Author's response to reviews

Title: Inhibition of STAT3-Interacting Protein 1 (STATIP1) promotes STAT3 transcriptional up-regulation and imatinib mesylate resistance in the chronic myeloid leukemia

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Author's response to reviews: see over
Dear editor,

We had previously submitted this manuscript to BMC Cancer Journal. However, as we considered reviewer’s comments pertinent and moreover, they pointed out that our manuscript needed a more specific focus, we decided to perform several additional experiments connecting STATIP1 and STAT3 expression with Imatinib resistance in chronic myeloid leukemia (CML). For this, we conducted real time PCR, apoptosis and cell cycle experiments in K562 and Lucena cells (Lucena is a multidrug resistance cell line also resistant to imatinib). Moreover, we added in vivo analyses by evaluating STATIP1 and STAT3 mRNA levels in CML patients’ samples with different response to Imatinib.

Below is the Reviewer’s comments and actual answers:

Reviewer’s report
Title: Inhibition of STAT3-Interacting Protein 1 (STATIP1) restores STAT3 transcriptional activation and promotes drug resistance in chronic myeloid leukemia K562 cell line
Answer: The manuscript’s title was modified because we performed new experiments using additional CML cell line and patients samples.

The manuscript “Inhibition of STAT3-Interacting Protein 1 (STATIP1) restores STAT3 transcriptional activation and promotes drug resistance in chronic myeloid leukemia K562 cell line” describes the role of STATIP1 in regulating STAT3 in K562 cell line. In general, this paper is interesting, however, several problems regarding the reported findings should be taken into consideration:

Major Compulsory Revisions
1. Is the observed phenomenon explored in any other Bcr-Abl positive cell lines besides K562?
Answer 1: In accordance to reviewer’s suggestion, we explored another CML cell line, so called Lucena. Lucena presents the Multidrug Resistance phenotype by overexpressing ABCB1/Pgp drug transporter and it is also resistant to Imatinib (Correa et al, Proteome Science, 2012). In this actual revised manuscript file, several analyses were conducted with K562 and Lucena cells, such as apoptosis activation, cell cycle, and real time PCR. Moreover, in order to strengthen our findings, we also analyzed CML patient’s samples with different response to Imatinib.

2. The Trypan Blue exclusion method used to determine cell viability was not convincing. Please provide other experiment results to further confirm the conclusions.

Answer 2: We would like to thank you for comment and for this reason we performed additional experiments using Anexin-PI labelling for apoptosis analyses on FACS instrument and colorimetric WST-1 Elisa assays were performed instead of Trypan blue exclusion.

3. The authors tested STAT3 and its target genes (BCL-XL and CCND1) expression change after STATIP1 depletion and concluded that STATIP1 was a negative regulator of STAT3. However, STATIP1 was not the only factor that influenced transcriptional activity of STAT3. As indicated in the paper, ZIPK, Pin1, INFAR-1 and EZI physically bind at STAT3 and enhance its transcriptional activity; NF-kB, p65, Grb2, Duplin and Daxx proteins down-regulate STAT3 transcriptional activation. Is there any possibility that STATIP1 depletion affects the expression of other regulators of STAT3 leading to its overexpression? Therefore, the specific mechanisms about how STATIP1 interacting with STAT3 (directly or indirectly) and influencing its transcriptional activity should be explored in the paper.

Answer 3: We would like to thank you for this observation and comment. We understand the complexity of cellular signaling pathways, specially STATs signaling. However in this paper we wanted to focus on STAT3 target genes
alteration in response to STATIP1 interference, and this relationship with CML resistance. As there are little information regarding STATIP1, we cannot (and actually do not) know the entire cross-talk signaling changes linked to STATIP1. In our manuscript we suggest a possible negative relationship between STATIP1 levels and increase of STAT3 target genes, and its association with resistance to Imatinib in CML. It is important to address that we do not exclude other proteins/pathways that could also regulate STAT1P1 and STAT3. This subject is extremely relevant for further investigation, however it is not the focus of this manuscript.

4. A paper entitled “A Stat3-interacting protein (StIP1) regulated cytokine signal transduction” (Collum et al. PNAS 2000, 97:10120-5) has demonstrated that StIP1 may regulate the activation of Stat3 as well as other STATs. The results suggest that STATIP1 tested in this paper may influence cell viability and sensitivity to imatinib through targeting other STATs, such as STAT5, which is also overexpressed in CML cell lines. The authors should provide related experiments to exclude this possibility.

Answer 4: In the original STATIP1 paper the author showed interaction with others JAK/STAT proteins, such STAT3 and, with less extent, STAT5, JAK2, and STAT1. However, we focused on the STAT3, which has been related to play an important role in CML. Due to fact of CML complexity and the potential relationship between STAT3 and CML resistance, we decided to focus on STAT3 in this paper exclusively, and used STAT3 inhibitor (LLL3) to assure we only be evaluating changes regarding STAT3 inhibition.

5. The authors concluded that decreased expression of STATIP1 could promote imatinib resistance in K562 cells. The results will be more convincing if authors provide any evidences that ectopic expression of STATIP1 in CML patient samples with imatinib resistance.

Answer 5: Thank you for suggestion. In order to test the STATIP1 relationship with Imatinib resistance phenotype, we included STATIP1 and STAT3 evaluation
in Lucena, a known Imatinib resistant CML cell line, and also evaluated both genes in Imatinib responsive and resistant CML patients' samples.

6. The authors should verify the effects of LLL-3, a specific inhibitor of STAT3, on K562 cells with STATIP1 depletion.

Answer: Thank to reviewer for this consideration, in this new submission we performed additional experiments as reviewer's suggestion. The STAT3 inhibition by LLL3 treatment in a STATIP1 depleted K562 cell showed a diminishing of loss of viability. As observed in others results, we suggesting this resistance due to increase of STAT3 levels.

Minor Essential Revisions

• There are many spelling faults in this paper.

Answer: Thank you for observation, we careful revised and performed English International Editor for the corrections.

• The references are not in BMC Cancer style.

Answer: Apologize for the mistake, we revised the bibliography and edited this new version according BMC Cancer style.

Overall, this paper provides limited information concerning how STATIP1 modulate STAT3 transcriptional activity.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.