Author’s response to reviews

Title: High number of CD45RO+ tumor infiltrating lymphocytes is an independent prognostic factor in non-metastasized (stage I-IIA) esophageal adenocarcinoma

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“High number of CD45RO+ tumor infiltrating lymphocytes is an independent prognostic factor in non-metastasized (stage I-IIA) esophageal adenocarcinoma” by the authors Sandra Rauser, Rupert Langer, Sebastian Tschernitz, Peter Gais, Uta Jütting, Marcus Feith, Heinz Höfler and Axel Walch

Ad reviewer 1 (Hans Nijman):
Q: Personally, I would not include data showing "a trend for an association" as brought forward on page 8 and 10. What is the limit for a trend?
A: The value of the limit for “a trend for an association” is given in the “Methods” section (p. 7). $P$ values less than 0.05 were considered statistically significant, whereas $P$ values of 0.05 to 0.1 were considered as a trend.

Ad reviewer 2 (Woo Ho Kim):
Q1: The findings shown here are not novel. There are many papers indicating the prognostic role of TILs in various types of cancer including esophageal cancer.
A1: The authors agree with the reviewer that there are many publications indicating the prognostic role of TILs in various types of cancer including esophageal cancer. Nevertheless, most of the studies concerning esophageal cancer are conducted in squamous cell carcinomas and not in esophageal adenocarcinoma, which are considered as two distinct entities due to various morphological, etiological, molecular and clinical differences. To our knowledge, at date of submission, our study was the first one which showed the prognostic influence of TILs in a large cohort of primary resected Barrett’s cancer (i.e. esophageal adenocarcinoma) patients ($n = 118$). There is a previous study from Schumacher et al. who analyzed the prognostic significance of CD8+ TILs in esophageal carcinomas ($n = 70$), but this patient cohort comprised both esophageal squamous cell carcinomas and adenocarcinomas and there was not made any difference between those both entities (Schumacher et al., Cancer Res 61, pp 3932, 2001). This study has already been mentioned in the discussion. There is a very recent publication (Zingg et al., Eur J Surg Oncol 36, pp 670, 2010), which comprised 106 primary resected esophageal adenocarcinomas, but failed to show any prognostic influence of CD3+ and CD8+ TILs. However, the method of assessing TIL density in this work differs from ours. We have implemented a comment on this in the discussion.
Q2: The number of subjects is too small to derive any conclusions in such a study using immunohistochemistry only.

A2: In contrast to other types of cancers the number of subjects analyzed in this study seems to be limited. However, considering that Barrett’s cancer is a rare tumor type, the study cohort with 118 patient samples is quite large and comparable to patient numbers in published studies.

Q3: It is necessary to describe the detailed method of determining cut-off points of various TIL densities.

A3: The cut-off points of TIL densities were determined by receiver operating characteristic (ROC) analyses as described in the “Methods” section. The ROC analysis is a commonly used method to find optimal parameter values and we selected the cut-off levels for the labeling indices of TILs (as achieved by image analysis) that correlate with the clinical outcome of the patients. This is now added in the manuscript (“Statistical Analysis” in the “Methods” section).

Q4: Data presentation and description are poor in result section. Also, Table 1 and 2 are in need of modification.

A4: The authors are still convinced that the data presentation and description as well. Table 1 and 2 are adequate and conform to similar work published. As the criticism is unspecific, we were not able to make specific changes.

Q5: Discussion section is too short and its contents are insufficient.

A5: The discussion is now extended in its contents.