Reviewer's report

Title: Activation of the human immune system by chemotherapeutic or targeted agents combined with the oncolytic parvovirus H-1

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Reviewer: Ulrich Lauer

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This article by Moehler et al. is of high interest since combinatorial treatment approaches employing virotherapeutics together with „conventional“ cancer therapeutics probably will pave the way for breaking resistance in therapy of solid tumors. The experiments in this work address this important setting most appropriately and help to unravel significant questions on this topic in the case of oncolytic parvoviruses.

However, some points should be clarified and worked over by the authors:

Introduction
The negative effects on intratumoral parvoviral replication by classical cytotoxic therapies should be described explicitly and referenced; so far, this seems to be more a hypothesis than being evidenced by primary research work.

Methods
It should be explicitly described whether there is a washing step performed after infection and before addition of chemotherapeutic agents or sunitinib.

This is unclear: cisplatin and vincristine were freshly dissolved in medium to a concentration of 0.1 µg/ml; however, concentrations up to 5 µg/ml were employed throughout the experiments.

Please correct:
For combined treatment, cells were firstly infected with H-1PV in complete medium and one hour OR 24 H after infection, chemotherapeutic agents or sunitinib were added and cells were incubated until analysis (see Results section: treatment 24 hours p.i. led to a 24% decrease in cell viability (data not shown)).

This is a little bit confusing; definition of MOI is given by two completely different methods; please clarify:

... The multiplicity of infection (MOI) is given by the number of plaque-forming units (PFU) inoculated per cell AND ... Infections were performed for 1 hour at a MOI of 10-2 RU/cell.

Results & Figures
Number formatting. Please format numbers according to the convention used in English language journals, namely period for decimal point and comma for thousands separator. Please check text, figures, and tables carefully for this change. Please also include blanks between numbers and units. In each figure legend, the MOI used for infection with H-1PV has to be given.

Figure 1A: please explain why there is no further reduction in cell viability over time comparing the results at days 4 and 6 p.i.; for further understanding, please provide data on the kinetics of viral replication of H-1PV in human melanoma cell lines being investigated in this study.

Figure 1B: please present loading control data in the Western blot picture; otherwise it is hard to follow up the conclusion that time-dependent expression of the viral non-structure protein NS1 was highest on day 2 p.i.; how do the data look on day 6 p.i.?

Figure 1C: how do the data look line on day 6 p.i.?

Figure 1D: number is partially cut and thereby not easy to read.

Figure 2A: please give the MOI used in this experiment; figure legend: include time point of measurement; please provide the interesting data for vincristine as supplementary material.

Since NS1 expression is essential for H-1PV-induced cell killing and for transgene expression driven by recombinant constructs, how is it influenced under combinatorial therapy with cisplatin, vincristine and sunitinib?

Figure 4B: A dramatic increase in TNF-alpha levels following co-culture of CTL with DC incubated with H-1PV-induced SK29 Mel-1.22 lysates was observed (Fig. 4B); please, explicitly give the factor of increase in the text.

This also should be applied for the next sentence: High levels of IL-6 were also observed in CTL co-cultures with immature DC incubated with H-1PV-induced SK29-Mel-1.22 TCL (Fig. 4B); please, explicitly give the factor of increase in the text.

References
years of publication are missing; examples for that are:


Furthermore, there are a couple of typing errors which should be removed in a revised version of the manuscript.

**Level of interest:** An article of outstanding merit and interest in its field

**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.