Reviewer’s report

Title: New uracil analog U-332 is an inhibitor of NF-κB in 5-fluorouracil-resistant human leukemia HL-60 cell line

Version: 0 Date: 08 Dec 2019

Reviewer: Sam Salman

Reviewer's report:

The authors outline the effects of a new uracil analog on the mRNA expression and activity (via ELISA) of components of the NF-κB family in vitro using a leukaemic cell line along with its uracil resistant form. Some control/comparisons are included in the experiments, however, appear limited. The conclusion that this pathway is responsible for the resistance in these cells may be better demonstrated by connecting these pathways with the upregulated transporters - for example using BGD to reinstate sensitivity to 5-FU in the resistant cell line.

Background:

- Page 3. The discussion of AML is out of place given 5-FU is not commonly used in this setting, while it (and its analogues) are more common in several solid organ malignancies.

- Page 3. It would be of value to understand is the other 5-FU analogues have been tested in this way - if not including them in these experiments would provide further context to the proposed novel compound.

- Page 4/5. The logic is difficult to follow here given the detailed presentation of the NF-κB system, which is complex and has a large number of effects. Suggest re-thinking the flow of logic so that the reader can more easily understand why these experiments were performed. For example, the mechanism of U-332 activity in resistant cells is already presented - how did this then lead to the presented experiments.

- Page 5/6. Using 5-FU as a control substance in the experiments would add value (see comment above regarding also testing other uracil analogues). Additionally, it is not clear why the effect of BGD was not also assessed (or presented) in both cell lines.

Results:

- Page 8. Section 3.1 may be more appropriate in materials/methods as the manuscript is not presenting a new synthesis methods.
- Page 9. Section 3.3 - It is unclear why BGD was not also tested on HL-60 cells (non-resistant).

Discussion

- Page 10. It is stated that all members of the NF-kB family are overexpressed in the resistant cell line, however, two of the five were no different in the resistant cells. This statement is not correct.

- The authors should consider a discussion between the canonical and non-canonical NF-kB pathways as it appears members of the canonical pathway are those that have significant findings in the presented experiments.

- Page 11. The final statement needs to be revised as the authors have presented no data that the discussed compound would not cause drug resistance.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:
1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal