PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	THE UTILITY OF KI-67 AS A PROGNOSTIC BIOMARKER IN PULMONARY NEUROENDOCRINE NEOPLASMS: A SYSTEMATIC REVIEW AND META-ANALYSIS.
AUTHORS	Naheed, Salma; Holden, Chloe; Tanno, Lulu; Pattini, Linda; Pearce, Neil; Green, Bryan; Jaynes, Eleanor; Cave, Judith; Ottensmeier, Christian H.; Pelosi, Giuseppe

VERSION 1 – REVIEW

REVIEWER	Loya, Asif Shaukat Khanum Memorial Cancer Hospital and Research Centre, Pathology
REVIEW RETURNED	18-Aug-2020

GENERAL COMMENTS	1. Major limitations. 2. Missing Prospective studies. 3. Limited number of studies and all retrospective. 4. Studies selected are not comparative. 5. Ki67 being evaluated is not compared to other variables like stage, grade, mitoses. 6. Should add proposed thresholds for Ki67 index and its correlational with grade, survival data.

REVIEWER	Tanaka, Yugo Kobe University Graduate School of Medicine School of Medicine
REVIEW RETURNED	13-Oct-2020

GENERAL COMMENTS	The authors reviewed previous reports about Ki67, a known biomarker of cellular proliferation, to present a convenient prognostic marker for bronchopulmonary neuroendocrine neoplasms. After my review I have the following comments to the authors of this review. Major 1. There are many contents of the “Introduction” section. I think some paragraphs should be moved to the “Discussion” section. Please summarize a little more briefly how you came to work on this review. 2. There are only two reports on LCNEC and SCLC in this review, and the number of cases is small. In addition, Ki-67 is considered to have little significance as a prognostic marker in both reports. Since LCNEC and SCLC are tumors with a very poor prognosis, I

	consider that Ki-67 may not be a prognostic marker for those histological type. 3. The authors have included LCNEC and SCLC in this review and analyzed them. Do you really think that Ki-67 is a prognostic marker in LCNEC and SCLC? Since the authors analyzed the results of other papers dealing only with TC and AC, it seems that those results influenced the entire NEN in your study. I am convinced that Ki-67 is a prognostic marker for TC and AC. However, from this result, I disagree that Ki-67 will be a prognostic marker for LCNEC and SCLC. Also, from this review, I am afraid that readers may misunderstand that Ki-67 is a prognostic marker for LCNEC, SCLC. 4. Some LCNECs and SCLCs have a better prognosis and there are several past reports about the association between NE markers and prognosis. From these previous reports, I think we should carefully consider whether to include HGNEC in this review. 5. I think HGNEC (SCLC and LCNEC) and carcinoid (TC and AC) should be considered as different group because they have very different clinical aspects (treatment policy and prognosis). Considering the above concerns, we strongly recommend that LCNEC and SCLC should be excluded from this review and the significance of the Ki-67 marker should be considered by focusing on TC and AC. Minor 1. The explanation of some abbreviations in Table and Figure is missing.

REVIEWER	Scoazec, JY Gustave Roussy
REVIEW RETURNED	17-Oct-2020

GENERAL COMMENTS	The manuscript by Naheed et al describes a meta analysis focusing on the potential role of Ki67 as a biomarker in lung neuroendocrine neoplasms. Conclusions are based on the only 7 papers found suitable for study in the literature. The work might be of interest to fuel the current debate among specialists about the inclusion, or not, of the Ki67 index in the evaluation of these neoplasms, but it might be not timely since the revision of the WHO classification has already been made. A number of issues might be raised. 1. Introduction: a/ the authors state that lung NENs "derive from pulmonary enterochromaffin cells" : the term "derive" might not be appropriate; well differentiated NENs recapitulate most of the phenotype of peptidergic cells but do they really derive from them ? moreover, it has been suggested that poorly differentiated NENs may derive from any lung cell type, if the appopriate genetic changes occur. In addition, the term "enterochromaffin" usually refers to serotonin-producing endocrine cells; is it fully appropriate here ?

	b/ a number of recent references about Ki67 functions are not cited, especially the role of Ki67 as "chromosomal surfactant" or "nucleolar organizer" c/ The techniques described for evaluating Ki67 are those recommended for digestive NENs; this must be acknowledged; recommendations are different for breast tumors d/ the authors must state clearly that Ki67 index is required for grading and classification of digestive NENs noty only to "assist oncologists to select the best treatment" e/ for GEP-NENs, only mitotic index and Ki67 index are required, not necrosis, unlike the authors'statement page 6 f/ beyond the 2017 WHO classification of pancreatic NENs, there is now a 2019 WHO classification for all digestive NENs; please acknowledge and cite ! 2. Methods and results a/ It is really unclear how the authors retrieve as many as 12,000 papers from their literature search. Were the criteria well selected ? b/ MIB1 is the recommended antibody for Ki67 evaluatiuon; if this is not precised in the paper analyzed, there might be a bias c/ the number of works selected is very low and it is probably difficult to draw definitive conclusions 3. Discussion a/ Why not cite GEP-NENs aming the tumors in which the prognostic value of Ki67 has been demonstrated and is even more thoroughly accepted than in some of the tumors cited ? b/ that Ki67 index is a continuous variable is obvious c/ the remark about the "fundamental biological diversity between the two tumor sites" which might prevent the use of the same grading system must be clarified and substantiated by references ... it is not so obvious 4. References Their presentation does not conform to journal instructions, is heterogeneous and sometimes incomplete (see for instance, 27)

REVIEWER	Bernasconi, Davide Università degli Studi di Milano-Bicocca, School of Medicine and Surgery
REVIEW RETURNED	20-Jan-2021

GENERAL COMMENTS	This systematic review and meta-analysis on the role of KI-67 as a prognostic marker in lung neuroendocrine neoplasms is well conducted and well reported. The PRISMA guidelines are fully accomplished. From the methodological point of view, I have only minor comments to point out: - page 7, line 38. Do the HRs of interest describe outcome variation due to a 1 point increase in the Ki-67 concentration units? In other words, do these HRs express association with the outcome of the continuous Ki-67 variable? Please specify. - page 7, line 41. Can you clarify the difference between RFS and DFS in this setting? I think it would be useful for readers to add a

	definition of the three endpoints of interest (e.g. RFS is time elapsed between event x and event y or z). - page 8, lines 3-4. This sentence does not sound clear to me. I^2 statistic is indeed a measure of heterogeneity between studies and not a way to calculate the pooled measure. I suggest rephrasing it, e.g. “In order to assess the heterogeneity of results between studies, Higgins I^2 statistic was used.” - Page 8, line 8. The Begg's method is known to be largely underpowered (Sterne JAC, Gavaghan D, Egger M. Publication and related bias in meta-analysis: Power of statistical tests and prevalence in literature. Journal of Clinical Epidemiology 2000;53:1119-1129.) and should not be mentioned, in my opinion. - Page 8, lines 32-33. Do you mean that the 5 year survival estimate was not reported in all studies and could only be detected from the Kaplan Meier plot? Please clarify. - Page 9, line 27. “…how hazard ratios are derived”. - Page 9, line 27. Consider moving the sentence “The pooled HR…” at the end of the paragraph (high heterogeneity --> random effects model --> pooled HR of the random effects model). - Page 9, line 39. Again, consider moving the sentence “The pooled HR…” at the end of the paragraph. - Page 11, line 14. It is more appropriate to say “is associated” instead of “correlates”. Consider changing this also in the conclusions of the abstract. - Page 23, figure 3. There is a typo “Cusamano” instead of “Cusumano”. Also, it is not nice to have the red square of the Cusumano study overlapping the vertical line HR=1 while the confidence does not include 1. I realize that this is due to the weight assigned to the study leading to a square larger than the amplitude of the confidence interval but maybe the problem can be fixed by rescaling the area of squares.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Asif Loya, Shaukat Khanum Memorial Cancer Hospital and Research Centre

Comments to the Author:

1. Major limitations.

Response: We thank you for this comment. As with any study, there will be limitations to the work. We have acknowledged the limitations of our study in the discussion (paragraph 4 and 5 of ‘Discussion’).

2. Missing Prospective studies.

Response: We thank you for this useful suggestion. We acknowledged this limitation in the discussion (paragraph 4).

3. Limited number of studies and all retrospective.

Response: We thank you for this useful suggestion. We acknowledged this limitation in the discussion (paragraph 4 and 5).

4. Studies selected are not comparative.

Response: Thank you for this comment. We have acknowledged the heterogeneity of the studies and, as the reviewer rightly point out, this heterogeneity is the reason why 5 year survival data was not included in the studies instead relying on hazard ratios

5. Ki67 being evaluated is not compared to other variables like stage, grade, mitoses.
Response: Thank you for this comment. We have acknowledged this in the paragraph 2 of the discussion and explained the reasons why – namely the limited number of good quality studies precludes such an analysis.

6. Should add proposed thresholds for Ki67 index and its correlational with grade, survival data.

Response: Thank you for this comment. We had initially intended to capture data regarding Ki-67 thresholds but unfortunately due to the heterogeneity of studies available, it was not possible to present the data in this way.

Reviewer: 2

Dr. Yugo Tanaka, Kobe University Graduate School of Medicine School of Medicine
Comments to the Author:
The authors reviewed previous reports about Ki67, a known biomarker of cellular proliferation, to present a convenient prognostic marker for bronchopulmonary neuroendocrine neoplasms.
After my review I have the following comments to the authors of this review.

Major
1. There are many contents of the “Introduction” section. I think some paragraphs should be moved to the “Discussion” section. Please summarize a little more briefly how you came to work on this review.
Response: Thank you for your suggestion. We have amended the manuscript reorganising the text as suggested. The remainder of your comments we have dealt with together.

2. There are only two reports on LCNEC and SCLC in this review, and the number of cases is small. In addition, Ki-67 is considered to have little significance as a prognostic marker in both reports. Since LCNEC and SCLC are tumors with a very poor prognosis, I consider that Ki-67 may not be a prognostic marker for those histological type.

Response: Please see the discursive text below.

3. The authors have included LCNEC and SCLC in this review and analyzed them. Do you really think that Ki-67 is a prognostic marker in LCNEC and SCLC? Since the authors analyzed the results of other

papers dealing only with TC and AC, it seems that those results influenced the entire NEN in your study. I am convinced that Ki-67 is a prognostic marker for TC and AC. However, from this result, I disagree that Ki-67 will be a prognostic marker for LCNEC and SCLC. Also, from this review, I am afraid that readers may misunderstand that Ki-67 is a prognostic marker for LCNEC, SCLC.

Response: Please see the discursive text below.

4. Some LCNECs and SCLCs have a better prognosis and there are several past reports about the association between NE markers and prognosis. From these previous reports, I think we should carefully consider whether to include HGNEC in this review.

Response: Please see the discursive text below.

5. I think HGNEC (SCLC and LCNEC) and carcinoid (TC and AC) should be considered as different group because they have very different clinical aspects (treatment policy and prognosis). Considering the above concerns, we strongly recommend that LCNEC and SCLC should be excluded from this review and the significance of the Ki-67 marker should be considered by focusing on TC and AC.

Response: Please see the discursive text below.

Discursive Text:

We thank the reviewer for these thoughtful, well-constructed and supported comments. We fully appreciate that there are different “schools of thought” as to the utility of Ki-67. This manuscript is an objective literature review which meets international standards as readily acknowledged by many of the other reviewers.

We appreciate that the number of studies including SCLC and HGNEC is small. However, the PROSPERO standard protocol – produced at the outset of this study planned to include the full spectrum of pulmonary neuroendocrine neoplasms (including the carcinomas). Any deviation from this, once we knew what studies were available, would be a retrospective amendment and by its very nature, an amendment that would be biased. One reason there for the limited number of studies available is the historical lack of interest in Ki-67 in these tumour types. We are also aware of the fact that SCLC is often diagnosed on cytology, making accurate assessment of Ki-67 challenging.

Having regard for the poor prognosis of SCLC and HGNEC the reviewer is absolutely correct for highlighting this. However, as the reviewer also acknowledges within HGNEC and SCLC there are some patients who fare better and there are indeed a number of biomarkers which have been identified which might have greater utility in identifying these patients; accordingly we have included this in the discussion

[paragraph 5 of the Discussion]. Nonetheless, we did not wish to ignore the possibility that Ki-67 may be of some value and it is only by doing work such as this study that clinician scientists can determine whether a biomarker has any clinical utility.

We agree with the reviewer that the evidence we have found thus far does not support the use of Ki-67 to subtype HGNEC and SCLC, possibly for some of the reasons identified above. We do not want to accidentally give our readers the impression that Ki-67 is a prognostic marker for LCNEC, SCLC, and we are grateful to you for pointing out that our paper might be misinterpreted in this way. We have changed [please see paragraph 5 of the discussion]. Nevertheless we did not want to preclude future research into Ki-67 in these tumour groups with so little evidence, especially considering that in high grade gastroenteropancreatic NETS, there is good evidence to suggest that Ki-67 is contributory with a cut off of 55% (ref Nordi NEC study https://pubmed.ncbi.nlm.nih.gov/22967994/). It is on the basis, we suggest that future studies should be carried out with larger numbers.

Minor

1. The explanation of some abbreviations in Table and Figure is missing.

Response: We have reformatted the Tables and Figures in keeping with the 'Guidance to Authors' issued by the Editorial Team

Reviewer: 3

Dr. JY Scoazec, Gustave Roussy

Comments to the Author:

The manuscript by Naheed et al describes a meta analysis focusing on the potential role of Ki67 as a biomarker in lung neuroendocrine neoplasms. Conclusions are based on the only 7 papers found suitable for study in the literature. The work might be of interest to fuel the current debate among specialists about the inclusion, or not, of the Ki67 index in the evaluation of these neoplasms, but it might be not timely since the revision of the WHO classification has already been made.

Response: Thank you for highlighting the importance of this work, particularly to those who work in this field. In so far as the WHO classification is concerned, this manuscript highlights an important aspect which appears to hae been overlooked and would warrant inclusion at the next iteration. By publishing this now, we are laying the foundations for a discussion amongst the scientific community, which can then be utilised at that, next, iteration.

A number of issues might be raised.

1. Introduction:

a/ the authors state that lung NENs "derive from pulmonary enterochromaffin cells" : the term "derive" might not be appropriate; well differentiated NENs recapitulate most of the phenotype of peptidergic cells but do they really derive from them ? moreover, it has been suggested that poorly differentiated NENs may derive from any lung cell type, if the appopriate genetic changes occur. In addition, the term "enterochromaffin" usually refers to serotonin-producing endocrine cells; is it fully appropriate here ?

Response: We thank you for this comment. We have removed this statement from our text and revised the sentence accordingly.

b/ a number of recent references about Ki67 functions are not cited, especially the role of Ki67 as "chromosomal surfactant" or "nucleolar organizer"

Response: We have included text to reflect this additional information (Page 4, Paragraph 2)

c/ The techniques described for evaluating Ki67 are those recommended for digestive NENs; this must be acknowledged; recommendations are different for breast tumors

Response: Thank you for this useful comment. We have now acknowledged this within the text.

d/ the authors must state clearly that Ki67 index is required for grading and classification of digestive NENs noty only to "assist oncologists to select the best treatment"

Response: Thank you for this useful comment. We have adjusted the introduction to reflect your comments.

e/ for GEP-NENs, only mitotic index and Ki67 index are required, not necrosis, unlike the authors' statement page 6

Response: Thank you for highlighting this error. We have amended the manuscript.

f/ beyond the 2017 WHO classification of pancreatic NENs, there is now a 2019 WHO classification for all digestive NENs; please acknowledge and cite!

Response: Thank you for highlighting this. We have amended the text appropriately.

2. Methods and results

a/ It is really unclear how the authors retrieve as many as 12,000 papers from their literature search. Were the criteria well selected?

Response: Thank you for this comment. The search strategy was designed with the help of a University of Southampton librarian. It was intentionally designed not to be too restrictive in order to ensure any papers of relevance were not missed. I have attached a copy of the precise search strategy used for Medline OVID and EMBASE as an additional supplementary file.

b/ MIB1 is the **recommended** antibody for Ki67 evaluation; if this is not precised in the paper analyzed, there might be a bias

Response: Thank you for this comment. We have amended the introduction to reflect this point. In the results section we have documented how many of the studies disclosed which antibody had been used for evaluation of the Ki-67 LI. Within the discussion we have also mentioned that there could be bias as a result of the differences in the dilution of primary antibody utilised.

c/ the number of works selected is very low and it is probably difficult to draw definitive conclusions

Response: We agree with this comment and have acknowledged this in the discussion and conclusion.

3. Discussion

a/ Why not cite GEP-NENs aiming the tumors in which the prognostic value of Ki67 has been demonstrated and is even more thoroughly accepted than in some of the tumors cited?

Response: We agree with this comment and have amended the discussion to reflect this suggestion.

b/ that Ki67 index is a continuous variable is obvious

Response: We agree but some readers may find this clarification helpful.

c/ the remark about the "fundamental biological diversity between the two tumor sites" which might prevent the use of the same grading system must be clarified and substantiated by references ... it is not so obvious

Response: Thank you for this comment. We have amended the text to provide further clarification as have substantiated this through the addition of the relevant references.

4. References

Their presentation does not conform to journal instructions, is heterogeneous and sometimes incomplete (see for instance, 27)

Response: We have amended the formatting to conform to the journal instructions; where it was wayward, we apologise.

Reviewer: 4

Dr. Davide Bernasconi, Università degli Studi di Milano-Bicocca

Comments to the Author:

This systematic review and meta-analysis on the role of KI-67 as a prognostic marker in lung neuroendocrine neoplasms is well conducted and well reported. The PRISMA guidelines are fully accomplished.

Response: Thank you for your positive comments highlighting the strengths of this review.

From the methodological point of view, I have only minor comments to point out:

- page 7, line 38. Do the HRs of interest describe outcome variation due to a 1 point increase in the Ki-67 concentration units? In other words, do these HRs express association with the outcome of the continuous Ki-67 variable? Please specify.

Response: Thank you for this comment. The papers of origin predominantly looked at Ki-67 as a categorical rather than a continuous variable.

- page 7, line 41. Can you clarify the difference between RFS and DFS in this setting? I think it would be useful for readers to add a definition of the three endpoints of interest (e.g. RFS is time elapsed between event x and event y or z).

Response: We have added this detail to the manuscript, as requested.

- page 8, lines 3-4. This sentence does not sound clear to me. I^2 statistic is indeed a measure of heterogeneity between studies and not a way to calculate the pooled measure. I suggest rephrasing it, e.g. “In order to assess the heterogeneity of results between studies, Higgins I^2 statistic was used.”

Response: Thank you for your comment – we have revised the text as per your suggestion.

- Page 8, line 8. The Begg's method is known to be largely underpowered (Sterne JAC, Gavaghan D, Egger M. Publication and related bias in meta-analysis: Power of statistical tests and prevalence in literature. Journal of Clinical Epidemiology 2000;53:1119-1129.) and should not be mentioned, in my opinion.

Response: Thank you for this comment. We had planned to use this method in our protocol and therefore in order to keep to the planned protocol of this study we have commented on this. However, we were unable to use Begg’s method at any rate due to the low number of studies included in the meta-analysis.

- Page 8, lines 32-33. Do you mean that the 5 year survival estimate was not reported in all studies and could only be detected from the Kaplan Meier plot?

Response: We agree with your comments and have rewritten this sentence to make it clearer.

- Page 9, line 27. “…how hazard ratios are derived”.

Response: We apologise for this typo and have corrected the error.

- Page 9, line 27. Consider moving the sentence “The pooled HR…” at the end of the paragraph (high heterogeneity --> random effects model --> pooled HR of the random effects model).

Response: We agree with your comment and have moved the sentence as per your suggestion.

- Page 9, line 39. Again, consider moving the sentence “The pooled HR…” at the end of the paragraph.
Response: As above, we agree with your comment and have moved the sentence as per your suggestion.

- Page 11, line 14. It is more appropriate to say “is associated” instead of “correlates”. Consider changing this also in the conclusions of the abstract.

Response: We agree with this comment and have revised the text accordingly.

- Page 23, figure 3. There is a typo “Cusamano” instead of “Cusumano”. Also, it is not nice to have the red square of the Cusumano study overlapping the vertical line HR=1 while the confidence does not include 1. I realize that this is due to the weight assigned to the study leading to a square larger than the amplitude of the confidence interval but maybe the problem can be fixed by rescaling the area of squares.

Response: Thank you for this comment. We apologise for the spelling error and have corrected this. We have attempted to rescale the squares but unfortunately we have been able to remedy this issue with the software we have utilised.

VERSION 2 – REVIEW

REVIEWER	Loya, Asif Shaukat Khanum Memorial Cancer Hospital and Research Centre, Pathology
REVIEW RETURNED	11-May-2021

GENERAL COMMENTS	1. No prospective studies included. 2. No multivariate analysis done. 3. Limited number of studies included. Perhaps, initial search needs to be better defined. 4. Extremely limited number of high grade NEN with a major chunk of low grade NEN making it difficult to draw a meaningful conclusion. 5. No well defined scoring criteria available for Ki67 indices in case of Pulmonary NEN. 6. Limited availability of survival data. 7. Recommend including more studies to answer/resolve the above described limitations and resubmitting.

REVIEWER	Tanaka, Yugo Kobe University Graduate School of Medicine School of Medicine
REVIEW RETURNED	11-May-2021

GENERAL COMMENTS	There are no further comments. Thank you for your response to my comments.

REVIEWER	Scoazec, JY Gustave Roussy
REVIEW RETURNED	30-May-2021

GENERAL COMMENTS	The manuscript by Naheed et al describes the results of a meta analysis of the prognostic relevance of Ki67 index in pulmonary neuroendocrine neoplasms (NEN). The possible role of Ki67 in lung NEN remains an unsolved issue and results in a major discrepancy in the grading systems used for digestive NEN, on one hand, ang lung NEN, on the other hand. The question

	addressed is therefore an important one in the field of NEN and a meta-analysis is an intersting strategy to address the point. However, the results presented are somewhat disappointing, not because of the authors, but because of the very few articles fulfilling the requirements stated by the study design. Nevertheless, despite its limited value, this study, even it does not reach a high priority, remains of interest, at least for a specialized readership Some minor issues: - introduction: lung NEN, if they include SCLC, are not "rare" diseases; only carcinoids and LCNEC are rare - introduction: it is not the heterogeneity of well differentiated digestive NEN which has been recognized by the new category NETG3, but the heterogenreity of G3 NEN, which contain both well- and poorly-differentiated neoplasms - results: is it possible to have more details about the method of Ki67 counting in each study ? is the information provided ? - discussion: a cut off of 55% has been suggested to stratify digestive G3 NEN and predict their response to platinum salts; is there any evidence in lung NENs supporting or suggesting something analogous ?

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Dr. Asif Loya, Shaukat Khanum Memorial Cancer Hospital and Research Centre
Comments to the Author:
1. No prospective studies included.

Response: Thank you for your these comments. As discussed in the response to the first revision, we have acknowledged this limitation in our discussion. Furthermore it is not a failure of the systematic review that there are a lack of prospective studies included but rather a product of the current state of the literature.

2. No multivariate analysis done.

Response: We thank you for the comment. As discussed in our PROSPERO protocol (previously published in the BMJ Open) and our current paper, the aim of this study is to carry out a systematic review and if the literature allows a meta-analysis. We have undertaken a meta-analysis with the literature available. It is simply not possible to undertake a multivariate analysis of a meta-analysis.

3. Limited number of studies included. Perhaps, initial search needs to be better defined.

Response: Thank you for this comment. The search strategy was designed with the help of a University of Southampton librarian. It was intentionally designed not to be too restrictive in order to ensure any papers of relevance were not missed. I have attached a copy of the precise search strategy used for Medline OVID and EMBASE as an additional supplementary file.

4. Extremely limited number of high grade NEN with a major chunk of low grade NEN making it difficult to draw a meaningful conclusion.

Response: Since the PROSPERO protocol had planned to include the full spectrum of pulmonary neuroendocrine neoplasms (including the carcinomas), we felt it was important not to deviate from the planned protocol once we knew what studies were available. We feel that it's likely that one reason there were few studies was because of the historical lack of interest in Ki-67 in these tumour types. We are also aware of the fact that SCLC is often diagnosed on cytology, making accurate assessment of Ki-67 challenging.

5. No well defined scoring criteria available for Ki67 indices in case of Pulmonary NEN.

Response: Yes we are aware that there are currently no well defined scoring criteria available for Ki-67 indices in the case of pulmonary NENs. We have acknowledged this in our discussion and hope that this paper will induce further discussion on this subject.

6. Limited availability of survival data.

Response: Yes we are aware of the limited survival data available to us. This was one of the reasons we were unable to complete the full analysis we had hoped to. The literature was simply not there to verify the survival data based on Ki-67.

7. Recommend including more studies to answer/resolve the above described limitations and resubmitting.

Response: Following a very thorough literature review using a PROSPERO devised protocol, we have concluded that these studies are what is available and fits the parameters of this prospectively designed literature review. It is simply not a case of being able to add more studies.

Reviewer: 2
Dr. Yugo Tanaka, Kobe University Graduate School of Medicine School of Medicine
Comments to the Author:
There are no further comments.
Thank you for your response to my comments.

Response:

Reviewer: 3

Dr. JY Scoazec, Gustave Roussy
Comments to the Author:

The manuscript by Naheed et al describes the results of a meta analysis of the prognostic relevance of Ki67 index in pulmonary neuroendocrine neoplasms (NEN). The possible role of Ki67 in lung NEN remains an unsolved issue and results in a major discrepancy in the grading systems used for digestive NEN, on one hand, ang lung NEN, on the other hand. The question addressed is therefore an important one in the field of NEN and a meta-analysis is an intersting strategy to address the point.

However, the results presented are somewhat disappointing, not because of the authors, but because of the very few articles fulfilling the requirements stated by the study design. Nevertheless, despite its limited value, this study, even it does not reach a high priority, remains of interest, at least for a specialized readership

Thank you for your thoughtful comments. We thank the author for recognising that this is an important area which needs to be addressed in the field of pulmonary NENs and a meta-analysis is a novel means of doing this.

Some minor issues:
- introduction: lung NEN, if they include SCLC, are not "rare" diseases; only carcinoids and LCNEC are rare.

Response: Thank you for this comment. We have amended the text accordingly.

- introduction: it is not the heterogeneity of well differentiated digestive NEN which has been recognized by the new category NETG3, but the heterogenreity of G3 NEN, which contain both well- and poorly-differentiated neoplasms

Response: Thank you for this suggestion. We have amended the text to reflect this suggestion.

- results: is it possible to have more details about the method of Ki67 counting in each study ? is the information provided ?

Response: Thank you for this comment. The methodology for calculating Ki-67 in each study included in the systematic review can be found in Table 1.

- discussion: a cut off of 55% has been suggested to stratify digestive G3 NEN and predict their response to platinum salts; is there any evidence in lung NENs supporting or suggesting something analogous ?

Response: Thank you for this thoughtful comment. Only 1 study looked at a cut-off of 55% (Rego et al, 2017) and this only included the small cell population. Therefore, currently there is insufficient evidence to support a similar cut-off in lung NENs.

VERSION 3 – REVIEW

REVIEWER	Loya, Asif Shaukat Khanum Memorial Cancer Hospital and Research Centre, Pathology
REVIEW RETURNED	29-Sep-2021

GENERAL COMMENTS	The article is well written and major limitations with the study have been highlighted clearly.