Peer Review File

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Reviewer A

Comment 1: The Author proposes a review of genomic alterations in thymic carcinoma starting with a concept which, in my opinion, is not acceptable. He says that at variance with the WHO classification, which consider TC in the spectrum of TET, the TCGA consider TC a separate entity. Both concepts are not in contradiction, it is misleading to use these absolutely consistent concepts as in contradiction. The same Author initially says that he doesn't want to use papers relating on both Thymoma and thymic carcinoma, but then he uses also these papers and explore also the concept of combined thymoma-thymic carcinoma and of sequential TC after thymoma.

Reply 1: I appreciate your frankly comment. I agree that my expression could mislead readers to consider thymic carcinoma is distinct concept completely separate from thymic epithelial tumors. As a result of an error in terminology, I changed the expression to regard thymic carcinoma as part of the spectrum of thymic epithelial tumors rather than being considered a distinct category.

Changes in the text 1: I have modified the text as advised (see Page 3, line 57).

Comment 2: Basically, I think that the paper requires to be rewritten completely and reviewed by an English mother-language scientific expert. The text is full of unclear statements or of grammar mistakes: I am providing only some examples.

Reply 2: According to your advice, I have outsourced to an English editing company, where English mother-language scientific expert exist.

Changes in the text 2: I have added the text (see Page 7, line 192).
According to Editage advice, I have modified the texts in manuscripts totally.

Comment 3: Moreover, it appears that the Author want to use a paper citing references until 2021, and then he want to refers only after 2022. The review should be complete and the interesting points exhaustively discussed in appropriate manner. Some important references which could be important to cite are lacking (see list at the bottom)

Reply 3: I thank you for your listing novel paper list. I rereviewed the papers which I cited from start point and your paper list. I have added the contents which relate with your paper list.

Changes in the text 3: I have added the text (see Page 3, line 49−54; Page 5, line 119−123).

Comment 4: First of all, I do not agree with the title: the alteration in thymic carcinoma are…several, so I would use the plural: “alterations”. Moreover: Frequent genomic alteration focused on thymic carcinoma: I do not understand why “focused”. I would render the title “more appealing “.

Reply 4: According to your advice, I have modified the title.

Changes in the text 4: I have modified the title to
“Genomic insights into molecular profiling of thymic carcinoma”. (see Page 1, line 2)".

Comment 5: I am providing some examples of phrases not clear or with some words probably lacking:

ABSTRACT: CYLD, regulating signaling relating with proliferation and interact (ing with) AIRE expression and T cell development, may predict immunotherapy response

Linie 34-35 Overall, analysis of genomic background enables personalized prediction of prognosis and therapy for this rare cancer.

Very poor phrase

Reply 5: I have modified the sentences.

Changes in the text 5: I have modified the text (see Page 2, line 27-28).

Comment 6:
Background:
Linie 45: Radvoch: the name is Radovich
Linie 45-46: While thymic carcinoma is typically classified as one of the thymic epithelial tumors, Radvoch et al (4) propose it as a distinct category

Radovich et al proposed it as a molecularly distinct entity, meanwhile TC is one of the types of TET. I do not see any contradiction neither diversity It is clear that TC and thymoma are different; however, it should be taken into account that there are combined examples

Reply 6: I agree your advice that Radovich et al suggested that thymic carcinoma is not a distinct category but a distinct entity of thymic epithelial tumors. I have changed the expression of the sentences.

Changes in the text 6: I have modified the text as advised (see Page 3, line 56−57).

Comment 7: Line 57-58: The report was not assessed as it did not differentiate between thymoma and thymic cancer.

This phrase is not understandable: Which (single) report do you refer to?

Reply 7: I wanted to express that I omitted the reports in which thymoma and thymic carcinoma were integrated when analyzing genomic profiling data and the characteristics of thymic carcinoma could not be extracted. However, when I rereviewed papers in detail, I could focus on the genomic data of subgroup of thymic carcinoma.
Then, I have deleted the sentences.

Changes in the text 7: I have deleted the sentences. ("The report was not assessed as it did not differentiate between thymoma and thymic cancer").

Comment 8: Line 63-64: In this study, this information was used as a reference NOT CLEAR

Reply 8: I would like to clarify that Table 2 was adapted from the table presented in Xu et al.'s report to avoid the plagiarism. Despite referencing the citation, the table submitted as Table 2 was deemed unoriginal by the editor. Consequently, I reconstructed the table from scratch, incorporating histological subtype data, to address the feedback provided by reviewer 2.

Changes in the text 8: I have deleted the sentences. ("Table 2. Xu et al. suggested the summary of mutation profile of thymic carcinoma and thymoma based on the literature up to 2021 Years. In this study, this information was used as a reference. I added additional information of papers involving publication after 2022 and sample information and focused only on thymic carcinoma.")

Comment 9: Linie 97-99: They indicated that a possible disruption of epigenetic regulation in thymic carcinoma (a part of the phrase is lacking, there is no verb) and this point is a characteristic of genome which is different to that of thymoma. NOT CLEAR at all

Reply 9: I modified the sentences to explain that the genome aberration of thymic carcinoma relating with epigenetic regulation was specific to thymic carcinoma.

Changes in the text 9: I have modified the sentences. (see Page 5, line 113−114)

Comment 10: Linie 106-107: Regarding KIT mutations, almost more ratio in thymic carcinoma patients. NOT CLEAR

Reply 10: I have changed the sentences relating with *KIT* mutations to explain in detail.

Changes in the text 10: I have modified the sentences. (see Page 4, line 103–Page 5, line 115)

Comment 11: Linie 127-128 This finding had been reported (22), which led the hypothesis that thymic carcinoma and thymoma, particularly type B3, is sequential pathology. Badly written

Reply 11: I have modified the sentences in order to show the possibility of sequential pathology in relationship with thymoma and thymic carcinoma.

Changes in the text 11: I have modified the sentences. (see Page 6, line 158–159)

Comment 12: Linie 130-132: This concept is supported by identification of only two combined TC and B3 thymomas in an independent cohort of over 600 thymomas and type B2 and B3 thymoma and TC (23) (??)

Reply 12: The text was changed to explain that there were only two mixed cases despite the large cohort of over 600 cases.

Changes in the text 12: I have modified the sentences. (see Page 6, line 162–163)

Comment 13: Line 136-137 Actually, although only two case reports, not mixed type but transformation from thymoma to TC after 15years and 40 years (25, 26). The phrase is incomplete

Reply 13: I have modified the texts to complete the phrase.

Changes in the text 13: I have modified the sentences. (see Page 6, line 169–171)

Comment 14: Some of the recent references which could be of interest to cite:

Thomas A, Rajan A, Berman A, Tomita Y, Brzezniak C, Lee MJ, Lee S, Ling A, Spittler AJ, Carter CA, Guha U, Wang Y, Szabo E, Meltzer P, Steinberg SM, Trepel JB, Loehrer PJ, Giaccone G. Sunitinib in patients with chemotherapy-refractory thymoma and

thymic carcinoma: an open-label phase 2 trial. Lancet Oncol. 2015 Feb;16(2):177-86. doi: 10.1016/S1470-2045(14)71181-7. Epub 2015 Jan 13. Erratum in: Lancet Oncol. 2015 Mar;16(3):e105. PMID: 25592632; PMCID: PMC4401497.

Sato J, Satouchi M, Itoh S, Okuma Y, Niho S, Mizugaki H, Murakami H, Fujisaka Y, Kozuki T, Nakamura K, Nagasaka Y, Kawasaki M, Yamada T, Machida R, Kuchiba A, Ohe Y, Yamamoto N. Lenvatinib in patients with advanced or metastatic thymic carcinoma (REMORA): a multicentre, phase 2 trial. Lancet Oncol. 2020 Jun;21(6):843-850. doi: 10.1016/S1470-2045(20)30162-5. PMID: 32502444.

Tateo V, Manuzzi L, Parisi C, De Giglio A, Campana D, Pantaleo MA, Lamberti G. An Overview on Molecular Characterization of Thymic Tumors: Old and New Targets for Clinical Advances. Pharmaceuticals (Basel). 2021 Apr 1;14(4):316. doi: 10.3390/ph14040316. PMID: 33915954; PMCID: PMC8066729.

Conforti F, Pala L, De Pas T, He Y, Giaccone G. Investigational drugs for the treatment of thymic cancer: a focus on phase 1 and 2 clinical trials. Expert Opin Investig Drugs. 2022 Sep;31(9):895-904. doi: 10.1080/13543784.2022.2113373. Epub 2022 Aug 19. PMID: 35961945.

Asselta R, Di Tommaso L, Perrino M, Destro A, Giordano L, Cardamone G, Rubino L, Santoro A, Duga S, Zucali PA. Mutation profile and immunoscore signature in thymic carcinomas: An exploratory study and review of the literature. Thorac Cancer. 2021 May;12(9):1271-1278. doi: 10.1111/1759-7714.13765. Epub 2021 Mar 11. PMID: 33704917; PMCID: PMC8088947.

Nicolì V, Coppedè F. Epigenetics of Thymic Epithelial Tumors. Cancers (Basel). 2023 Jan 5;15(2):360. doi: 10.3390/cancers15020360. PMID: 36672310; PMCID: PMC9856807.

Reply 14: I appreciate introducing of the attracting reports. I cited these papers in the manuscripts.
These citations deepen the discussion of the biology of thymic carcinoma.

Changes in the text 14: I have added the text (see Page 3, line 49–54; Page 5, line 119–123).

Reviewer B

Overall, this is a good review of thymic carcinoma genetics. The reviewer would like to comment on the following points.

Major:

Comment 1:

1) Thymic carcinoma is subclassified into many histological subtypes, although 80% is squamous cell carcinoma. The reviewer assumes that the author introduces the genetics of thymic carcinoma regardless of the subtypes. However, the reviewer would like to know whether genetic abnormalities of thymic carcinoma may differ among the subtypes.

Reply 1: I appreciate the attracting discussion point. I agree that reviewer and readers are interested in the differences of genomic findings based on histological subtypes. I rereviewed the manuscript and I have reconstructed Table 2 in order to add the factors of histological subtypes. However, the description of genomic findings of each patient with histological subtype was limited. Most reports integrated the results. Then, I described the genomic findings specific to sub-categories without statistical analysis.

Changes in the text 1: I have added the sentences (see Page 5, line 121−126) and Table 2.

Comment 2:

2) Since the reviewer focuses on CYLD, he may add its location (16q) and its general role as a tumor suppressor.

Reply 2: I agree that *CYLD* mutation should be described in detail such as its location and central role as a tumor suppressor. I added these points.

Changes in the text 2: I have added the sentences. (see Page 4, line 89−91)

Comment 3:

Minor:

1) Line 118: the reviewer is unsure whether thymic carcinoma (not thymoma) definitely has higher frequencies of IRAEs than other cancers, even after reading ref.21.

Reply 3:

I agree your advice, then I changed the sentences in order to discuss frequencies of IRAE of thymic carcinoma distinct from thymoma.

Changes in the text 3: I modified the texts. (see Page 6, line 147−152)