Reviewer's report

Title: A-770041 reverses paclitaxel and doxorubicin resistance in osteosarcoma cells

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Reviewer: Emmy Fleuren

Reviewer's report:

Thank you for the opportunity to review this manuscript. This is an interesting, nicely written and well structured study addressing the issue of multidrug resistance (MDR) in osteosarcoma. The figures are in general of good quality, although some clarifications are necessary as indicated below. The authors screened a kinase specific inhibitor compound library in two MDR osteosarcoma cell lines to identify compounds capable of reversing chemoresistance to doxorubicin and paclitaxel. A-770041, a potent Src-family kinase inhibitor, was identified as the most effective kinase inhibitor to overcome MDR. Combination of a Src inhibitor with conventional chemotherapy could therefore be a worthwhile treatment option to further explore in osteosarcoma patients.

I have a few comments/questions which could aid in improving the manuscript:

Major Compulsory Revisions:

1. Results, first paragraph. After screening of 3000 compounds, the authors identified 18 small molecule inhibitors that can increase chemotherapy effectiveness in two MDR osteosarcoma cell lines. Next, they further verified efficacy of those 18 compounds by serially titrating drug combinations with paclitaxel, of which 8 showed an improved effect in combination with paclitaxel. Further studies validated A-770041 as the most potent MDR reversing agent. I have some comments/questions regarding this part:

a. Although the authors mention some of the identified compounds in the text, it would be interesting to have an overview of all 18 identified small molecule compounds in a (supplemental) table including their target-kinases, also highlighting the 8 compounds identified after the second screen.

b. Please define ‘further studies’ in regard to A-770041. Why exactly was A-770041 selected for further analysis over the other 7 compounds remaining after the titration studies?

c. Why did the authors use the two doxorubicin-resistant cell lines U-2OSMR and KHOSR2 for the initial screening, and not also the taxol/paclitaxel-resistant cell line U-2OSTR?

d. Why did the authors perform the serial titrating combination studies with paclitaxel (and not with doxorubicin) on the two doxorubicin-resistant cell lines?
2. General comment: throughout the manuscript, the authors have conducted various experiments to examine effectiveness of certain compounds and combinations. However, I cannot find any statistics or p-values indicating significant differences. Although the data look sound, statistical tests are necessary to confirm the findings. Figures 3 and 5A will also be more convincing when statistics are performed.

3. How did the authors assess drug synergy? Figure 3 shows that the combinations are indeed more effective than the monotherapies, but it would be more appropriate to use a formula to officially assess drug synergy, for instance using the combination index (CI) method (e.g. Zhao 2004 Clin Cancer Res;10:7994-8004 and van Gaal 2013 Eur J Cancer 49(16):3462-70).

4. Results, fifth paragraph ('effect of... Src, Lck'): ‘Src kinase...osteosarcoma phenotype.’ Please provide references for this statement.

Minor Essential Revisions:
1. Figure 1: Please indicate what the green, blue and red boxes represent, and also what the red and blue line represent.
2. Figure 2B, 2C and 5A: Please indicate what type of error bars were used. Standard deviations?
3. The experiments in figure 3 were performed multiple times as indicated in the figure legend, please give error bars. Did the authors also measure the absorbance of untreated cells?
4. Figure 5: Do the authors also have data about cytokeratin 18, PARP and Pgp levels in untreated U-2OSMR, KHOSR2 and U-2OSTR cells? It also would have strengthened the results if these assays were performed upon A-770041 monotherapy, since A-770041 itself might already exert effects on for instance apoptosis.

Discretionary Revisions:
1. Discussion, final sentence "these preclinical... treat osteosarcoma”. Although I agree with the authors that the combination of Src inhibitors with chemotherapy could definitely be a promising approach to treat osteosarcoma patients in the future, I would not state that clinical studies would be the next step. It would in my opinion be appropriate to include a sentence like ‘further (in vivo) research is warranted’.

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.