Reviewer's report

Title: Molecular Mechanisms of Action and Potential Biomarkers of Growth Inhibition of Dasatinib (BMS-354825) On Hepatocellular Carcinoma Cells

Version: 2 Date: 26 March 2013

Reviewer: Jorge Martin-Perez

Reviewer's report:

Comments to revised version

Major Compulsory Revisions

If I asked you to determine the ration p-EGFR/EGFR, pFak/Fak, p-Stat3/Stat3, pAkt/Akt, pMapk42-44/Mapk42-44 is because, as a reviewer, I think that is the correct method to determine activation of these molecules, and also, because your scientific document will improve in quality. However, you have not modified the figures to include the statistical analyses. This would be something that it will greatly help the reader to interpret the date. For the ratio pSrc/Src the result could be indeed misleading as the anti-pY418-Src recognizes all members of the Src Family of Kinases (SFKs) and unless you immunoprecipitate each one of them and do in vitro kinase reaction, you may not know which member(s) are activated in your model system. Therefore, it is probably more correct to set the ration pY418-Src/beta-actin, assuming that beta-actin does not change upon your treatment. For the other phospho-proteins the appropriated is to determine the ration between the phosphorylated forms versus the total protein.

In Figure 9 A Co II it is surprising that no standard deviation is detected. In this figure as well as in Figure 10, to show statistical differences an eventually significance among treatment within the graph would also be very useful.

The invading analyses should quantified and results presented in a graphic (number of assays, SD and significance) together with the pictures.

Thank you very much for considering our publication on J Biol Chem 2006, 281(30):20851-64 (reference 30 in you document), nevertheless the title of that publication is not “A non-catalytic function of the Src family tyrosine kinases controls prolactininduced Jak2 signaling”, it is: “Role of c-Src in Human MCF7 Breast Cancer Cell Tumorigenesis”. In that scientific document we described the observation that conditional expression of SrcDN in MCF7 human breast cancer cells reduces adhesion, migration and spreading; concomitantly, SrcDN expression inhibits activation of Src (pY418-Src), Fak (pY925-Fak) and p130CAS. Because expression of SrcDN alters the shape of MCF7 cells, immunofluorescence confocal analyses showed concentrated focal adhesion proteins. However, the adhesion of cells was reduced. Furthermore, in MDA-MB-231 human metastatic breast cells, we have shown that Dasatinib inhibits proliferation, migration and invasion, as well as inhibits activation of Src,
Fak (Y925), paxillin, caveolin-1 and p130Cas phosphorylation/activation [Cellular Signaling 2012, 24(6):1276-86]. In contrast, in Huh-7 hepatocellular carcinoma cells (your reference 29), which you showed that are the most resistance cells to Dasatinib, increased adhesion and migration occurs at the same time upon treatment with prostaglandin E2 by increasing activation of Fak, paxillin and Erk2. One would have expected that increased adhesion would reduce migration, however, the nature of cell origin may determine specific cellular responses.

Minor Essential Revisions
In the text, Figure 7 should be now Figure 8, Figure 8 should be now Figure 9, Figure 9A,B should be Figure 10A,B and Figure 10 should be Figure 11. Please, check again orthographic and typographic errors

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

II declare that I have no competing interests