

Peer Review File

Article information: <https://dx.doi.org/10.21037/jtd-23-1349>

Reviewer A

Huang, et al. present a series of pre-clinical studies to provide proof of principal data regarding the use (and mechanism) of pazopanib for idiopathic pulmonary fibrosis. The impetus for this work is a dearth of viable treatment options for this deadly disease. The experimental approach is straightforward and appears to be sound. Briefly, the authors compared pazopanib to nintedanib *in vivo*, since the latter is already used for IPF, and the former has a similar alleged mechanism of action. Pazopanib seemed to work as well and sometimes better than nintedanib for the outcome measures presented. They also performed *in vitro* experiments with pazopanib using immortalized and primary lung cells, and the drug appeared to have desired effect in these assays as well. The studied drug seems to work by promoting pulmonary cell apoptosis, likely through a mechanism involving Akt suppression, and, separately, the inhibition of TGF- β signaling, at least *in vitro*. These results warrant further study into pazopanib for IPF. I think this paper could be accepted pending minor or major revisions. My comments are as follows, and mostly have to do with reporting of methods:

1. Overall, the paper needs to be carefully copyedited by a non-AI native English speaker. There are many grammatical errors, terminology that is not standard in modern American or British English, and misspellings throughout the manuscript. There are too many errors to list here, which is why this will need professional copyediting.

Reply: The grammar and sentences have been revised and improved.

2. In the abstract, the last sentence under methods (lines 32-34) should be moved to results, and then the methods portion should be expanded with more detail.

Reply2: We have moved the last sentence of the summary method to the conclusion (see page 2, lines 50-51); moved the results of the *in vivo* experiment in the method to the results section, and summarized the method part (see page 2, lines 38-47).

Changes in the text: (see page 2, lines 50-51)、(see page 2, lines 38-47)

3. “mpk” is not a standard unit reporting. I believe the authors mean “mg/kg” and so this should be corrected throughout and in all figures. This is one example of the issues mentioned in point 1, above.

Reply3: We have replaced mpk with the standard international unit mg/kg (see page 11, line 307)

Changes in the text: (see page 11, line 307)

4. Include the actual parameters and instrumentation for qPCR.

Reply 4: We have added the model and brand of QPCR instrument in this article.(see page 6, lines 153-156)

Changes in the text:(see page 6, lines 153-156)

5. What was the seeding density of cells for the scratch assays?

Reply 5: We have added the cell density of the scratch experiment to the article.
(see page 7, lines 167-169)

6. For the lung function measurements, there was no mention of how stimulus for measurement was controlled. For example, was there a methacholine stimulus?

Reply 6: During the measurement of lung function, we did not carry out external substance stimulation, and the mice were anesthetized and placed in the lung function measuring machine for measurement.

7. Only male mice were used in the studies. Ideally, this should be justified if it cannot be replicated in both sexes.

Reply 7: Estrogen in female mice can affect the construction of pulmonary fibrosis model, references are as follows:

Jenkins, R Gisli et al. "An Official American Thoracic Society Workshop Report: Use of Animal Models for the Preclinical Assessment of Potential Therapies for Pulmonary Fibrosis." *American journal of respiratory cell and molecular biology* vol. 56,5 (2017): 667-679. doi:10.1165/rcmb.2017-0096ST

Gharaee-Kermani, Mehrnaz et al. "Gender-based differences in bleomycin-induced pulmonary fibrosis." *The American journal of pathology* vol. 166,6 (2005): 1593-606. doi:10.1016/S0002-9440(10)62470-4

8. It was never actually directly mentioned how much TGF- β was used with the cells.

Reply 8: The concentration of TGF- β stimulation used by the cells was 5ng/ml, which was marked on page 8 and line 193.

Changes in the text:(see page 8, lines 193)

9. I was surprised to not see any detailed discussion on the known pharmacology of pazopanib and nintedanib. This is essential information and literature synthesis. Furthermore, and related, there was no substantive discussion of how this translates (dosage) in vitro to in vivo to human.

Reply 9: We have introduced the known mechanisms of Nintedanib and Pazopanib in the discussion section, adding on lines 320-334 on page 12. (see page 12, line 320-334);Instructions on the conversion of drug doses between humans and laboratory animals are supplemented on page 4, lines 109-113. (see page 4, lines 109-113)

Changes in the text:(see page 12, line 320-334); (see page 4, lines 109-113)

10. There seem to be selective comparisons to nintedanib. This is particularly noticeable by the lack of comparison for the experiments detailed in Fig. 2, etc. I think it would be useful to do these mechanistic comparisons since the two drugs were compared in vivo. Should the editor choose to require this, then the revision should be MAJOR.

Reply 10: Both nidanib and pazopanil inhibit tyrosine kinase. Even though their targets are identical, their mechanisms differ in vitro. Our research will look into the efficacy of nidanib and pazopanil in humans, as well as the pharmacological mechanism of

pazopanil in vitro.

Reviewer B

Please indicate the meaning of asterisk (*) in the figure legend of Figure 5.

Please check if any descriptions for “#” in Figure 5 is needed.

Please add the citation of Table 1 in the main text.

P2 Line 1: “It has the same target as that of Nintedanib...”

P2 Line 5: delete “explored” here

P2 Line 6: “showed” (simple past tense for Methods and Results presentation)

P2 Line 20-21: “...lung disease and its pathogenesis is unknown.” (presentation)

P2 Line 24: “Due to the aging population and ...”

P3 Line 1: please first define “IPF” (abbreviations have to be defined in both the Abstract and the Main Text.)

P3 Line 5-6: “In recent studies, ...agree that...are vital for the ...” (plural; simple present tense would be fine for general facts or beliefs description)

P3 Line 7: “is well-known” (simple present tense would be fine for general facts or beliefs description)

P3 Line 8: “is extremely...” (simple present tense would be fine for general facts or beliefs description)

P3 Line 9: “in the lungs of IPF patients.” (arrangement)

P3 Line 9: “ware” => “are” (tense; spelling mistake)

P3 Line 11-12: “The phosphorylation of Smad2 and Smad3 is were motivated by ... and mainly works as a ...” (for singular subject “The phosphorylation” and simple present tense)

P3 Line 13: space issue, add a space before the opening bracket

P3 Line 15: “could” => “can”

P3 Line 18: “proteins” (better to keep plural)

P3 Line 18: “suppresses” (simple present tense would be fine for general facts or beliefs description); please review the adverb “extremely” (does it mean “suppresses ... to a great extent”?)

P3 Line 20: delete “restricted”; please first define “iNOS” (abbreviations have to be defined in both the Abstract and the Main Text.)

P3 Line 21: “the decline of ...”

P3 Line 21: space issue, please review the space before “Therefore”

P3 last paragraph Line 1: “Pazopanib is a multi-tyrosine kinase inhibitor with the same targets as those of Nintedanib and has been approved by FDA...” (please first define “FDA” here)

P3 last paragraph Line 4: “Pazopanib also had a positive impact on solitary fibrous tumor(SFT)...” (space issue at the highlighted part)

P3-4 Line 5-6: “In a murine xenograft, Pazopanib worked as a possible agent against synovial sarcoma growth mainly via suppressing suppresses the PI3K-Akt pathway[18].” (main verb is missing; verb usage)

P4 Line 8-9: “resulting in of which results reveal a reduction of tumor volume[20].”

P4 Line 9-10: “and a well believed mechanism main reason is via the induction of apoptotic cell death[21].”

P4 Line 11-12: “have has not been reported.”

P4 Line 12: please consider delete “firstly” as there is nothing afterwards

P4 Line 14: “plays” (simple present tense would be fine for general facts or beliefs description)

P4 Line 17-19: “This study provides will provide a theoretical and experimental basis for the clinical treatment of Pazopanib in pulmonary fibrosis with Pazopanib.”

P4 last paragraph Line 4: “purchased from ...”

P4 last paragraph Line 6: “animal care” (no “s” for “animal” here)

P4 last paragraph Line 9: “Animal studies” (no “s” for “animal” here); “were reported” (simple past tense for Methods and Results presentation)

P4 last paragraph Line 10: “5” => “five” (better to spell out number <10 for formal writing; please check across the whole paper)

P4 last paragraph Line 11: please first define “BLM” (abbreviations have to be defined in both the Abstract and the Main Text.)

P4 last paragraph Line 13: use full stop instead of comma before “Nintedanib were purchased from...”

P4 last paragraph Line 13, 14: space issue, add a space before the opening bracket; please check across the whole paper

P5 Line 16: for 7-14 days.” (plural)

P5 Line 17-18: please review “at a dose of 2U/kg body weight for analysis of the fibrotic response.” Please review if the highlighted part is necessary here as this part describes the Methods only. Or please revise the presentation, e.g., “at a dose of 2U/kg of the body weight of each mouse for the analysis of the fibrotic response.”

P5 Line 19: “of the same amount of saline.” => “of saline with the same dose as that of the bleomycin in the BLM group.” (better to be specific, please review)

P5 Line 19-21: “The drug administration group began to be administered daily from the seventh day of the modeling to the 14th day. Mice were sacrificed at Day 14 and lung tissues were was harvested.”

P5 Line 23-24: “Mlg, NIH-3T3 cell lines were kindly gifted from Professor Wen Ning, Nankai University.” => “Professor Wen Ning from the Nankai University kindly provided the Mlg and NIH-3T3 cell lines.” (presentation)

P5 Line 25: “was isolated” => “were isolated” (subject-verb alignment)

P5 Line 26: “are cultured” => “were cultured” (simple past tense for Methods and Results presentation)

P5 Line 27, 29: please check and define “DMEM”, “CO2” first

P6 Line 44: “Transcription was performed using...” (main verb is missing)

P8 Line 105: “Two-tailed student t test” (spell out the number if it starts the sentence)

P8 Line 3 the subheader: “attenuated” (simple past tense for Methods and Results

presentation)

P8 Last paragraph Line 8: “has shown” => “showed” (simple past tense for Methods and Results presentation)

P8 Last paragraph Line 9: “compared with the model group”

P8 Last paragraph Line 10-11: “are illustrated” (subject-verb alignment)

P9 Line 16: “is worse” => “was less obvious” (tense, word choice)

P9 Line 18: please review the term “fibrotic percent” (it seems this is not the natural presentation)

P12 Line 4: “A wide idea” => “A well-known idea” (word choice); “is mainly secreted” (simple present tense would be fine for general facts or beliefs description)

P12 Line 5: “is difficult” (simple present tense would be fine for general facts or beliefs description)

P12 Line 6: please review “in present study” (meaning is not clear for its purpose in the sentence)

P12 Line 7-8: please review “fibroblasts can sustain an high activation and uncontrolled state to repair injured lung tissues” to see if the presentation is valid or clear

P12 Line 9: “decrease” (simple present tense would be fine for general facts or beliefs description)

Reply: Revised as suggested.