Reviewer’s report

Title: Cidofovir selectivity is based on the different response of normal and cancer cells to DNA damage

Version: 2 Date: 7 May 2013

Reviewer: Tohru Kiyono

Reviewer’s report:

Reviewers are asked to provide detailed, constructive comments that will help the editors make a decision on publication and the author(s) improve their manuscript. A key issue is whether the work has serious flaws that should preclude its publication, or whether there are additional experiments or data required to support the conclusions drawn. Where possible, reviewers should provide references to substantiate their comments.

Reviewers should address the points below and indicate whether they consider any required revisions to be 'major compulsory revisions', 'minor essential revisions' or 'discretionary revisions'. In general, revisions are likely to be 'Major compulsory revisions' if additional controls are required to support the claims or the interpretations are not supported by the data, if further analysis is required that may change the conclusions, or if the methods used are inadequate or statistical errors have been made.

1. Is the question posed original, important and well defined?
   The research question posed by the authors should be easily identifiable and understood.
   It is useful to both the editors and authors if reviewers comment on the originality and importance of the study within the context of its field. If the research question is unoriginal because related work has been published previously, please give references.
   Reviewers should ask themselves after reading the manuscript if they have learnt something new and if there is a clear conclusion from the study.

2. Are the data sound and well controlled?
   If you feel that inappropriate controls have been used please say so, indicating the reasons for your concerns, and suggesting alternative controls where appropriate. If you feel that further experimental/clinical evidence is required to substantiate the results, please provide details.

3. Is the interpretation (discussion and conclusion) well balanced and supported by the data?
   The interpretation should discuss the relevance of all the results in an unbiased manner. Are the interpretations overly positive or negative?
   Conclusions drawn from the study should be valid and result directly from the data shown, with reference to other relevant work as applicable. Have the
authors provided references wherever necessary?

4. Are the methods appropriate and well described, and are sufficient details provided to allow others to evaluate and/or replicate the work?

Please remark on the suitability of the methods for the study, which should be clearly described and reproducible by peers in the field.

If statistical analyses have been carried out, specify whether or not they need to be assessed specifically by an additional reviewer with statistical expertise.

5. What are the strengths and weaknesses of the methods?

Please comment on any improvements that could be made to the study design to enhance the quality of the results. If any additional experiments are required, please give details.

If novel experimental techniques were used please pay special attention to their reliability and validity.

6. Can the writing, organization, tables and figures be improved?

Although the editorial team may also assess the quality of the written English, please do comment if you consider the standard is below that expected for a scientific publication.

If the manuscript is organized in such a manner that it is illogical or not easily accessible to the reader please suggest improvements.

Please provide feedback on whether the data are presented in the most appropriate manner; for example, is a table being used where a graph would give increased clarity? Are the figures of a high enough quality to be published in their present form?

7. When revisions are requested.

Reviewers may recommend revisions for any or all of the following reasons: data need to be added to support the authors’ conclusions; better justification is needed for the arguments based on existing data; or the clarity and/or coherence of the paper needs to be improved.

8. Reviewers are reminded of the importance of timely reviews.

If reviewers encounter or foresee any problems meeting the deadline for a report, they should contact editorial@biomedcentral.com.

9. Confidentiality

Any manuscript sent for peer review is a confidential document and should remain so until it is formally published.

10. Are the included additional files (supplementary materials) appropriate?

Online publishing enables the inclusion of additional files with published articles. Additional files of many types can be submitted, including movies, tabular data and mini-websites. Reviewers are encouraged to comment on the appropriateness of the types of additional files, included with the manuscript, for publication with the final article. Additional files pertaining to original/raw data files that support the results reported in the manuscript can be included. It is not
expected that reviewers should reanalyze all supporting data as part of their peer review, but the availability of supporting data enables more detailed investigation of particular aspects of the study if the reviewer or editor feels it is necessary.

The authors revised the manuscript without including any experimental data requested by the reviewers. As a result, what the revised manuscript suggests is that anti-proliferative effect of CDV on SiHa, HeLa and HaCaT is based on its DNA damaging activity probably through incorporation of CDV as a nucleotide analogue into chromosomal DNA but not through the well-known antiviral activity. All the DNA damaging agents somehow have selectivity to tumor cells over normal cells. Though the microarray data might be useful in the future as a database, they do not support well the conclusions of the current manuscript. Their results never explain selectivity of CDV to HPV+ tumor cells over HPV negative tumor cells, or selectivity of CDV over other DNA damaging agents including 5-FU or other ANPs, which should be important goals of the authors’ group. To this end, the experimental design is not adequate.

The authors responded by saying that as evidence for biological responses, such as apoptosis and cell cycle arrest, has been published previously in 1998 and 2000 in most cases, such experimental data requested by the reviewers are not required. However, the previous reports did not necessarily use the same set of the cells with the same protocol of drug treatment. As the authors described, cellular responses depend on the protocol of CDV treatment. Therefore, most of the conclusions are not based on substantial evidence but on speculation based on microarray data and historical data.

As described in the rebuttal letter, this manuscript might be the first study that CDV functions as a DNA damaging agent. However, this conclusion is nothing novel and pretty much anticipated, and can be more directly and more easily proved by other methods, such as Tunnel assay and Western blots for activation of ATM/ATR, accumulation of gamma-H2AX, than the macroarray analyses (see below).

Though this reviewer appreciates their nice publications suggesting selectivity of CDV to tumor cells and the thorough pathway analyses from the microarray data set in this manuscript, most of the interpretations are not supported directly by the data shown but based on the previous reports.

Major Compulsory Revisions
1. Ideally CDV selectivity for HPV-positive cancer cells over HPV-negative cancer cells or the selectivity over other ANPs or DNA damaging agents should be examined by microarray.
2. Alternatively, the authors should include direct evidence for DNA damage, apoptosis and cell cycle arrest by other methods, such as Tunnel assay, Western blots and FACS analyses, in the same protocol used for the microarray analyses. With these data, the discussion and conclusions should be well balanced and adequately supported. Even if the authors omit some of the direct evidence for biological responses, the limitations of the work should be clearly stated. The authors should distinguish the conclusion directly based on the current
experiments and speculation. For example, the authors stated in the Abstract that CDV induced “cell cycle arrest” in HPV- immortalized cells. However, no data presented in the manuscript directly support this statement. Same is true for statements about “activated cell cycle regulation”, “genomic stability” and “survival and apoptosis”.

3. Amounts of key proteins, such as E6, E7 and p53, which are not regulated by transcriptionally or not detected by microarray, should be examined by Western blots. Though availability of good anti-E6 antibodies might be limited, clone C1P5, which is commercially available, can detect HPV18 E6 expressed in HeLa cells. Anti-E7 antibodies which can detect endogenous expression of HPV16 E7 in SiHa cells and CaSki cells or HPV18 E7 in HeLa cells are available from several companies, e.g. clone 8C9 for HPV16 E7 and Santa Cruz (sc-1590) for HPV18 E7.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.