Author's response to reviews

Title: Enhancing SHP-1 expression by 5-Azacytidine may inhibit STAT3 activation and confer sensitivity in Lestaurtinib (CEP-701) resistant FLT3-ITD positive Acute Myeloid Leukemia

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Author's response to reviews: see over
Reviewer: Prof Friedrich Stölzel

Thank you for your valuable comments and please refer to the following:

1- Microarray part in statistical analysis has been deleted

3-

b- Figure 1-A not only edited but also the other concentrations have been added. Please note that:

- The concentrations are presented as 0, 100, 200, 300…..1000 and 2000 nM for all cell lines.
- The concentrations 3000, 4000 and 5000 nM were applied only for resistant cells.

However, in chart area, the axis was presented as: 0, 500, 1000, 1500…..5000 to avoid overlap values.

c-The resistant cell lines only inhibited at higher (more than 10 times higher) concentration of CEP-701 compared to that on parental as well as 5-Aza treated cells and the highest dose that was applied for resistant cells was 5000 nM at which 30% of resistant cells still alive.

d-We interested to look for the sensitivity of resistant cells to CEP-701 before and after treatment with 5-Aza. However, the parental cells were used for verification of the acquired resistance in resistant cells.

4- Although the conclusion has been edited, here are some reports that support the roles of SHP-1 expression and STAT3 inactivation on resistance and sensitivity response towards tyrosine kinase inhibitors as the following:

Depending on experiment performed by Esposito et al, 2011 [51], CML cells resistant to imatinib (KCL22-R) was transfected with human full-length SHP-1 sequence (NCBI NM_002831.4). The SHP-1 sequence in p-IRES2-eGFP retroviral expression vector (p-IRES-SHP1-neo) was transfected the KCL22-R cell line with either p-IRES-SHP1-neo (KCL22-RSHP-1+) or the empty p-IRES-neo vector (KCL22-RControl). They found that, SHP-1 expression in transfected KCL22-R induces a significant reduction in BCR-ABL protein. Consequently they have concluded that, SHP-1 expression could be a new biological indicator at baseline of imatinib sensitivity in CML patients.

Additionally, the activation of STAT3 is an important mechanism of resistance to imatinib (Bewry et al., 2008) [27]. Moreover, Han et al., 2006 concluded that,
restoration of \textit{SHP-1} expression by 5-Aza correlates with a significant down-regulation of the JAK3/STAT3 signaling in both ALK\textsuperscript{+} ALCL cells [25]. Furthermore, it was reported that, overexpression of \textit{SHP-1} (PTPN6) is able to completely dephosphorylate STAT3 in diffuse large B cell lymphoma \cite{Witzig2014} [55].

\textbf{Editor's comments:}

Dear Dr Magdalena Chechlinska

Thank you very much for your valuable comments and please be informed that we have revised according to your recommend and please refer to the following:

1- Microarray part has been deleted.

2- The legend of Figure 2 has been edited accordingly.

3- Same as that of reviewer 3-d [our interest was to look for the sensitivity of resistant cells to CEP-701 before and after treatment with 5-Aza. However, the parental cells were used for verification of the acquired resistance in resistant cells. So we didn’t treat parental cells with 5-Aza].

4- We interested to look for the expression of \textit{SHP-1}, \textit{SOCS-1}, \textit{SOCS-3}, \textit{STAT5a} and \textit{JAK2} in resistant cells before and after treatment with 5-Aza. However, the results showed that, after treatment of the resistant cells with 5-Aza only \textit{SHP-1} was re-expressed. Therefore, methylation profile using MS-PCR followed by pyrosequencing analysis was studied for \textit{SHP-1} gene only.

5- Whole manuscript has been revised.

Thank you very much.