Reviewer's report

Title: Engineered Measles Virus Edmonston Strain Used as a Novel Oncolytic Viral System Against Human Hepatoblastoma

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Reviewer: Pavlos Msaouel

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This is an appropriately conducted study showing the in vitro and in vivo efficacy of MV-CEA again hepatoblastoma cell lines and a xenograft model. Below are some issues that I believe the authors should address.

Major revisions:

1) Given the fact that the average age of hepatoblastoma diagnosis is one year and most cases will occur before two years of age this means that a significant number of patients may not be immune to measles prior to MV-CEA treatment (children in the west are vaccinated at 12-15 months of age, although in other areas with higher measles prevalence the target age may be ~9 months). This issue deserves to be addressed in the discussion section.

2) In the “materials and methods” section (page 7) there is a “Western blot analysis and ELISA” subsection which does not look like it corresponds to any of the results mentioned in the manuscript.

3) In the “statistical methods” subsection, the authors should also mention the statistical test (and software) used to obtain the p values in their flow cytometry apoptosis comparisons.

4) In the “results” section (page 9) in the “MV-CEA successfully replicated in human HB cells and induces cell lysis” the authors should also provide data (with figures) on the actual intracellular and supernatant viral titers (not only CEA concentration). The title of this subsection should also be modified as this particular experiment may show successful replication of infectious MV-CEA but not cell lysis induction.

5) It appears that relatively high CEA concentrations (~100ng/ml) were also detected in mice injected with inactivated MV-CEA. Where did that CEA come from? Given the fact that Hep2G is known to intrinsically produce CEA, these cells themselves may be the source of the CEA although I would expect the serum CEA levels to actually increase (at least somewhat) along with the tumor burden over time. On that note, the authors should also mention the percentage of hepatoblastoma patients that are found to have increased CEA levels and how this may affect the potential clinical application of MV-CEA in such patients.

6) The authors should also discuss the potential role of Nectin 4 (PVRL4), which was recently identified as the epithelial cell receptor for MV, in engineered
MV-Edm tumor selectivity.

Minor revisions:
1) In the “introduction” section the authors cite reference #33 when discussing that CD46 is highly expressed in tumor cells. However, that study did not involve tumors. A paper by Jurianz et al. (Complement resistance of tumor cells: basal and induced mechanisms. Molecular immunology 1999, 36, 929-39) may be more relevant in addition to the already appropriately cited reference #32.

2) A few typos in the “introduction” section: page 4, beginning of 2nd paragraph: “advantages” should be “advantage”; On the same paragraph, in the sentence “Detection of CEA in the serum is a widely available via clinical assay” the word “via” should be removed.

3) In the “Materials and methods” section (page 6) in the “Assessment of MV replication in human HB cells” subsection it is stated that 2.0x10^4 cells well plated per 6-well. This is quite a low density and I suspect that the authors meant to write “2.0x10^5”.

4) In the “Materials and methods” section (page 6) in the “In vivo experiments” subsection female SCID mice are mentioned but, as far as I could gather, only nude mice were used in this paper. Also, please mention in the main text the number of mice used per group (it is only mentioned in the figure legends).

5) The authors mention on a figure legend that MV-CEA was given every other day for a total of 5 doses. On the last paragraph of page 10, they mention that “The CEA concentration reached its maximum on day 15 after the last viral dose. This coincides with peak viral replication in vitro.” I am not sure however that the in vitro and in vivo data do indeed coincide since it appears that in the in vitro experiments the CEA levels peak between 3-4 days after the (single) MV-CEA administration whereas in the xenograft model the serum CEA levels peak 15 days after the last (out of 5 total) MV-CEA administration.

6) In the discussion section (page 14) the authors state that “…using a variety of techniques we have demonstrated extensive apoptosis after transfection of MV-Edm in human HB cell lines. These results are in agreement with prior work that has indicated that apoptosis is the main mechanism of death for MV-induced syncytia”. However, I believe that the authors used only one technique (flow cytometry) to demonstrate apoptosis. The other techniques reported in this manuscript demonstrate cell death without distinguishing apoptosis from necrosis. In addition, I would use the word “infection” of the cell lines rather than “transfection” since a replicating oncolytic virus was used.

**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: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I have been employed within the last 5 years in the Department of Molecular Medicine at the Mayo Clinic where MV-CEA was developed and is currently being tested in clinical trials and I have used and received funding for the preclinical testing of this virus in cancer models.