Reviewer’s report

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

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Reviewer: Narendra v Sankpal

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Review of resubmitted manuscript

In the current resubmitted manuscript authors describe soluble EpCAM presence in body fluids can neutralize Catuxumomab antibody therapy.

This is very interesting and important concept in treatments with antibody therapies.

Readers will expect to see
i). sEpCAM presence patients samples. Authors have demonstrated in 100 samples.

ii. Although logically it’s acceptable that sEpCAM binding to Catuxumomab may loose its ability to bind cancer cells. It’s important to demonstrate this concept with more experiments to accept this important hypothesis.

Major Compulsory Revisions

Although manuscript is still weak to meet the authors hypothesis can be improved. To prove that sEpCAM from body fluid neutralizes Catuxumomab and fail to target tumor tissue. Authors need more experiments to convince.

Authors have raised question with data in Figure 6. With previously reported observation that soluble EpCAM can also induce EpIC cleavage from surface EpCAM and activate wnt signaling. In this situation blocking sEpCAM with Catuxumomab mediated therapy can help cancer patients?: need to discuss.

Following experiments can improve manuscript.

1. Experiments with HEK293 in Figure 6 cells are convincing, but need to show in at least two cancer cell lines with secreted EpCAM (EpCAM positive cells) or spiked EpCAM (EpCAM negative cells).

2. Need to show Catuxumomab binds sEpCAM in ascites. As Catuxumomab is bi-specific. (is there data cleaved or sEpCAM and surface EpCAM have different binding affinities to Catuxumomab?)

3. Cell death assays need to be done with appropriate and independent techniques.

4. Need to cite literature on clinical trial catumaxomab and their results, and
discuss how your observation can help in future trials.
5. Reduce discussion part appropriate to manuscript data.

Minor Essential Revisions
1. Flow cytometry data for dying YFP can be misleading as dead cells fluoresce.
2. Data can be added in supplementary Figure 1, Figure 2.
3. Appropriate references on clinical trials and EpCAM in body fluid/ serum.