Differential expression of SHP-1 in chronic myeloid leukemia


Published in:
Leukemia & lymphoma

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2014 Informa UK, Ltd.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person’s rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
Dear Author,

Please check these proofs carefully. It is the responsibility of the corresponding author to check against the original manuscript and approve or amend these proofs. A second proof is not normally provided. Informa Healthcare cannot be held responsible for uncorrected errors, even if introduced during the composition process. The journal reserves the right to charge for excessive author alterations, or for changes requested after the proofing stage has concluded.

The following queries have arisen during the editing of your manuscript and are marked in the margins of the proofs. Unless advised otherwise, submit all corrections using the CATS online correction form. Once you have added all your corrections, please ensure you press the “Submit All Corrections” button.

[AQ1] Please review the table of contributors below and confirm that first and last names are structured correctly and that the authors are listed in the order of contribution.

<table>
<thead>
<tr>
<th>Contrib. No.</th>
<th>Given name(s)</th>
<th>Surname</th>
<th>Suffix</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Jaspal</td>
<td>Kaeda</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Daniel</td>
<td>Neuman</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Simone</td>
<td>Bonecker</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ken</td>
<td>Mills</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Christian</td>
<td>Oberender</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Leila</td>
<td>Amini</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Frauke</td>
<td>Ringel</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Anna</td>
<td>Serra</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Michaela</td>
<td>Schwarz</td>
<td></td>
</tr>
<tr>
<td>Page No.</td>
<td>Query Details</td>
<td>Author Reply</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>[AQ2] Affiliations are correct? Please check and advise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>[AQ3] We have inserted a running head. Please approve or provide an alternative.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>[AQ4] Please check table as it has been re-keyed.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>[AQ5] Please provide better quality of Image for Figure1.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
LETTER TO THE EDITOR

Differential expression of SHP-1 in chronic myeloid leukemia

Jaspal Kaeda1, Daniel Neuman1, Simone Bonecker2, Ken Mills3, Christian Oberender1, Leila Amini1, Frauke Ringel1, Anna Serra4, Michaela Schwarz1, Bernd Dörken1, Ilana Zalcberg2 & Philipp le Coutre1

1Hämatologie, Onkologie und Tumorimmunologie, Medizinische Klinik m.S., Campus Virchow Klinikum, Charité, Universitätsmedizin Berlin, Berlin, Germany, 2Bone Marrow Transplant Center (CEMO), INCA, Rio de Janeiro, Brazil, 3Haematology Research Group, CCRCB, Queens University Belfast, Belfast, UK and 4Department of Clinical and Biological Sciences, University of Turin, Italy

Despite the unprecedented success of tyrosine kinase inhibitors (TKIs), the clinical management of 20–30% of patients with chronic myeloid leukemia (CML) experiencing primary or secondary resistance to imatinib mesylate (IM) continues to be challenging [1–3]. Early identification of these patients would indicate a more potent agent upfront, or alternative drug following the initial suboptimum response, or stem cell transplant (SCT) prior to the subject becoming refractory to further treatment. Therefore, a biomarker with proven clinical utility of predicting patients’ response to IM would assist considerably in optimizing clinical management for such patients. Recently, investigators reported that Srchomology 2 domain-containing phosphatase-1 (SHP-1) expression levels at diagnosis were prognostic and predictive of TKI response in patients with CML [4]. Previously, others suggested that down-regulation of SHP-1 contributes to constitutive activation of Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling and disrupts protein phosphatase 2A (PP2A) mediated BCR-ABL11 elimination, thereby triggering CML transformation [5].

Therefore, we retrospectively studied 97 cDNA samples from patients with highly heterogeneous CML to assess the clinical utility of measuring SHP-1 mRNA levels in patients with CML (Table I). The samples were collected at various time points, reflected by the overlap in BCR-ABL1 transcript numbers for those who achieved a major molecular response (MMR) and those who did not (Table I). Of the 97 patients, 24 had advanced disease (AD), i.e. accelerated phase (AP) n = 6 and blast crisis (BC) n = 18, and 73 patients were in highly heterogeneous chronic phase (CP) treated with different modalities. For 35 of the 73 patients in CP the MMR status was available for assessing the clinical utility of SHP-1 levels. Among the 24 patients in AD, at least five archived serial mRNA samples were available for each of the five patients for longitudinal studies. Of these five patients, four had been treated with one or more TKIs and one had undergone allogeneic SCT. We also included a cohort control of 77 diagnostic samples from a group of patients with heterogeneous acute myeloid leukemia (AML) and 18 normal control samples from adult volunteer blood donors, whose characteristics are detailed in Table I.

SHP-1, BCR-ABL1 and endogenous control gene, GUSβ, transcripts were quantified by real-time polymerase chain reaction (Q-PCR) as previously reported [6]. Standard curves were constructed for each assay using serial log dilutions of plasmid, ranging from 1×10⁶ to 1×10⁵, with target gene specific insert. BCR-ABL1 and GUSβ target sequences were included in one plasmid and the other included the SHP-1 insert (a kind gift from Professor F. Pane, Naples, Italy). Only those samples with ≥ 5500 GUSβ transcripts were evaluated for this report. Non-parametric Mann–Whitney tests were performed using PRISM software.

Briefly, 38 of the 73 patients in CP were prescribed single agents: (interferon and cytarabine [n = 1]), IM (n = 30), nilotinib (n = 6) or dasatinib (n = 1). The remainder were treated with two or more agents, as were the 24 patients with AD. SHP-1 mRNA was detectable in all samples screened by Q-PCR (Table I). However, a significant differential in mRNA expression (p < 0.0001) was observed between patients in CP and the normal control group. Furthermore, the SHP-1 transcripts were significantly lower (p = 0.0001) in patients with AD, with a median of 14.0 (range 0.8–211.9), in comparison to patients in CP, median 35.7 (range 5.2–675.1). Similarly, we observed a significant difference between patients with CML with AD and normal control samples (p < 0.0001). However, we observed no significant difference in SHP-1 levels between AML and normal control samples (p = 0.801). This is probably explained by the molecular heterogeneity among the patients with AML, in contrast to the single genetic lesion associated with CML, and that SHP-1 is reported to bind to BCR-ABL1.

In contrast to published data [4] we found no significant difference (p = 0.0966) between patients who failed to achieve a MMR within 18 months (n = 22) and those...
patients who did ($n = 13$). To exclude the possibility that the statistical value might have been influenced by either the highly variable collection time-points or the diverse therapeutic agents administered, a restricted analysis of 15 patients treated with IM alone and for whom we had samples collected at diagnosis was performed. Even within this group we found no significant difference ($p = 0.4527$), i.e. between those who did ($n = 6$) and failed to ($n = 9$) achieve MMR within 12 months. This did not change even when the criterion was extended to 18 months. This variance from published data may reflect differences in the timing of sample collection during the course of treatment in this study and that reported by Esposito et al. [4]. However, these data do not exclude the possibility that assessing SHP-1 activity at the protein level would be predictive. Nevertheless, protein analysis is too complex for a clinical laboratory to perform, in contrast to Q-PCR analysis, and therefore not within the scope of this assessment.

In addition we noted no significant difference in SHP-1 mRNA levels between those patients in CP who had been prescribed one ($n = 37$), two ($n = 7$) or $\geq 3$ TKIs ($n = 8$), which generally correlated with optimal, suboptimal and/or failed response.

The kinetics data were consistent with overall CP and AD results, showing that SHP-1 levels decreased as the BCR-ABL1 transcript numbers increased, i.e. an inverse relationship (Figure 1), implying that regulatory control of the two is directly or indirectly linked. We did note that for patient 4, including the period when the subject was in CP (Figure 1), this relationship was not observed. However, there was no difference of note in this patient's clinical history compared to the other four subjects. More importantly, BCR-ABL1 transcripts in these five patients were not preceded by a decrease in SHP-1.

Given the relatively low levels of SHP-1 in comparison to BCR-ABL1 expression, we confirmed that our assay could...
reproducibly detect a five-fold change in SHP-1 mRNA levels by titrating, in duplicate, the SU-DHL-1 cell line with the LAMA-87 hematopoietic cell line. This is consistent with the generally accepted view that Q-PCR assays have a dynamic range of 5-log, although up to an 8-log range is achievable.

Therefore, the kinetics and MMR data suggest that measuring the SHP-1 mRNA level does not provide additional information for identifying patients at risk of disease progression or predicting response to TKIs beyond that gleaned from close regular monitoring by measurement of disease-specific BCR-ABL1 transcripts. However, the differential expression of SHP-1 between CP and AD observed in this study was consistent with earlier reports suggesting that phosphatase antagonizes BCR-ABL1 ability to block differentiation [7,8]. Reduced expression of SHP-1 might free BCR-ABL1 to recruit and activate JAK2. Active JAK2 has been reported to enhance β-catenin activity and inactivate PP2A mediated degradation of BCR-ABL1, thus triggering BC [9].

In conclusion, our data imply that SHP-1 levels fail to predict TKI response. However, in keeping with previous reports, our data provide further evidence to support the notion that SHP-1 plays a role in CML disease progression.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

References