Author's response to reviews

Title: Early increase in circulating carbonic anhydrase IX during neoadjuvant treatment predicts favourable outcome in locally advanced rectal cancer

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Version: 4 Date: 2 June 2015

Author's response to reviews:

Responses to the concerns raised by Reviewer 1:

Major Compulsory Revisions:

1. Responding to the reviewer’s request, more information about the sample collection procedure and processing is given in L113-117 of the revised manuscript.

2. The reviewer seeks clarification to the number of patients in Figure 1 versus the numbers given for the remaining analyses.

Response:

Please, see the below response.

3. The reviewer recommends that patients who had samples at baseline but not at the post-NACT sampling point are excluded from the initial analyses since we use the parameter [delta]NACT in all of the outcome analyses.

Response:

We thought this was a brilliant idea and have implemented it throughout. The exclusion of patients that did not have a post-NACT sample has resulted in altered numbers of cases at baseline (now the same 66 cases as at post-NACT) and also at post-CRT (n = 54 now) and evaluation (n = 50 now). The corresponding edits can be seen in L95, L97, L109, L111-113, L137, L182-183, L184, L185-186, L197, Figure 1 and its legend and Table S1. The amendments of the Table S1 data resulted in a statistical correlation between the absolute level of serum CAIX at post-NACT and progression-free survival, as indicated in L189-191.

4. The reviewer encourages us to discuss more about limitations of the study—that the results are preliminary observations of a small number of
samples and the use of the Nordic FLOX regimen.

Response:
Limitations associated with the small patient cohort are discussed in L252-261. Possible concerns related to the FLOX regimen are discussed in L312-317.

5. Responding to the reviewer’s recommendation, the ROC graph is included as Additional file 2: Figure S1.

Minor Essential Revisions:
1. Information of the PFS rate of 56% for patients below the [delta]NACT threshold is included in the Abstract L39-40.
2. The wording of the conclusion in the Abstract is amended in L44.
3. In the revised Table 1, the definition of the two patient groups (below and above the [delta]NACT threshold) is included. Moreover, the type of statistics used for each calculation is clearly indicated.
4. The expression “were balanced” is specified in L213.
5. The reviewer suggests the title of the article should reflect the specific use of the Nordic FLOX regimen in this study.

Response:
After careful consideration, we have chosen to maintain the title unamended. With reference to findings by others (Reference no. 16), we believe it is not clear whether the [delta]NACT response was contingent on the Nordic FLOX regimen as such or would have been observed as an early CAIX response irrespective of the therapeutic modality. This is discussed in L301-317. Please, do also refer to the above Major Revision no. 4 response.

Discretionary Revisions:
Yes, we originally considered to perform immunohistochemical analysis but were not able to retrieve tumour tissue from all of the study patients.

We had already included CEA in our analysis; cf. Table 1 and Table 2.

Responses to the concerns raised by Reviewer 2:
Minor Essential Revisions:
1. The recommended wording is implemented in L70.
2. Assay performance metrics are reported in L130-134.
3. The wrong word is deleted from L231-232.
4. A proposed mechanism for the increased shedding of CAIX extracellular domain is discussed in L301-317.
5. The sentence is improved in L280-281.
6. The inadvertently omitted word is inserted in L335.
7. The sentence is improved in L350.