Author’s response to reviews

Title: Putative contribution of CD56 positive cells in cetuximab treatment efficacy in first-line metastatic colorectal cancer patients

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Author’s response to reviews: see over
Dear Editor,

Please find in attachment the revised manuscript intitled “Putative contribution of CD56 positive cells in cetuximab treatment efficacy in first-line metastatic colorectal cancer patients” that we conformed to the journal style.

All named authors have agreed to the submission and have participated in the study to a sufficient extent to be named as authors.

Thank you in advance for your attention and I am looking forward to receiving some news from you soon.

Yours sincerely

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Reviewer comments

We thank the four reviewers for their helpful comments and we provide now the answers as well as the revised manuscript.

• Reviewer #1: Niels Halama

1. We used the term CD56 positive cells instead of NK cells since CD56 does not produce a homogeneous cell population of NK cells but a mixture of cells.
2. The quantification of CD56 cells has been more widely explained (pages 6-7).
3. The prognostic role of FOXP3 positive immune cells has been mentioned in the introduction (page 5) and adequate references reported.
4. Abbreviations LTCD8, LTCD4 (page 7) have been defined.
5. CD56 positive tumors are defined on page 10 as tumor with positive CD56 staining while CD56 negative tumors are tumors with undetectable CD56 staining.
6. The CD56 lymphocyte density was found to be not correlated with the KRAS status. This result has been added. (results section, page 11).
7. The difference in the ability of intratumoral and peripheral NK cells to elicit ADCC is discussed in the discussion (page 15).
8. We discussed the fact that ADCC against colorectal cancer cells require the expression of the EGFR at the cell membrane and the binding of CTX to the EGFR.
9. We mentioned that CD56 positive cells is expressed by activated T cells and the Ohkawa reference has been added (page 15).
10. The mean number of CD56+ cells has been mentioned directly in the discussion (page 14).
11. We change the focus of the paper: the experimental findings and additionally clinical data. We clarified in the methodology paragraph, the quantification of CD56. The cell density has not been evaluated only on 1/mm², but the cell count was expressed per millimeter square.
12. The figure label has been modified.

• Reviewer #2: Frederica Di Nocalantonio:

Major compulsory revisions

1. No correlation between intratumoral CD56+ cells count and the KRAS status was found. This result is reported in the results section (page 11).
2. One aim of our study was to evaluate in a KRAS mutated CRC cell line over-expressing the EGFR whether the cetuximab can activate ADCC, the role of CD56 positive cells in ADCC activity and if the impact of intratumoral immune infiltrate on cetuximab-based therapy efficacy. We used a K-ras mutated cell to assess the impact of ADCC in such condition because the K-ras mutation is the only validated predictive marker for the clinical practice and ADCC property of cetuximab could be of interest in K-ras mutated tumor. Furthermore, the cetuximab-induced ADCC has been reported to be independent of the K-ras status (Barrière J et al. ASCO 2009).
3. We globally discussed about the role of ADCC in the clinical activity of CTX and mentioned data that argue for and those suggesting that ADCC has no impact in the clinical efficacy of cetuximab (discussion section, pages 12-14).
4. We mentioned that the small number of analysed sample is a limitation to our conclusions (discussion section).
5. We stated about the potential of translating our preliminary findings to clinical trial (discussion section, page 16).

Minor essential revisions

6 + 7: The first paragraph of the discussion that contains general consideration and no relevant informations has been deleted as suggested by the reviewer #3.
8: synonym of evaluate has been added
9: The figure legends have been reviewed and modifications have been amended through the text.

Minor points:

Page 6: space between the following words “tumors” and “were”, has been added
Page 7: histologically instead of histologicaly
Page 7: dot has been deleted in “American culture type”
Page 7: sodium pyruvate instead of pyruvate sodique
Page 12: we deleted extra punctuation mark at the end of the sentence
Hazard ratios have been included
Page 9: saturating instead of saturing

• Reviewer # 3: Frederic Di Fiore

1. The manuscript has been shortened as well as possible. Some sections of the discussion has been deleted.
2. The first part of the discussion has been deleted
3. The results of the table 2 of the appendix have been included in the manuscript.
4. We notified the characteristics of the DLD1 cell lines and the reason of the choice of this cell lines (Patients and methods section, pages 7-8)
5. We mentioned that we choose the PFS rather than the OS as end-point of our study, because we focused our work on the potential predictive markers of ctx activity. To this end, the PFS is a more appropriate end-point than the OS which could be influenced by the efficacy of second-line and third-line chemotherapy but also by performing or not surgical metastasis resection (page 6).
6. The criteria of choice for variables selection in the multivariable model has been added in the methodology section (pages 9-10).
7. Discussion section has been modified to include sentences about the main results of the study.
Reviewer #4: Anthony Goncalves

Major Compulsory revision

1. The hazard ratios have been added in the table. We mentioned in the discussion that CD56 tumor status could be prognostic
2. We rule out panitumumab for ADCC
3. We provided statistical tests for the *in vitro* study
4. The level of CD16 on peripheral NK cells has been evaluated for the two groups (health volunteers and mCRC patients) and no significant difference was observed. We did not add this result in the manuscript.
5. We discussed the impact of polymorphonuclear cells in ADCC
6. We discussed the fact that phenotype and properties of intratumoral and blood CD56+ cells are different but we did not performed the CD16 immunostaining.

Minor revisions

1. The figure numbers have been controlled
2. We mentioned that cytotoxic CD8 and gamma delta T cells harbour CD56 (discussion section, page 15)
3. The discussion section has been modified