Author's response to reviews

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

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Comments to reviewers:

First reviewers:

Point 1 and 3: The working concentrations for AKIs were chosen according to our previous experiments that identified a working concentration of each AKI that showed minimal effect when used alone using cells carrying the native Bcr-Abl construct. In experiments shown in Figure 1, we identified concentration of 0.2 µM as suboptimal concentration that showed minimal effect of Imatinib on proliferation inhibition of Ba/F3 cells carrying native p185 Bcr-Abl. However, with Dasatinib that drug concentration was identified as 2 nM. Since all AKIs are un-effective in inhibiting proliferation of Ba/F3 carrying mutated T315I Bcr-Abl, a concentration of 1µM was chosen for all treatments and in all cases inhibition observed by AKIs only was between 10-15% inhibitions of proliferation.

Point 2. The mistake in Figure 1D was corrected. % of viable Ba/F3 p185 Bcr-Abl T315I cells in the presence of 1µM Imatinib is 87.04 +/- 4.5. Exposure time was 72 hrs and the legends were corrected accordingly to include exposure time. Errors bars and P values were added to Figure 1B of the Dasatinib column.

Point 4: Figure 2 examining the effect of GNF-2 on clonogenicity of the Ba/F3 p185 Bcr-Abl T315I cells in the presence of AKIs. As expected the AKIs alone at 0.1µM or 1µM exhibited minimal activity on clonogenicity of the T315I mutated cells. AKIs exhibited different potency toward cells carrying the native Bcr-Abl, however they exhibited similar low activity against the T315I mutated d cells.

Figure 5: There is no Figure 6. Corrections were made.

Second Reviewer:

Point 1: Experiments in Figure 2 and Figure 5 utilizes colony forming assay to examine effect of GNF-2 and AKIs in Bcr-Abl and JAK2 transformed Ba/F3 clonogenicity. The parental Ba/F3 cells cannot be used in the colony forming assay because they do not form colonies. After performing the experiment shown in Figure 2, we asked whether the observed effect is selective for Bcr-Abl and thus the rational for using JAK2 transformed Ba/F3 cells. Our data argue the observed effect is mediated in Bcr-Abl dependent (in Bcr-Abl native condition) as well as by Bcr-Abl independent mechanisms (mainly in Bcr-Abl mutated forms and probably in Bcr-Abl negative cells). In this study, we identify JAK2 as a target of GNF-2/AKIs action, however, we believed that other un-identified kinases might be the target of GNF-2/AKIs since MBP is found in many kinases (manuscript in preparation) and thus they might be a target for its action. This makes it difficult to select an appropriate control.

Point 2: In Figure 1 and others we added statistical analysis, we added p values in Figure 1 and we used SD since the experiment shown is a representative experiment and not the average of 3 experiments. However, the other 2 experiments showed comparable outcome.
**Point 3:** Quantitation of experiments in Figure 3 and Figure 4 was carried out and relative amounts of pBcr-Abl and pSTAT5 were shown.

**Point 4:** In this study we did not go into details to determine the nature of the cooperation between GNF-2 and AKIs. However, our follow-up manuscript that also under evaluation in the Journal BMC Cancer (Mian et al., 2012, Allosteric inhibition enhances the efficacy of ABL kinase inhibitors to target unmutated BCR-ABL and BCR-ABL-T315I) performed in depth analysis using three dimensional model of Prichard and Shipman (1990) and MacSynergy software. The conclusion is a synergistic effect exists between Dasatinib and GNF-2. Regarding Imatinib and Nilotinib, it seems to be cell line –dependents.

Minor essential revisions:

Point 1: We intentionally used the acronym AKIs to refer to Abl kinase inhibitor and not using the general term of TKI (tyrosine kinase inhibitors) since our study was limited to Abl kinase and its inhibitors.

Point 2: The text was corrected and referred to Figure 1 in the correct order.

Point 3: Description of Figure 1C was added to the text.

Point 5: Mistake in Figure 1D (number of viable cells following Imatinib treatment) was corrected.

Point 6: The name of each cell used was added on top of each figure. The different parts of Figure 2 and Figure 5 were separated to improve resolutions.

Point 7: Error bars were added in Figure 1A.

Point 8: The correction was made in the text. No figure 6 instead it refers as Figure 5 (D-E).