordinate the study
John Goldman, Richard E Clark, Jane Apperley, Dragana Milojkovic, Christopher Pocock and Stephen O’Brien conducted the clinical trial, provided patient care and commented on the manuscript
Letizia Foroni commented on the manuscript and performed the molecular studies. David Marin performed statistical analysis and wrote the manuscript.
Reference List


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Table 1. Patients characteristics at diagnosis and Relative risks for the achievement of CCyR, MR3 and MR_{4.5} in the univariate analysis

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>CCyR</th>
<th>MR3</th>
<th>MR_{4.5}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>79 (55.6)</td>
<td>p= 0.77</td>
<td>p=0.88</td>
</tr>
<tr>
<td>female</td>
<td>63 (44.4)</td>
<td>1</td>
<td>1.030</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td>p=0.40</td>
<td>p=0.09</td>
</tr>
<tr>
<td>median (range)</td>
<td>54.4 (18-82)</td>
<td>1.004</td>
<td>1.011</td>
</tr>
<tr>
<td><strong>Sokal risk group, n (%)</strong>*</td>
<td></td>
<td>p= 0.005</td>
<td>p=0.002</td>
</tr>
<tr>
<td>Low</td>
<td>35 (29.9)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate</td>
<td>51 (43.6)</td>
<td>0.813</td>
<td>0.789</td>
</tr>
<tr>
<td>High</td>
<td>31 (26.5)</td>
<td>0.510</td>
<td>0.350</td>
</tr>
<tr>
<td><strong>EUTOS risk group, n (%)</strong>**</td>
<td></td>
<td>p= 0.09</td>
<td>p=0.04</td>
</tr>
<tr>
<td>Low</td>
<td>86 (83.5)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>17 (16.5)</td>
<td>0.608</td>
<td>0.451</td>
</tr>
<tr>
<td><strong>Spleen size at diagnosis (cm)</strong></td>
<td></td>
<td>p=0.11</td>
<td>p=0.02</td>
</tr>
<tr>
<td>median (range)</td>
<td>2 (0-32)</td>
<td>0.976</td>
<td>0.953</td>
</tr>
<tr>
<td><strong>Haemoglobin level (g/dl)</strong></td>
<td></td>
<td>p&lt; 0.001</td>
<td>p&lt; 0.001</td>
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<td>median (range)</td>
<td>11.0 (4.2-15.8)</td>
<td>1.225</td>
<td>1.267</td>
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<tr>
<td><strong>White blood cell count (x 10^9/l)</strong></td>
<td></td>
<td>p= 0.04</td>
<td>p= 0.05</td>
</tr>
<tr>
<td>median (range)</td>
<td>56.3 (2-428)</td>
<td>0.998</td>
<td>0.997</td>
</tr>
<tr>
<td><strong>Platelet count (x 10^9/l)</strong></td>
<td></td>
<td>p=0.8</td>
<td>p=0.77</td>
</tr>
<tr>
<td>median (range)</td>
<td>425 (100-2433)</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Blasts in peripheral blood (%)</strong></td>
<td></td>
<td>p=0.045</td>
<td>p=0.09</td>
</tr>
<tr>
<td>median (range)</td>
<td>0.6 (0-14.5)</td>
<td>0.898</td>
<td>0.895</td>
</tr>
<tr>
<td><strong>Basophils in peripheral blood (%)</strong></td>
<td></td>
<td>p=0.54</td>
<td>p=0.74</td>
</tr>
<tr>
<td>median (range)</td>
<td>3.9 (0-19.0)</td>
<td>0.989</td>
<td>0.993</td>
</tr>
</tbody>
</table>

* 25 patients had missing data
** 39 patients had missing data
Figure 1. Two year cumulative incidence of CCyR, MR₃, and CMR₄.₅ according to the 3 months BCR-ABL1 transcript level

Panel A and B show the cumulative incidence of CCyR according to whether the transcript level is higher or lower than 10% (panel A) or 2.26% (panel B). Panel C and D show the cumulative incidence of MR₃ according to whether the transcript level is above or below 10% (panel C) or 0.92% (panel D). Panel E and F show the cumulative incidence of MR₄.₅ according whether the transcript level is higher or lower than 10% (panel E) or 0.57% (panel F). 2.26%, 0.92% and 0.57% are the transcript level cut-offs identified using the ROC curves that optimally predict for each specific outcome (see text).
A

BCR-ABL/ABL ≤10%, n=117

BCR-ABL/ABL >10%, n=11

p<0.001

Time from onset of therapy (months)

Cumulative incidence of CCyR

B

BCR-ABL/ABL ≤2.26%, n=88

BCR-ABL/ABL >2.26%, n=40

p<0.0001

Time from onset of therapy (months)

Cumulative incidence of CCyR
Cumulative incidence of MR3

Time from onset of therapy (months)

BCR-ABL/ABL \( \leq 10\% \), \( n=117 \)

BCR-ABL/ABL >10\%, \( n=11 \)

\( p<0.001 \)

Time from onset of therapy (months)

BCR-ABL/ABL \( \leq 0.92\% \), \( n=72 \)

BCR-ABL/ABL >0.92\%, \( n=56 \)

\( p<0.0001 \)
**E**

Cumulative incidence of MR4.5

- Time from onset of therapy (months)
- \(p<0.001\)

- **BCR-ABL/ABL ≤10\%, n=117**
- **BCR-ABL/ABL >10\%, n=11**

**F**

Cumulative incidence of MR4.5

- Time from onset of therapy (months)
- \(p<0.0001\)

- **BCR-ABL/ABL ≤0.57\%, n=62**
- **BCR-ABL/ABL >0.57\%, n=66**
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