

Point-by-point response reviewers

Point 1: - The problem with this study, as the authors acknowledge, is the sample size. This is probably the underlying cause for the lack of significance of the described differences. Since the role of chance in the found differences cannot be ruled out, it seems a bit strong to say that “Serrated pathway associated molecular features are more common in FIT-interval CRCs”. I would rephrase it to “seem to be” or “may be”.

Response: we have rephrased the sentence in the abstract to “seem to be” as requested.

Point 2: The lack of statistical significance does not automatically mean a lack of clinical significance. But we cannot figure this out because the confidence intervals are not provided. Knowing the upper and lower bound of the difference in proportion (e.g. 7% in MSI FIT-interval CRC vs 14% SD-CRC) may give us information about how big or small the true difference might be.

Response: we agree and we have added the mean of the difference in proportion and 95% CI for each item (MSI, CIMP and all mutations). We added this extra analysis also in the methods section.

Point 3: In 14% (8/54) and 7% (2/27) of cases, DNA was not available. In other cases, the quality of reading for mutational analysis was not enough and they were excluded. In 22% of cases, DNA was not of enough quality. Despite using a manual extraction method these are quite high figures when dealing with low sample sizes and could have impacted the final results. This should be discussed in the “Discussion” section.

Response: In 14% (8/54) and 7% (2/27) of cases, tissues were not available, therefore no DNA could be isolated (12% of cases). From the 46 and 25 cases, from which we could retrieve the tissues, we obtained good quality DNA in all cases. However, in downstream analysis, the quality of the reads was not always sufficient and therefore, some cases were excluded (See material and methods section and Supplementary Figure 1). The reason for this could be the existence of DNA cross links, frequently present in formalin-fixed, paraffin-embedded material, for which some sequencing techniques are sensitive to.

We have added now a sentence in the discussion:

“Moreover, due to inherent formalin-fixed, paraffin-embedded associated artifacts, like DNA cross-links, the quality reads of some of the downstream analyses was poor and therefore some of the selected cases were further excluded from the final analysis (supplementary Figure 1).”

Point 4: We do not know the familiar history of patients with cancers included in the study. Albeit rare, some of the interval CRCs might be related to some kind of familial cancer.

Response: we agree with the reviewer. We have added this limitation to the discussion.

Point 5: Change the comma for a dot in the values of the last row in table 1, page 17.

Response: we have adjusted as requested.