Reviewer’s report

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

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Reviewer: Jorge Martin-Perez

Reviewer’s report:

This manuscript intends to evaluate the effect of Dasatinib, a SFKs tyrosine kinase inhibitor, as a potential therapeutic agent in hepatocellular carcinoma (HCC). To this end, authors tested the effect of Dasatinib on 9 different HCC cell lines. This is an interesting approach. As in other solid tumors, not all cell lines that have activated Src (pSrc416) respond to Dasatinib treatment in terms of growth inhibition.

Major Compulsory Revisions:

In M&M section author indicated: “For dasatinib inhibition study, serum-starved cells were treated with various concentrations of dasatinib for 24 h prior to the addition of 20% FBS stimulation, and then were collected for western blotting analysis”. Could you please show that this treatment does not affect cellular viability?

According to Figure 1, only Li-7, PLC/PRF/6 and Sk-Hep1 appear to be sensitive to this compound. Nevertheless, it is surprising that authors established correlation between sensitivity and the levels of p-Src416, t-Src or t-EGFR. Dasatinib is considered as an inhibitor of SFKs tyrosine kinase activity, therefore it would be more appropriate to set correlations between specific activity of Src, that is, the ratio of p-Src416/t-Src, similarly of p-EGFR/t-EGFR (Figure 2). There are not, as far as we know that Dasatinib regulates Src expression. If so, it is difficult to understand that authors conclude (page 7): “sensitivity of HCC cells to Dasatinib was associated with over expression of Src protein, low expression of EGFR and low expression percentage of p-Src in t-Src”. Furthermore, there is not quantitation of the results presented in the Western blots and, as indicated above, p-Src/Src, p-EGFR/EGFR, pFak/Fak, p-Stat3/Stat3, pAkt/Akt, pMapk42-44/Mapk42-44 are needed to properly determine the alteration of the state of activation of these molecules upon Dasanitib treatment (Figure 3).

In Figure 4 correlations need to be done for pSrc/Src, pAkt/Akt, pFak/Fak. Similar corrections are needed in Figure 5.

In Figure 6 only results for one of the Dasatinib-sensitive cell line (PLC-PRF/6) were shown, what happens with the others? Why authors did not make quantitation for p-Src/Src and pEGFR/EGFR? The image of pSrc appears to be the same as in Figure 3. It looks like pSrc416 signal increases up to 0.01 µM Dasatinib and then decreases. Is that correct? If so, how it can be explained?
In Figure 7, why different targets were measured in panel A versus panel B?

In Figures 8 and 9 there are not statistical analyses of the data.

In Figure 10, microscopy images of invasion assay were shown; quantification and statistical analyses are needed.

In the third paragraph of the discussion authors reported: “Huh-7 expresses escalated levels of activated FAK576/577 and increases cell adhesion and migration after dasatinib treatment”. How authors explain that cell adhesion and migration increase at the same time?

Minor comments:
There are some typographic errors.

Conclusion:
Although this scientific document represents an interesting approach to define sensitivity of HCC cell lines to Dasatinib, the results need to be strengthened to establish useful conclusions for future therapeutic applications.

**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:**
I declare that I have no competing interests