Peer Review File

REVIEWER A

This is a very nice and succinct review of rare subtypes of lung cancer. Very comprehensive accounts of the entities are presented in an easy to read and concise fashion. I enjoyed reading it and found it helpful. I have only two very minor suggestions for amendments:

Comment 1: Line 67: I would say that sarcomatoid carcinomas show features 'suggestive of both epithelial and mesenchymal differentiation'. This is to reflect that fact that the 'mesenchymal' components of at least some of these entities may not actually be mesenchymal, but are instead likely very poorly differentiated epithelial components.

Reply 1: We thank the review for his comment. We have modified our text as advised.

Changes in the text: "Pulmonary sarcomatoid carcinomas (PSC) are a rare and poorly differentiated subtype of NSCLC, showing features suggestive of both epithelial and mesenchymal differentiation." (see page 5, lines 76-78).

Comment 2: Line 313: I think that it is unlikely that NUT carcinoma would be misdiagnosed as acute leukaemia. I would recommend not including this.

Reply 2: We appreciate reviewer's suggestion. We modified the text as advised.

Changes in the text: NCs often stain for CD34 and occasionally they show positivity also for chromogranin, synaptophysin, and even TTF1 (see page 14, line 293-294).

REVIEWER B

The writing is well written, but overall, there are some insufficiencies in the use of figures and tables. Therefore, I would like to recommend as following.

Comment 1: It needs one table that contains and summarizes the type and subtype of rare histologic lung cancer, columns describing histologic characteristics, frequency, markers, and genetic changes.

Reply 1: We really appreciate review's suggestion. We created a table summarizing main characteristics of rare lung tumor entities discussed in this review (see table 1).

Changes in the text: see table 1.

Comment 2: Also it would better be to presents another table summarizing treatment options.

Reply 2: We thank the reviewer for his comment. We decided to create a unique table summarizing histologic characteristics, frequency, markers, genetic changes and treatment options of rare lung tumor entities discussed in this review (see table 1).

Changes in the text: see table 1.

Comment 3: Every photo needs magnification and scale on the figures

Reply 3: We thank the reviewer for this comment. We modified figures as suggested.

Changes in the text: All the figure magnifications and staining (as suggested by Reviewer C) were added to figures and to figures' legends.

Comment 4: A table in the figure 4D need to be separately presented

Reply 4: We thank the reviewer for his comment. The figure 4D was the graphical representation of the fusion detection in the NGS software. The first line showed the fusion product detected. The other lines were the control genes that are always present in the run. As also suggested by the Reviewer C, this last part could make confusion, so we decide to remove it and to show only the positive fusion detection.

Changes in the text: Positive control genes deleted.

Comment 5: Characteristic radiographic photo would help understanding of the manuscript, esp PSC, p-SGTs.

Reply 5: We appreciate reviewer's suggestion. We added some radiological images to support the understanding of the text.

Changes in the text: see figures 5-9.

REVIEWER C

Comment 1: English is not my native language, nevertheless some errors are scattered within the manuscript that need to be reviewed: ie l307 "iperexpression"

Reply 1: We thank the reviewer for his suggestion. We conducted a thorough reading of the text and corrected linguistic inaccuracies.

Changes in the text: A new revision of the English was made.

Comment 2: this review excludes non-epithelial tumors, which is not explained

Reply 2: We appreciate reviewer's comment. In the introduction section, we specified that this review is focused on rare epithelial types of cancer and that it will exclude rare non-epithelial neoplastic entities.

Changes in the text: In the introduction section we explained why we excluded sarcomas and we substituted the word "tumor" with "cancer" to be clearer. (see page 4, lines 58-63)

Comment 3: The SMARCA4-deficient tumor is not addressed even though it is a separate entity in the WHO 2021 classification.

Reply 3: We thank the reviewer for his suggestion. In accordance with the WHO 2021 classification, we added a paragraph focused on undifferentiated SMARCA4-deficient tumors, even if it is a borderline entity, more similar to mesenchymal tumors, as for frequent morphological similarities with poorly differentiated lung cancer, even if this review is not focused on mesenchymal tumors. We excluded SMARCA4-deficient NSCLCs considering that their occurrence is far from rare.

Changes in the text: A paragraph on undifferentiated SMARCA4-deficient tumors was added (see page 16).

Comment 4: Large cell carcinoma, an entity that is certainly in the process of being dismembered and almost a diagnosis of exclusion, is not addressed.

Reply 4: We thank the reviewer for his comment. We agree with the Reviewer that pure large cell carcinoma (i.e. "null" phenotype) is becoming a very rare and dismembering entity. Since this review is focused on therapeutic options different from conventional and more frequent lung cancers, we decide to exclude from rarer cancer this entity that is more similar to the other more common histologies in terms of management and treatment. We therefore avoided to confuse the reader since the treatment of large cell carcinoma at early stages is the same of other NSCLCs, while, at advanced stages, large cell carcinoma is a diagnosis of exclusion.

Changes in the text: we added an explanatory sentence in the introduction section (see page 4, lines 58-63).

Comment 5: while a large part of the manuscript is based on histopathological data and the authors have provided histopathological pictures there is no pathologist.

Reply 5: We thank the reviewer for his comment. We apologize for the misunderstanding. Luisella Righi is Associate Professor of Pathology who has an extensive experience in thoracic malignances and publications in this field. Gianluca Witel is a fellow of the Surgical Pathology School at the University of Turin, Italy. We modified our affiliations to include each author's unit for clarity as we acknowledge that is was not clear on the first version of the manuscript.

Changes in the text: We modified authors'affiliations.

Comment 6: Fig1b: I am not convinced by the squamous cell carcinoma in the picture. Can you provide a more representative photo?

Reply 6: Thank you for this suggestion, we agree with the Reviewer.

Changes in the text: The figure 1b was changed with a more representative one and squamous features were pointed with specific arrows.

Comment 7: Fig2: I am not convinced by the photograph, the squamous cell carcinoma contingent here may represent epithelial metaplasia of the bronchus

Reply 7: Thank you for this suggestion, we agree with the Reviewer

Changes in the text: The figure 2 was changed with a more representative one.

Comment 8: Fig4 C: the anti-NUT labeling is of low intensity, do you have a better image to show? Ideally at x400 magnification

Reply 8: Thank you for this suggestion, we agree with the Reviewer.

Changes in the text: The figure 4C was changed with a more representative one

Comment 9: For all histological photographs specify magnifications and staining.

Reply 9: We thank the Reviewer for this suggestion.

Changes in the text: All the figure magnifications, staining and scale bars (as suggested by Reviewer B) were added in the figure and figure legends.

Comment 10: Because of its multiple objectives between treatment, diagnosis and main treatments each tumor is treated superficially. This is certainly sometimes useful for daily practice but some major references are missing. I suggest to the authors to limit the parts concerning the tumorigenesis of some tumors, for example adenosquamous carcinomas, since this is out of the objectives set by the authors.

Reply 10: We appreciated reviewer's comment. As stated in the intent of the review and emphasized by the reviewer himself, the goal of our manuscript is to provide a user-friendly and quick-to-understand guide for the reader. We believe that the conciseness in dissertation of these rare entities can provide valuable support in daily clinical practice. We also believe that knowledge of tumorigenesis can help the reader in understanding nature and biological behavior of these neoplasms.

Changes in the text: No changes in the text have been made.

Comment 11: The different parts are not treated in relation to their frequency, e.g. the part concerning NUT carcinomas is almost as long as the part concerning sarcomatoid carcinomas which are much more frequent.

Reply 11: We thank the reviewer for his help. In accordance with the intent of our review, we tried to make a short but, at the same time, thorough dissertation of rare lung cancer histologies. The amount of reported information reflects not so much frequency as the amount of evidence found in the literature. To date, evidences about these entities are rather scanty, in-depth and separate discussion of each rare histology will certainly be welcome in the future.

Changes in the text: No changes in the text have been made.

Comment 12: Fig4D: unnecessary figure, to be deleted, or show a more representative figure.

Reply 12: the figure 4D was the graphical representation of the fusion detection in the NGS software. The first line showed the fusion product detected, while the other lines were the control genes that are always present in the run. As suggested by the Reviewer, this last part could make confusion, so we decide to remove it and to show only the positive fusion detection.

Changes in the text: Positive control genes deleted.

Comment 13: Tables would be welcome to synthesize the authors' remarks

Reply 13: We appreciated reviewer's comment. As also suggested by another reviewer, we created a table summarizing histologic characteristics, frequency, markers, genetic changes and treatment options of rare lung tumor entities discussed in this review.

Changes in the text: see table 1.

REVIEWER D

Comment 1: Title. In the title, please clearly identify this manuscript as a Narrative Review (lines 3-5). E.g. "xxx: a narrative review. "

Reply 1: We thank the reviewer for his comment. We modified the title as suggested.

Changes in the text: Moving through rare lung cancer histologies: a narrative review on diagnosis and treatment of selected infrequent entities. (see page 1)

Comment 2: **Abstract**. Please arrange the abstract as structured with

(1) Background and Objective--describe relevant background, reasons for conducting this review and primary objectives of this review

(2) Methods--briefly describe the search strategy, including databases, time frame, and language considerations

(3) Key Content and Findings--describe what the literature review will mainly contain and any key findings

(4) Conclusion--describe the main conclusions and how the review may potentially impact future researches, clinical practice and policy making

This revision may further specify the contribution of this review.

Reply 2: We thank the reviewer for his suggestion. Since we wrote a narrative review, and not a systematic review, with the intention of being a guide for the clinician's daily practice, we decided to lighten the text by leaving only information of clinical utility, specifically we structured the abstract in free form. To transparently report the process, writing methodology according to the narrative review reporting checklist has been uploaded separately in the supplementary materials.

Changes in the text: No changes in the text have been made. See supplementary materials.

Comment 3: **Introduction**. Please add a statement at the end of the Introduction: "We present the following article in accordance with the narrative review reporting checklist".

Reply 3: We thank the reviewer for his help. We modified the text as suggested.

Changes in the text: We present the following article in accordance with the narrative review reporting checklist (see Supplementary materials) (see pag 4, line 64-65).

Comment 4: **Methods**

(1) We suggest that the authors add a separate paragraph about "Methods" after "Introduction" in the text, including date of search, search terms, timeframe, inclusion and exclusion criteria, and selection process.

(2) To further make the information more easy-going and self-explaining, please also include a completed table (https://pcm.amegroups.com/pages/view/guidelines-for-authors, content 2.2.2 Narrative Review--Table X) in the Methods, which includes an independent supplement table to present detailed search strategy of one database as an example.

For authors' reference:

Table 1 The search strategy summary

Items	Specification
Date of Search (specified to date, month and year)	… (Please add it, e.g. "October 1, 2021")

Databases and other sources searched	… (Please add it, e.g. "PubMed, Google Scholar, …"
Search terms used (including MeSH and free text search terms and filters) Note: please use an independent supplement table to present detailed search strategy of one database as an example	Search terms: … (Please add it) Search strategy of XX database: … (Please add it)
Timeframe	… (Please add it, e.g. "from origin until September 31, 2022"
Inclusion and exclusion criteria (study type, language restrictions etc.)	Inclusion and exclusion criteria: … (Please add it, e.g. (1) Articles languages: English; (2) Article types: …)
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	… (Please add it)
Any additional considerations, if applicable	None

This part is essential as it reflects the sources of evidence (even though it is not a systematic review). This is to transparently report the process, not to judge it.

Reply 4:

1) We appreciate reviewer's suggestion. After the introduction section, we added a "methods" paragraph summarizing the methodology of research.

2) We thank the reviewer for this suggestion. Since we wrote a narrative review, and not a systematic review, with the intention of being a guide for the clinician's daily practice, we decided to lighten the text by leaving only information of clinical utility. To transparently report the process, writing methodology according to the narrative review reporting checklist has been uploaded separately in the supplementary materials.

Changes in the text: see page 5, line 67-73 ("Methods"); see supplementary materials.

Comment 5: **Narrative**

(1) Please cite the reference for this sentence: "In a minority of cases the glands have more atypical features, being similar to conventional ADC." (Lines 83-84).

(2) Line 181: "Indeed, Tzuhako et al found …[50]". "Tzuhako" should be "Tsuhako".

(3) As the title says, this article summarizes the diagnosis and treatment of rare lung cancers and evaluates the limitations of these studies. Only two suggestions.

- Could the authors consider dividing the Narrative section into several subsections and number them. For example

  1 ADENOSQUAMOUS LUNG CANCER

  1.1 Diagnosis (Histopathology, immunohistochemistry, and Molecular Biology)

  1.2 Treatment

  2 ADENOSQUAMOUS LUNG CANCER

  2.1 …

- For readers' convenience, could the authors consider drawing a three-line table to summarize the similarities and differences in the histological features of patients with different types of rare lung cancers and the corresponding treatments (including possible adverse effects). We do believe that this would help clinicians to analyze, diagnose and give appropriate treatments options.

Reply 5:

1) We thank the reviewer for his comment. We specified the reference to the WHO Classification of Tumours, 5th ed. 2021;5. Available from: https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/Thoracic-Tumours-2021

2) We thank the review for his correction. We modified the text as suggested.

3) We really appreciate reviewer comment. Instead of subdividing paragraphs into sections, we created a table summarizing histologic characteristics, frequency, markers, genetic changes and treatment options of rare lung tumor entities discussed in this review. We hope that this will be judged of help for the reader of Precision Cancer Medicine.

Changes in the text: page 5-6, lines 81, 89, 92 and 97; and page 10, line 193; see table 1.

Comment 6: **Footnote**

Please add a Reporting Checklist statement in the footnote: "The authors have completed the Narrative Review reporting checklist."

Reply 6: We thank the reviewer for his suggestion. We added this statement in the footnote section.

Changes in the text: see page 19.