

Dear Editor,

Please kindly consider these responses to the reviewers comments.

Reviewer's code: 00057665

I have the following comments and questions:

Why were 160 patients included?

The aim was to obtain over 100 specimens for analysis and we were fortunate to obtain more than this and hence all available were included. We had access to archived pathological specimens treated in our unit for a 10 year period and all available specimens were retrieved and analysed.

Were they consecutive patients?

All patients were treated in our tertiary referral unit over a period of 10 years. All eligible/available patient specimens were assessed for suitability for inclusion in this study. Some histological biopsy specimens did not contain >60% adenocarcinoma tissue and hence were not included in the analysis. It is considered therefore that all patients were treated consecutively except where samples were not available for analysis or excluded for other reasons described in the text.

2. The percentage for males and females should be included. Is the distribution what one would expect in your population?

The percentage have now been included in the text. The male to female ratio is representative of our current practice.

3. It would be appropriate to have a better description of the group of patients with a complete response

Additional descriptions of this patient group has been added to the text

4. Could you provide data related to the quality of mesorectal excision for all cases?

Four patients (3%) had a demonstrated involved circumferential resection margin and this is included in the text. This is significantly lower than the UK national average of 5% reported in 2015 by the NBOCAP group.

<http://www.acpghi.org.uk/members/groups/nbocap/>

5. What was the statistical power of non-statistically significant comparisons?

A power calculation was not performed as it was not possible to predict the size of effect (if any) expected between responders and non responders. Furthermore, the sample size was dictated by the availability of histological specimens and it was considered that >100 would be reasonable to detect a clinically significant effect.

Reviewer's code: 00186128

Comments of the manuscript entitled "Extramural vascular invasion and response to neoadjuvant radiotherapy in rectal cancer: influence of DNA Methylation and the CpG island methylator phenotype": -

Title: extramural vascular invasion and response to neoadjuvant chemoradiotherapy (not only radiotherapy).....: influence of the CpG island methylator phenotype (why DNA Methylation) -

Thankyou for your comment, the title has been amended to reflect this

Table 1: the number of sex don't correspond with text (113 males and 47 females) -

Thankyou for your comment, this was a typo which has been amended

Table 2 and 3: The number of RCpath score is 159 and not 160 -

Thankyou for pointing out this typo which has been adjusted in the tables.

Results: the results of this study are very curious: no one relationship between CIMP status and survival. However CIMP status was significantly associated

with EMVI positivity which itself was associated with worse survival. It's not comprehensible.

The relationship between CIMP status and survival is complex and conflicting published evidence has been discussed in this paper in this regard. The interaction of the many clinicopathological and molecular features of individual rectal cancers may explain this complexity, however, the relationship between CIMP status and EMVI has not previously been identified and is therefore worthy of note. We have put forward putative mechanistic explanations for this relationship, however, these require further experimental investigation and may further explain the complex relationship between EMVI, CIMP and survival however, this is beyond the scope of the current paper.

Discussion: there are many paragraph and many information which are the same in the review of the authors in : Williamson et al. Clinical Epigenetics (2015) 7:70

The discussion has been reworked to modify this.

References: 42 and 26 ; 39 and 40 are the same.

Duplicate references have been addressed in the text and reference list

There is no recent references (2015 and 2016);

Further relevant references have been added to the discussion

Why there isn't your preliminary results edited in Gut 64(Suppl 1):A354.1-A354 ? June 2015

The report from our research group referred to in the reference above was performed in a separate group of patients who did not receive neoadjuvant chemoradiotherapy and therefore could not be directly compared to the patients in the present study

Reviewer's code: 00041468

In the basic study of Williamson et al. the authors aimed to identify whether CIMP status is predictive of response to neoadjuvant chemoradiotherapy and outcomes in rectal cancer. They found that a novel association of CIMP status with extramural vascular invasion which represents an adverse prognostic indicator and provides a novel insight into potential mechanisms for the association of poor survival with CIMP-H rectal cancers. The study is well-designed and presented. The results are all clear and understandable, the descriptions of methods and materials are also clear. However, some -mainly format- issues need revision: -

Introduction is too long, it must be shortened.

The introduction has been made more concise

Regarding histopathological reports, how was the interobserver variability among the pathologists?

The variability was low with almost perfect agreement. Within the manuscript text we have commented "When examining tumour regression scores, to ensure there was agreement between the two pathologists scoring the regression, Cohen's Kappa statistic was utilised to measure agreement between both raters. For the Royal College of Pathologists tumour regression score there was almost perfect agreement ($k=0.856$ $P<0.001$)."

Core tip and keywords are missing, they must be inserted into the text. - The acknowledgement and conclusion must be changed. - Some typos need correction. After major revision, I suggest to accept the manuscript for publication.

Core tip and Keywords have been inserted into the manuscript.