Peer Review File

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Reviewer A:

Major comments

Comment 1: Abstract line 26: which different conclusions?

Reply 1: Thank you very much for your valuable comments. The different conclusions we want to express here are that some previous studies have shown that Ki-67 has predictive value for tumor prognosis（Turri-Zanoni M, Maragliano R, Battaglia P, Giovannardi M, Antognoni P, Lombardi D, Morassi ML, Pasquini E, Tarchini P, Asioli S, Foschini MP, Sessa F, Nicolai P, Castelnuovo P, La Rosa S. The clinicopathological spectrum of olfactory neuroblastoma and sinonasal neuroendocrine neoplasms: Refinements in diagnostic criteria and impact of multimodal treatments on survival. Oral Oncol. 2017 Nov;74:21-29. doi: 10.1016/j.oraloncology.2017.09.010. PMID: 29103747.）, but some studies have also shown that Ki-67 is not related to the prognosis of some types of tumors（Böger C, Behrens HM, Röcken C. Ki67--An unsuitable marker of gastric cancer prognosis unmasks intratumoral heterogeneity. J Surg Oncol. 2016 Jan;113(1):46-54. doi: 10.1002/jso.24104. Epub 2015 Dec 28. PMID: 26709194; PMCID: PMC4736456.）， and there are different conclusions between studies regarding the correlation between Ki-67 and tumor prognosis. Meanwhile, studies on the correlation between Ki-67 and prognosis of GEP-NENs need to be further investigated. We have added the detailed presentation to the manuscript. The following is our revised content. "Several previous studies proved that Ki-67 was related to tumor prognosis, but others still reported that Ki-67 had no predictive value for tumor prognosis. There are different conclusions between studies regarding the correlation between Ki-67 and tumor prognosis, and there is a lack of studies about this correlation of GEP-NENs. Further analysis is still needed to evaluate the prognostic value of Ki-67 in GEP-NENs, to provide reference for clinical decisions."

Changes in the text: Page 2, line 26-31.

Comment 2: Abstract lines 30-33: ki67 become compulsory with 2010 WHO, this time could be considered as discriminatory in your study

Reply 2: Thank you very much for your valuable comments. Our intention in this manuscript is not to deny the lack of Ki-67 index in the pathological diagnosis of gastroenteropancreatic neuroendocrine tumors by other authors, only because the raw data were not presented in the article for meta-analysis. We changed the expression in the origin manuscript to "303 studies were retrieved that included Ki-67, GEP-NENs, prognosis, survival, and other subject terms and keywords. We excluded studies that did not show complete Ki-67 index, number of patients and five-year survival data available for meta-analysis, non-cohort studies, articles published before 2000 or not published in English. 15 studies were finally included to assess the value of Ki-67 in the prognosis of patients with GEP-NENs using a random-effects model."

Changes in the text: Page 2, line 33-37.

Comment 3: Abstract lines 35-38: Ki-67 cut since WHO 2017 is 3%, instead 2% is the old one proposed in WHO 2010

Reply 3: Thank you very much for your valuable comments. By reviewing the literatures we found WHO updated the pathological grading criteria for pancreatic neuroendocrine tumors in 2017 to revise the Ki-67 cut-off value to <3% for pancreatic NET G1 grade（Scoazec JY, Couvelard A; Réseau TENpath. Classification des tumeurs neuroendocrines pancréatiques : nouveautés introduites par la classification OMS 2017 des tumeurs des organes endocrines et perspectives [Classification of pancreatic neuroendocrine tumours: Changes made in the 2017 WHO classification of tumours of endocrine organs and perspectives for the future]. Ann Pathol. 2017 Dec;37(6):444-456. French. doi: 10.1016/j.annpat.2017.10.003. Epub 2017 Nov 21. PMID: 29169836.）and applied this pathological criteria to GEP-NENs in 2019.（Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of

tumours of the digestive system. Histopathology. 2020 Jan;76(2):182-188. doi: 10.1111/his.13975. Epub 2019 Nov 13. PMID: 31433515; PMCID: PMC7003895.）.

We added the statement that WHO revised the criteria for pancreatic tumors in 2017 and modified the expression in the manuscript as follows. "The cumulative five-year survival rate for GEP-NEN G1 (Ki-67 <= 2%), G2 (Ki-67 2%-20%) and G3 (Ki-67 >20%) was 86%, 65%, 25% respectively. The five-year survival rate of GEP-NEN G1 (Ki-67 <3%, first revised in WHO classification 2017, redefined WHO classification 2019) and G1 (Ki-67<=2%, WHO classification 2010) was 97% and 84% respectively."

Changes in the text: Page 2, line 39-42.

Comment 4: Highlight box: sentence with Ki67 >55% is difficult to understand

Reply 4: Thank you very much for your valuable suggestion. By reviewing the literatures we found that GEP-NENs with Ki-67 > 55% are more likely to develop lymph node metastasis and patients above and below the 55% cut-off value have different responses to chemotherapy, so whether there is any difference in prognosis for patients with Ki-67 20%-55% and Ki-67 > 55% needs to be further explored（Yan S, Liu T, Li Y, Zhu Y, Jiang J, Jiang L, Zhao H. Value of computed tomography evaluation in pathologic classification and prognosis prediction of gastric neuroendocrine tumors. Ann Transl Med. 2019 Oct;7(20):545. doi: 10.21037/atm.2019.09.114. PMID: 31807527; PMCID: PMC6861764.）. The following is our revised content. "The Ki-67 is important for the prognosis of GEP-NENs. In the future, a large homogeneous population-based cohort study is needed to validate the findings of this study. Meanwhile, some studies have shown that GEP-NENs with Ki-67 > 55% are more likely to develop peritoneal and lymph node metastases, and patients above and below the 55% cut-off value have different responses to chemotherapy, so it is worthy to further investigate whether there is any difference in prognosis between patients with Ki-67 20%-55% and Ki-67 > 55%."

Changes in the text: Page 4, line 54.

Comment 5: Introduction line53: not all neuroendocrine neoplasm for sure originate by neuroendocrine cells in fact neuroendocrine carcinoma could originate by glandular stamina cells.

Introduction lines 54-59: epidemiology is not your target, so please erase this part

Reply 5: Thank you very much for pointing out our mistakes. We have revised the definition of neuroendocrine tumor as follows: Neuroendocrine neoplasms (NEN) are rare tumors that originate from peptidergic neurons or neuroendocrine cells that are clustered in various endocrine glands (pituitary, parathyroid, pancreatic islets, adrenal medulla, and other glands) or scattered in the skin, gastrointestinal tract, bronchial and pulmonary airway mucosa. Therefore, theoretically, neuroendocrine neoplasms can occur in all organs and tissues of the body (except fingernails, toenails and hair). According to the reviewer's suggestion, we have removed the section relating to epidemiology, as described in the manuscript.

Changes in the text: Page 4, line 57-61. Page 5, line 62.

Comment 6: Introduction lines 61-76: it's not clear if authors would to compare all the classes of 2010 WHO with the same of 2019 WHO or if would like to compare only G1 class, please clarify.

Reply 6: We appreciate your suggestions and we revised the statement in the manuscript. The aim of this study is to evaluate the predictive value of the Ki-67 index based on WHO 2010 grading criteria for the prognosis of patients with GEP-NENs by meta-analysis, and to compare the impact of the change of NET G1 cut-off value in WHO 2019 with WHO 2010. The expression has been modified in the manuscript and the following is our revised content. "In 2019, WHO updated the grading criteria for GEP-NENs and remodified the Ki-67 index cut-off for NET G1 from the original <=2% to <3%. But whether the revision of this cut-off value affects the predictive value of Ki-67 for the prognosis of GEP-NENs patients remains unclear. In this study, we evaluated the predictive value of the Ki-67 index based on the WHO 2010 grading criteria for the prognosis of patients with GEP-NENs by meta-analysis, and compared

the impact of the change in the NET G1 cut-off value in WHO 2019 with the WHO 2010 grading criteria to provide references for clinical diagnosis and treatment."

Changes in the text: Page 5, line 78-82.

Comment 7: Study selection: if the investigator disagree

Reply 7: Thank you very much for your suggestion, and a description of the solution for the disagreement between the two independent investigators has been added. The following is our revised content. "The retrieved literatures were initially screened by two independent investigators by viewing the literature abstracts. If the literature met the inclusion criteria, the full-text content and its citations were evaluated in detail, and the useful data were extracted. If two investigators hold different opinions, the disagreement is resolved through negotiation with a third investigator reassesses. The extracted content included the study name, author, year of publication, study type, population size, general information about the study population, tumor site, tumor grade, Ki-67 index, 5-year survival rate, and other indicators."

Changes in the text: Page 6, line 94-100.

Comment 8: Discussion lines 165-166: this is discussion non preliminary information are requested, please move this sentence in introduction

Reply 8: Thank you very much for your suggestion, we have moved this sentence to introduction as you suggested.

Changes in the text: Page 5, line 69-70.

Comment 9: Discussion lines 170-193: Ki-67 is the best prognosis predictor in fact the last 3 WHO classifications are built on Ki-67. Rephrase all the paragraph.

Reply 9: Thank you very much for your suggestion, we have rephrased this paragraph completely and emphasized the importance of Ki-67. The following is our revised content. "In the previous grading criteria for GEP-NENs (2010 and 2019 grading criteria), WHO used Ki-67 index combined with morphological index to achieve stratification of GEP-NENs, showed the importance of Ki-67 for the diagnosis of GEP-

NENs. Several studies have shown that Ki-67 is correlated with the prognosis of patients with neuroendocrine tumors, but this association may have some differences in terms of different locations and types of tumors. This study supported the predictive value of Ki-67 on the prognosis of patients with GEP-NENs through literature search and meta-analysis, and confirmed that the higher the Ki-67 index, the worse the prognosis of patients, providing a clinical reference for the preliminary classification of patients with GEP-NENs according to Ki-67 index. In this study, we found that the overall five-year survival rate of patients showed a decreasing trend as the Ki-67 index increased, and the prognosis of patients decreased significantly when Ki-67 > 20%, which was consistent with previous studies, indicating that the higher the Ki-67 index, the higher the malignancy of the tumor, the higher the risk of recurrence and metastasis in patients and a relatively poorer prognosis."

Changes in the text: Page 10, line 172-184.

Comment 10: Discussion lines 192-193: Why Ki-67 cut off should be controversial?

Reply 10: Thank you very much for your comment. The most commonly used GEP-NENs grading is the WHO grading criteria based on Ki-67 index, the cut-off value for G1 pancreatic neuroendocrine tumors was modified from <=2% to <3% in the WHO 2017 pancreatic neuroendocrine tumor grading criteria（Scoazec JY, Couvelard A; Réseau TENpath. Classification des tumeurs neuroendocrines pancréatiques : nouveautés introduites par la classification OMS 2017 des tumeurs des organes endocrines et perspectives [Classification of pancreatic neuroendocrine tumours: Changes made in the 2017 WHO classification of tumours of endocrine organs and perspectives for the future]. Ann Pathol. 2017 Dec;37(6):444-456. French. doi: 10.1016/j.annpat.2017.10.003. Epub 2017 Nov 21. PMID: 29169836.）, and is fully applied in the WHO 2019 GEP-NENs grading criteria（Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of

tumours of the digestive system. Histopathology. 2020 Jan;76(2):182-188. doi: 10.1111/his.13975. Epub 2019 Nov 13. PMID: 31433515; PMCID: PMC7003895.）. The Ki-67 was calculated by microscopic counting of 500-1000 tumor cells, and the percentage of Ki-67 positive cells was used as the proliferation index, so the index is a continuous variable and the patient's Ki-67 can be kept a decimal in the measurement（Burman P, Casar-Borota O, Perez-Rivas LG, Dekkers OM. Aggressive pituitary tumors and pituitary carcinomas: from pathology to treatment. J Clin Endocrinol Metab. 2023 Feb 28:dgad098. doi: 10.1210/clinem/dgad098. Epub ahead of print. Erratum in: J Clin Endocrinol Metab. 2023 Apr 27;: PMID: 36856733.）, therefore we believe that this change is significant and may be the result of extensive clinical experience. It has been a long-standing effort of clinicians to more precisely set the optimal Ki-67 cut-off value to gradepatients with GEP-NENs and to select the appropriate treatment, and some studies have shown that in ileal NETs, each unit increase in Ki-67 leads to an 18% increase in the risk of death（Panzuto F, Campana D, Fazio N, Brizzi MP, Boninsegna L, Nori F, Di Meglio G, Capurso G, Scarpa A, Dogliotti L, De Braud F, Tomassetti P, Delle Fave G, Falconi M. Risk factors for disease progression in advanced jejunoileal neuroendocrine tumors. Neuroendocrinology. 2012;96(1):32-40. doi: 10.1159/000334038. Epub 2011 Dec 28. PMID: 22205326.）. Therefore, we believe that this change needs to be supported by more research evidence, and we have modified the language expression in this section. The following is our revised content. “The most commonly used grading criteria for GEP-NENs is the WHO grading criteria based on Ki-67 index and mitotic rate, which was first proposed in 2010. The WHO first updated the pathological grading criteria for pancreatic NETs in 2017, changing the cut-off value for G1 grade from <= 2% to < 3% and proposing a relatively better prognosis for NET G3 with high proliferative activity. The WHO fully applied this pathological grading criteria to GEP-NENs in 2019. According to the clinical method of Ki-67 detection, Ki-67 index should be a continuous variable, and a study showed that in pulmonary ACTH-secreting NETs, 1% increase in Ki-67 index increased the

risk of recurrence by 1.41. In ileal NETs, each unit increase in Ki-67 index increased the risk of death by 8%. Therefore, we believe that this change is significant and the result is based on extensive clinical experience. More evidence is needed to support the definition of the optimal cut-off value for GEP-NENs G1."

Changes in the text: Page 10, line 193. Page 11, line 194-203.

Comment 11: What does mean GC patients?

Reply 11: Thank you very much for your suggestion, GC patients means gastric cancer patient, we have revised all the descriptions in the full manuscript.

Changes in the text: Page 5, line 74-75. Page 12, line 216.

Comment 12: Discussion lines 215-222: NEC diagnose is by definition associated to poor morphology no more clarifications deserve

Reply 12: Thank you very much for your suggestion, according to your suggestion we have deleted the redundant description part, the following is our revised content. "The limitation of this study was that the included studies were retrospective, therefore, the study population, tumor staging, and grading were heterogeneous to some extent. A larger cohort study with a homogeneous population is still needed to validate the results of this study. In the comprehensive estimation of the five-year survival rate for patients with GEP-NEN G3 (Ki-67>20%), because of the lack of original patient pathology data, this study did not classify the cytological nature according to the latest WHO 2019 criteria. We only graded NETs according to Ki-67 labelling index, which means that this population contained both NET G3, which had relatively good differentiation and prognosis, and NEC, resulting in a lower prognosis for this group of patients. Therefore to further assess the prognosis of patients with the GEP-NENs（Ki-67 >20%）should consider the impact of the type of pathology."

Changes in the text: Page 12, line 227-228.

Comment 13: Discussion lines 226-227 Ki-67 55% cut off has been proposed to distinguish therapy response: less 55% no response to platinum, more 55% response to platinum

Reply 13: Thank you very much for your suggestion, we have added the discussion of the content of Ki-67 cut-off values on treatment response in this section and further elaborated on the research direction of 55% cut-off values in future studies. The following is our revised content. "Some studies have shown that GEP-NENs with Ki-67 >= 55% respond well to platinum chemotherapy drugs, while GEP-NENs with Ki-67 less than 55% respond poorly to platinum drugs. Moreover, some studies have shown that tumors with Ki-67 index >= 55% are more likely to involve the plasma membrane and to have lymph node metastases, Therefore, whether there is a difference in the prognosis of GEP-NENs patients with Ki-67 20%-55% and those Ki-67>55% needs to be further investigated."

Changes in the text: Page 12, line 232-237.

Reviewer B:

Major issues:

Comment 1: Why were patients with co-morbidities listed as excluded in the methods.

Reply 1: Thank you very much for your valuable comments. The aim of our study was to assess the predictive value of Ki-67 on the prognosis of patients with GEP-NENs, and its assessment as a prognostic predictor is easier to observe in a homogeneous population, because they are more similar in terms of clinical characteristics and are well comparable. The endpoint selected for this study was five-year survival, so if any of the included patients had a combination of other types of tumors or severe diabetic complications, hypoxic disease, cardiovascular disease, or other serious systemic diseases, this group of patients may die because of the comorbidities before reaching the follow-up endpoint, and there is a possibility of bias in the study results and conclusions due to competing risks. Therefore, we excluded patients with comorbidities

from the exclusion criteria to ensure homogeneity and representativeness of the study population and reliability of the findings.

Comment 2: Why were pregnant women excluded in the methods and how could this have been identified from the collected studies?

Reply 2: Thank you very much for your valuable comments, we initially set this exclusion criterion considering pregnant women as a vulnerable group and chose to exclude them based on ethical considerations. After deliberation, since the included studies were retrospective and during the literature search, we found that the retrieved literatures did not mention this special population, the final included literatures were not affected by this exclusion criterion during the inclusion and exclusion phase, and the combined five-year survival rates did not change, so we have modified this part of the exclusion criteria by removing the pregnant population and reorganizing the expression. The following is our revised content. "Exclusion criteria: 1. Patients pathologically diagnosed with combined subtypes of GEP-NENs,（other pathological types of tumors combined with GEP-NENs）; 2. Patients with severe diabetic complications, hypoxic diseases, cardiovascular diseases or other serious systemic diseases; 3. Studies lacking complete raw data such as Ki-67 index and five-year survival rate; 4. Studies lacking corresponding accurate data on the axes through Kaplan-Meier survival curve response grading and 5-year survival rate; 5. Studies published before 2000; 6.Studies not published in English."

Changes in the text: Page 6, line 104-105. Page 7, line 106-109.

Comment 3: The forest plot for figure 2 - 6 are not clear about which groups are being compared in each curve. This needs to be clarified in order to interpret the data.

Reply 3: Thank you very much for your comment. This study is a single-group rate meta-analysis and the study was conducted on the five-year survival of patients with GEP-NENs of different Ki-67 classifications, therefore no control group was set. In Figures 2-6, the five figures represent the combined five-year survival rates obtained by meta-analysis in the five Ki-67 grades. For each figure, the line after each

experiment represents the five-year survival rate and its 95% CI for patients in that Ki-67 grade in each experiment. Total (95% CI) represents the combined effect value of the five-year survival rate for that grade obtained by meta-analysis, and the corresponding five-year survival rate for the specific group is obtained by the upper and lower data conversion formula P=OR/(1+OR). We have added the above description of the forest plot content in the results section of the manuscript. The following is our revised content. "Data analysis were performed using Review Manager 5.4, and the random-effect model was selected to generate forest plots for each level（Figure 2-6）. The calculation of ratio type information was taken, with the outcome indicator being OR, and 95% CI confidence intervals. The line after each trial in the forest plot represents the five-year survival rate and its 95% CI for patients in the Ki-67 grade the figure belongs to. Total (95% CI) represents the combined effect value of the five-year survival rate for that grade obtained by meta-analysis. Overall five-year survival rates were obtained by conversion of the outcome indicator."

Changes in the text: Page 8, line 140-143.

Comment 4: Table 2 needs to be reformatted as it is difficult to understand what it is saying. Acronyms also need to be defined.

Reply 4: Thank you very much for your suggestion. We have reformatted Table 2 to make it easier to understand and redefined the acronyms in the table as you suggested. We would like to explain to you the meaning of this table, which contains the original information registry of studies with complete and analyzable information according to WHO 2010 Ki-67 classifications. The table contains information of the patient characteristics of all study populations, as well as the original Ki-67 classification for each study, the number of patients in the respective classification, and the five-year survival rate before processing. Because the grading criteria of each study were different and could not be analyzed, we combined the above-mentioned studies according to the WHO 2010 GEP-NENs grading criteria within the study subgroups in the meta-analysis and classified them into three grades of Ki-67 <=2%, 2%-20%, and >20% for the subsequent analysis, which is described in the results.

Changes in the text: Revised Tables

Comment 5: Please provide more justification and analyses comparing the two cut points of 2 and 3%. This may be present but unclear in the current way the tables and figures are shown.

Reply 5: Thank you very much for your comment, currently the most commonly used pathological grading for GEP-NENs is developed by WHO, which contains the main grading indexes Ki-67 as well as mitotic rate. The WHO 2010 edition of GEP-NENs guidelines defines the Ki-67 index for grade G1 as <=2%, and in 2017 WHO first updated the pathological grading criteria for pancreatic neuroendocrine tumors, modified the cut-off value of G1 grade to <3%, and introduced the concept of NET G3 with high proliferative activity, and the WHO 2019 GEP-NEN guidelines applied this criterion comprehensively. We believe it is a change based on extensive clinical practice, and we believe this change is meaningful for several main reasons as follows. Firstly, Ki-67 index is an indicator of proliferative activity of tumor cells, and the main method of clinical measurement is to microscopically examine 500-1000 tumor cells and count the proportion of Ki-67 positive cells by staining, so Ki-67 is a continuous indicator, and the decimal number can be kept during Ki-67 measurement（Burman P, Casar-Borota O, Perez-Rivas LG, Dekkers OM. Aggressive pituitary tumors and pituitary carcinomas: from pathology to treatment. J Clin Endocrinol Metab. 2023 Feb 28:dgad098. doi: 10.1210/clinem/dgad098. Epub ahead of print. Erratum in: J Clin Endocrinol Metab. 2023 Apr 27;: PMID: 36856733.）. A study has also shown that for each unit increase in Ki-67 in ileal NETs, there is an 18% increase in the risk of death in patients（Panzuto F, Campana D, Fazio N, Brizzi MP, Boninsegna L, Nori F, Di Meglio G, Capurso G, Scarpa A, Dogliotti L, De Braud F, Tomassetti P, Delle Fave G, Falconi M. Risk factors for disease progression in advanced jejunoileal neuroendocrine tumors. Neuroendocrinology. 2012;96(1):32-40. doi: 10.1159/000334038. Epub 2011 Dec 28. PMID: 22205326.）.Therefore, we believe that this change is very important

for GEP-NENs grading criteria. It has always been the goal of clinicians to set more appropriate Ki-67 cut-off values for GEP-NENs, so we believe that this change needs to be supported by more evidence and deserves to be further investigated. The following is our revised content. "The most commonly used grading criteria for GEP-NENs are the WHO grading criteria based on Ki-67 index and mitotic rate, which were first proposed in 2010. The WHO first updated the pathological grading criteria for pancreatic NETs in 2017, changing the cut-off value for G1 grade from <= 2% to < 3% and proposing a relatively better prognosis for NET G3 with high proliferative activity. The WHO fully applied this pathological grading criteria to GEP-NENs in 2019. According to the clinical method of Ki-67 detection, it should be a continuous variable, and a study showed that in pulmonary ACTH-secreting NETs, 1% increase in Ki-67 index increased the risk of recurrence by 1.41. In ileal NETs, each unit increase in Ki-67 index increased the risk of death by 8%. Therefore, we believe that this change is significant and the result is based on extensive clinical experience. More evidence is needed to support the definition of the optimal cut-off value for GEP-NENs G1." We have also added the explanation of the figures in the manuscript and reformatted the table to make it easier to understand. The following is our explanation for the figures. "The line after each trial in the forest plot represents the five-year survival rate and its 95% CI for patients in the Ki-67 grade the figure belongs to. Total (95% CI) represents the combined effect value of the five-year survival rate for that grade obtained by meta-analysis."

Changes in the text: Page 10, line 193. Page 11, line 194-203. Page 8, line 140-143. Revised Tables

Minor issues:

Comment 1: In the abstract and paper there are minor formatting and grammar errors that could be corrected, such as extra spaces between words etc.

Reply 1: Thank you very much for your valuable suggestions. We have corrected all the formatting errors and grammatical errors we found in the manuscript.

Changes in the text:

Page 2, line 25.

Page 2, line 36.

Page 2, line 39-40.

Page 5, line 65.

Page 6, line 94.

Page 8, line 146.

Page 8, line 147.

Page 8, line 148.

Page 8, line 149.

Page 9, line 164.

Comment 2: Please remove the word predict from the conclusion of the abstract as this is purely being assessed as prognostic and not predictive.

Reply 2: Thank you very much for your suggestion and we have removed the word predict from the abstract and final conclusions based on your suggestion.

Changes in the text: Page 3, line 45. Page 13，line 241.

Comment 3: I do not think the conclusion about changing the cut off in the conclusion of the abstract is correct as 97% and 84% are quite different.

Reply 3: Thank you very much for your comment. For the change in the GEP G1 cut-off value although the WHO did not explain this change in detail, we believe it is based on extensive clinical experience. We obtained five-year survival rates of 84% and 97% before and after the cut-off value change, respectively, based on a single-group rate meta-analysis. The number of patients included in both GEP-NENs G1 classification and the prognosis of the corresponding patients were correlated with the results obtained, which are descriptive and do not imply a significant increase in survival after raising the cut-off value, so we used a very cautious description in drawing our conclusions. We believe that this change in the cut-off value did not significantly reduce the five-year survival rate, because it has been shown that the risk of death and

recurrence of NET patients increases significantly with an increase in Ki-67, but this change in the WHO cut-off value did not significantly reduce the five-year survival rate, So we applied this expression in the conclusion.（Burman P, Casar-Borota O, Perez-Rivas LG, Dekkers OM. Aggressive pituitary tumors and pituitary carcinomas: from pathology to treatment. J Clin Endocrinol Metab. 2023 Feb 28:dgad098. doi: 10.1210/clinem/dgad098. Epub ahead of print. Erratum in: J Clin Endocrinol Metab. 2023 Apr 27;: PMID: 36856733.）

Comment 4: The implication section of the practice points is unclear with respect to the 55% cut point. Please revise this statement.

Reply 4: Thank you very much for your suggestion and we have revised the expression in this section as you suggested. By reviewing the literature, we found that GEP-NENs with Ki-67 > 55% are more likely to develop lymph node metastasis, and this cut-off value has been documented to reflect the sensitivity of patients to chemotherapy, so whether there is a difference in prognosis between patients with Ki-67 between 20%-55% and those with Ki-67 > 55% needs to be followed up with further studies.(Garcia-Carbonero R, Sorbye H, Baudin E, Raymond E, Wiedenmann B, Niederle B, Sedlackova E, Toumpanakis C, Anlauf M, Cwikla JB, Caplin M, O'Toole D, Perren A; Vienna Consensus Conference participants. ENETS Consensus Guidelines for High-Grade Gastroenteropancreatic Neuroendocrine Tumors and Neuroendocrine Carcinomas. Neuroendocrinology. 2016;103(2):186-94. doi: 10.1159/000443172. Epub 2016 Jan 5. PMID: 26731334.) The following is our revised content. "The Ki-67 is important for the prognosis of GEP-NENs. In the future, a large homogeneous population-based cohort study is needed to validate the findings of this study. Meanwhile, some studies have shown that GEP-NENs with Ki-67 > 55% are more likely to develop peritoneal and lymph node metastases, and patients above and below the 55% cut-off value have different responses to chemotherapy, so it is worthy to further investigate whether there is any difference in prognosis between patients with Ki-67 20%-55% and Ki-67 > 55%."

Changes in the text: Page 4, line 54.

Comment 5: In the intro: "highly differentiated" should be changed to well differentiated.

Reply 5: Thank you very much for your suggestion, we have changed the highly differentiated to well differentiated on your suggestion.

Changes in the text: Page 5, line 66.

Reviewer C:

Comment 1: The authors perform a systematic review and meta-analysis of studies including cases of GEP NEN which reported on Ki67 values and 5 year survival, to analyze impact of Ki67 on survival, specifically looking at the impact of Ki67 cutoff of <2 and <3% for survival. However, I think the premise on which the authors based their comparison of <2 and <3% is flawed. The major change in the 2019 WHO guidelines was division of grade 3 tumours into G3 well differentiated NET (Ki67 >20%) and neuroendocrine carcinoma (poorly differentiated into Ki67 >20%) into separate entities. In the 2019 guidelines, for grade 1 tumours Ki-67 is specified as <3% whereas in the 2010 guidelines it was specified as <2% but my understanding is that these cutoff values are completely equivalent and do not reflect a change in the guidelines. See: Nagtegaal et al The 2019 WHO classification of tumours of the digestive system in Histopathology 2019:76;2:1820188 https://onlinelibrary.wiley.com/doi/full/10.1111/his.13975

Reply 1: Thank you very much for your comments. Here is what we think about the significance of the <=2% compared to the <3% cut-off modification. After reviewing the literatures, we found that WHO proposed the grading of GEP-NENs based on two important indicators, Ki-67 index and mitotic rate in 2010, and defined the cut-off value of Ki-67 for GEP-NENs G1 patients as <=2% for the first time (Li ZS, Li Q. [The latest 2010 WHO classification of tumors of digestive system]. Zhonghua Bing Li Xue Za Zhi. 2011 May;40(5):351-4. Chinese. PMID: 21756837.) In 2017, WHO revised the

pathological grading criteria of pancreatic neuroendocrine tumors for the first time, changing the cut-off value of G1 grade from <=2% to <3%, and proposed NET G3 with high proliferative activity to make a distinction from histology（Scoazec JY, Couvelard A; Réseau TENpath. Classification des tumeurs neuroendocrines pancréatiques : nouveautés introduites par la classification OMS 2017 des tumeurs des organes endocrines et perspectives [Classification of pancreatic neuroendocrine tumours: Changes made in the 2017 WHO classification of tumours of endocrine organs and perspectives for the future]. Ann Pathol. 2017 Dec;37(6):444-456. French. doi: 10.1016/j.annpat.2017.10.003. Epub 2017 Nov 21. PMID: 29169836.）, and fully applied this guideline to the pathological grading of GEP-NENs in 2019（Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020 Jan;76(2):182-188. doi: 10.1111/his.13975. Epub 2019 Nov 13. PMID: 31433515; PMCID: PMC7003895.）. The change of the cut-off value of G1 was not explained in detail, we believe that this is a conclusion based on extensive clinical experience, and in our study, we further analyzed this change mainly for the following reasons. Firstly, Ki-67 is measured clinically by microscopic observation of 500-1000 tumor cells, and the percentage of Ki-67 positive cells is counted by staining, so this indicator is a continuous variable, and there are cases between 2% and 3% (Burman P, Casar-Borota O, Perez-Rivas LG, Dekkers OM. Aggressive pituitary tumors and pituitary carcinomas: from pathology to treatment. J Clin Endocrinol Metab. 2023 Feb 28:dgad098. doi: 10.1210/clinem/dgad098. Epub ahead of print. Erratum in: J Clin Endocrinol Metab. 2023 Apr 27;: PMID: 36856733.), so we believe that the change in the cut-off value is very meaningful. The second point is that several studies have evaluated Ki-67 for tumor-related biological risk. In pulmonary ACTH-secreting NETs, the risk of recurrence increased by 1.41 for every 1% increase in Ki-67 index (La Rosa S, Volante M, Uccella S, Maragliano R, Rapa I, Rotolo N, Inzani F, Siciliani A, Granone P, Rindi

G, Dominioni L, Capella C, Papotti M, Sessa F, Imperatori A. ACTH-producing tumorlets and carcinoids of the lung: clinico-pathologic study of 63 cases and review of the literature. Virchows Arch. 2019 Nov;475(5):587-597. doi: 10.1007/s00428-019-02612-x. Epub 2019 Jul 1. PMID: 31264037.). In ileal NETs there is an 8% increase in the risk of death for every unit increase in Ki-67 index（Panzuto F, Campana D, Fazio N, Brizzi MP, Boninsegna L, Nori F, Di Meglio G, Capurso G, Scarpa A, Dogliotti L, De Braud F, Tomassetti P, Delle Fave G, Falconi M. Risk factors for disease progression in advanced jejunoileal neuroendocrine tumors. Neuroendocrinology. 2012;96(1):32-40. doi: 10.1159/000334038. Epub 2011 Dec 28. PMID: 22205326.）.

Therefore, we believe that the WHO has made an important change in the cut-off value for the least malignant G1 grade in GEP-NENs and our study provides support for this change accordingly, and we have added more descriptions of the implications of this study in the discussion section as specified in the revision of the manuscript. The following is our revised content. "The most commonly used grading criteria for GEP-NENs is the WHO grading criteria based on Ki-67 index and mitotic rate, which was first proposed in 2010. The WHO first updated the pathological grading criteria for pancreatic NETs in 2017, changing the cut-off value for G1 grade from <= 2% to < 3% and proposing a relatively better prognosis for NET G3 with high proliferative activity. The WHO fully applied this pathological grading criteria to GEP-NENs in 2019. According to the clinical method of Ki-67 detection, Ki-67 index should be a continuous variable, and a study showed that in pulmonary ACTH-secreting NETs, 1% increase in Ki-67 index increased the risk of recurrence by 1.41. In ileal NETs, each unit increase in Ki-67 index increased the risk of death by 8%. Therefore, we believe that this change is significant and the result is based on extensive clinical experience. More evidence is needed to support the definition of the optimal cut-off value for GEP-NENs G1. In this study, we calculated the combined five-year survival rate estimates using two different ki-67 cut-off values as G1 grading criteria. We found that the combined five-year survival rate estimates for the group with a Ki-67 cut-off value of <3% for G1 GEP-NEN did not significantly decrease compared with the group with a

cut-off value of <=2%, so the increase in the cut-off value did not affect the accuracy of Ki-67 for prognosis prediction. The results of the meta-analysis showed that the combined five-year survival rates for GEP-NEN G1 (Ki-67 <3%) and GEP-NEN G1 (Ki-67 <=2%) were 97% and 84%, respectively. This result might due to the small number of patients observed with a <3% cut-off value and does not indicate an improved prognosis for patients with an elevated Ki-67 index. Unlike the previous tertiary comparisons, this part of the comparison has an overlapping area of Ki-67 index, and also reflected that the overall definition of well differentiated and good prognosis for G1 grade was more consistent using <3% as the cut-off value, providing some evidence for the change in the WHO 2019 GEP-NEN grading criteria. Lee HE et al. found an increase in value-added ki-67 index indicated that tumor cells were more proliferative, but highly proliferating tumor cells had less invasive subclones and therefore had less metastatic potential, resulting in a better prognosis for gastric cancer patients. However, based on our results, we suggested that elevating the cut-off value of ki-67 in GEP-NEN G1 with no significant decrease in patient prognosis. That means a similar mechanism may exist for GEP-NEN, which needs to be further confirmed by subsequent studies."

Changes in the text: Page 10, line 193. Page 11 , line 194-203.