Manuscript # NO: 54484 - Manuscript revision "Differential regulation of JAK/STAT signaling in patients with ulcerative colitis and Crohn`s disease"

Dear editorial team, dear reviewers,

thank you for considering our manuscript for publication in the *World Journal of Gastroenterology* and your fair and respectful comments, which distinctly improved the quality of the manuscript.

We have addressed all issues raised by the reviewers and are very much looking forward to hearing from you. Please find below our point-by-point response to your remarks.

Yours sincerely,

F. Cordes (for all authors)

Reviewer comments:

Reviewer#03478404:

Conclusion: Minor revision
Scientific Quality: Grade B (Very good)
Language Quality: Grade C (A great deal of language polishing)

In this manuscript, the authors analysed available data on differential JAK/STAT-signaling in ulcerative colitis and Crohn's disease and it resulted in a very comprehensive and detailed review. Up-to-date references regarding JAK/STAT signaling were used, others are old. Obviously, there was a lot of work involved in collecting the data and writing the manuscript.

Comments/suggestions:

Comment 1. English language should be revised and corrected all over the manuscript. Even the abbreviations contain errors – e.g. CD, Crohn`disease; RA, rheumatoid arthirits
Answer: We apologize for the errors in English language. For the revision, a native speaker has carefully revised the entire manuscript.

Comment 2. Core tip: Please rewrite the sentence "Clinical implications include more specific JAK/STAT targeting as well a cell-subset-specific JAK/STAT inhibition.", in order to be clearer.
Answer: As suggested, the sentence has been re-written as follows (page 4-5, lines 66-68): "Development of JAK/STAT-inhibitors with specific targeting of associated inflammatory pathways might further improve efficacy and safety profiles of this drug class."

Comment 3. Introduction:
Comment 3a. lines 59-62: Authors should mention that IBD comprise also IBD-U (unclassified). Please also correct - strictures and fistulas appear only in CD. There are more recent references than 1-4.
Answer: The paragraph has been modified according to the reviewer's suggestion. We included the following references:

1. Bettenworth D, Lopez R, Hindryckx P, et al. Heterogeneity in endoscopic treatment of Crohn's disease-associated strictures: An international inflammatory bowel disease specialist survey. J Gastroenterol 2016;51:939-48.
2. Molendijk I, Nuij VJ, van der Meulen-de Jong AE, et al. Disappointing durable remission rates in complex Crohn's disease fistula. Inflamm Bowel Dis 2014;20:2022-8.
3. Mooiweer E, van der Meulen-de Jong AE, Ponsioen CY, et al. Incidence of Interval Colorectal Cancer Among Inflammatory Bowel Disease Patients Undergoing Regular Colonoscopic Surveillance. Clin Gastroenterol Hepatol 2015;13:1656-61.
4. Pariente B, Hu S, Bettenworth D, et al. Treatments for Crohn's Disease-Associated Bowel Damage: A Systematic Review. Clin Gastroenterol Hepatol 2019;17:847-856.
5. Zhu Z, Mei Z, Guo Y, et al. Reduced Risk of Inflammatory Bowel Disease-associated Colorectal Neoplasia with Use of Thiopurines: a Systematic Review and Meta-analysis. J Crohns Colitis 2018;12:546-558.

Furthermore, the indicated sentence was corrected as follows (page 6, lines 74-78):
"Inflammatory bowel diseases (IBD) comprise the entities ulcerative colitis (UC) and Crohn`s disease (CD) as well as ***unclassified IBD***, which are chronic remittent diseases characterized by intestinal inflammation and the risk of uncontrolled disease activity which may lead to severe complications such as fistulas and strictures ***in CD***, and colorectal neoplasia in ***both entities.***"

Comment 3b. line 63 "medical treatment " is repeated twice.
Answer: The sentence was corrected to avoid repeat of "medical treatment".

Comment 3c. References 5 and 6 should be replaced by more recent ones. (i.e. instead of reference 5 – use "Wong DJ, et al. Surgery in the age of biologics. Gastroenterology Report 2019: 7(2): 77–90.
Answer: We thank the reviewer for bringing up these points. References 5 and 6 were replaced by more recent and/or appropriate ones including the suggested reference by Wong DJ et al. (Wong DJ, Roth EM, Feuerstein JD, et al. Surgery in the age of biologics. Gastroenterol Rep (Oxf) 2019;7:77-90) as well as references by Kirchgesner, J. et al. (Kirchgesner J, Lemaitre M, Carrat F, et al. Risk of Serious and Opportunistic Infections Associated With Treatment of Inflammatory Bowel Diseases. Gastroenterology 2018;155:337-346 e10) and Ben-Horin S. et al. (Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther 2011;33:987-95).

Comment 3d. Authors should carefully revise all references in the manuscript and use the correct ones. As an example, they wrote – lines 65-66 "Population based studies reported that 44% and 34% of patients with CD and UC…" and inserted reference 4. In fact, reference 4 is only a review of the data known in 2007 (OLD!). The population-based study the authors referred to is Faubion WJ, Loftus EJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. Gastroenterology 2001; 121: 255–60. This is just one example. And there are many instances. The original article should be referenced and not the review.
Answer: We agree with the reviewer. All references have been carefully evaluated and updated, where appropriate and possible.
Furthermore, we updated the indicated sentence, including two more recent original articles by Burisch et al. on the natural course of UC (Burisch J, Katsanos KH, Christodoulou DK, et al. Natural Disease Course of Ulcerative Colitis During the First Five Years of Follow-up in a European Population-based Inception Cohort-An Epi-IBD Study. J Crohns Colitis 2019;13:198-208.) and CD (Burisch J, Kiudelis G, Kupcinskas L, et al. Natural disease course of Crohn's disease during the first 5 years after diagnosis in a European population-based inception cohort: an Epi-IBD study. Gut 2019;68:423-433), as follows (page 6, lines 81-83):
"Population based studies reported that still 46% of patients with CD and 14% of patients with UC are still being treated with systemic corticosteroids for more than 6 month to achieve remission[1, 2]."

Comment 3E. Lines 69-72 "maintenance of remission is still challenging: While approximately 22% of CD and one third of UC patients were classified as primary non-responder or had to stop treatment due to severe side effects, up to 18% of IBD patients revealed a secondary loss of response to biological therapy." This long sentence is not clear and has no reference. Where are these data taken from? According to a very recent review (Papamichael K, et al. Clinical Gastroenterology and Hepatology 2019), up to one-third of patients with Crohn's disease (CD) and ulcerative colitis (UC) show primary non-response

(PNR) to biologic therapies, and up to 50% of patients after an initial clinical response stop therapy for either secondary loss of response (SLR) or a serious adverse event. Correct references of these studies are “Ben-Horin S, Chowers Y. Loss of response to anti-TNF treatments in Crohn’s disease. Aliment Pharmacol Ther 2011;33:987–995”. AND “Papamichael K, et al. Therapeutic drug monitoring during induction of anti-tumor necrosis factor therapy in inflammatory bowel disease: defining a therapeutic drug window. Inflamm Bowel Dis 2017;23:1510–1515”. These references are not cited in this manuscript.
Answer: We apologize for not including references. We thank the reviewer for bringing up the recent review of Papamichael K, et al. (Clinical Gastroenterology and Hepatology 2019) and the recent studies on primary and secondary loss of response . We updated response rates according to the recent reports (Papamichael K, Vande Casteele N, Ferrante M, et al. Therapeutic Drug Monitoring During Induction of Anti-Tumor Necrosis Factor Therapy in Inflammatory Bowel Disease: Defining a Therapeutic Drug Window. Inflamm Bowel Dis 2017;23:1510-1515. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther 2011;33:987-95.).
Furthermore, as suggested, the sentence was rephrased for better understanding including update of primary and secondary response rates as follows (page 6, lines 84-89):
“The introduction of biological therapy has improved the spectrum of anti-inflammatory treatment. Nevertheless, the induction and maintenance of remission can still be challenging due to primary non response and secondary loss of response to biological therapy. Indeed, approximately one third of patients with CD and UC were classified as primary non-responder, up to 50% of patients with IBD have a secondary loss of response to biological therapy or had to stop treatment due to severe side effects[3, 4].”

Comment 3f. Paragraph “The JAK/STAT-pathway” contains pertinent data; however, it should be shortened and made clearer. A figure showing the mechanisms would be very useful.
Answer: The paragraph has been shortened as suggested (please see the marked version of the revised manuscript). Furthermore a figure (entitled Figure 1 in the revised version of the manuscript) showing the general mechanism of JAK/STAT signaling has been included.

Comment 3g. Paragraph “Clinical efficacy of JAK inhibition in RA and IBD”:
Comment 3g1. Please define DMARDs before the abbreviation (for readers).
Answer: A definition has been added.

Comment 3g2. When referring to a study, the reference should be inserted there (e.g. lines 128-129 - In a recently published phase III RCT, tofacitinib demonstrated efficacy for induction and maintenance of remission in patients with UC – reference 55).
Answer: We apologize for this error and corrected the placement of the indicated reference.

Comment 3g3. REFERENCE 60 HAS ALREADY BEEN PUBLISHED. It should show all coordinates: Inflamm Bowel Dis. 2020;26(3):391-406. This remark is valid for other references not having all compete data.
Answer: We thank the reviewer for carefully correcting the references and updated the indicated reference as well as other references with missing data.

Comment 4. Paragraph “JAK/STAT SIGNALING IN IBD: T-CELLS” Fig.1 is explanatory and contains most important data.
Answer: We thank the reviewer for this comment.

Comment 5. Paragraph STATs – lines 200-201 – "Mudter et al. found increased IFNγ-induced STAT1 but not phospho-STAT1 in lamina propria T-cells of CD compared to UC patients." Reference should be inserted here.
Answer: The reference has been inserted as suggested by the reviewer.

Comment 6. Paragraph "JAK/STAT": Lines 312-313 "Oppositely to that, Soendergaard et al. found SOCS1 and additionally SOCS3 mucosal RNA level…" please insert reference 26. Figure 2 shows most important data and connections between pathways.
Answer: We apologize for the misplaced reference and corrected it as suggested.

Comment 7. Paragraph "GENETIC ASSOCIATION OF IBD AND THE JAKS/STAT PATHWAY". Table 1 – reference 142 is missing from "Prager 2014"
Answer: The indicated reference has been inserted.

Comment 8. Paragraph "JAKs" – references 130-133 should be after those used in Table 1, as they are referred later in the manuscript, after Table 1.
Answer: We thank the reviewer for bringing this to our attention. Table 1 has been included in the manuscript text, so former references 130-133, which are referred later in the manuscript after Table 1, are now listed as 151-154.

Comment 9. Conclusion is too long and too detailed. Please shorten it and make it crispier. Conclusion should not contain redundant data.
Answer: The conclusion has been shortened substantially.
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Reviewer#00503587

Conclusion: Minor revision
Scientific Quality: Grade A (Excellent)
Language Quality: Grade C (A great deal of language polishing)

This review manuscript focuses on JAK/STAT signalling and related pathways in the context of IBD. The manuscript describes the topic in great detail and is comprehensive.

SPECIFIC COMMENTS

Comment 1. There are many errors of grammar and/or inappropriate phraseology. As some examples line 47, line 144, line 203 and many others
Answer: The manuscript was carefully read and corrected for English language by a native speaker, specifically errors of grammar and inappropriate phraseology were corrected (see revised version of the manuscript).

Comment 2. the term "CD patients" should be rewritten to read "patients with CD" And similar other such phrases also
Answer: We changed the wording as suggested by the reviewer.

Comment 3. Some referencing is misplaced. When utilising the term Author et al, the relevant reference should follow immediately after e tal
Answer: Thank you. The changes have been made.

Comment 4. Some of the paragraphs are very long.
Answer. We thank the reviewer for highlighting this point and agree. We therefore shortened some of the paragraphs, i.e. "The JAK/STAT-pathway", and "Conclusion" in the revised version of the manuscript (please see the marked version of the revised manuscript).

Comment 5. At line 430, should this be Clinical Implications?
Answer: We changed the paragraph title to Clinical Implication.

Reviewer#00058695

Conclusion: Minor revision
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)

This review of differential regulation of JAK/STAT-signaling in patients with ulcerative colitis and Crohn's disease summarize the latest published evidence of JAK/STAT signaling in immune cells of IBD as well as a genetic association between the JAK/STAT pathway and IBD. I have some comments.

General Comments:

Comment 1: In general, the authors should provide references for key issues mentioned in the text. Thus, in the Introduction it is recommended that the authors among others give a reference for the 22 and 18 percentages, and the reference 6 seems to be placed rather awkwardly and should preferably be placed by end of the sentence.
Answer: Key references were added to the revised version of the manuscript.

Comment 2: Moreover, it is preferable if the authors consequently could add reference numbers when dealing with specific publications, e.g. Soendergaard et al. on page 15 (line 309-312).
Also, the statement that "previous data from our own group" as mentioned on page 8, line 148, should have a reference number affiliated. The authors should check the entire manuscript once more regarding these matters.
Answer: The specific reference of Soendergaard et al. (Soendergaard C, Bergenheim FH, Bjerrum JT, et al. Targeting JAK-STAT signal transduction in IBD. Pharmacol Ther 2018;192:100-111.) as well as the specific reference of our own data (Cordes F, Lenker E, Spille LJ, et al. Tofacitinib Reprograms Human Monocytes of IBD Patients and Healthy Controls Toward a More Regulatory Phenotype. Inflamm Bowel Dis 2020;26:391-406.) has been added. Furthermore, the entire manuscript has been double-checked for the correct inclusion and placement of references.

Comment 3: It is recommended that the authors briefly explain for the readers the difference between canonical and non-canonical pathways, e.g. p. 6, line 106.
Answer:
We agree with the reviewer and added the suggested explanation to the manuscript (page 7-8, lines 122-125):
"Besides these classical canonical pathways, which include subsequent JAK/STAT activation, non-canonical pathways with independent activation of either JAKs or STATs for signal transduction has been described[5-7]."

Comment 4: When describing the various STATs, perhaps the authors should consider mentioning the difference between STAT5a/STAT5b.
Answer: Thank you for this important comment. We added a sentence depicting the difference between STAT5a and STAT5b as follows (page 7, lines 108-109):
"STAT5a and STAT5b represent two proteins with almost identical amino acids but are encoded by different genes[8]."

Comment 5: In general, the paper provides a nice summary that more selective JAK/STAT inhibitors are needed, which might increase efficacy and at the same time decrease side effects as observed with first JAK pan-inhibitor marketed for ulcerative colitis, i.e., tofacitinib.

Answer: We thank the reviewer for this answer.

Specific comments

Comment 1. When focusing on IBD, the first sentence of the abstract should be in 2018… for the treatment of ulcerative colitis, as treatment of rheumatoid arthritis is out of scope in this context.

Answer: We agree with the reviewer and corrected the first sentence of the abstract as suggested.

Comment 2. Also, in the abstract the authors should delete "human" (x 2) as IBD is a pure human disorder. In animal models you can talk about experimental colitis only, as there is absolutely no "animal IBD".

Answer: We thank the reviewer for this answer and agree. We deleted the word "human" prior to IBD as suggested.

Comment 3. In keywords generic drugs should not be capitalized, including tofacitinib.

Answer: Generic drugs were checked and decapitalized in the entire manuscript including tofacitinib in the keywords.

Comment 4. Add comma in 10,292

Answer: Could the reviewer please further specify this remark?

Comment 5. The authors should consider mentioning that the IL-23/IL-12 receptor stimulation might mimic that of ustekinumab (p. 20, line 427)

Answer: The potential interaction between IL-12/IL-23 receptor stimulation and ustekinumab has been added as follows (page 22, lines 449-451):

"The relevance of IL-23 and IL-12 signaling on IBD pathogenesis is further confirmed by therapeutic efficacy of the IL-12/IL-23 blocker ustekinumab in both entities."

Comment 6. The authors should check all references "Epub Ahead of Print" if they have been recently published.

Answer: We thank the reviewer for this comment. We checked all references for "Epub Ahead of Print" and corrected it, where appropriate.

Reviewer#00054993

Conclusion: Accept (General priority)
Scientific Quality: Grade A (Excellent)
Language Quality: Grade A (Priority publishing)

Based on the observation that patients with ulcerative colitis treated with the pan-Janus kinase (JAK) inhibitor tofacitinib showed a positive healing response while patients with Crohn-Disease did not, the authors present a comprehensive review on the current knowledge of the interplay of the JAK/STAT pathway and inflammatory bowel disease (IBD). They address the JAK/STAT pathway in general, JAK/STAT signaling in T-cells, monocytes and monocyte-derived cells and genetic associations of IBD and the JAK/STAT pathway. The authors also reflect on clinical implementations and in their conclusion make several valid points as to how more specifically targeted personal treatment approaches might improve therapeutic outcomes in patients with IBD. One table and 2 figures help to comprehend the complex text.

Comments:

Comment 1: The statements on page 13, lines 260 - 262 should be referenced.
Answer: We apologize for the missing reference and the statements on former page 13, lines 260 – 262 have been referenced now.

Comment 2: A few typing errors need attention, for instance on page 5, line 77 (omit one "has"): page 11, line 225 (mechanism needs a plural "s"), etc.
Answer: We apologize for the typing errors and thank the reviewer for bringing up these errors. The entire manuscript was carefully corrected for typing errors including the indicated errors.

References

1. Burisch J, Katsanos KH, Christodoulou DK, et al. Natural Disease Course of Ulcerative Colitis During the First Five Years of Follow-up in a European Population-based Inception Cohort-An Epi-IBD Study. J Crohns Colitis 2019;13:198-208.

2. Burisch J, Kiudelis G, Kupcinskas L, et al. Natural disease course of Crohn's disease during the first 5 years after diagnosis in a European population-based inception cohort: an Epi-IBD study. Gut 2019;68 423-433.

3. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther 2011;33:987-95.

4. Papamichael K, Vande Casteele N, Ferrante M, et al. Therapeutic Drug Monitoring During Induction of Anti-Tumor Necrosis Factor Therapy in Inflammatory Bowel Disease: Defining a Therapeutic Drug Window. Inflamm Bowel Dis 2017;23:1510-1515.

5. Nan Y, Wu C, Zhang YJ. Interferon Independent Non-Canonical STAT Activation and Virus Induced Inflammation. Viruses 2018;10:196.

6. Pfeffer SR, Fan M, Du Z, et al. Unphosphorylated STAT3 regulates the antiproliferative, antiviral, and gene-inducing actions of type I interferons. Biochem Biophys Res Commun 2017;490:739-745.

7. Majoros A, Platanitis E, Kernbauer-Holzl E, et al. Canonical and Non-Canonical Aspects of JAK-STAT Signaling: Lessons from Interferons for Cytokine Responses. Front Immunol 2017;8:29.

8. Grimley PM, Dong F, Rui H. Stat5a and Stat5b: fraternal twins of signal transduction and transcriptional activation. Cytokine Growth Factor Rev 1999;10:131-57.