Reviewer's report

Title: Putative contribution of Natural Killer cells in cetuximab treatment efficacy in first-line metastatic colorectal cancer patients.

Version: 1 Date: 29 January 2010

Reviewer: Niels Halama

Reviewer's report:

Dear editors,

I was given the opportunity to review the work by Raphael Marechal and colleagues on the topic of CD56+ immune cell density in colorectal cancer primary tumors and the relation to cetuximab-based treatment outcome. The authors use experimental data from experiments with PBMCs to support the results from the clinical analysis. The authors propose a very interesting hypothesis and present interesting experimental and clinical findings. In conclusion I recommend publication after revision. Below are the major issues the authors need to address before the article is suitable for publication.

1. (MAJOR COMPULSARY REVISION, key point) The ambiguity with CD56: staining with CD56 does not produce a homogeneous cell population of NK cells. Instead there is a mixture of cells, which makes it extremely difficult -especially in cancer- to pinpoint the real culprit. As the authors do not have used other means to delineate the major contributing cell type, I would recommend re-phrasing some passages in the manuscript, as they relate to NK cells (e.g. the title could be: Putative contribution of CD56 positive immune cells in cetuximab treatment efficacy in first-line metastatic colorectal cancer patients). The authors are obviously aware that this ambiguity exists, but do not comment on it and are somewhat hesitant to address this problem. I also would suggest to include references related to CD56+ immune cell populations (Ortaldo et al. 1991 Cell Immunol, Ohkawa et al. 2001 Immunology, etc.). That problem with the ambiguity of CD56 cell populations is not solved by the IL-2 stimulation, as also activated T cells are stimulated and the NK-like subtype (CD3 / CD8 positive, CD16 negative) likewise.

2. (MAJOR COMPULSORY REVISION, key point) The quantification of CD56 positive immune cells is not adequately explained. The authors state that they
analyzed
a given area, but the precise size of this area per patient is not explained. Given
the small cohort of patients, the
basis for solid evaluations is the reproducible quantification. As a sideline: the
cohort size itself is not a problem.
The Evaluation of CD56 could be a problem. To assess the precision (and allow
criticism on that point),
the reader should have a tissue surface area that was evaluated.

3. (MINOR ESSENTIAL REVISION) On page 5 the authors discuss the possible
role of T regs (FOXP3 positive), but do not reference the body of work that shows
a positive effect for higher T reg densities (e.g. Salama et al. 2008, Nagorsen et
al.). That should be added.

4. (MINOR ESSENTIAL REVISION) Use of undefined abbreviation: LTCD8,
LTCD4, LT on page 7.

5. (MINOR ESSENTIAL REVISION) Not precise: "CD56 positive tumors" on page
10. I assume the authors wanted to say: intratumoral CD56+ lymphocytes.

6. (MAJOR COMPULSORY REVISION) The authors need to adress on
equestion more clearly: is the KRAS mutation status related to the CD56+
lymphocyte density? It remains a little vague throughout the manuscript and
therefore should be made clear to the reader.

7. (MINOR ESSENTIAL REVISION) On page 11 again: "These data suggest that
CTX was able to activate ADCC against colorectal cancer cells..." is meant for
the periphery, the intratumoral situation is not clear. The sentence should be
corrected to make that point clear.

8. (MAJOR COMPULSORY REVISION) In the discussion the authors should
discuss, that they only used an EGFR+ cell line and no control EGFR- cell line. In
principal, the EGFR antibody could induce an activation of effector immune cells
regardless of EGFR status. The authors have not discussed that aspect.

9. (MINOR ESSENTIAL REVISION) On page 12 the authors state that CD56 is
expressed on NK, NKT and polymorphonuclear cells. They should also mention
that CD56 is expressed on activated T cells (Ortado et al. 1991, Ohkawa et al.
2001).

10. (MINOR ESSENTIAL REVISION) On page 13 the authors discuss the low
CD56 cell number. Here the observed cell numbers should be mentioned directly
to give the reader a feeling for the situation.

11. (MAJOR COMPULSORY REVISION) The focus in that paper should (see
commentary 1+2) be on the experimental findings of PBMCs and then
additionally the clinical data. If the evaluation of CD56+ cells is robust (from a
statistical point: imagine a cell density of median 0 cells / mm² measured only on
1 mm²!) then the phrasing can be left unaltered, if the evaluation basis however is shaky, then this should be addressed as an addition.

12. (MAJOR COMPULSORY REVISION) There is a mistake in labelling figure 2 (which exists twice) and also the labels in the bar graph are misleading or erroneous. That should be checked.

GENERAL COMMENT: There are so many typos in that manuscript that I strongly recommend corrections by the authors. There are so many typos that I will not present all here. As examples: page 4 "in third-line the anti-EGFR therapies" should be "in third-line of the anti-EGFR therapies", page 5 "analyze" should be "analysis", "Darmstadt" should be "Darmstadt", "administered" should be "administered", etc.

Summary: I believe that the authors addressed a interesting hypothesis and that this manuscript should be published after a thorough revision. The patient cohort is small but still informative, given that the evaluation is based on enough tissue areas. The implications for clinical benefit are possible and the results spark novel investigations, so that I encourage the authors to invest time in a thoughtful revision.

Level of interest: An article of importance in its field

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests