Author’s response to reviews

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

Authors:

Markus Moehler (moehler@mail.uni-mainz.de)
Maike Sieben (maike.sieben@unimedizin-mainz.de)
Susanne Roth (rothsusi@gmx.de)
Franziska Springsguth (franziska.springsguth@gmx.de)
Barbara Leuchs (b.leuchs@dkfz-heidelberg.de)
Maja Zeidler (zeidler@1-med.klinik.uni-mainz.de)
Christiane Dinsart (c.dinsart@dkfz-heidelberg.de)
Jean Rommelaere (j.rommelaere@dkfz-heidelberg.de)
Peter R. Galle (galle@1-med.klinik.uni-mainz.de)

Version: 3 Date: 5 October 2011

Author’s response to reviews: see over
Reviewer's report

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

Version: 1 Date: 12 August 2011
Reviewer: Ulrich Lauer

Reviewer's report:

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 paroviruses. 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.

Reviewer’s comment is correct: although a negative effect on intratumoral parvoviral replication by classical cytotoxic agents (in particular genotoxic drugs) may be expected from the strict dependency of parvovirus life cycle on cellular factors whose expression requires the S phase of the cell cycle, this was not evidenced so far. Hence this sentence has been deleted in the revised version.

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:

Virus titers are determined by plaque assays (wild-type virus) or infected cell hybridization assays (recombinant viruses), and are expressed as plaque forming units (PFU) or replication units (RU) per milliliter of virus suspension.

... The multiplicity of infection (MOI) is given by the number of plaque-forming units (PFU) ... Infections were performed for 1 hour at a MOI of 10-2 RU/cell.
Please format numbers according to the convention used in English language journals, namely period for decimal point and comma for thousands separator. → revised
Please check text, figures, and tables carefully for this change.
Please also include blanks between numbers and units. → revised

In each figure legend, the MOI used for infection with H-1PV has to be given. → included

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.; → included
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.; → not available
how do the data look line on day 6 p.i.? → included

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

Figure 1D: number is partially cut and thereby not easy to read. → revised
This has been revised accordingly to the reviewer. Numbers can be read now fully

Figure 2A: please give the MOI used in this experiment; figure legend: include time point of measurement; → included
please provide the interesting data for vincristine as supplementary material.
This is enclosed in the results with full details...

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?
⇒ Good point, therefore we reviewed again the literature and cited and included the data on page 16.

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. → included

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. → included

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

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.