Reviewer's report

**Title:** Overcoming Bcr-Abl T315I mutation by combination of GNF-2 and ATP competitors in an Abl-independent mechanism.

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**Reviewer:** Paolo Neviani

**Reviewer's report:**

This manuscript has some strong and original points; in particular the authors use a novel approach in targeting CML cells in a BCR/ABL kinase independent manner, with the goal of overcoming resistance to the current tyrosine kinase inhibitors. The concept of using Abl allosteric kinase inhibitors is promising and surely needs to be further developed. In this report the authors show how the combination of such an inhibitor, GNF-2, and current first and second generation Abl tyrosin kinase may potentiate the effect of the latter in inhibiting BCR-ABL, particularly the form bearing the T315I mutation. Unfortunately, while the data presented support their claim to some extent, the lack of sound statistical analysis (no p-values anywhere), the lack of densitometric analysis (in some western blots it is difficult to truly assess this “cooperation” effect), and, most importantly, the lack of data on parental non-BCR-ABL+ cells make it difficult to extrapolate whether the combination of such compounds may be truly beneficial and clinically translatable. Also the quality of some of the figures is really poor as far as details and resolution.

**Major Compulsory Revisions:**

1. The experiments in Fig 2 and Fig 5, should be repeated with parental Baf3 cells and other BCR-ABL negative cells. In the 2006 paper Adrian et al in Nature Chemical Biology, the cytotoxicity of GNF-2 was tested on a variety of parental cell and BCR-ABL negative cell lines, there they didn’t see any effect for GNF-2 concentrations up to 10uM. Here the authors show that GNF-2 impairs the clonogenic potential of BaF3-p185-T315I cells with an IC50 of 25uM and that the combination with TKIs reduces it to values above or around 10uM (except the combination with 1uM Dasatinib). It is therefore of great importance to determine whether these concentrations may be toxic on untransformed cells and whether a therapeutic window can be achieved.

2. Throughout the manuscript the statistical analysis is missing. Experiments should be complemented by statistical analysis and p-values. For example fig 1A-B: it is not clear if the results shown are representative of one experiment or rather the average of the three experiments; in this case they should plot the SEM rather than the SD. This should be made clear for every experiment in the manuscript.

3. Fig3D. There doesn’t seem to be cooperation between GNF-2 and Imatinib and only marginally with Nilotinib. All of the panels in figure 3 should be
quantitated by densitometric analysis, some changes in band intensity are hard to grasp, especially given that the authors want to demonstrate synergism/addition between compounds.

4. Is the cooperation between the GNF-2 and TKIs synergistic or additive? This needs to be evaluated.

Minor Essential Revisions
1. the Abl kinase inhibitors are usually referred in the literature as tyrosin kinase inhibitors; I suggest changing AKI to TKI for consistency.
2. The panels in figure 1 are not referenced in the correct order in the text.
3. Fig 1C is not described in the text.
4. Fig 1C and 1D should include untreated control.
5. Fig 1D: Imatinib is reducing the number of viable cells, not according to the text. Is it a typo?
6. Figures should be improved; they should state clearly what kind of cells are being used for each experiment; since different cell lines are used within the same figures this would make the manuscript easier to follow. The resolution of fig 2 and 5 is also unacceptable.
7. Fig 1A is missing an error bar.
8. A figure 6 is referenced in the manuscript but there is not such a figure or legend included.

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

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests