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Institut national de la santé et de la recherche médicale
Centre Hospitalier Universitaire de Nice
Université Nice Sophia Antipolis
EA 6302 Tolerance Immunitaire

Nice, 24th August 2016

Dear Profs Ze-Mao Gong and Jin-Lei Wang,

RE: ESPS Manuscript No 28653, Original Study

Following our initial submission to the journal, we thank you for giving us an opportunity to re-submit a revised version of our paper titled: **'Dysregulation of innate immunity in ulcerative colitis patients who fail anti-TNF therapy"** by Baird and colleagues for publication in *The World Journal of Gastroenterology*.

We have taken all the reviewers comments on board and have now revised our manuscript accordingly. In addition, all minor grammatical and spelling errors have now been addressed and final version of the manuscript read and approved by native speakers (first and senior authors, AB, IL and MKT). Together, these new suggested changes strengthen our message illustrating previously undiscovered role for innate immunity in UC patients who fail to respond to anti-TNF therapy. These new data are likely to have important implications on future strategies for treatment of patients with gut dysfunction.

We thank you in advance for your thoughtful consideration of our manuscript.

Yours sincerely,

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EA 6302 Tolerance Immunitaire

Prof Meri K Tulic

RESPONSE TO REVIEWER 1- **00068574**

Comment: The objective of the paper is to identify alterations of immune functions susceptible to predict the lack of response to anti-TNF therapy in IBD patients. Major points In the first part of the paper the title: effects of anti TNF therapy, inflammation, medication and disease type on innate immunity does not correspond to the results. Why did the authors divide the patients into responders and non-responders?; it is confusing compared to the second part of the study.

Response: The main aim of the paper was to investigate if there were differences in innate immune function between the patients who responded and those that did not respond to anti-TNF therapy. Differences may be a useful tool in clinic to predict the success of such expensive therapy. In the first part of the study we examined whether inflammation, medication use, and patient demographics, were predictive of patient outcome (*response* or *non-response* to TNF therapy). These results suggest that whether an IBD patient will respond or not to anti-TNF therapy is not pre-determined by the ESR, CRP, CDAI or partial Mayo score, nor their medication use; hence a need for better marker of outcome. In the main part of the study we examined whether measuring innate immune function in these two groups could give us an insight into differential mechanisms operating and therefore a key to unraveling why certain individuals respond and others dont (part 2). This has now been made clearer in both the Methods and Results section of the paper (title of results section changed as well).

Comment: In addition, the number of non-responders is too small to be able to draw conclusions. For example, in table1b, the conclusion that ESR and CRP are not significantly different between responders and non-responders is not reliable.

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Moreover, analyzing the effect of anti-TNF therapy may be influenced by several confounding variables in particular in a small and heterogeneous sample of patients who showed different outcomes at the time of the second blood draw.

Response: We agree that the number of patients is not large however we have used stringent statistics analyses of non-parametric data (and not parametric t-tests for normally distributed data) to account for this as its routinely used in research. We do agree however that heterogeneity may be reduced with larger samples and therefore this may be one of the weaknesses of our study. As a result, we have included reference 28 which suggests that elderly patients may have more severe disease although in our cohort this is unlikely to explain the results as ages were smilar between test groups. We have also now discussed these in our manuscript (pages 27-28).

Comment: The second part of the study is correctly conducted and I suggest to remove the first part from the manuscript.

Response: Thank you for the positive comment regarding the second part of our study. However, we have refrained from removing the first part of the study as this forms an integral part of the paper; the results which support that medication use, patient demographics, surgery and anti-TNF therapy itself are not predictive of patient outcome (part 1) but their innate immune response to TLR agonists is (part 2). The data in part 1 support (and significantly add to) the message of the paper and suggest that it is not simply patients' behaviour, prior medication use, their environment or the use of other therapy that determines who is likely to respond but it is their constitutive ability to mount a correct innate immune response that determines their efficacy of anti-TNF treatment. Results of part 1 show that whether a patient will respond or not to anti-TNF therapy is not pre-determined by their ESR, CRP, CDAI or Mayo score, nor their medication use and other factors are responsible hence the need to assess TLR function. To help clarify the importance of this first section of the paper, we have clarified these Results (page 14) and have discussed

their importance in the Discussion (page 25) as well as in the Comments section (page 29).

Comment: Minor point -Please indicate the year in ref 9

Response: Thank you for pointing this out. The year of reference 9 is 2009. This has now been added on page 31.

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RESPONSE TO REVIEWER 2- **00227403**

Comment: In the section abstract, please specify the acronyms TNF, IBD and ELISA.
Response: TNF is tumor necrosis factor, IBD stands for inflammatory bowel disease and ELISA is enzyme-linked immunosorbent assay. These have now been added in the abstract (page 4).

Comment: In the section introduction the first sentence should be deleted.
Response: This has now been done (page 6).

Comment: In the section introduction, please clarify better "All TLRs signal through MyD88-dependent pathway except TLR3 while TLR4 can signal through both but requires CD14 (Figure 1)."
Response: We thank the reviewer for pointing this out as it was not clear enough. 'Both' signifies MyD88-dependent and MyD88-independent pathway and this has now been clarified on page 6.

Comment: The authors should write a simple and direct aim "Aim of our study was……". The remnant part should be deleted or reported before or after.
Response: The aim of the study is simplified and its potential clinical use mentioned in discussion of abstract.

Comment: In the text, please clarify the acronym, CD.
Response: CD stands for Crohn's disease. We have now added this explanation at its first mention (page 6) in the text.

Comment: In the section "Basal and stimulated PBMC cytokine production pre- and post-anti-TNF therapy" the authors should report, the differences (or the lack of differences) on the basis of IBD type (CD and UC).

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Response: Although this is a great suggestion, the numbers we have are too small to do extensive group analysis with multiple variables as it has been done in the second part of this study. This was not the subject of our current communication but would be great value to do in a separate, larger study.

Comment: I suggest to delete table 1c Pag 18, sentence "When compared to preanti-TNF therapy, medication use, ESR, CRP and partial Mayo scores was similar between responders and non-responders (Table 2a)." Was? Is it "were"?

Response: Yes, this sentence was superfluous as similar information is given in the previous sentence. We have therefore, as suggested by the reviewer now deleted this sentence.

Comment: Page 18, please could the authors clarified the sentence" Colectomy status at time of blood draw had no significant effect on response to anti-TNF therapy or the levels of basal or stimulated cytokine production (data not shown)."

Response: We apologize for this confusion. This sentence was to state that a number of patients have had a colectomy by the time of the second blood draw. The patient's who did not respond to anti-TNF therapy and had a colectomy and those who did not respond and did not have a colectomy were not different in any of the investigations undertaken in the study. On reflection, this sentence has now been removed as it does not add a lot to the results and does not contribute to interpretation of our data (page 19).

Comment: The authors should report as limitations of their study the small sample size with the mixed population (13 patients with CD and 5 with UC). Since the pathogenesis of CD and UC could be different, with the former conceived as the result of a prevalently increased membrane permeability, as opposed to the latter, which is thought of as an immune deficiency state (see for example Ardesia et al. The

aged gut in inflammatory bowel diseases. Minerva Gastroenterol Dietol 2015;61:235-47), this could influence the results.

Response: We have now acknowledged in discussion (pages 27-28) that a small sample size with the mixed populations is a limitation of our study and have now included the reference mentioned. Furthermore, the aim of this study was not to examine the difference between UC and CD patients but between *responders* and *non-responders* to anti-TNF therapy. This has been clarified throughout the text.

RESPONSE TO REVIEWER 3- **00068278**

Comment: In the presented article the authors aimed to predict anti-TNF response in IBD patients by means of alterations in immune functions. There are two parts of the study. The effects of disease, treatment and inflammation on innate immunity were evaluated in 18 patients. In the second part, the differences between responders and non-responders were evaluated in 24 patients. The study may add new knowledge to the current literature.

Response: We thank the reviewer for the positive feedback of the value of our study.

Comment: 1-There are grammar, vocabulary and spelling errors throughut the article; "expansive" used throuhout the test must be corrected as "expensive" Page 22, Differences in stimulated …… In general, responders had similar TNF, IL-beta … Reference number 9; the year of publication is 2009

Response: Thank you for pointing these out. We have now thoroughly read the manuscript and corrected any spelling and grammatical errors. The final version of the manuscript was read and approved by three native speakers (ACB, IL and MKT). As suggested, we have now also added the year for reference 9 (2009).

Comment: 2-In Table 1b: there are 6 and 4 patients receiving prednisone and 9 and 2 patients receiving corticosteroids in responders and non-responders respectivley. What is the difference between prednisone and corticosteriods? The small number of the patients in both groups may cause type 2 statistical error (for ESR and CRP, particularly. This situation must be discussed. The same criticisms are valid for Tables Ic and Table 2a.

Response: There is no difference between prednisone and corticosteroids but are shown separately as some patients who were taking prednisone were also taking

theopurine. The small sample size has now been discussed as a study limitation and a sentence added in discusson regarding the need to confirm these findings in a larger cohort of UC patients. In addition, all Tables have been amended accordingly.

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RESPONSES TO EDITOR

Comment: You'd better offer us one paper with word format next (such as, .doc or .docx), which is easy to be edited and helpful to accept earlier. Thank you very much!

Response: All files provided have now been given in .docx format for easier editing. We apologize for previous inconvenience.

Comment: Author listings, affiliations, postal codes etc …

Response: All of these comments have now been addressed and changed according to specifications.

Comment: Write a core tip summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Response: Core tip summary has now been added (page 5) (96 words).

Comment: Make an audio file describing your final core tip.

Response: This has been made an uploaded with the revised version of the manuscript.

Comment: Please provide all authors abbreviation names and manuscript title here. *World J Gastroenterol* 2016; In press.

Response: This has now been added as requested on page 5.

Comment: Structing of the abstract and word limits for each section.

Response: All comments have now been addressed and abstracts conforms to word limits (Design 110 words, Results 125 words and Conclusion 26 words).

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Comment: Comments section to be added.

Response: This section has now been added (pages 28-29).

Comment: Please check there is no repeat references.

Response: This has now been checked.

Comment: Please provide scientific research project answers to 5 questions.

Response: This has now been done and uploaded with revised document.

Comment: Please subject the manuscript to *CrossCheck* analysis and the final title to Google Scholar search, and store screenshot images of the results.

Response: After contacting the WJG office and I understand this will be done by the editor (corresponded with Jin-Lei Wang, 19th August). Thank you.

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