Reviewer's report

Title: Soluble EpCAM levels in ascites correlate with positive cytology and inhibit catumaxomab activity in vitro

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Reviewer: Uwe Zangemeister-Wittke

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The MS of Seeber et al. describes an assay system to detect tumor cells and EpCAM in the ascites fluid of carcinomatosis patients. Ascites from liver cirrhosis was used as control. The majority of experiments describes cloning, transfection, biochemical purification, biophysical analyses and standardization procedures for EpCAM quantification. Statistical analyses were included too. Altogether, the technical part is sound, the measurements were carefully done and include the necessary controls.

The information that, like other epithelial antigens, EpCAM is shedded into the environment and may serve as a marker to predict tumor targeting with anti-EpCAM ligands is of interest. Apart from this finding the cancer-relevant part of the study, however, is weak. For the ascites measurements, only small patient cohorts were used where positive cytology and sEpCAM only infrequently occurred in the aspirates. Moreover, EpCAM levels were often below the detection threshold of the ELISA system and the background noise by inflammatory degenerative events like liver remodeling was comparatively high. It also remains unclear if all measured sEpCAM was produced by cleavage and shedding from the surface of viable cells or resulted from massive tumor cell death in the ascites.

It is unclear what the authors mean with the prognostic value of EpCAM in ascites. The trigger of EpCAM cleavage and how this reflects biologically-relevant alterations is unknown. At least the authors demonstrate that at amounts achieved in the ascites in at least some of the malignant conditions like ovarian cancer were sufficient to inhibit catumaxumab targeting and ADCC. Surprisingly, the amount of sEpCAM produced by tumor cell lines in vitro was far below the levels required for catumaxumab inhibition. Although the ADCC experiments were done with only one effector to target ratio and without a time course, it suggests that carcinomatosis patients with high sEpCAM titers should be precluded from local cancer immunotherapy. The question remains to what extent the efficacy of other EpCAM targeting drug delivery systems will be affected by such competition binding. Only an in vivo tumor xenograft model in mice, e.g. ovarian cancer cells growing i.p., could provide a clue how sEpCAM in biological fluids affects tumor targeting and destruction, and whether its quantification may be useful for therapy decisions.

Despite proof of concept that sEpCAM can be produced by cytologically positive ascites and may compete anti-EpCAM ligand binding on the cell surface, it would
be of interest to measure circulating sEpCAM levels as a marker for tumor targeting and malignant progression also in the blood as this is the more common clinical condition. However, I assume that this will be far below the detection limit.

In the whole Fig. part incl. Fig. legends the name catuxumumab (?) is used.

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

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

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

**Declaration of competing interests:**

I declare no competing interests.