Reviewer's report

Title: A Novel Oncolytic Adenovirus Targeting Cyclin E Overexpression Repressed Lung Tumor Growth

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Reviewer: Dana M Brantley-Sieders

Reviewer's report:

Overview:

In this study, Cheng et al. investigate the therapeutic potential of oncolytic adenoviruses harboring cyclin E2 in an immunocompetent pre-clinical model of lung cancer. Comparison of previous preclinical studies versus clinical trials suggests that data from mouse models may not reflect the efficacy of oncolytic adenoviral therapies in cancer treatment. The authors of this study seek to validate their previous data showing that replication of human adenovirus depends on cyclin E dysregulation or overexpression in target cancer cells as well as to test the efficacy of oncolytic therapy in an immunocompetent model in vivo. They tested the efficacy of oncolytic virus therapy in a cyclin E-dependent syngeneic transplantable model of lung cancer. This model was responsive to adenoviral cyclin E therapy and their data suggest that cell lysis was restricted to target cancer cells rather than healthy host tissue. They conclude that their virus targeting cyclin E represses growth in a syngeneic mouse model and that capsule structures encircling target cells post-infection might prevent spread of the virus.

As discussed below, the manuscript could be significantly strengthened by the following:

Major Compulsory Revisions:

- Preliminary analysis of infiltrating immunocytes, including macrophage and CD8+ T cells as noted in the Discussion. As a major goal of this study is to establish and validate an immunocompetent model in which to assess the efficacy of oncolytic virus therapy in cancer, these data should be included.

- Also, showing alterations in cyclin E-CDK2-Rb signaling between treatment groups would validate their proposed mechanism through which viral replication specifically in cancer cells (versus normal host tissue) is mediated.

Discretionary Revisions:

Though perhaps beyond the scope of this study, looking at growth and therapeutic efficacy in the context of the lung microenvironment (e.g. orthotopic allograft) would add more clinical relevance to their study.
Specific Questions:

1. Is the question posed by the authors well defined? The authors pose two clear questions in their study: 1) Does targeting cyclin E by oncolytic adenoviruses effectively block cancer cell growth/mediate destruction of cancer cells? 2) Is there a benefit/recapitulation of efficacy from human trials using an immunocompetent pre-clinical model?

2. Are the methods appropriate and well described? The methods are well-described and seemed appropriate for the most part. One concern is the subcutaneous xenograft approach for analysis of ED-1 lung adenocarcinoma growth in vivo. Though perhaps beyond the scope of this study, looking at growth and therapeutic efficacy in the context of the lung microenvironment (e.g. orthotopic allograft) would add more clinical relevance to their study.

In addition, since they emphasize the advantage of using a model with an intact immune system (e.g. noting that ‘Ads have complex interactions with host immune response effectors [2, 8]. In the presence of the immune system, the oncolytic effects of the virus may be reduced due to the immune responses against viral particles.’), the manuscript would be significantly strengthened by some analysis of immune cell infiltration into the tumors in the presence/absence of virus, as well as exploring if the immune-mediated effects enhance or inhibit oncolytic effects in their model.

Minor concern – they cite previous studies in which their virus induces CDK2 activity to promote viral replication, but they do not present data showing CDK2 activation or Rb phosphorylation from their virus-treated tumors.

3. Are the data sound? The data presented are sound, but as noted above, some essential data (e.g. analysis of immune system contributions in their model and confirming the cyclin E2-CDK2-Rb mechanism of viral replication) are missing and would strengthen the story if included.

4. Do the figures appear to be genuine, i.e. without evidence of manipulation? The figures do appear to be genuine. Bright field photomicrographs presented in Figure 2 were of poor quality/too small and therefore difficult to interpret.

5. Does the manuscript adhere to the relevant standards for reporting and data deposition? Not applicable.

6. Are the discussion and conclusions well balanced and adequately supported by the data?

As noted above, conclusions about how the immune system may influence oncolytic viral replication and/or therapeutic efficacy (included in the Discussion) cannot be drawn without at least initial analysis of immune cell infiltration into tumors across treatment groups.

Also, while the authors speculate about the nature of the capsule structures formed around infected cells within the tumor, they do not present data
characterizing these structures. Are they similar to what is observed in human tumors treated with oncolytic virus? Have those components been identified. A more thorough discussion of this would strengthen the manuscript.

7. Are limitations of the work clearly stated? No limitations of the work appear to be discussed.

8. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes.

9. Do the title and abstract accurately convey what has been found? Yes.

10. Is the writing acceptable? Yes. The manuscript is well-written and clear, but could benefit from including a rationale for some of their studies. For example, the first paragraph of the Results section deals with comparing growth rates between ED-1 cells and A549 cells without providing a clear reason why this comparison was necessary. Presumably it was to compare growth rates between cyclin E high versus lower expressing cells, but this approach does not seem to be the best way to address this. Perhaps comparing ED-1 lines versus ED-1 lines in which cyclin E is knocked down (as well as parental versus cyclin E knockdown A549) would be better.

As noted above, a more detailed discussion of capsule structures would improve the manuscript.

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.