Differential expression of SHP-1 in chronic myeloid leukemia

Kaeda, J., Neuman, D., Bonecker, S., Mills, K., Oberender, C., Amini, L., ... Coutre, P. L. (2015). Differential expression of SHP-1 in chronic myeloid leukemia. Leukemia & lymphoma, 56(5), 1547-1549. DOI: 10.3109/10428194.2014.969258

Published in:
Leukemia & lymphoma

Document Version:
Peer reviewed version

AUTHOR QUERY SHEET

Author(s): Kaeda, Neuman, Bonecker, Mills, Oberender, Amini, Ringel, Serra, Schwarz, Dörken, Zalcberg, le Coutre

Article title: Differential expression of SHP-1 in chronic myeloid leukaemia

Article no: 969258

Enclosures: 1) Query Sheet

3) Article Proofs

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.

Contrib. No.	Given name(s)	Surname	Suffix
1	Jaspal	Kaeda	
1	Daniel	Neuman	
2	Simone	Bonecker	
3	Ken	Mills	
1	Christian	Oberender	
1	Leila	Amini	
1	Frauke	Ringel	
4	Anna	Serra	
1	Michaela	Schwarz	

1	Bernd	Dörken	
2	Ilana	Zalcberg	
1	Philipp	le Coutre	

Page No.	Query Details	Author Reply
1	**[AQ2]** Affiliations are correct? Please check and advise	
3	**[AQ3]** We have inserted a running head. Please approve or provide an alternative.	
2	**[AQ4]** Please check table as it has been re-keyed.	
2	**[AQ5]** Please provide better quality of Image for Figure1.	

Leukemia & Lymphoma, 2014; Early Online: 1–3

ISSN: 1042-8194 print / 1029-2403 online
DOI: 10.3109/10428194.2014.969258

informa
healthcare

LETTER TO THE EDITOR

Differential expression of *SHP-1* in chronic myeloid leukemia

[AQ1] Jaspal Kaeda[1], Daniel Neuman[1], Simone Bonecker[2], Ken Mills[3], Christian Oberender[1], Leila Amini[1], Frauke Ringel[1], Anna Serra[4], Michaela Schwarz[1], Bernd Dörken[1], Ilana Zalcberg[2] & Philipp le Coutre[1]

[AQ2] [1]*Hämatologie, Onkologie und Tumorimmunologie, Medizinische Klinik m.S., Campus Virchow Klinikum, Charité, Universitätsmedizin Berlin, Berlin, Germany,* [2]*Bone Marrow Transplant Center (CEMO), INCA, Rio de Janeiro, Brazil,* [3]*Haematology Research Group, CCRCB, Queens University Belfast, Belfast, UK and* [4]*Department 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 Src homology 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 were 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 1×10^3 to 1×10^6, 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

Correspondence: Jaspal Kaeda, PhD, FRCPath, Medizinische Klinik m.S. Hämatologie und Onkologie, Charité Campus Virchow Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany. Tel: + 49(0)30-450-559438. Fax: + 49(0)30-450-559929. E-mail: jaspal.kaeda@charite.de

Received 2 June 2014; revised 15 September 2014; accepted 20 September 2014

Table I. Summary of sample groups.

Subjects*	*n*	Sex, M/F	Age (years) (median)	*BCR–ABL/GUSβ* (median)	*SHP-1/GUSβ* (median)
Normal controls	18	8/10	35–61 (44)	—	1.40–6.36 (3.66)
AML	77	42/35	8–85 (63)	—	0.56–13.29 (3.50)
CML: CP	73	44/29	19–75 (63)	0–1053 (18.38)	5.18–675.1 (35.69)
CML: AD†	24	16/8	34–74 (61)	0.40–1947 (182.7)	0.82–211.9 (14.0)
CML: MMR	13	8/5	20–66 (52)	0.24–140.30 (7.30)	15.46–318.9 (35.69)
CML: f-MMR	22	17/5	19–72 (32)	0.0–197.60 (62.33)	6.31–162.1 (26.72)

AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CP, chronic phase; AD, advanced disease; MMR, major molecular response; f-MMR, failed MMR.
*From among the total 97 patients with CML, 13 were classified as having achieved MMR and 22 did not.
†Six accelerated phase, 18 blast crisis.

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 ≥ 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

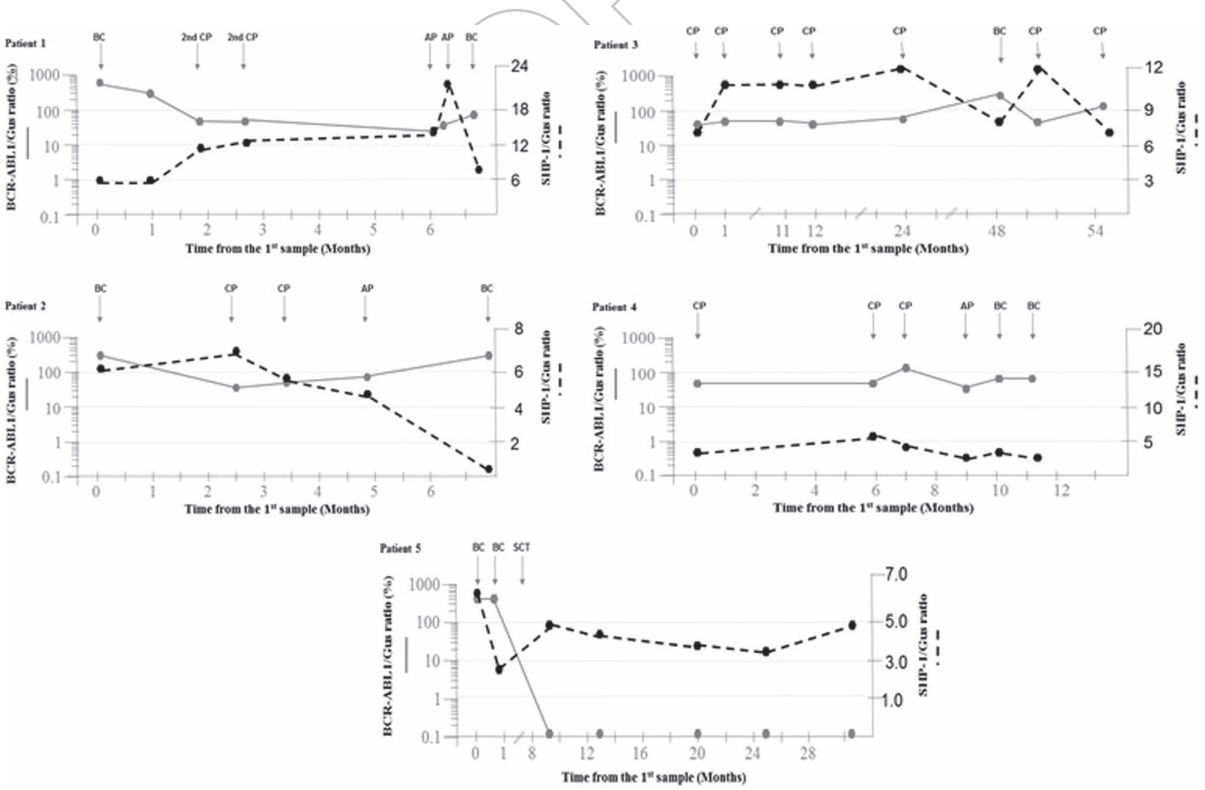

Figure 1. *SHP-1* and *BCR–ABL1* kinetics. Kinetics data for *SHP-1* (dashed lines) and *BCR–ABL1* (solid lines) are shown for five patients with CML included in the longitudinal study. *Y*-axis for *SHP-1* levels is on the right of each graph. *SHP-1* mRNA was detected in all samples tested for the five patients and reflected the *BCR–ABL1* kinetics. *SHP-1* levels did not predict a change in patients' disease status, such that an increase or decrease in its expression did not precede a change in *BCR–ABL1* transcript levels. Therefore, we conclude that its predictive value is not superior to that of the disease specific marker, *BCR–ABL1*.

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

[1] Hughes TP, Kaeda J, Branford S, et al. International Randomised Study of Interferon versus STI571 (IRIS) Study Group. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med 2003;349:1423–1432.

[2] Jabbour EJ, Cortes JE, Kantarjian HM. Resistance to tyrosine kinase inhibition therapy for chronic myelogenous leukemia: a clinical perspective and emerging treatment options. Clin Lymphoma Myeloma Leuk 2013;13:515–529.

[3] Crews LA, Jamieson CH. Chronic myeloid leukemic stem cell biology. Curr Haematol Malign Rep 2012;7:125–132.

[4] Esposito N, Colavita I, Quintarelli C, et al. SHP-1 expression accounts for resistance to imatinib treatment in Philadelphia chromosome-positive cells derived from patients with chronic myeloid leukemia. Blood 2011;118:3634–3644.

[5] Neviani P, Santhanam R, Trotta R, et al. The tumour suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL regulated SET protein. Cancer Cell 2005;8:355–368.

[6] Kaeda J, O'Shea D, Szydlo RM, et al. Serial measurement of BCR-ABL transcripts in the peripheral blood after allogeneic stem cell transplant for chronic myeloid leukemia. An attempt to define patients who may not require further therapy. Blood 2006;107:4171–4176.

[7] Bruecher-Encke B, Griffin JD, Neel BG, et al. Role of the tyrosine phosphatase SHP-1 in K562 cell differentiation. Leukemia 2001;15:1424–1432.

[8] Amin HM, Hoshino K, Yang H, et al. Decreased expression level of SH2 domain-containing protein tyrosine phosphatase-1 (Shp1) is associated with progression of chronic myeloid leukemia. J Pathol 2007;212:402–410.

[9] Neviani P, Harb JG, Oaks JJ, et al. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. J Clin Invest 2013;123:4144–4157.