Reviewer’s report

Title: Long term survival following the detection of circulating tumour cells in head and neck squamous cell carcinoma

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Reviewer: Thomas Brunner

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This manuscript highlights the question of the predictability of local or distant relapses in head and neck cancer. It would be extremely helpful to predict such relapses at the time of primary treatment. The manuscript is interesting, however it suffers from a sufficiently large number of patients investigated to draw conclusions on survival. It is rather a pilot study which should be expanded on more patients to get an idea for survival effects. It can seen as a starting point to further explore the questions.

Abstract

1. Major: Rephrase the first sentence: “14 patients were positive to circulating tumour cells either prior to or immediately after surgery …”. This is confusing as one good thing is that some of the patients have only been checked after surgery.

2. Major: Conclusions: “the clinical application of techniques for detection of spreading disease, such as the new magnetic enrichment RT-PCR analysis used in this study, should be explored further.” From the reading of the abstract of the lack of significant differences in overall disease-free survival is hard to understand this conclusion. Why should something that does not matter be further explored?

Minor: Material and Methods:

Page 8, “reconstruction experiments…”: please mention if the final concentrations of 10, 50, 100 and 500 cells per 10 mL are representative for what is typically found in patients.

Results:

Minor, Table 1:

The adjuvant therapy of the patients was very in-homogenous. This should be taken into consideration when making any conclusions on the results obtained in the small number of patients investigated.

Major, Table 3:

The names of the studied markers should be consistent throughout the manuscript. Use Elf3 or ESX but not both (e.g. Table 4). In Table 3 the reader is
confronted with just two more markers without having any explanation of what BerEP4 or EMA is. EpCam or ESA is a much more common name used for BerEP4 and so is Muc-1 for EMA. Give the synonyms in the text accompanying the table. While the manuscript focuses on Elf3, CK19, EGFR and EphB4 it is not clear from the text why not these but BerEP4 and EMA are used for labelling.

Major: Figure 1, Table 4:
The understanding of figure 1 could be much enhanced by using arrows to show the relevant information. From the figure and the legends is very difficult to conclude what was suggested to be shown. Indicate how the counting was done in the figure legend: it is not clear if it was done by eye under the light microscope. If this is the case, what was the magnification? What were the negative control cell lines used? Did you also consider to do to a FACS analysis which would have allowed to count a large number of cells automatically.

Minor: Page 12, Immunobead RT-PCR analysis of blood samples: was the blood drawn from an artery or from a vein?

Major: Page 13, third line: did you check for the influence on the overall survival in the six patients who were positive for three markers?

Major: Aged 13, immunobead RT-PCR analysis: calculate the statistical degree of agreement between the expression of markers in the duplicate samples.

Major, Page 14, comparison of individual marker expression prior to and after surgery: the relevance of this section is quite limited since there is not a very high degree of agreements between the expression of markers in the duplicate samples at one given time. It thus cannot be expected to be higher when comparing pre- and post-surgery values. This section could be taken out completely.

**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.