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Ying Dou
Science Editor
World Journal of Gastrointestinal Surgery

Dear Ying Dou and esteemed reviewers,

Thank you kindly for your thoughtful review of our manuscript entitled "Molecular Therapeutic Strategies Targeting Pancreatic Cancer Induced Cachexia (Manuscript NO: 41454)". We believe the reviewers have brought up important points that have significantly improved the quality of this manuscript. Additionally, we would also like to thank you for the opportunity to revise and resubmit our work.

As you will note below, we have addressed each point raised by the reviewers and performed additional analysis per your suggestions. Changes in the revised manuscript are tracked. We have provided both a 'tracked' and a 'clean' version with correct reerences of the manuscript for your kind re-review. Each point raised by the reviewers is discussed below:

Reviewers' comments:

- **Please provide the author contributions. See the format in the attachment file-revision policies.**

Answer: *Dear Reviewer, thank you so much for your thorough review of this manuscript. We neglected to include author contributions in the manuscript and we added authors contributions in the revised manuscript. We have included it below for your reference.*

Author contributions: Yakovenko A and Trevino J designed research; Yakovenko A performed research; Yakovenko A, Cameron M, and Trevino J analyzed data; Yakovenko A critically interpreted the data; Yakovenko A and Cameron M wrote the manuscript; Yakovenko A, Cameron M and Trevino J performed critical revisions.

- **Core tip should less than 100 words.**

Answer: *Thank you so much for bringing this to our attention. We addended and condensed the core tip to 98 words. We have included edited core tip below for your reference.*

Core tip: Pancreatic cancer induced cachexia is a complex metabolic syndrome associated with increased morbidity, mortality and reduced quality of life. The complex pathophysiology of cachexia involves muscle wasting, systemic inflammation, and metabolic alterations. Molecular signaling pathways responsible for muscle wasting include TGF-β, myostatin/activin, IGF-1/PI3K/Akt, and JAK-STAT. IL-6, TNF-α, and INF-γ are the most well studied pro-cachectic cytokines that promote systemic inflammation. Metabolic alterations such as increased energy expenditure and glycolytic pathway dysfunction could be potentially improved with ketonemia, silibinin, and ω3-polyunsaturated fatty acids. Targeting molecular signaling pathways in pancreatic cancer induced cachexia could lead to discovery of effective therapies.

- **Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI.**

Answer: *Thank you so much for bringing this to our attention, we neglected to include PMID and DOI in the references. We edited our references to include PMID and DOI. For the references from ClinicalTrials.gov we included study ID number. All the authors have been listed in each reference.*

- **Figure 1 is not clearly.**

Answer: *Thank you so much for pointing this out and allowing us to address Figure 1 to make it more clear. We apologize for the lack of detail in the explanation below the figure. In hope to provide more clarity we addended the description below the figure and we edited the explanation. Please let us know if this is not sufficient and we will readdress this issue. The revised manuscript reflects the changes. We have included the revised Figure 1 below for your reference.*

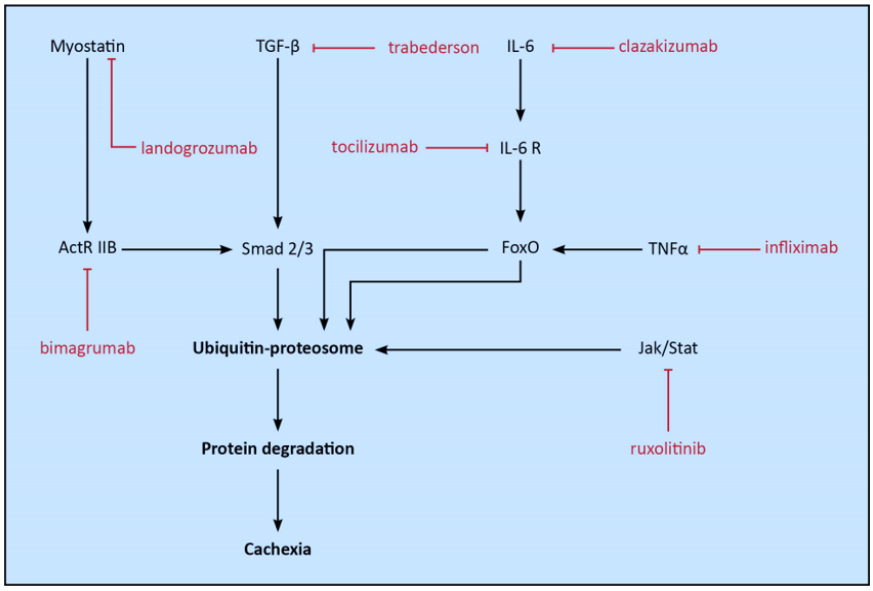

Figure 1. Signaling pathways involved in the pathophysiology of cachexia and targeted therapies. Multiple molecular signaling pathways and mediators lead to protein degradation and cancer cachexia including myostatin/ActRIIB, TGFβ, Smad 2/3, IL-6, TNFα, FoxO and JAK-STAT. These molecular signaling pathways serve as therapeutic strategies for treatment of cachexia. Pharmacologic inhibitors that have been used clinically or experimentally are labelled in red and specific targets are notated.

Reviewer 03464737: This article reviews molecular aspects in pancreas cancer cachexia. Indeed, this is a very relevant emerging topic. Understanding the molecular mechanisms and draggability for pancreatic cancer cachexia is very important in both pathology and therapeutics. However, the review lacks some pieces of recent important information. This would require improvement prior to publication.

#1. Insulin resistance and fat tissue browning: Here the authors mention these important aspects of cachexia, however, a critical discussion emphasizing the possible molecular therapeutic strategies is needed.

Answer: *Thank you so much for your thoughtful review of our manuscript. We agree with you; insulin resistance and fat tissue browning are important aspects of cachexia that we failed to address in our manuscript. We have added a new section (page 13) on fat tissue browning and metabolic alterations related to fat tissue browning and insulin resistance. We appreciate your suggestion and believe this will enhance the quality of this manuscript. The revised manuscript reflects these changes. We have included a new section below for your reference.*

> **Fat tissue browning:** Lipolysis and adipose tissue wasting play a key role in the development of cachexia syndrome. An interesting phenomenon of fat tissue browning has been observed in animal models of cancer cachexia[1, 3, 70]. It is hypothesized that a combination of pro-inflammatory microenvironment derived from factors such as IL-6, TNF-α and parathyroid-related peptide (PTHrP) secreted by the tumor and the host as well as metabolic dysregulation leads to white adipose tissue browning as cachexia syndrome progresses[70, 71]. Brown adipose tissue is characterized by high mitochondrial content and increased uncoupling protein 1 (UCP1) which is responsible for thermoregulation by uncoupling electron transport from ATP generation. This causes increased energy expenditure, increased heat production, and lipolysis which leads to exhilarated weight loss and contributes to the cachexia syndrome progression[70-72]. White adipose tissue wasting and fat browning seems to occur early during development of cachexia syndrome and independently from skeletal muscle wasting[70]. However, the complex molecular signaling pathways implicated in fat tissue browning and lipolysis are not well described. Further investigation to understand the mechanisms of how systemic metabolic and inflammatory alterations leads to switching of white adipose tissue to brown adipose tissues is necessary to advance our knowledge and treatment options for cachexia. Inhibition of fat tissue browning should be explored as a possible molecular therapeutic strategy for pancreatic cancer induced cachexia.

#2. Anabolic and appetite stimulant effects of ghrelin agonists: Here a major discussion point missing is the possibility of ghrelin agonists' use to treatment of cachexia. Lancet Oncol. 2016 Apr;17(4):519-531.

Answer: *Thank you for your insight and bringing up such an important point and we apologize for failing to include discussion about ghrelin agonists in our manuscript. The reference article you provided contributed significantly to improving our manuscript and addressing your suggestion. We agree with your concern and added a new section (page 14) on anabolic and appetite effects of ghrelin agonists. We reviewed several sources and added pertinent information including results from ROMANA 1,2 and 3 trials. We believe that the additional section on ghrelin will enhance the quality of our manuscript. The manuscript and references have been amended accordingly to reflect these changes. We have included a new section below for your reference.*

> **Ghrelin:** Ghrelin is a peptide hormone secreted by the stomach and pancreas and modulates energy homeostasis, increases appetite, and stimulates growth hormone (GH) secretion[76]. Ghrelin constitutes a promising novel therapeutic strategy since it plays a key role in appetite and energy expenditure regulation. Multiple studies reported that administration of ghrelin or ghrelin receptor agonists such as anamorelin improved food intake, appetite, adiposity, and lean body mass in cachectic patients[76-78]. Three Phase 3 RCTs (ROMANA 1 - NCT01387269, ROMANA 2 - NCT01387282 and ROMANA 3 - NCT01395914) reported that anamorelin significantly increased lean body mass in cachectic patients with NSCLC [76, 79]. A multicenter Japanese study

examined the efficacy and safety of anamorelin in patients with NSCLC and concluded that anamorelin was safe, well tolerated and it significantly increased lean body mass, improved anorexia and nutritional state (mainly seen as an increase in prealbumin)[80]. In a recent Cochrane systematic review, Khatib *et al.* stated that there is insufficient evidence to be able to support or refute the use of ghrelin in cancer cachexia and further investigation with adequately powered RCTs is warranted [81]. Furthermore, no clinical trials to date have been conducted to evaluate effectiveness of ghrelin or ghrelin agonists in pancreatic cancer associated cachexia. Given the promising results of anamorelin in treatment of cancer cachexia across multiple studies, it is worth exploring this further as a treatment option for pancreatic cancer induced cachexia.

#3. Current FDA approved drugs: The manuscript would be enriched contrasting the possible wasting muscle effects of corticosteroids and progestational drugs during cachexia treatment.

Answer: *We sincerely appreciate your time and insight in the review of our manuscript. We agree with the reviewer. You bring up an excellent point regarding corticosteroids and progestational drugs use during cachexia treatment. However, the discussion about corticosteroids and progestational drugs is so broad and would lead away from the main focus of the manuscript on the molecular signaling pathways. Discussion of the effects of corticosteroids on muscle and its relevance to cachexia could serve as a separate topic for a manuscript. Corticosteroids do not have a specific molecular target and affect many signaling pathways that would be difficult to discuss/address in this paper. Contrasting the muscle wasting effects of corticosteroids and progestational drugs during cachexia treatment would open up a whole new topic for a discussion that is beyond the scope of our minireview manuscript. As such, we decided to focus on more specific molecular signaling pathways involved in muscle wasting such as Myostatin/activin, IGFBP-3 and IGF-1/PI3K/AKT and TGF-β signaling.*

Reviewer 03664493: 1 Title. Does the title reflect the main subject/hypothesis of the manuscript? Yes. 2 Abstract. Does the abstract summarize and reflect the work described in the manuscript? Yes. 3 Key words. Do the key words reflect the focus of the manuscript? Yes. 4 Background. Does the manuscript adequately describe the background, present status and significance of the study? Yes. 5 Methods. Does the manuscript describe methods (e.g., experiments, data analysis, surveys, and clinical trials, etc.) in adequate detail? Yes. 6 Results. Are the research objectives achieved by the experiments used in this study? What are the contributions that the study has made for research progress in this field? Yes. This review provides a meaningful direction for further research on the applicable therapies for cachexia in pancreatic cancer patients. 7 Discussion. Does the manuscript interpret the findings adequately and appropriately, highlighting the key points concisely, clearly and logically? Are the findings and their applicability/relevance to the literature stated in a clear and definite manner? Is the discussion accurate and does it discuss the paper's scientific significance and/or relevance to clinical practice sufficiently? Yes. 8 Illustrations and tables. Are the figures, diagrams and tables sufficient, good quality and appropriately illustrative of the paper contents? Do figures require labeling with arrows, asterisks etc., better legends? 9 Biostatistics. Does the manuscript meet the requirements of biostatistics? 10 Units. Does the manuscript meet the requirements of use of SI units? 11 References. Does the manuscript cite appropriately the latest, important and authoritative references in the introduction and discussion sections? Does the author self-cite, omit, incorrectly cite and/or over-cite references? 12 Quality of manuscript organization and presentation. Is the manuscript well, concisely and coherently organized and presented? Is the style, language and grammar accurate and appropriate? Yes. 13 Research methods and reporting.

Authors should have prepared their manuscripts according to manuscript type and the appropriate categories, as follows: (1) CARE Checklist (2013) - Case report; (2) CONSORT 2010 Statement - Clinical Trials study, Prospective study, Randomized Controlled trial, Randomized Clinical trial; (3) PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review, Meta-Analysis; (4) STROBE Statement - Case Control study, Observational study, Retrospective Cohort study; and (5) The ARRIVE Guidelines - Basic study. Did the author prepare the manuscript according to the appropriate research methods and reporting? 14 Ethics statements. For all manuscripts involving human studies and/or animal experiments, author(s) must submit the related formal ethics documents that were reviewed and approved by their local ethical review committee. Did the manuscript meet the requirements of ethics?

Answer: *Thank you very much for your detailed review of our manuscript. We sincerely appreciate your time. We did not identify any specific questions or concerns to address from your comments. We prepared our manuscript based on the guidelines according to the specific category. If we failed to address something, please let us know and we will revise things accordingly.*

We sincerely thank the reviewers and the editor for their thoughtful and careful review of our work. We have made every effort to address their concerns, including the incorporation of additional sections. We feel that the suggested changes greatly improve the quality of the manuscript and hope that the reviewers agree. We are honored for the consideration to publish our work in *World Journal of Gastrointestinal Surgery* and look forward to your comments.

Sincerely,

Anastasiya Yakovenko
Miles Cameron
Jose Trevino