Reviewer's report

Title: Comparative analysis of novel and conventional Hsp90 inhibitors on HIF activity and angiogenic potential in clear cell renal cell carcinoma: Implications for clinical evaluation

Version: 1 Date: 4 August 2011

Reviewer: Chann Lagadec

Reviewer's report:

I reviewed the manuscript from Bohonowych et al. untitled: “Comparative analysis of novel and conventional HSP90 inhibitors on HIF activity and angiogenic potential in CCRCC: Implications for clinical evaluation. The authors tested the efficiency of 3 HSP90 inhibitor drugs, 17-AAG, EC154 and LBH589, on HIF# expression, HIF dependent targets expression, cell biology (Viability, Migration) and on endothelial cells/structures (tubulogenesis and permeability). The authors observed interesting and promising results. While, the paper in really dense, it’s sometime confusing and hard to follow... certainly due to a none homogeneous effects of the drugs.

Major comments:

1. Authors analyzed the effects of the drugs on expression of HIF1# and HIF2#, interestingly they found a decrease of expression but in some condition an increase... nevertheless the transcriptional activity is still drastically decrease... the authors referred to other papers to explain that the HIF# expression is not directly correlated with his activity, but lack to give other explanation... What about the regulatory subunit expression in grugs treated condition: HIF# expression? or ARNT expression?

2. Figure 3. In 786-O, drugs repress HIF1# specific targets (CADK and GLUT1), while those cells do not express HIF1#... Do HIF2# compensate the HIF1# lack of expression?

3. p16/17. Mobility is inhibited as low concentration as 10 pM. At this dose there is a weak or no effect on ERK, FAK and Src phosphorylation, this pathway has to be exclude as an explanation... the other pathway suggested is uPA, but as described in figure 5, while LBH589 decrease uPA secretion, 17-AAG increase it, however both drugs reduce mobility! VEGF expression is not affected neither... how to explain this massive mobility reduction?

4. Figure 5. While the authors mention a possible increase of expression of uPA at low doses in discussion, nothing is described in the results section.

5. To verify the specificity of drugs effect on HIF#, it’s important to verify it, using HIF1#-/-/HIF2#-/- cells, and/or a model where HIF is constitutively active. Could the authors observe any expression modification of non-HIF target genes but
HSP90 targets.

6. p19. What about the dramatic drop of impedance with 17-AAG and EC154 at 1-2 hour? Could the drugs charged explain this change of impedance? Do the drugs modify ions efflux of the cells and not the permeability? To confirm these results it can be interesting to perform a quick dye diffusion experiment at different time point.

7. p19. LBH589 does not restore endothelial barrier function, one explanation will be a non-HSP90 effect of the drug... to verify non-HSP90 dependent effects of the 3 drugs, HSP90 deficient (HSP90/-, siRNA) cells have to be used... could that explain some inconsistency between drugs?

8. While authors claim to analyze the “implication for clinical evaluation”, no in vivo experiment has been done!!!!

Discretionary Revisions:

1. p18. “via” and not “via”

2. p22. “via” and not “via”

3. Figure 1B. Standard deviation of the western Blot replicates.

4. Impedence or impedance? Have to be verified...

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests