Reviewer's report

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

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Reviewer: Anthony Goncalves

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General comments:

This is an interesting study, the objective of which was to investigate a potential role for NK cells, critical components of ADCC process, in cetuximab-activity in metastatic colorectal cancer (mCRC). Authors retrospectively assessed by IHC staining the prognostic impact of tumor-infiltrating immune cells on primary tumor, in 33 chemonaive synchronous mCRC patients receiving cetuximab-based chemotherapy as first-line treatment, following surgical resection of the primary. No significant link was observed between macrophages (CD68+ cells) and T lymphocytes (CD3+, CD4+, CD8+, Foxp3+ cells) cell-infiltrates and clinical outcome. However, authors found a subset of tumors with a low number of immune cells strongly positive for CD56, CD56 being a marker of NK cells. CD56+ tumors were significantly more frequent in patients responding to cetuximab-based treatment versus non responders. Moreover OR and PFS were significantly higher in patients with CD56+ versus CD56- tumors. In a control population of 35 synchronous mCRC patients receiving chemotherapy without cetuximab following surgical resection of the primary and that had been matched to the cetuximab group on main clinical parameters, no such associations were observed, suggesting a specific predictive value for cetuximab benefit. While KRAS mutational status was also found to be associated with OR and PFS, a multivariate model including KRAS and CD56+ infiltrate revealed that both parameters had an independent prognostic value. Finally, an in vitro ADCC activity was observed with PBMC from mCRC patients incubated with CRC cells and cetuximab, which was significantly reduced after PBMC depletion of CD56+ cells.

Since only less than 50% of WT-KRAS mCRC patients seem to benefit from cetuximab-based treatment, it is a very relevant issue to identify other biomarkers allowing to predict cetuximab efficacy. The question they posed is well defined and relatively appropriately assessed by the experimental design, even though there are some limitations to be stressed. Methods are clearly described. Title and abstract accurately convey what has been found and the writing is acceptable.

Data look sound but need to be further clarified and some limitations have to be discussed in the discussion section (see below).
- Major Compulsory Revisions

1- The essential question raised by these results comes from the nature (prognostic or predictive ?) of the potential biomarker they found (tumor-infiltrating CD56+ cells). The absence of significant impact on clinical outcome of CD56 infiltrate in the control group, not receiving cetuximab, makes the authors preferring a cetuximab-specific predictive rather than prognostic value. However, table 2 lacks some data to be more convinced. Specifically, only p-values and not HR are shown for OR and PFS, according to CD56 status. I think HR should be presented (or at least cited in text for CD56). Actually, since p-value for PFS is 0.14, I think it would be interesting to have an idea of the HR: there could be a trend for better OR and/or PFS in CD56 + tumors in this control group, as in the cetuximab group, but that does not reach significance, putatively due to the small sample size.

On a more general level, and whatever the value of the HR, this comparison with such a limited matched-control group should be considered as hypothesis generating and this should be clearly stated in the discussion section. As should be stated that the data presented here do not exclude a prognostic effect not related to cetuximab, and that only analysis of prospective randomized trials may solve this issue.

2- Another general question relates to the putative role of ADCC in anti-EGFR mab activity. How can the authors conciliate a putative major role for ADCC with the observation of a similar activity for cetuximab and panitumumab, in spite of the theoretical low ability of IgG2 (panitumumab) to recruit immune effectors compared to IgG1 (cetuximab) ?

3- Regarding to the in vitro ADCC activity data, no statistical tests are provided, whereas differences are considered as significant. These data should be provided.

4- It would be interesting to see data (which are probably already available) about the level of CD16 on peripheral NK cells: it could be altered in colorectal patients compared to controls before IL2+ cetux stimulation. If there is initially less CD16 express on NK cells isolated from the patient, that could explain the slightly lowest response observed.

3- No markers for polymorphonuclear cells have been evaluated despite their potential implication in ADCC. This should be discussed.

5- ADCC is performed on PBMC but authors do not prove that PBMC reflect what’s happening in the tumor. Indeed, we now know that the phenotype of NK cells infiltrating the tumor is totally different from the peripheral NK cells (Ti NK cells are usually CD56 bright CD16 NEGATIVE and are unable to do ADCC). This should be discussed. Regarding to this question, it would have been interesting to look at CD16 on IHC package to prove that there is a correlation between that what is observed in the blood might occur in situ.
- Minor revisions

1- There is a problem with figure numbers: there are 2 “figure 2”. In the first figure 2, the diagram on top refers to “health volunteers”: it is probably a mistake since this seems to present CD56+ cells/mm²; that should be changed for “control group”

2- 1- Even though CD56 is a recognized marker of NK and NKT cells, it has to be noted that it is also expressed on cytotoxic CD8 and Gamma delta T cells.

3- The paragraph on the other immune cells is rather confusing because it does not bring additional information regarding the implication of NK cells in cetux efficiency. It would have been more interesting to look at the correlations between NK and Treg infiltrates… => More Treg, less NK…

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.

Declaration of competing interests:

I declare that I have no competing interests