Author's response to reviews

Title: Disruption of Focal Adhesion Kinase and p53 Interaction with Small Molecule Compound R2 Reactivated p53 and Blocked Tumor Growth.

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Author's response to reviews:

Dr. Chistna Chap
Dr. Amancio Carnero
BMC Cancer Editorial Office
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May 9, 2013

Dear Dr. Chap and Dr. Carnero,

Thank you for your letter of 4.30.2013 and for reviewing our manuscript “Disruption of Focal Adhesion Kinase and p53 Interaction with Small Molecule Compound R2 Reactivated p53 and Blocked Tumor Growth.”

We appreciate the reviewers’ comments and addressed all comments by point-to-point response to both reviewers. We are glad that the reviewers found manuscript important to the field. We significantly improved the quality of all low-resolution scanned images and revised the manuscript according to all suggestions. We added suggested the additional data for Octet binding and addressed the computer modeling concerns.

We identified a small molecule R2 that disrupted complex of FAK and p53 proteins, blocked cancer cell viability and clonogenicity and inhibited xenograft tumor growth in a p53-dependent manner. This can be effectively used for development of future FAK-p53 targeted anti-cancer therapy approaches.

We include the revised manuscript with changes marked by yellow color and revised figures. We think that timely publication of this report is important for the
field of cancer research.

Thank you for your consideration.

Sincerely yours,

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Answer to Reviewer #1 (Juan Martinez)
1. The reviewer suggested improving the quality of Figure 2B with less saturation.

We improved the quality of the Figure 2B, as suggested. The revised Figure Was prepared in black and white. There is a significant decrease of clonogenicity at
10 microM R2 in HCT116p53+/+ cells compared with untreated and
HCT116p53-/-
cells.

2. The reviewer suggested improving Figures 3 C, D, E, 5A,B and Figure 6B, 7B with higher resolution and less saturation. The reviewer suggested where it applied to use densitometry to quantification.

We appreciate the reviewer’s comments. We rescanned the Figures with higher resolution and improved the quality of these Figures. We included densitometry
for Figure 5A to quantify protein level relatively to beta-actin control.
3. Figure 3B. The reviewer suggested including several dilutions of compound R2 to control the binding.

We appreciate the reviewer’s comment. We performed an additional experiment by Octet of FAK and p53 disruption of binding (dissociation) at different doses of R2 by Octet and show that R2 disrupts binding of FAK and p53 in a dose-dependent manner. We included a supplemental Table S1 to show dose-dependent increase of constant of dissociation of FAK and p53 complex by R2.

4. In Figure 5A, left panel, it was not clear to the reviewer that the level of Bax protein was increased.

We marked by arrow the Bax protein increase, which was increased at 10 microM dose, and then the level decreased to basal level due to decreased cell viability and cell death. We added densitometry of protein expression relatively to beta-actin to show protein increase.

5. Fig. 5A, right panel. The reviewer commented that at higher drug concentration p21 and Mdm-2 are not increased, and FAK is also decreased.

At small doses R2 caused an increase of p21 and Bax. The decreased level of these proteins p21, Mdm-2, FAK and uncleaved caspase-8 at higher doses is caused by apoptosis and protein degradation. The apoptosis is shown by cleavage of PARP. We included densitometry of each protein level relatively to beta-actin.

6. Fig. 5C. The reviewer suggested increasing the image size for Flow cytometry. The reviewer suggested explaining the behavior of HCT116p53-/- cells treated with R2 compound at 20 microM dose.

We agree with the reviewer and increased the size of the Flow cytometry image. The R2 increased G2 arrest of HCT116p53-/- cells at a 20 microM dose, which will be studied in the future. The most important conclusion is that R2 increased G1 arrest in HCTp53+/+ cells at 100 microM, while it did not in HCT116p53-/- cells.

Minor comment.

The reviewer suggested using IAUPAC nomenclature rather than Chemical abstracts nomenclature.

Both nomenclatures are acceptable. We prefer to use IAUPAC nomenclature for consistency, as we used this in previously published manuscripts (J. Med. Chem., v.51, 2008, 7405-7416; J. Med. Chem., 2009, 52, 4716-4724).

We appreciate the reviewer’s comments, which significantly improved the quality of the manuscript.

To reviewer 2 (Narcis Fernandez-Fuentes)
1. The reviewer suggested that we clearly explain how the modeling of the p53 Nt region was done. The reviewer asked what template was used for the modeling, and what the precision value was.

We appreciate the comment. The modeling was performed by an expert of in silico molecular modeling and our collaborator, Dr. David Ostrov, who is the head of the structure-design Core at the University of Florida. We included additional explanation on the modeling of the p53Nt region and a reference that modeling of the p53Nt was performed, as described in Anti-Cancer Agents in Medicinal Chemistry, 13, 2013, 532-545. Phyre used to identify the most likely templates for the existing peptide. The template for the p53 was 2GS0. We included template for p53 peptide to Materials and Methods. The E value was equal to 4.5x10^-4 and the estimation precision of the model was 95%.

2. It was not clear to the reviewer why information about the peptide was needed, as the filtering of the docking solutions (p53-FAK) was based on the region in FAK involved in the recognition of p53 (ref.19).

Ref. 19 shows that the FAK FERM -p53 binding site likely spans the F1 and F2 FERM lobe regions. To define potentially druggable sites in this region, we used macromolecular docking to define protein-protein interaction site amendable to binding small molecules. We showed that p53 peptide bound FAK at the same lobes, as was shown in ref.19 (F1 and F2) by Lim et al. We added to the Results and to the Figure 1 C legend amino-acids of FAK involved in interaction with p53 peptide. We included to results that the small molecules (purple color) targeted the p53 peptide (blue color)-FAK-FERM binding area (Figure 1D).

3. The reviewer commented that the in silico screening lacked basic details such as the details of the library that was screened.

We added to Materials and Methods that we used the National Cancer Institute Development Therapeutics Program NCIDTP collection of small molecule compounds (http://dtp.nci.nih.gov) and included a references to this database (Holbeck et al., Eur. J Cancer, 6,785-793,2004; Leukemia, 16, 520-526, 2002) and also ZINC UCSF (university of California, San Franscisco) database and included a reference (J. Chem Inf Model, 2012).

4. The reviewer had a concern about the details of the grid and parameters for screening.

We included in the Materials and Methods section that scores were based on a grid spaced five angstroms from the spheres selected for molecular docking. Each compound was docked in 100 orientations, and grid-based energy scores were generated for the highest scoring orientations. Scores approximate predicted delta G values based on the sum of polar electrostatic interactions and Van der Waals contacts.

5. The reviewer asked us to clarify the meaning that hydrogen atoms and partial charges were created using the SYBDB program.
We included in Materials and Methods the reference to the program SYBDB, which allowed us to calculate partial atomic charges and Van der Waals parameters (Noorwez et al, IOVS, 2008, v 49, 2008).

6. The reviewer commented that the quality of Figures 3, 6 and 7 is poor.

We appreciate the comment. We improved the quality of Figures 3 C, D,E, 6B, and 7B and present improved figures. In addition, we included densitometry for Fig 5A. We revised Figure 6B and don’t present Western blot on tumor xenografts with p21, FAK and p53, as we showed the same analysis in Fig 6A by immunohistochemical staining of xenograft tumors. We increased the font on Fig 6A for immunohistochemistry staining for better presentation. In the revised Figure 6B we present immunoprecipitation of FAK and p53 in xenografts and confirm that R2 disrupts the complex of p53 and FAK. We improved the quality of Figure 7B.

7. The reviewer had a concern about some language corrections.

The manuscript is checked for spelling and professionally edited by native English speaking colleagues (Dr. Cance and other co-authors).

We appreciate the reviewer’s comments, which significantly improved the quality of the manuscript.