Reviewer’s report

Title: IL-6 secreted by Ewing Sarcoma tumor microenvironment confers anti-apoptotic and cell-disseminating paracrine responses in Ewing Sarcoma cells

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Reviewer: Ryan Roberts

Reviewer’s report:

The authors of this manuscript outline a clear hypothesis that IL-6 expression can induce mediate chemoresistance in Ewing’s sarcoma. The data presented generally address the hypothesis well and are easy to follow. As recognized by the authors in the manuscript, the work presented does have two major weaknesses:

1. As presented, the work is largely agnostic of the tumor microenvironment. This is despite the fact that the only contextually-accurate data shown (IHC) suggests that within a tumor, the contribution of stroma to the IL-6 effect through paracrine IL-6 stimulation is much, much greater than that of autocrine IL-6. Most of the paper focuses on autocrine cytokine as a primary source.

2. All experimental (not descriptive) data is in vitro and in monoculture. There is no animal experimentation, no co-culture, and investigation into the human samples is very limited.

That said, the methods used could yield data making these investigations appropriate for publication in a journal such as BMC cancer if the authors will address a few major issues:

MAJOR COMPULSORY REVISIONS

1. Inconsistency of cell lines used. In the data presented, it is not clear why the authors show one set of cell lines in Figure 1B, then only some of those same cell lines in 1C, then bring in some of the same and some new lines in 2A and on through the rest of the paper. This makes it look like they have selected only the data that “worked.” This data has value even if not everything “works” for all of the cell lines on everything. In paneled reports, the authors should be consistent in the collection of cell lines used. If a smaller panel is used in some experiments, they should present a logical rationale for why this was done.

2. For patient samples analyzed, there should be appropriate normal tissue controls shown. How does this expression compare to normal tissues?

3. Please provide a table showing relevant patient data for human samples used. Location, age, sex, whether samples are pre-or post-chemo, biopsy/resection/metastasis, primary or recurrence should all be reported.

4. It is very hard to make any conclusions with the human samples as shown given to small numbers, not paired, not all data shown (only 2 stains, no
quantitative or qualitative analysis. Conclusions made in the paper should be supported by data shown.

MINOR ESSENTIAL REVISIONS
1. The authors claim that a “plateau has been reached” since 2000 for Ewing’s sarcoma. This is misleading and suggests a lack of familiarity with the clinical literature. For example, the implementation of interval compression in AEWS0031 improved survival by almost 10% (Womer et al 2012).
2. Were samples obtained through the EURO-Ewing study? This is not clear.
3. Were specific statistical methods used to determine whether samples were not normally distributed? If so, this should be stated, including testing used and results obtained.
4. The authors state that “compared to ES cell lines, primary tumors showed a similar IL-6 mRNA expression pattern. Is this supported by the data? If the authors feel so, this should be explained.
5. The authors state that IL6 is most richly produced by tumor stroma, but make no attempt to characterize this further. Which are the cells of origin? Which parts of the tumor? This is one of the most interesting aspects and is overlooked.
6. Pg 15 line 5: please described how this was scored or compared patient to patient. Seems speculative and non-scientific.
7. A smaller sub-G1 population does not necessarily prove a decrease in apoptosis. Please provide a more robust assessment, if even just by the addition of Annexin V. Additionally, there is no data to suggest that this is not proliferation. BRDU or other proliferation assay would be helpful.
8. Figure 5 – please show some high power images of the positive cells.

DISCRETIONARY REVISIONS
1. It would be helpful to reference the hexameric structure with 2:2:2 stoichiometry for the IL-6 functional signaling complex, about which a great deal is known (such as Boulanger et al Science 2003).
2. This reviewer would recommend adherence to standardized nomenclature when referring to specific genes and gene products, such as IL6R and IL6ST instead of gp80 and gp130.
3. While the English of the document is generally understandable and well-written, there are a number of places where word choice is suboptimal. Examples include:
   a. Pg 5 line 15: 1990 through 2012
   b. Pg 6 line 5: unspecific >> nonspecific
   c. Pg 6 line 8: polymere>> polymer
   d. Pg 6 line 10: digitalized >> digitized
   e. Pg 8 line 3: dissolved >> resuspended
   f. Pg 8 line 9: thereafter >> afterward
g. Others

4. It would be helpful to perform in vitro PK to justify the repeat dosing experiments, especially given the fact that single doses had very little effect in most.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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.