Author's response to reviews

Title: Enhancing SHP-1 expression with 5-azacytidine may inhibit STAT3 inactivation 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
Dear Editor of BioMed Central Journal

Thank you very much for valuable review and please have a look to the following statements that have been edited. Please note that, the statements after the line numbering (highlighted with yellow) indicate the original statement and the below of each are the edited statements which highlighted with red color. Please also note that, those statement which only edited with "comma" of "the" are underlined and highlighted only the word beside was the editing.

Thank you very much

36 The aim of this study, therefore, was to assess the effect of

Therefore, the aim of this study, was to assess the effect of

45-46 The cytotoxic dose of CEP-701 on resistant cells was significantly higher than parental and MV4-11R-cep+5-Aza cells

The cytotoxic dose of CEP-701 on resistant cells was significantly higher in comparison with parental and MV4-11R-cep+5-Aza cells

46-47 The resistant cells showed significant higher viability and lower levels of apoptosis compared with other cells

The resistant cells showed a significant higher viability and lower apoptosis compared with other cells

53-55 Our findings support the hypothesis that the tumor-promoting effect of SHP-1 loss is reversible and its re-expression might play an important role in re-inducing sensitivity to TKIs

Our findings support the hypothesis that, the tumor-suppressor effect of SHP-1 is lost due to epigenetic silencing and its re-expression might play an important role in re-inducing sensitivity to TKIs

82 The loss of its suppressor function results in
The loss of *SHP-1* suppressor function results in de-methylation.

Resistance to imatinib in chronic myeloid leukemia is conferred by the activation of STAT3 signaling, and the sensitivity is restored by STAT3 inactivation.

We hypothesized that JAK/STAT negative regulators may lose their tumor suppression function in TKI-resistant AML cells due to epigenetic silencing, and that re-expression of these genes could re-induce sensitivity to CEP-701.

Upon incubation of cells in the presence of 300 nM CEP-701, a significant reduction of cell viability of 90% (down to 37) and 33% was detected.

This showed significant up-regulation of *SHP-1* in...
These findings suggest that the up-regulated

The findings suggest that activated STAT3 could be involved in the acquisition of resistance to CEP-701 in MV4-11R-cep cells, which is consistent with previous reports in which the activation of STAT3 was associated

We also found that increasing CEP-701 concentration caused a significantly greater increase of apoptosis in 5-Aza-treated resistant cells than in the untreated resistant cells

Taken together, our results indicate that re-expression

Our data suggest a crucial role for STAT3 in the development of resistance to TKIs and support inhibition of STAT3 phosphorylation as an effective means of re-inducing sensitivity.

We also found that increasing CEP-701 concentration caused a significant increase of apoptosis in 5-Aza-treated resistant cells compared with the untreated resistant cells.
We thank all staffs.

A significantly higher level of methylation was observed in A significant higher level of methylation was observed in.

In contrast, the increase in apoptosis in the resistant cells without 5-Aza exposure was only 10% at 300 nM PKC-412 (p<0.001).

In contrast, the increase of apoptosis in the resistant cells was only 21% at 300 nM PKC-412 (p<0.001).

A, B, E, and F indicate other cell lines not included in this study and are in agreement to the findings of this study.

However, STAT1 and STAT5 showed no phosphorylation in all cells; a, b, c, d, e and f indicate other cell lines not included in the present study but they are in agreement with the findings of this study.

Apoptotic cells increased significantly in (A) MV4-11 and (C) MV4-11R-cep+5-Aza cells compared with (B) MV4-11R-cep cells with increasing concentrations of

Apoptotic cells increased significantly in (A) MV4-11 and (C) MV4-11R-cep+5-Aza cells compared with (B) MV4-11R-cep cells by increasing concentrations of

in MV4-11 and MV4-11R-cep+5-Aza cells, respectively, in association with 58 and 65% apoptotic cells, respectively.

in MV4-11 and MV4-11R-cep+5-Aza cells, in association with 58 and 65% apoptotic cells, respectively.
vitality induced by CEP-701 was concentration dependent and was greater in MV4-11R-cep compared with MV4-11 and MV4-11R-cep+5-Aza cells.

vitality induced by CEP-701 was concentration dependent and was greater in MV4-11 and MV4-11R-cep+5-Aza cells compared with MV4-11R-cep.

C) Pyrosequencing analysis revealed low methylation of SHP-1 in MV4-11R-cep+5-Aza cells.

C) Pyrosequencing analysis revealed low methylation levels of the CpG islands in the promoter region of SHP-1 in MV4-11R-cep+5-Aza cells.

The box blot showed a significant lower (p=0.023) methylation in CpG islands of SHP-1 gene in MV4-11R-cep+5-Aza cells compared with that in MV4-11 and MV4-11R-cep cells.

The box blot showed a significant lower (p=0.023) of methylation in the CpG islands of SHP-1 gene in MV4-11R-cep+5-Aza cells compared with that in MV4-11 and MV4-11R-cep cells.

Lastly, labels for the figures have been edited as the followings:

-Figure 1-B; MV4-11-R and MV4-11-R+5-Aza changed to MV4-11R-cep and MV4-11R-cep+5-Aza, respectively.

-Figure 1-C [the capital letters ; A, B, C, D, E and F have been changed to small letters a,b,……to avoid confusion with the original Figure 1-A,………

-Figure 2 the labeling A, B and C have been edited to be A: MV-4-11, B: MV4-11R-cep and C: MV4-11Rcep+5-Aza