Reviewer's report

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

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Reviewer: Andrea Lampis

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Major Compulsory Revisions

1 Serum sampling row 111: The authors should provide more information of sample collection procedure and processing (handling time before processing, tubes type, methods and storage time before analysis) in order to give an indication of methods and storage stability of the biomarker analysed.

2 Results rows 170-172: The authors state that fig 1 Baseline illustrates the analysis on 87 patients and subsequently in row 172 n=85 as in row 107. This should be clarified.

3 From baseline to post-NACT and post-CRT there is a loss of 17-23 patients’ serum analysis. Authors should consider excluding from baseline level analysis patients that do not have paired serum at post-NACT as the proposed parameter #NACT has been calculated as a cut-off generated from a baseline matched median level.

4 The authors should discuss more about limitations of the study: a) The results are preliminary observations as overall analyses include only a small group of matched timepoints patients b) the use of Nordic FLOX regimen as neoadjuvant chemotherapy before chemoradiotherapy is not a standard of care outside northern countries.

5 Row 190: ROC analysis graph should be added as it generates the threshold proposed.

Minor Essential Revisions

1 Row 39: The value below threshold (56%) should be included.

2 Row 44: “Absolute changes” as is not clear, should be substituted by “increase”

3 In the table 1 given is missing, in the two groups “#NACT”, who is above/below the threshold (< or > 224pg/ml). In the same table three types of statistics has been used but it should be specified or marked where in each part of the table.

4 Row 201-203: The verb “were balanced” is not clear. The sentence should be reformulated.

5 The title should be reformulated including the specific treatment after neoadjuvant word as: (Nordic FLOX regimen) to point the result is clearly associated to that specific treatment.
Discretionary Revisions

Even though the authors found significant association of higher #NACT as better PFS predictor they did not find association of the #NACT with TGR score but only with tumour down-staging and node sterilization: did the authors consider in performing an analysis on patients tumours (Immunohistochemistry) for CAIX in order to possibly correlate expression in serum with that of the tumour? Moreover the source of CAIX is not entirely clear as they mentioned in the discussion, so this can give an indication of cell source expressing CAIX that contribute to therapy response assessment.

Should the authors have considered to add another parameter in the analysis as for instance CEA, already showed as a good indicator of PFS in LARC (i.e: Buijsen J et al 2014) at the time points used to attempt improving the sensitivity?

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