Author's response to reviews

Title: Fast selection of maribavir resistant cytomegalovirus in a bone marrow transplant recipient

Authors:

Axel Schubert (axel.schubert@uniklinik-ulm.de)
Karoline Ehlert (karoline.ehlert@ukmuenster.de)
Susanne Schuler-Luettmann (Susanne.Schuler-Luettmann@ukmuenster.de)
Eva Gentner (eva.gentner@uni-ulm.de)
Thomas Mertens (thomas.mertens@uniklinik-ulm.de)
Detlef Michel (detlef.michel@uniklinik-ulm.de)

Version: 2 Date: 10 June 2013

Author's response to reviews: see over
Dear Ms. Ramos,

we thank the reviewers for their detailed comments and questions which have all been addressed in the submitted revised version of our manuscript as you may see also from the following (see next page) point to point answers.

We believe that by following the reviewers instructions the manuscript has been substantially improved and hope that it is now regarded suitable for publication in BMC Infectious Diseases.

Sincerely

Detlef Michel
Reviewer 1:

1.) For strict exclusion of any confusion about the title, the authors should cite that Strasfeld et al., 2010 first reported on the same genotypic MBV resistance (T409M, H411Y) in a clinical isolate from an HTX recipient. In context of BMT the statement is correct. (title and last sentence in the abstract/background)

Background, page 5, last sentence in the first section:
“To our knowledge no other cases of genotypic MBV resistance in treated patients have been published so far”

Please see also: Hakki, Chou, 2011, Curr Opin Infect Dis 24:

We agree with the reviewer. To avoid confusion, we have rewritten this section and have made it more clear that our case is the first bone marrow recipient and that genotypic MBV resistant HCMV has been observed in a solid organ recipient before.

2.) Was the virological analysis performed retrospectively? What was the outcome of the patient? Did she really receive a BMT in 2010 or SCT? Did the girl suffer from organ disease under peak viral loads for HCMV and ADV prior to MBV administration? (case presentation page 6, Fig 1)?

The patient received indeed a bone marrow graft. The virological analyses have been performed routinely before and after the period of bone marrow transplantation. Only the cloning of UL97 fragments has been done retrospectively with remaining material. We have further described the fate of the patient during the different periods of the illness, the outcome until today, therapy and have included the requested information concerning an organ disease under high viral loads. At this time the girl suffered from pneumonia.

3.) Clinical data should also be implemented into Fig 1. (infusion of CD34+ and ADV-spec T cells). What was the therapeutic rationale for giving ribavirin, which has no evident anti ADV potential in vivo?

We agree with the reviewer and have implemented clinical data concerning the administration of CD34 cells and ADV-specific T-cells in both Fig.1 and the text section. The therapeutically benefit of ribavirin against adenovirus is indeed controversially discussed in the literature, however, in the situation to deal with a patient at high-risk all possible therapeutic options have to be considered.

4.) Did the authors identify ADV species and subtype responsible for disseminated ADV infection?

Yes, adenovirus genotyping has been performed and the species “adenovirus A” could be identified. We have implemented this information into the respective text section.
5.) In the abstract and on page 8, beside UL97 H411Y, also T409M and H411N are given, but in the Fig only T409A and no T409M.

We have corrected these points. The T409A was a scribal error and should read T409M.

6.) The interference of the UL54 mutations T700A and A809V with GCV/PFA and/or CDV drug resistance with UL97 L595S and M460V and H411Y should be discussed. What is known about contribution to antiviral hyper/hyposensitivity like interference of MBV H411Y and M460V?

Indeed, limited in-vitro results suggest that the GCV-resistance mutations M460V/I confer MBV hypersensitivity as shown with one recombinant HCMV strain [Hakki and Chou, 2011]. We have included and discussed this information as suggested by the reviewer. The mutation H411Y has no influence on the CDV resistance or the polymerase mutations T700A and A809V.

7.) How the authors did estimate the individual ratios between wildtype and mutant strains? (Page 7, section 3)

We estimated the ratios by using the electropherograms from the direct sequencing procedure as published by our group (Michel et al., 2003; Fast genotypic identification and estimation of ganciclovir-resistant cytomegalovirus from clinical specimens, Monogr. Virol. Basel, Karger).

However, to avoid confusion we deleted this information from the Fig. 1 and eliminated the values from the text section.

As suggested by Reviewer 2 we includes additional information concerning the estimation of the ration between wild type and mutated clones.

8.) Did the authors try to receive a viral isolate, because plaque purification could confirm the findings of UL97 cloning for direct sequencing?

Unfortunately, we were not able to isolate HCMV from patient specimens, although several attempts have been done. As suggested, we have included the information into the text section.

Reviewer 2:

Major:

1. The percentage of virus variants should be calculated on the number of UL97 clones carrying the different aa changes, rather than estimating it on the basis of electropherograms.

See above.

We sequenced 33 independent clones: 22 were UL97 wild-type (67%) and 11 carried a single mutation.

2. The patient outcome should be briefly described. Was the patient treated with HCMV-specific T cell clones as she was for adeno? Did she recover?

Done. See above.
3. The word "obviously" should not be used. Indeed, the initial high viral load could be a major factor for fast emergence of drug resistant strains, but it is not demonstrated. How many pts with similar HCMV DNA level clear the virus instead of developing drug resistance? Moreover, what about the role of immune suppression? Again, "...obviously several mutated viruses were selected separately...". Indeed, this event is more common, but selection of strains with multiple drug resistance mutations has also been recently reported.

The referee is right and we followed his suggestions.

Minor.

1. dosage for FOS, GCV, CDV and leflunomide should be provided.

Done. We have further described the fate of the patient and have included the requested information concerning the dosages of all mentioned antiviral compounds.

2. the para "Consistent with MBV acting..." can be deleted.

We followed the suggestion of the reviewer and skipped this part.

3. "UL97 is not an essential gene...". However, M. Pritchard showed that UL97-deficient strains are highly impaired in their replication capacity.

We included the information and have rewritten the sentence.