Author's response to reviews

Title: TRAIL receptor I (DR4) polymorphisms C626G und A638C are associated with an increased risk for hepatocellular carcinoma (HCC) in HCV-infected patients

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Author's response to reviews: see over
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Dear Danrolf de Jesus

Here we resubmit our revised manuscript "TRAIL receptor I (DR4) polymorphisms C626G and A638C are associated with an increased risk for hepatocellular carcinoma (HCC) in HCV-infected patients" to be considered for possible publication in BMC Cancer. We are grateful for the reviewers’ comments, which helped us to considerably improve the quality of our manuscript. All changes in the revised version of our manuscript are marked in bold.

In detail, we respond to the referees’ comments as follows:

**Editor**

1) Approval by the Bonn university ethics committee (including its reference number) is now fully described in the Methods section (patients and methods sections, page 4).

2) Second, the editor was interested in whether polymorphism of both alleles (C626G) and (A638C) or one of the alleles is sufficient to develop HCC?

The manuscript now clearly explains that each of the two polymorphisms was associated with an increased risk of HCC. The simultaneous presence of the risk allele in either polymorphism corresponds to a further increased risk of HCC, so that in multivariate analysis only the simultaneous presence of risk alleles in both polymorphism was confirmed as an independent risk factor (together with age).

(page 8, 1st paragraph)

**Specific comments of Reviewer: Saumitra Das**

1. What is the significance that these genetic variants (C626G, A683C) matched Hardy-Weinberg equilibrium?

The Hardy-Weinberg equilibrium controls for possible inadvertent typing errors and also checks if the sample is in equilibrium with the population rather than reflecting selection bias. As stated in the manuscript the distribution of DR4 C626>G and A683>C genetic variants matched the Hardy-Weinberg
equilibrium in all analysed groups and subgroups. We now also provide the $p$ values for all analysed groups and subgroups in table 1 (Fisher’s Exact Test)

2. The authors didn’t attempt to address whether C626G and A638C polymorphism is specific for HCV induced HCC or true for even Hepatitis B virus induced HCC.
To address this issue we analyzed the distribution of the alleles in the C626G and A638C polymorphisms in 56 new patients with HBV-associated HCC (all HCV-negative). The genotype distribution of this group is shown in an additional column of table 1 and indicates that unlike HCV-associated HCC differences to controls and HCV-positive patients without HCC were only minor. In particular, the high risk constellation of the simultaneous presence of a 626C allele in combination with the 683AA genotype was similarly frequent in HBV-associated HCC (50.0%), HCV-infected patients without HCC (49.4%) and healthy controls (47.0%) while the difference to HCV-associated HCC (65.4%) was significant ($p=0.042$). Thus, the effect of the DR4 polymorphisms on the risk of HCC development appears to be rather specific for HCV infection. This information is given on page 8, 2nd paragraph and is briefly discussed on page 9 2nd paragraph.

3. It is not clear that distribution of A683C is identical in healthy control and HCV infected patient without HCC. However, HCV virus load tend to increase in case of A683C and considered as risk factor-why?
In order to be more precise, we changed our statement from “... was identical ...” to “... did not differ ...” (results section, page 7). The observed genetic associations probably reflect less efficient immune control via TRAIL-mediated mechanisms. This effect seems to affect tumour surveillance of transformed HCV-infected cells by the immune system and viral loads, once HCV infection has occurred. On the other hand neither of the DR4 polymorphisms appears to affect susceptibility to HCV infection. This constellation can be explained by the fact that DR4 expression is rather low on normal liver cells while up-regulated DR4 expression following HCV infection sensitizes liver cells towards TRAIL-mediated apoptosis (Zhu et al. Gastroenterology 2007, Lan et al. J Immunol 2008). This interpretation has been incorporated into the discussion (page 10, 1st paragraph)

4. Also, it is not clear whether the C626G transition happens with the gradual progress towards HCC in the HCV infected patients.
As explained above HCV infection up-regulates expression of DR4, and sufficient DR4 expression on transformed liver cells appears to be a pivotal prerequisite for efficient tumour surveillance by the immune system (Kriegl et al. Clinical Cancer Research 2010). (page 10, 1st paragraph)

5. It appears from the results that presence of both the variations (C626G, A683C) together is significant for developing HCC-this should be clarified further.
As explained in the comments to the Editor, either of the two polymorphisms was associated with an increased risk of HCC. The simultaneous presence of the risk allele in each of the polymorphic sites corresponded to a further increased risk of HCC. In the multivariate analysis the simultaneous presence of risk alleles at both sites was identified as the only independent risk factor together with age, probably because each single polymorphism alone contributed rather similarly to the combined effect of two simultaneous risk variants. (page 8, 1st paragraph)
We also followed the reviewer’s advice to thoroughly proof read our manuscript in order to improve its style and spelling.

**Reviewer: Muhammad Idrees**

No comments to respond.

In summary, we hope now that our revised manuscript has been satisfactorily improved as to warrant its publication in BMC Cancer.

Sincerely,

H.D. Nischalke    C. Koerner