May1, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 17416-review.doc).

Title: Intensified Intensity Modulated Radiotherapy in Anal Cancer with prevalent HPV p16 positivity.

Authors: Liliana Belgioia, Stefano Vagge, Dario Agnese, Stefania Garelli, Roberto Murialdo, Giuseppe Fornarini, Silvana Chiara, Fabio Gallo, Almalina Bacigalupo, Renzo Corvò

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 17416

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer n° 17416
It is an interesting paper about anal canal cancer treatment but it is not the first publication in this field. The follow-up is a little bit short.

a) We need more information about the population: histologic characteristics etc…
ANSWER: Thank you for the observation. We have added more information about population in Table I.

b) How are defined low-risk and high-risk categories? Is it important because, all the patients seem to be in the high-risk category?
ANSWER: Thank you for this observation; this point was clarified, we have eliminated the division in high and low risk and we have tried to better explain the dose prescription system to target volumes as described in the text.

c) Paragraph population: Is the last sentence at the good place?
ANSWER: Thank you for your observation, we have added a new paragraph untitled "HPV detection".

d) Toxicity : I don't understand the results, how more than 50% of patients need major

analgesic therapy without grade 3 toxicity? we need for more information about pain.
ANSWER: Thank you for the comment, we checked again all data about toxicity, we have not registered grade 3 pain, but major analgesic is linked also to minor grade of pain (for example grade II) and especially to breakthrough pain at the time of defecation.

e) I don't agree with the authors when they state that the increase in radiotherapy dose is useful (page 10 and last sentence) (D Peiffert J Clin Oncol 2012). The authors must justify this opinion.
ANSWER: Thank you for this interesting comment: we agree with the reviewer because there are not randomized controlled trial that clearly demonstrate a benefit of high dose radiotherapy in anal cancer, but we consider that a positive trend in this direction is showed in some casistics (D Peiffert J Clin Oncol 2012). We have modified whole discussion and justified this aspect in the text.

Reviewer n° 02962220

Thank you for the opportunity to review your paper. Overall this is a good article but I found it difficult to read because of grammatical errors - this will need to be proof read again please. Some other comments to help improve your paper.

MAJOR ESSENTIAL CHANGES:

a) I found it difficult to follow the discussion. As it stands it is one big paragraph with no obvious structure. Can I suggest you please break this up into several paragraphs using topic sentences to introduce each paragraph. A good discussion will include the following points 1) Statement of principal findings 2) Strengths and weaknesses of the study 3) Strengths and weaknesses in relation to other studies, discussing particularly any differences in results 4) Meaning of the study: possible implications for clinicians and policymakers 5) Unanswered questions and future research
ANSWER: Thank you for this interesting suggestion; whole discussion has been revised and we have divided it in paragraphs. We hope that this changes can make it clearer.

MINOR ESSENTIAL CHANGES:

b) Abstract: Aim - could you define if you are looking at intracanal or perianal cancer?
ANSWER: thank you for the observation, all patients presented intracanal cancer.

c) Introduction - has SIB been used for other cancers besides anal cancer? Can you please reference?
ANSWER: thank you to the reviewer for the comment, in the introduction section we have added some references about SIB in other disease like head and neck or gynecologic cancer.

d) Materials and methods - population - could you please describe your Institute - is it a tertiary hospital? private? public? urban? regional? pg 6 under toxicity and follow up - what sort of follow up investigations were done at 3 monthly intervals and then 6 monthly intervals? pg 7- can you please reference the Common Toxicity Criteria for Adverse Events and RTOG criteria?
ANSWER: thank you for these observations, all this points have been precised in the text and we have added references where required.

e) Results - there seems to be an unusually large skew of anal cancer to women. Is this because the males did not want to participate in the research?
ANSWER: Anal canal cancer incidence is major in female than male gender, as reported for example by NCCN guidelines, female prevalence in our study is comparable to that reported in literature.

f) Please clarify. pg 8 - under chemoradiotherapy - could you specify the hematological toxicity and cardiovascular disease you mention in the last line?
ANSWER: thank you for the comment; we have clarified this points in the text.

Reviewer n° 17416
This paper reports on the 2-years outcome of 41 consecutive patients with anal cancer treated with HT using a simultaneous integrated boost technique. I have some comments:

a) Material and methods: The authors need to clarify if the study represents a retrospective series or data analyzed have been prospectively collected.
ANSWER: thank you for the comment, our study is based on a retrospective analysis.

b) Dose constraints for OARs used for inverse planning are not mentioned in the text. The authors observed a relatively low rate of hematological toxicities compared to other studies: did they apply dose constraints for iliac crests to reduce hematological side effects? A table illustrating the OARs dose constraints and dosimetric results would be welcomed.
ANSWER: thank you for the comment, we agree with the reviewer, we have added a table with constraints (Table I).

c) The authors should clarify if local failure concerned the relapse in the anal canal only or in the pelvic nodes too. This point is not clear (paragraph toxicity and follow-up). ANSWER: thank you for the comment, local failure concerned anal canal and/or pelvic nodes relapse.

d) Were the tumors located in the anal canal only or some lesions were found in the anal margin too?
ANSWER: thank you for the comment, all tumor originated from anal canal, we have clarified this in the text.

e) The authors did not find in their cohort of patients any prognostic value of the HPV p16 positivity. However, recent literature showed a clear prognostic value of HPV infection with better prognosis in positive patients. As this point constitutes a major point of the paper (cfr title of the manuscript), the author should better comment on that on the discussion section.
ANSWER: thank you for the comment, whole discussion has been modified and HPV role better discussed.

f) Why late toxicities were assessed only at the 6 month follow-up endpoint?
ANSWER: thank you for the comment, we modified results showed in table IV and referred to late toxicity registered at last follow up.

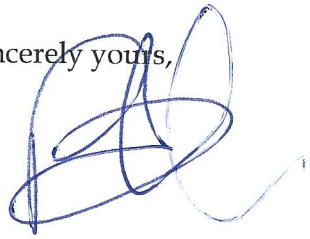
g) Kaplan-Meier curves: Please report on colostomy-free survival rates and KM estimates for stage I-II vs stages IIIA and IIIB.
ANSWER: thank you for the observation, we have added this points in the manuscript.

h) Minor comments: - Results: some data (age, gender) are repeated twice in the manuscript (result section and Table I). - - Tables II and III: please add Grade 0 toxicity and report on the total number of patients analyzed - - Figure 1. Please add numbers of patients at risk to the three figures.
ANSWER: thank you for the observations, all these points have been modified.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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