**Name of journal:** World Journal of Gastroenterology

**Manuscript NO:** 87228

**Title:** Thioridazine reverses trastuzumab resistance in HER-2 positive gastric cancer by inhibiting Skp2-mediated aerobic glycolysis

We thank the reviewers greatly for their thorough reviews and highly appreciate their comments and suggestions, which are all valuable and very helpful for improving the quality of our paper. We tried our best to revise the manuscript. These changes will not influence the content and framework of the paper. Please find below the detailed point-to-point response to each comment.

**Responds to the reviewers' comments:**

Reviewer #2 (SPECIFIC COMMENTS TO AUTHORS):

I have now reviewed your paper and recognize the importance of your research question. Manuscript NO. 87228 aimed to identify a clinical drug capable of inhibiting Skp2 and reversing trastuzumab resistance in HER-2-positive gastric cancer. ABSTRACT: [The "BACKGROUND" subsection is well-written.] ①**[The "AIM" subsection should be more concise and appealing to the reader.]** ②**[Consider expanding upon the "METHODS" subsection to provide a more detailed account of the procedures used to evaluate the impact of thioridazine, trastuzumab, and lapatinib on resistant cells. This could involve specifying the dosages administered, the duration of treatments, and the specific outcome measures that were assessed.** ③**Additionally, it's important to ensure that the METHODS subsection remains distinct from the RESULTS subsection to maintain clarity in the manuscript.]** ④**[The "CONCLUSION" subsection requires improvement to emphasize the significance of the results within the framework of a thorough critical analysis, with a specific focus on the clinical relevance of the findings and a discussion of the study's inherent limitations.]** INTRODUCTION: [The "INTRODUCTION" section effectively provides context for the reader and serves as a strong point of the manuscript.] METHODS: The

"MATERIALS AND METHODS" section provides a comprehensive description that effectively supports the results and conclusions presented in the preceding sections. RESULTS: The "RESULTS" section furnishes ample experimental evidence, enabling the formulation of intriguing and promising scientific conclusions regarding the role of Thioridazine in mitigating trastuzumab resistance in HER-2 positive gastric cancer. ⑤**DISCUSSION: In the "DISCUSSION" section, it is essential that the findings are expounded upon through a systematic theoretical analysis of the results. Additionally, I recommend that the authors delve into future prospects for their research, expanding on potential avenues for continued investigation.**

Response:

① In **page 4 of 87228_Auto_Edited**, we have have rewritten **the "AIM" subsection** as "To discover a Skp2 blocker among currently available medications and provide a potenitial therapeutic strategy for HER2-positive gastric cancer patients who have experienced progression following trastuzumab-based treatment."

② In **pages 4 and 5**, **the "METHODS" subsection** were expanded as "The association between Skp2 expression levels and trastuzumab sensitivity was examined in HGC27 cells transfected with Skp2 exogenous overexpression plasmids and HGC27-R cells transfected with SKP2-siRNA vectors by CCK-8 assays. Q-PCR and western blot analyses were employed to assess the regulatory impact of various concentrations of thioridazine (0, 1.25, 2.5, or 5 μM) administered for a duration of 24 hours on Skp2 expression. Flow cytometry was utilized to measure levels of apoptosis produced by lapatinib (1 μM or 2 μM) with or without thioridazine (5 μM). The Amplex Red Glucose/Glucose Oxidase Assay Kit and the Lactate Assay Kit were adopted to quantify the glycolytic activity of thioridazine (5 μM) and lapatinib (5 μM) individually and in combination in vitro. The efficacy of thioridazine (25 mg/kg) alone, as well as in combination with trastuzumab (0.5 mg/kg) or lapatinib (70 mg/kg), was evaluated in vivo using xenografted HGC27-R and SGC7901-R cells in mice."

③ In **page 5**, **the RESULTS subsection** was corrected to "In the parental HGC27 cells, an observed positive correlation exists between the overexpression of Skp2 and

an increase in trastuzumab resistance. Conversely, the sensitivity of HGC27-R cells to trastuzumab is enhanced through the knock-down of Skp2 expression. Treatment with 5 μM thioridazine leads to a notable decrease in Skp2 expression at both the mRNA and protein levels. Compared to the individual effects, simultaneous administration of thioridazine (5 μM) and lapatinib (1 μM or 2 μM) can improve the apoptosis rates of SGC7901-R cells by about five-fold and ten-fold, respectively. Moreover, the combined treatment of 5 μM thioridazine and 5 μM lapatinib demonstrates enhanced suppression of glucose uptake rate and lactate production through the inhibition of the Skp2/Akt/mTOR/Glut1 signaling pathways. Furthermore, the co-administration of thioridazine at a dosage of 25 mg/kg alongside lapatinib at a dosage of 70 mg/kg demonstrates a heightened and notable anticancer efficacy in an in vivo setting."

④ In **page 6**, **the "CONCLUSION" subsection** was improved as "In conclusion, our data highlight the therapeutic potential of thioridazine in combination with lapatinib by restraining Skp2 expression. This study provides an experimental foundation for the application of thioridazine in surmounting the resistance of trastuzumab in HER-2-positive gastric cancer. Additional pharmacodynamic investigations, aiming to optimize the drug ratio to minimize dosage, enhance effectiveness, and/or mitigate adverse reactions, will warrant the initiation of clinical trials."

⑤ In **page 24**, the last paragraph of **the "DISCUSSION" section** was revised as "Based on our findings, Skp2 may serve as a predictor of trastuzumab response in HER-2 positive gastric cancer patients, thereby aiding in identifying the patient subgroups most likely to benefit from HER-2 targeted therapies. Moreover, our study underscores the advantageous impacts of thioridazine when used in combination with synergistic drugs for the management of gastric cancer. These findings lend valuable

support to future clinical investigations targeting thioridazine as a possible treatment option for gastric cancer. It is noteworthy that HER-2 targeted drugs may cause cardiac toxicity that is independent of dosage. It has also been reported that thioridazine is associated with arrhythmias in a minority of schizophrenia patients. Consequently, when considering the administration of these drugs individually or in combination, it becomes imperative to exclude individuals with pre-existing heart conditions and implement vigilant cardiac surveillance."

We appreciate for the Editors' and Reviewers'warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.

Yours sincerely,

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