

## Response to the Reviewer's Comments

**Manuscript number:** 107610

**Title:** Genetically engineered mouse models in precancerous lesions of gastric cancer research: A review

Dear Editors and Reviewers,

We appreciate the opportunity to revise our manuscript titled " Genetically engineered mouse models in precancerous lesions of gastric cancer research: A review "and are grateful for the insightful comments provided by the reviewers. Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. In the following, we have provided detailed responses to each of the reviewers' comments.

Reviewer #1.

**Comment 1:** *While the article is well-structured, the language contains minor grammatical and syntactical errors that can be refined for better clarity. A thorough language review or professional proofreading will improve the readability and fluency of this manuscript.*

**Reply:** Thank you for your valuable feedback. We have already revised the manuscript to address the grammatical and syntactical issues. Additionally, we have once again engaged a professional proofreading service to further refine the language and ensure the highest level of clarity and fluency. We appreciate your attention to detail and hope these improvements meet your expectations.

**Comment 2:** *The review does an excellent job of summarizing GEMMs. Still, it*

*would benefit from a more critical evaluation of their limitations and how they compare to other available models (e.g., organoid models or patient-derived xenografts).*

**Reply:** Thank you for raising such a valuable point. We have already addressed this in the conclusion section by adding a critical evaluation of the limitations of GEMMs and a comparison with patient-derived xenografts (PDX) and patient-derived organoids (PDO).

**The revised content of the paper is as follows:** And certain rare or complex genetic background tumors are difficult to establish using GEMMs. The emergence of patient-derived xenograft models (PDX) and organoid models (PDO) has effectively addressed this issue. PDX and PDO can be directly obtained from patient tumor tissues, and they are capable of preserving the tumor's tissue structure and cell-to-cell interactions while partially simulating the tumor microenvironment. Moreover, they hold promise for evaluating treatment responses in individual patients, thereby facilitating the development of personalized treatment regimens. However, the considerable individual differences in PDX and PDO models may lead to inconsistencies between models, which in turn pose challenges for the reproducibility of experiments. In contrast, GEMMs do not have this issue. Additionally, there is a scarcity of GEMMs specifically designed for studying PLGC. The creation of GEMMs demands considerable time and resources and it cannot be guaranteed that all GEMMs will develop PLGC. To overcome this problem, some researchers have proposed combining PDOs with CRISPR screening. PDOs are rapid and efficient in assessing drug sensitivity, while CRISPR screening can identify novel therapeutic targets. The combination of these two approaches is expected to accelerate the development of more effective and personalized treatment strategies

**Comment 3:** Revise the keywords section and do not add abbreviations in the keywords.

**Reply:** corrected as suggested.

**Comment 4:** *Suggesting specific improvements in GEMMs (e.g., refining genetic modifications to mimic human conditions better) would strengthen the review.*

**Reply:** Thank you for your suggestion. We have proposed combining patient-derived organoid (PDO) models with CRISPR screening to accelerate the development of more effective and personalized therapeutic strategies. Additionally, we have suggested the development of more humanized mouse models to specifically improve GEMMs.

**The revised content of the paper is as follows:**

And certain rare or complex genetic background tumors are difficult to establish using GEMMs. The emergence of patient-derived xenograft models (PDX) and organoid models (PDO) has effectively addressed this issue. PDX and PDO can be directly obtained from patient tumor tissues, and they are capable of preserving the tumor's tissue structure and cell-to-cell interactions while partially simulating the tumor microenvironment. Moreover, they hold promise for evaluating treatment responses in individual patients, thereby facilitating the development of personalized treatment regimens. However, the considerable individual differences in PDX and PDO models may lead to inconsistencies between models, which in turn pose challenges for the reproducibility of experiments. In contrast, GEMMs do not have this issue. Additionally, there is a scarcity of GEMMs specifically designed for studying PLGC. The creation of GEMMs demands considerable time and resources and it cannot be guaranteed that all GEMMs will develop PLGC. To overcome this problem, some researchers have proposed combining PDOs with CRISPR screening. PDOs are rapid and efficient in assessing drug sensitivity, while CRISPR screening can identify novel therapeutic targets. The combination of these two approaches is expected to accelerate the development of more effective and personalized treatment strategies [96].

Moreover, there is a need to develop more humanized mouse models,

especially in terms of simulating human immune responses, which will provide a valuable platform for immunotherapy and targeted therapy.

**Comment 5/6:** *The Figures are of excellent quality, and the legends are self-explanatory. Add a detailed title for Table 1.*

**Reply:** Thank you for noticing this detail, which has indeed contributed to the completeness of the article. We have already added titles to Table 1 and Figure 1/2.

**The revised content of the paper is as follows:**

Table1 Genetically engineered mouse models of precancerous lesions of gastric cancer

Figure1 Working principle of CRISPR/Cas9

Figure2 Working principle of Cre/Loxp system

**Comment 7/8:** *The review mentions sex-based differences but does not explore mechanistic explanations. Expanding on hormonal influences or immune system variations contributing to gender disparities in PLGC would strengthen this section.*

**Reply:** Thank you for your valuable suggestion. We have incorporated discussions on the mechanisms underlying gender differences in precancerous gastric lesions in the INS-GAS model, focusing on the roles of estradiol and the gastrointestinal microbiota.

**The revised content of the paper is as follows:**

Furthermore, ovariectomized(OVX) INS-GAS mice develop more severe gastric mucosal diseases than intact mice, suggesting that estradiol may have a protective effect on the gastric mucosa of female INS-GAS mice [15]. Further findings indicate that exogenous estradiol exerts a protective effect against PLGC by stimulating IL-10 activity, enhancing Th2-mediated immune responses, and inhibiting epithelial cell proliferation [16].

The research on the microbiota also confirmed the impact of sex differences

on the development of PLGC: Male mice and OVX female mice exhibited more severe gastric lesions, while intact female mice had a higher abundance of beneficial bacteria. In contrast, pathogenic bacteria were more abundant in male mice and OVX female mice. Moreover, exchanging gut microbiota (through co-housing) significantly reduced the differences in gastric lesions between OVX and intact female mice [19].

**Comment 9/10:** *Some GEMMs have limited translational value due to species differences. A discussion on which GEMMs are most predictive of human PLGC and their clinical applicability would be valuable.*

**Reply:** Thank you for your suggestion. We have added this discussion in the Discussion section. We agree that each mouse model has its own advantages for studying specific mechanisms. However, the INS-GAS mouse model, due to its stable development of PLGC, predictable progression, and high similarity to human lesions, is currently the most widely used and suitable GEMMs for this purpose.

**The revised content of the paper is as follows:**

The INS-GAS mouse model can stably develop PLGC, with predictable progression and high similarity to human lesions, making it a widely used model.

**Comment 11:** *While the references are extensive, consider including more recent studies (2023–2024) on novel GEMMs, CRISPR-based models, and microbiome research.*

**Reply:** Thank you for your suggestion. We agree that incorporating recent studies enhances the timeliness and novelty of the article. We have added content on the application of GEMMs in microbiome research as well as the use of CRISPR screening.

**The revised content of the paper is as follows:**

The research on the microbiota also confirmed the impact of sex differences

on the development of PLGC: Male mice and OVX female mice exhibited more severe gastric lesions, while intact female mice had a higher abundance of beneficial bacteria. In contrast, pathogenic bacteria were more abundant in male mice and OVX female mice. Moreover, exchanging gut microbiota (through co-housing) significantly reduced the differences in gastric lesions between OVX and intact female mice [19].

When using this model to explore the treatment of intestinal metaplasia (IM) with metformin, it was found that metformin significantly reduced the progression of IM lesions in the *Atp4a* mouse model, possibly by downregulating the NF- $\kappa$ B and PI3K/AKT/mTOR/HIF-1 $\alpha$  signaling pathways [31].

To overcome this problem, some researchers have proposed combining PDOs with CRISPR screening. PDOs are rapid and efficient in assessing drug sensitivity, while CRISPR screening can identify novel therapeutic targets. The combination of these two approaches is expected to accelerate the development of more effective and personalized treatment strategies [96].

**Comment 12:** *Some terms are inconsistently used (e.g., "PLGC," "GEMMs of PLGC," "precancerous lesions"). Consider standardizing these throughout the text.*

**Reply:** corrected as suggested.

**Comment 13:** *Ensure uniformity in the formatting of gene names (italicized where appropriate) and abbreviations (e.g., "Cldn18" should consistently be "CLDN18" in uppercase).*

**Reply:** corrected as suggested.

We are grateful to you for allowing us to revise our manuscript and look forward to hearing from you soon.

Yours sincerely,

Jiang-Hong, Ling

**Revision reviewer 1**

**Comment:** After a thorough re-review and re-evaluation, I am pleased to recommend this manuscript for further consideration for publication. The authors have successfully addressed all the concerns and comments raised during the first round of revision. Therefore, I accept the manuscript in its revised form and believe it is suitable for publication in this journal.

**Reply:** Thanks for your comments.