Reviewer's report

Title: Oleanane triterpenoid CDDO-Me induces apoptosis in multidrug resistant osteosarcoma cells through inhibition of Stat3 pathway

Version: 2 Date: 23 October 2009

Reviewer: Cheryl A London

Reviewer's report:

This manuscript by Ryu et al is interesting and follows on the findings of others that STAT3 activation appears to be common in osteosarcoma. In the present work, the authors investigate the potential activity of oleanane triterpepenoid CDDO-Me against osteosarcoma cells. Comments are listed below.

Major Compulsory Revisions

1. The authors refer to multi-drug resistant osteosarcoma lines and the potential role of STAT3 in this process. However, data from their laboratory indicate that GADD45a expression may be responsible for this phenomenon. Is it known that STAT3 also contributes definitely to chemotherapy resistance in the MDR cell lines? How does PGP1 (expressed only in the two MDR osteo lines) affect the observed drug resistance?

2. In the methods it is stated that doxorubicin resistant cell lines were periodically cultured in doxorubicin to confirm drug resistance; there is no indication as to how often this was done, concentrations used, etc.

3. In the methods (and throughout the manuscript), the authors have mischaracterized the MTT assay as a cytotoxicity assay. This test only measures cell viability, and cannot be used to directly assess cytotoxicity or actual cell numbers. This should be changed throughout the text to represent cell viability rather than cell death/growth. This is particularly relevant for the results when CDDDO-Me is used in combination with doxorubicin; the investigators are not assessing whether these cells underwent apoptosis or died when using the MTT assay; they would need to use assays that directly assess apoptosis, such as flow cytometry with AnnexinV staining.

4. The method used for assessing drug combination effects is not technically valid. To determine whether two drugs are antagonistic, additive or synergistic in their effects on cell viability, a true combination index (CI) would need to be performed.

5. The authors should provide IC50 calculations for the CDDO-Me against the various cell lines (Figure 2).

6. For the responses to IL-6 in U2OS cells, the authors should demonstrate that treatment of cells with IL-6 alone results in enhanced STAT3 phosphorylation prior to the EGFP-STAT3 translocation experiments. Additionally, in Fig 3, there should be a treatment group with CDDO-Me alone. Lastly, it would be nice to see
the effects of IL-6 treatment on downstream STAT3 targets (i.e., survivin, etc) and show that the CDDO-Me can prevent upregulation of these targets (i.e., is actually preventing STAT3 transcriptional activity).

7. For the drug combination experiments (Fig 6), studies should be performed with both the drug sensitive and drug resistant lines to demonstrate a true difference in the resistant vs sensitive lines.

Minor Essential Revisions

1. There are several grammatical errors throughout the entire text. The manuscript should be thoroughly reviewed for spelling and grammar and all errors should be addressed. Also, the authors need to insure that the references are correct. For example, the references on page 4 for the U2OS line are incorrect.

Discretionary Revisions

1. With respect to the tumor samples used, it is assumed that these were all collected prior to neoadjuvant chemotherapy administration?

Level of interest: An article of importance in its field

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.