ANSWERING REVIEWERS

April 16, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2836-review.doc).

Title: "Somatic molecular changes and histo-pathological features of colorectal cancer in Tunisia"

Author: Sana AISSI, Marie Pierre BUISINE, Farid ZERIMECH, Nadia KOURDA, Amel MOUSSA, Mohamed MANAI, Nicole PORCHET.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 2836

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
(1) 00053417
(2) 00057100

3 References and typesetting were corrected

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

Sincerely yours,

Sana Aissi,
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Pr. J L Wang
Director, Editorial Office
World Journal of Gastroenterology

Tunisia, 15 April 2013

RE: Manuscript number 2836, Version 1: "Somatic molecular changes and histo-pathological features of colorectal cancer in Tunisia" by Sana Aissi et al.

Dear Professor Jin-Lei Wang,

Please find in attached file a copy of our revised manuscript entitled: "Somatic molecular changes and histo-pathological features of colorectal cancer in Tunisia" by Sana Aissi et al submitted to be published in World Journal of Gastroenterology in the category "original article".

To facilitate further review, here are our responses to the comments from the Reviewers.

We hope that, in this revised version of our manuscript, all points of criticism will have been discarded to the satisfaction of the reviewers.

Sincerely yours,

S. Aissi

RE: Manuscript number 2836, Version 1: "Somatic molecular changes and histo-pathological features of colorectal cancer in Tunisia" by Sana Aissi et al.

Responses to the comments from the Reviewers:

Reviewer *00053417*:

There was no suggestion.

Reviewer *00057100*:
Please find in the revised manuscript a response to your suggestions:

1. It is not clear why the authors selected normal mucosa from different patient who underwent CRC resection. Why they did not selected normal mucosa from the same patient? Are the samples from the 51 primary colorectal carcinomas from family members?

Response: No, we have collected the primary colorectal carcinoma and the corresponding normal bowel of 51 not related Tunisian patients who had undergone colonic resection for the treatment of colorectal cancer. 25 of these 51 patients belonged to families fulfilling the Amsterdam criteria[19] for the clinical definition of HNPCC or fulfilled at least one criterion of the revised Bethesda criteria for the identification of HNPCC patients[15].

2. The designed primers for the TP53 mutation screening must be described. It will allow future authors to use them if necessary. The length of the exon and introns should also be described.
Response: Primer sequences and PCR conditions are available on request. In other hand we have submitted an article describing in detail the type and the frequency of TP53 mutations which is under review.

3. Mutation screening for KRAS, CTNNB1 and BRAF genes were screened using sequencing. The primers used or the reference should be described.

Response: Primer sequences and PCR conditions are available on request. In addition we have submitted an article describing in detail the type and the frequency of KRAS mutations which is under review.

4. The first sentence in BRAF mutations results should be re written.

Response: The sentence " The $BRAF^{V600E}$ (c.1796A>T, p.Val600Glu) activating mutation in exon 15 shown to be specific to CRC sporadic tumors due to *MLH1* promoter hypermethylation and absent in MSS CRC tumors (37) and patients with *MLH1* or *MSH2* germline mutations (11) was found in only one (1/51, 2%) stage II non- mucinous and non-invasive tumor of the proximal..." become "The *BRAF* activating mutation c.1796A>T, p.Val600Glu was found in only 1 (1/51, 2%) stage II non- mucinous and

non-invasive tumor of the proximal colon of a 79 years old man with no cancer family history. This mutation was shown to be specific to sporadic CRC tumors due to *MLH1* promoter hypermethylation and absent in CRC tumors with MSS phenotype[31] and patients with *MLH1* or *MSH2* germline mutations[11].

5. Table 2, I recommend to write only the significant results. The other option is to change NS for the actual p value. It is not appropriate to write numbers and NS. It seems that all results are significant using chi2. In that case to write in the table chi2 is unnecessary.

Response: According to you can I omit this table 2 without affecting the clarity of my paper as variable's values are listed in the text?

6. The authors can write a small sentence in the legend of the table remarking that (as they did in table 4, 5 and 6) Table 4. As in Table 2 Table 5. As in Table 2

Response: The legends of:

Table 2. Statistical analysis of clinicopathological parameters of the 51 CCR studied tumors as a function of tumoral phenotype

Table 3. Comparison of the somatic phenotype and genotype as a function of the patient's clinical characteristics

Table 4. Comparison of MSI phenotype as a function of tumoral parameters

Table 6. Comparison of *TP53* somatic mutations as a function of tumoral parameters

Becomes

Table 2. Statistical analysis of clinicopathological parameters as a function of tumoral phenotype

Table 3. Somatic phenotype and genotype as a function of the patient's clinical characteristics

Table 4. MSI phenotype as a function of tumoral parameters

Table 6. *TP53* somatic mutations as a function of tumoral parameters

7. It will be interesting to see some pictures of the immunohistochemistry

Response: I agree with you but unfortunately I can't pay for a color picture of immunohistochemistry and the black and white photo will not be clear for the readers.