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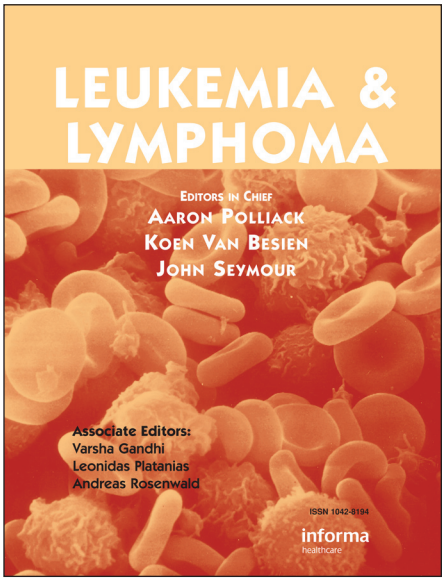

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Letter to the Editor

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

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Despite the unprecedented success of tyrosine kinase inhibitors (TKI), clinical management of 20-30% of chronic myeloid leukemia (CML) patients 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 initial sub-optimum response or 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 Src homology 2 domain-containing phosphates-1 (*SHP-1*) expression levels at diagnosis were prognostic and predictive of TKI response in CML patients [4]. Previously others suggested down regulation of *SHP-1* contributes to constitutive activation of Jak/Stat signaling and disrupts protein phosphatase 2A (PP2A) mediated *BCR-ABL11* elimination thereby triggering CML transformation [5] (Neviani *et al,* 2005).

Therefore we retrospectively studied 97 cDNA samples from highly heterogenous CML patients to assess the clinical utility of measuring *SHP-1* mRNA levels in CML patients (Table I). The samples were collected at various time points, reflected by the overlap in *BCR-ABL1* transcript numbers for those who achieved major molecular response and those who did not (Table I). Of the 97 patients 24 were in advanced disease phase (AD), i.e. accelerated phase (AP); n=6 and blast crisis (BC); n=18 and 73 highly heterogenous patients in CP treated with different modalities. For 35 of the 73 CP patients the major molecular responses (MMR) status was available for assessing the clinical utility *SHP-1* levels. From among the 24 patients in AD at least 5 archived serial mRNA samples were available for each of the 5 patients for longitudinal studies. Of these 5 patients four had been treated with one or more tyrosine kinase inhibitors (TKI) and one had allogenic stem cells transplant (SCT). We also included a cohort control of 77 diagnostic samples from heterogeneous group of AML patients and 18 normal controls samples from adult volunteer blood donors' characteristics are detailed in Table I.

SHP1, *BCR-ABL1* and endogenous control gene, *GUSβ* transcripts were quantified by real time polymerase chain reaction as previously reported [6]. Standard curves, were constructed for each assay using serial log dilutions of plasmid, ranging from $1x10^3$ to $1x10^6$, with target gene specific insert. *BCR-ABL1* and *GUS* target sequences were included in one

plasmid and the other included the *SHP-1* insert (*kind gift from Professor F. Pane, Naples, Italy*). Only those samples with ≥5500 *GUSβ* transcripts were evaluated for this report. Non-parametric Mann Whitney were performed using PRISM software.

Briefly, 38 of the 73 CP patients were prescribed single agents; interferon and cytarabine (n=1), Imatinib (n=30); nilotinib (n=6) and dasatinib (n=1). The remainder were treated with 2 or more agents, as were the 24 AD patients. *SHP-1* mRNA was detectable in all the samples screened by Q-PCR (Table I). However, 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 AD patients with median of 14.0 (range 0.8 to 211.9), in comparison to patients in CP, median 35.7 (range: 5.2-675.1). Similarly, we observed a significant difference between CML patients in AD and normal control samples, $p<0.0001$. But we observed no significant difference in *SHP-1* levels between AML and NC samples ($p=0.801$). This is probably explained by molecular heterogeneity among the AML patients in contrast to the single genetic lesion associated with CML and SHP-1 is reported to bind to BCR-ABL1.

In contrast to published data [4] we found no significant difference, $p=0.0966$, between the patients who failed to achieve 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. not significant between those who did (n=6) and failed (n=9) achieve MMR within 12 months. This did not change even if the criterion was extended to 18 months. This variance from published data may reflect the differences in timing of the sample collection during course of the treatment in this study and that reported by Esposito *et al* [4]. But these data to not exclude the possibility that assessing SHP-1 activity at protein level would be predictive. But protein analysis are 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 1 (n=37), 2 (n=7), or ≥ 3 TKI (n=8), which generally correlates with optimal, sub-optimal and/or failed response.

The kinetics data was consistent with overall CP and AD results, showing *SHP-1* levels decrease as the *BCR-ABL1* transcript numbers increased, i.e. an inverse relationship (Figure 1), implying regulatory control of 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 4 subjects. More importantly, *BCR-ABL1* transcripts in these 5 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 our assay could reproducibly detect a 5 fold change in SHP-1 mRNA levels by titrating, in duplicate, SU-DHL-1 cell line with LAMA-87 haematopoietic cell line. Consistent with the generally accepted view that Q-PCR assays have a dynamic range of 5 logs, although up to 8 logs range is achievable.

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

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

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Figure Legends

Fig. 1. *SHP-1* and *BCR-ABL1* kinetics. The Kinetics data for *SHP-1* (dashed lines) and *BCR-ABL1* (solid lines) are shown for the 5 CML patients included in the longitudinal study. The Y axis for the *SHP-1* levels are on the right of each graph. *SHP-1* mRNA was detected in all samples tested for the 5 patients and reflected the *BCR-ABL1* kinetics. *SHP-1* levels did not predict a change in the patients' disease status, such that an increase or decrease in its expression did not precede a change in *BCR-ABL1* transcript levels. Therefore, we concluded its predictive value was not superior to that of disease specific marker, *BCR-ABL1*.

Fig. 1

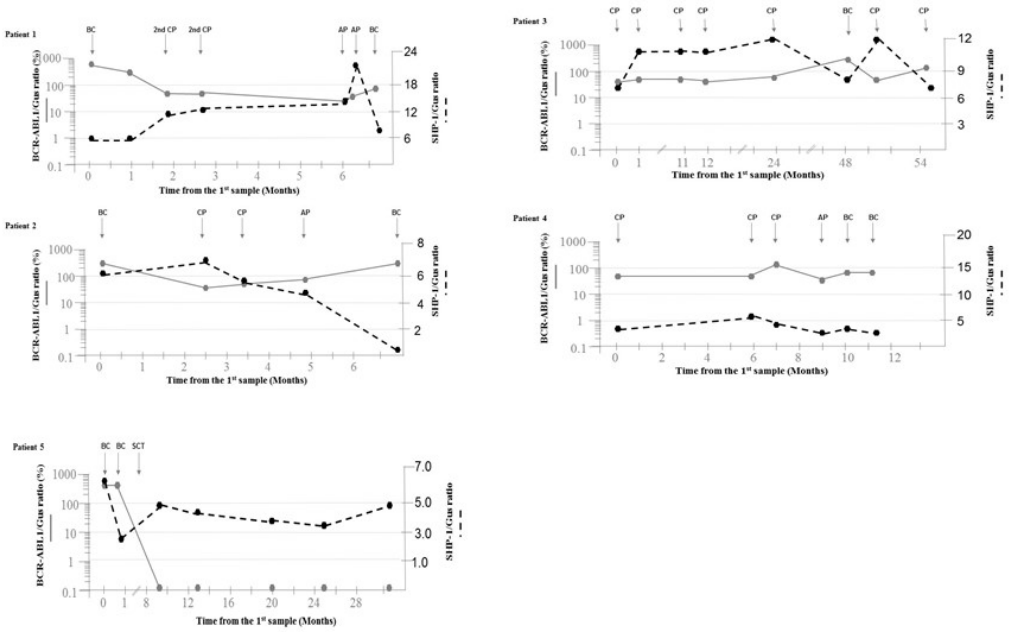

Table I. Summary of the sample groups

Subjects	n	Sex M/F	Age (years) (median)	BCR-ABL/GUSß(%) (median)	SHP-1 /GUSß (median)
Normal control	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	34-75 (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)

From among the total 97 CML patients 13 were classified as having achieved MMR and 22 who did not. CML: Chronic myeloid leukaemia; CP: Chronic phase ; AD : advanced disease ; *: 6 accelerated phase ; 18 BC; MMR: Major molecular response; f-MMR failed major molecular response; AML: Acute myeloid leukaemia.