Author's response to reviews

Title: Prognostic impact of tumor infiltrating CD8+ T cells in association with cell proliferation in ovarian cancer patients - A study of the OVCAD consortium

Authors:

Anna Bachmayr-Heyda (anna.bachmayr-heyda@meduniwien.ac.at)
Stefanie Aust (stefanie.aust@meduniwien.ac.at)
Georg Heinze (georg.heinze@meduniwien.ac.at)
Stephan Polterauer (stephan.polterauer@meduniwien.ac.at)
Christoph Grimm (christoph.grimm@meduniwien.ac.at)
Elena I Braicu (ioana@braicu.de)
Jalid Sehouli (sehouli@aol.com)
Sandrina Lambrechts (sandrina.lambrechts@uzleuven.be)
Ignace Vergote (ignace.vergote@uz.kuleuven.ac.be)
Sven Mahner (s.mahner@uke.de)
Dietmar Pils (dietmar.pils@meduniwien.ac.at)
Eva Schuster (eva.schuster@meduniwien.ac.at)
Theresia Thalhammer (theresia.thalhammer@meduniwien.ac.at)
Reinhard Horvat (reinhard.horvat@meduniwien.ac.at)
Carsten Denkert (carsten.denkert@charite.de)
Robert Zeillinger (robert.zeillinger@meduniwien.ac.at)
Dan Cacsire Castillo-Tong (dan.cacsire-castillo@meduniwien.ac.at)

Version: 2 Date: 3 July 2013

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

Title: Prognostic impact of tumor infiltrating CD8+ T cells in association with cell proliferation in ovarian cancer patients - A study of the OVCAD consortium

Authors:
Anna Bachmayr-Heyda (anna.bachmayr-heyda@meduniwien.ac.at)
Stefanie Aust (stefanie.aust@meduniwien.ac.at)
Georg Heinze (georg.heinze@meduniwien.ac.at)
Stephan Polterauer (stephan.polterauer@meduniwien.ac.at)
Christoph Grimm (christoph.grimm@meduniwien.ac.at)
Elena Ioana Braicu (ioana@braicu.de)
Jalid Sehouli (sehouli@aol.com) Sandrina Lambrechts (sandrina.lambrechts@uzleuven.be)
Ignace Vergote (ignace.vergote@uz.kuleuven.ac.be)
Sven Mahner (s.mahner@uke.de)
Dietmar Pils (dietmar.pils@meduniwien.ac.at)
Eva Schuster (eva.schuster@meduniwien.ac.at)
Theresia Thalhammer (theresia.thalhammer@meduniwien.ac.at)
Reinhard Horvat (reinhard.horvat@meduniwien.ac.at)
Carsten Denkert (carsten.denkert@charite.de)
Robert Zeillinger (robert.zeillinger@meduniwien.ac.at)
Dan Cacsire Castillo-Tong (dan.cacsire-castillo@meduniwien.ac.at)

Version: 2 Date: 03 July 2013

Author’s response to reviews: see over
Dear Editor,

Thank you very much for processing the reviewing of our manuscript.

According to your editorial requests,

1) We included a Competing interests section, where we declared no competing interests of the authors (page 17).

2) We confirm: there is no trial registration number as the study did not include a clinical trial.

We also thank both reviewers for their valuable opinions on our manuscript. Below are our point-to-point answers (in italic).

We hope that this revision will meet your criteria and we are looking forward to hearing from you soon.

I) Reviewer’s report (Hans Nijman)

Title: Prognostic impact of tumor infiltrating CD8+ T cells in association with cell proliferation in ovarian cancer patients - A study of the OVCAD consortium

Version: 1 Date: 22 April 2013

Reviewer: Hans Nijman

Reviewer’s report:

The authors have studied the prognostic impact of CD8+ T cells in ovarian cancer in association with cell proliferation in ovarian cancer. They did so by studying tumor tissue from 203 ovarian cancer patients. They performed IHC on TMA and RT-qPCR on the same material. Main findings are (i) CD8+ T cells are positively correlated to overall survival; (ii) Ki67 negative tumors have a reduced overall survival, (iii) no relation between CD8+ infiltration of tumors and Ki67 expression.

Comments

1. The authors could have described more clear why the combination of a proliferation marker (Ki67) and infiltrating T cells is of interest to be studied. The hypothesis is not clear.

   *We agree this is a very important point. We hypothesize that the outcome of cancer patients is a result of the interaction between tumor proliferation and immune reaction. So far, there are few studies on this topic in ovarian cancer. We added and modified the description in the “Introduction”, page 6 to make this point more clear.*

2. Finally 203 tumors were tested. This seems a low number of patients knowing the number and size of the clinics involved in this study. How unbiased is this cohort?

   *Ovarian cancer, in comparison to breast cancer, has a very low incidence and belongs to the “rare cancer” according to the definition of the WHO. Therefore, multicenter studies are needed to get reasonable numbers of tumor samples. The OVCAD study, as indicated in the title, is a FP6 EU project including 5 well established ovarian cancer centers in Europe (www.ovcad.eu). The whole study was carried out with standard protocol and the samples were centralized and examined for their quality by experienced pathologists in order to reduce the bias to the minimum.*
3. The construction of the TMA should be described within the Methods section.

*We added a section “Tissue Microarray” in the “Methods” on page 7 including the construction of the TMA.*

4. Why was chosen for two cores of 1.0 mm?? How representative are these cores for the whole tumor?

*In a previous study, we compared and evaluated the IHC data for tumor infiltrating CD8+ cells generated from two TMA cores of 1.0 mm² and whole tumor tissue sections. Even though there were variations between the two cores, their mean values correlated highly with the results from the whole tissue sections, indicating that the mean value of the two scores is representative. The reference was added to the section “Tissue Microarray” in “Methods”, page 8.*

5. Was the tumor material all from primary surgery before chemotherapy? So no interval surgery or tumor from recurrent disease?

*As indicated above, the whole study used standard processing protocol. All tumors were collected before chemotherapy and only primary tumors were included in the study. We indicated this in “Methods, Patient Information”, page 7.*

6. How well is the automatic counting of CD8 cells validated?

*The automatic TissueFAXS/HistoQuest counting method was published and validated in various journals (OncoTargets and Therapy, Nature methods, J Immunol Methods). We added the description and two references in “Methods, Immunohistochemistry”, page 9.*

7. What exactly is meant by “false positive cell counting was avoided by a specific gate for cell size and CD8 intensity staining”?

*This means: we set a gate, within which the cells were randomly controlled by visualizing the cells on the digital picture. We added an explanatory sentence to “Methods, Immunohistochemistry” on page 9.*

8. For qPCR the authors used homogenized whole tumor. Was this material from the same tumor spot as used for TMA construction? As mentioned by the authors in the discussion part, it could be envisioned that whole tumor encompasses stroma tissue as well, therefore not reflecting tumor tissue only. Why was chosen to persue with this approach?

*This is a very good point of view. The final aim of our approach is to establish clinical useful tools for prognosis. Although the automatic quantification of IHC-CD8 data is comparably easy and straightforward, it requires persons with expertise and experiences. RT-qPCR would be more easily automatized. So we wanted to see if we could get comparable results from RT-qPCR as those from IHC. Unfortunately, this was not the case. Molecular biology has provided some advantages for pathological examinations. Nevertheless, new approaches must be always evaluated with established and reliable methods before they can be further used.*

9. For IHC the mean value of the two cores was calculated. What was the variation between two cores?

*Please refer the answer to point 4.*

10. The authors compared optimally debulked patients with those who had remaining residual disease for CD8 infiltration. What was the hypothesis behind this?

*We assume that infiltrating CD8+ cells have a greater effect in patients with residual tumor after surgery than in patients without residual tumor since infiltrating cells are removed together with the tumor mass. We added this rationale and a reference to “Results, Better overall survival of ovarian cancer patients with Ki67+ tumors and high density of tumor infiltrating CD8+ lymphocytes”, page 13.*

11. The results are shown in a Kaplan Meier curve. How comparable are the two patient groups? Is the difference for CD8 infiltration tested in a multivariate analysis?
As outlined in the statistical methods sections, pairwise interactions of CD8+ cell density and other variables were screened and, as stated in the “Results, Better overall survival of ovarian cancer patients with Ki67+ tumors and high density of tumor infiltrating CD8+ lymphocytes”, page 13, the interaction with residual appeared to close-to-significant (p=0.0522). We further investigated this finding by KM plots, and by computing hazard ratios for CD8+ in optimally debulked patients and in patients with residual tumor, confirming significant association of CD8+ cell density in patients with residual tumor only. This generated a new hypothesis that CD8+ cell density may be a prognostic marker in patients with residual tumor only. The power of interaction testing in our study was too low to confirm this hypothesis. However, our study generated this hypothesis which future studies may be able to confirm. We added this explanation to “Results, Better overall survival of ovarian cancer patients with Ki67+ tumors and high density of tumor infiltrating CD8+ lymphocytes”, page 13 and “Discussion”, page 16.

12. Figure 3 can be skipped.

We skipped Figure 3 from our manuscript.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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

Declaration of competing interests: 'I declare that I have no competing interests

II) Reviewer's report (Nadya Tarasova)

Title: Prognostic impact of tumor infiltrating CD8+ T cells in association with cell proliferation in ovarian cancer patients - A study of the OVCAD consortium

Version: 1 Date: 30 May 2013

Reviewer: Nadya Tarasova

Reviewer's report:

Major Compulsory Revisions: None

Minor Essential Revisions:

1. The authors use the term “platinum” incorrectly on page 14. It should be replaced with "platinum compounds" or "platinum-based alkylating agents" because platinum itself is an inert metal and is not cytotoxic.

We replaced the term “platinum” with “platinum compounds” in “Discussion”, page 15.

Discretionary Revisions: None

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: I declare that I have no competing interests