Author's response to reviews

Title: VEGFR-3 and CXCR4 as predictive markers for treatment with fluorouracil, leucovorin plus either oxaliplatin or cisplatin in patients with advanced esophagogastric cancer: a comparative study of the Arbeitsgemeinschaft Internistische Onkologie (AIO).

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Version: 3 Date: 26 September 2013

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

Title: VEGFR-3 and CXCR4 as predictive markers for treatment with fluorouracil, leucovorin plus either oxaliplatin or cisplatin in patients with advanced esophagogastric cancer: a comparative study of the Arbeitsgemeinschaft Internistische Onkologie (AIO).

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Version: 2 Date: 04 August 2013

Author's response to reviews: see over
Title: VEGFR-3 and CXCR4 as predictive markers for treatment with fluorouracil, leucovorin plus either oxaliplatin or cisplatin in patients with advanced esophagogastric cancer: a comparative study of the Arbeitsgemeinschaft Internistische Onkologie (AIO).

Version: 2 Date: 10 June 2013

Reviewer: Edwin Bölke

Reviewer's report:
minor language corrections before being published

The manuscript was once again carefully inspected for possible language errors. Corrections have been made and have been highlighted in the text.

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

We thank the reviewer for his positive comment.

Quality of written English: Needs some language corrections before being published

After critical inspection we found out and corrected some minor language errors.

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

Declaration of competing interests:
I have no financial or non-financial competing interests in relation to this paper.
Reviewer's report
Title: VEGFR-3 and CXCR4 as predictive markers for treatment with fluorouracil, leucovorin plus either oxaliplatin or cisplatin in patients with advanced esophagogastric cancer: a comparative study of the Arbeitsgemeinschaft Internistische Onkologie (AIO).

Version: 2 Date: 14 June 2013

Reviewer: Alicia Okines

Reviewer's report:
- Major Compulsory Revisions
1. At present, this study is not compliant with REMARK guidelines. Please revise in line with these guidelines.
   We are sorry that we did not show a clear compliance of the main purpose and results of our trial with the REMARK guidelines in the submitted version of our manuscript. In order to conform to the REMARK guidelines, we summarized the main assays, findings and conclusions of our trial in Table 3.

2. Abstract; your results show trends rather than statistically significant differences; this must be clearly stated. The results from the (presumably) post-hoc subgroup analysis of patients aged >60 must be stated to be an exploratory analysis. The study is underpowered by the small sample size and this limitation should be mentioned.
   We thank the reviewer for her remark. The abstract was modified according to the instructions of the reviewer.

3. Background; this should state why you selected these particular biomarkers for this study; what was your hypothesis and why? Is this part of a wider biomarker analysis investigating other potential biomarkers (such as those involved in DNA repair or fluoropyrimidine metabolism, which might seem a more logical initial choice in a study of platinum plus fluoropyrimidine)?
   We regret that the purpose of our study did not become clear in the Background section. VEGFR-3 and CXCR4 are two molecules of great importance for the lymphangiogenic and metastatic potency of human cancers. Unlike other cancers, their role as potential biomarkers in advanced esophagogastric cancer is not clearly understood. For instance, despite the encouraging results of the addition of bevacizumab in phase II trials in metastatic and locally advanced esophagogastric cancer, a significant benefit in terms of OS was not observed in the phase III AVAGAST trial. The aim of our study was to investigate whether the expression of these markers on tumor tissues of advanced esophagogastric cancer could "predict" the clinical outcome of the established palliative chemotherapy. This could serve a more stratified treatment, since both of the standard regimes, FLO and FLP, have been proved as equally effective in patients with advanced esophagogastric cancer. We modified the Background section according to the suggestions of the reviewer. The references 27-29 were also added in the text.

4. Patients; please provide a patient flow diagram detailing why only 72 patients from the phase III study were included, full baseline characteristics (in particular previous surgery) and how they compare to the overall study population (e.g using Kaplan Meier curves). Also please state clearly the nature of the
specimens (biopsies or resections).

Availability of adequate material for staining was the main reason why only 72 patients from the phase III trial were included. A complete list of patients' baseline characteristics as well a direct comparison with the overall study population are shown in Table 1 and suppl. 1. The examined material was mainly obtained from tumor resections. The suggested changes have been made in the session of Methods.

5. Immunohistochemistry; please explain your choice of antibodies ideally referencing the studies that have used these successfully previously (e.g.I see ref 17 used the same primary CXCR4 antibody).

We thank the reviewer for that useful comment. We worked a lot of years with these antibodies and we had excellent experience with them. Moreover we evaluated their application also in different indications (like colon or pancreatic cancer) and got always successful results.

Thus, we added the following other supportive references from our group:

“new” reference 30, 31, 32:

Cetuximab with irinotecan, folinic acid and 5-fluorouracil as first-line treatment in advanced gastroesophageal cancer: a prospective multi-center biomarker-oriented phase II study.

An open-label, multicentre biomarker-oriented AIO phase II trial of sunitinib for patients with chemo-refractory advanced gastric cancer.

Strong expression of chemokine receptor CXCR4 by pancreatic cancer correlates with advanced disease.

6. Statistical analysis; please explain how you chose the scoring method, ideally referencing other studies which have used this method for these markers. Please state what p-value is considered significant (presumably <0.05) and any correction for multiple testing. State any pre-planned sub-group analyses and the clinic-pathological parameters selected.

We agree with the reviewer and added these details also in the manuscript to enhance the understanding.

The scoring method was the same we used before in other studies (see references above). The advantages of this method were predominant. If we used a combination of intensity and stained tumor area, the scoring was more specific. Furthermore, in case of biopsies the variations to resections were lesser. We also verified the scoring with our pathologists.

“The CXCR4-CXCL12 axis in Ewing sarcoma: promotion of tumor growth rather than metastatic disease
p values < 0.05 were considered to indicate significant differences. All statistical analyses were done by using SPSS and MedCalc statistical analysis software in close cooperation with the IMBEI. The clinic-pathological parameters were defined by the study team in 2008. We kindly asked the study coordinator Dr. Al-Batran to do post-hoc analysis. The research idea was created after the end of the study.

7. Results; all survival curves should have the number of patients at risk below the curves. The results which are not statistically significant (p>0.05) should be clearly stated as such.

We agree to this comment and checked the manuscript for these statements. In Fig 2, 3b, 4 and 5b were shown statically significant results (p<0.05). Furthermore, we added the number of patients at risk below all curves.

8. Discussion; limitations of your study, in particular the small sample size should be discussed.

A statement about the limitations of our study was added in the conclusions section.

- Minor Essential Revisions

1. Background; the result that oxaliplatin appeared more beneficial in patients aged >65 was obtained from a post hoc sub-group analysis therefore this must be stated or the sentence removed altogether.

We changed the specific sentence in the Background-section as indicated above.

2. Results; Your choice of a sub-group aged >60 years is not explained and should ideally be removed.

According to the suggestion of the reviewer we stated in our results that this subgroup consists an exploratory analysis. Our results indicate that VEGFR-3 and CXCR4 positive patients in this post hoc sub-group have a significant prolonged OS under FLP treatment. It is an exploratory observation which could lead to further trials focusing on this clinical important subgroup. Therefore we would prefer not to remove this report from the manuscript.

- Discretionary Revisions

1. Ideally please include a table detailing your compliance with REMARK
We thank the reviewer for her suggestion. Table 3 was added in the manuscript summarizing the main points and results of our study according to the REMARK guidelines.

2. Figure 2A and 2B are confusing and should ideally be removed; these show the same data as 2C and 2D but the choice of a different x-axis scale is misleading.

We removed the figures 2A and 2B as suggested by the reviewer.

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

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests
Reviewer's report
Title: VEGFR-3 and CXCR4 as predictive markers for treatment with fluorouracil, leucovorin plus either oxaliplatin or cisplatin in patients with advanced esophagogastric cancer: a comparative study of the Arbeitsgemeinschaft Internistische Onkologie (AIO).

Version: 2 Date: 19 June 2013
Reviewer: suayib yalcin

Reviewer's report:
Dear Editor,
Thank you very much for giving me the opportunity to review this interesting study investigating the role of VEGFR-3 and CXCR4 as predictive markers for survival in patients treated with fluorouracil, leucovorin plus either oxaliplatin or cisplatin for advanced esophagogastric cancer. The question of the study is simple and very well defined by the authors and the methods used are appropriate and well described. However only overall survival was investigated. I believe that the response rates, and progression free survival should also have been analysed. However the data still seems to be sound and the manuscript adheres to the relevant standards for reporting and data deposition. The discussion and conclusions are well balanced and adequately supported by the data. However limitations of the work are not clearly stated. Such as the size of the study is small and the statistical power is not high. The authors have clearly acknowledged the work upon which they are building, both published and unpublished and the title and abstract accurately convey what has been found. The writing is definitely acceptable.
My conclusion is that despite the limitations of the study, the manuscript can be published with its current form.
If they may include RR and PFS it would be great, however may not be possible.
With sincere regards
Prof. Suayib Yalcin
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We thank the reviewer for the useful comment. In deed, the main aim of our study was to investigate possible correlations of VEGFR-3 and CXCR4 expression in relation to overall survival in patients with advanced esophagogastric cancer. Since the number of examined patients was small, we focused on overall survival as an end point. We believe that our results state clearly that VEGFR-3 and CXCR4 may play a role in personalisation of treatment in patients with advanced esophagogastric cancer. We agree with the reviewer that future studies with a larger number of patients are necessary, where progression free survival and response rates will be also investigated. Finally, a comment about the limitations of the study was added at the conclusion part according to the suggestion of the reviewer.

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 have no conflict of interest related to the manuscript or the topic of the study.