Peer Review File

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Reviewer A

Very well written review with all relevant publications covered. The role of the TIME has been reviewed in great detail and summerizes the current of the art in this field.

It is a pleaure to read this article.

Minor: A few topos should be eliminated.

Reply: Thank you for your time and effort reviewing this article. We appreciate your positive comments on our review. Following your advice, we double-checked the article for typos and grammatical errors, and other reviewers also commented on few grammatical errors which we corrected.

Reviewer B

In this manuscript, the authors reviewed elements of tumor immune microenvironment (TIME) and therapeutic strategies to target them in advanced NSCLC.

The review appears to be thorough, but I am not certain whether the description of heterogeneity in TIME is relevant. The authors repeatedly mentioned the importance of TIME heterogeneity from a therapeutic strategy perspective, but I don't think that its importance is well described. Consequently, the sections in page 4 line 7 – page 5 line 28 has become irrelevant. The authors should consider either deleting this part, making it significantly more concise, or better describing the relation between heterogeneity in TIME and the reviewed therapeutic strategies.

Reply: Thank you for sparing the time and efforts reviewing the manuscript. Your comments had been really helpful. We agree that flow of the manuscript may seem unnatural and link between the subsections need more polishing. We have made some key changes following your and other reviewer's comments.

#1. Title was changed to *"Current literatures on the tumor immune micro-environment, its heterogeneity and future perspectives in treatment of advanced non-small cell lung cancer"* to avoid similar concerns from potential readers.

#2. Sentence such as *"The heterogeneity of TIME can be spatial and temporal and significantly influence efficacy of anti-cancer treatment modalities, especially ICIs."* was removed from the Abstract. Overall, abstract was corrected so that it does not create unnecessary confusion.

#3. (Page 4, Line 7 Overview of heterogeneity in the TIME) Following paragraph was added to elaborate the importance of TIME heterogeneity

"In management of advanced NSCLC, inhibiting cancer cell proliferation and if possible, killing them are the most important objectives. Therapeutic targeting of tumor cells becomes difficult, if cells within the same tumor exhibit various

phenotypes, and all phenotypes simultaneously show different responses to anti-tumor treatment. Heterogeneity in TIME is important, because bigger the heterogeneity, more likely that tumor cells be irresponsive to anticancer treatment. Furthermore, when cancer progresses, more genetically and molecularly divergent lineages will come to exist, augmenting the TIME heterogeneity. For these reasons, it is important to understand the TIME heterogeneity and overcome it in order to increase treatment responses."

#4. (Page 5 Line 46) Subsection title "*Temporal heterogeneity in the TIME*" was removed, and the regarding two sentences were shortened to a following sentence *"When the tumor is exposed to systemic and local anti-cancer treatment and cancer cells survive, diverse subclones develop, temporal heterogeneity can occur"* and combined with the following subsection *"Changes in the TIME after anti-cancer treatment."* We hope that this change improved the flow of the review, making it more natural for the readers to follow.

#5. The main body of the manuscript was divided into large three sections *Overview of TIME, TIME heterogeneity, Current Strategies and Future Perspectives for TIME targeting treatment*

#6. We also updated the tables.

Table 2: We have edited the table with description of the each cyto- and chemokine with sources and roles. We added over 30 new citations to not miss out important role.

Table 3: We appreciate your helpful comment. Following your suggestions, we have categorized the immune cells into myeloid and lymphoid groups. We have specified the immune cell populations in order to illustrate them in more organized ways.

Table 4: We have added a brief summary of the content described on Page 6 and presented it as Table 4.

Additional comments:

1. Page 2 line 39: by blocking "immune checkpoint" activation – activation of inhibitory immune checkpoints will likely promote malignant progression; thus, this description is not appropriate.

Reply: Thank you for your comment on the sentence. We corrected the sentence following your comment

Text: The sentence was changed to "The TIME is dynamic, and involved cells participate in treatment resistance and show complex interactions during malignant progression by activation of inhibitory immune checkpoints"

2. Page 5 line 50 – page 6 line 2: PD-L1 expression after various therapies does not always increase in tumor tissues. There are multiple studies that have shown variable changes in PD-L1 expression between pre-treatment biopsies and post neoadjuvant chemo +/- radiation resections.

Reply: We agree that this sentence has oversimplified the studies, and not correct. The sentence was corrected accordingly.

Text: "Reportedly, after various anti-cancer modalities such as chemotherapy, radiotherapy, and targeted therapies, PD-L1 expression show variable changes in tumor tissues, indicating a possibility of unpredictable immune-mediated cancer cell killing activities."

3. There are numerous problems with the text; thus, it is worth editing by a native English speaker in the field. A few examples are as follows.
Reply: I apologize for the shortcomings regarding the text. We have double-checked for the typos and inappropriate sentences. All of the following comments had been reflected in the revised version, in addition, we have made corrections following the comments by the other reviewers.

a. Page 3 lines 4-7: The two sentences are in part redundant and could be combined in one sentence.
Reply: Thank you very much for the point
Text: The TIME is comprised of both tumor cells and nonmalignant cells, including fibroblasts, pericytes, adipocytes, vascular endothelial cells, various immune cells, carcinoma-associated fibroblasts (CAFs), ECM, and blood and lymphatic vessels.

b. Page 6 lines 18-19: Furthermore, EGFR-TKI-resistant cancer cells promoted EMT (64). Promoted -> exhibited
Reply: The text was changed following your suggestion.
Text: Furthermore, EGFR-TKI-resistant cancer cells exhibited EMT

c. Page 9 lines 20-22: In this phase 1 study, combination treatment was shown to induce polyclonal immunity to overcome heterogeneity in tumor antigens and a potential synergistic effect of combined regional CAR-T cells and PD-1 blockade (110). - I don't understand how the mesothelin-targeted CAR-T cell therapy can induce polyclonal immunity.
Reply: Thank you for your comment. We agree that an additional explanation is necessary for clarification. We added following sentence after the regarding sentence.
Text: *The study showed that combination of CAR T cells and pembrolizumab further expanded endogenous T-cell clones which can contribute to overcoming tumor antigen heterogeneity.*

d. Page 10 lines 34-35: Cancer nanomedicine has an advantage of controlled delivery with modular flexibility that can co-exist with the surrounding environment. - I don't understand this sentence.
Reply: We agree that this sentence needs further clarification. I think "can co-exist with the surrounding environment" is not accurate enough to be stated in the review, so this segment was deleted in the revised version. We further corrected the sentence, so it can be more comprehensible to the readers.
Text: Cancer nanomedicine has several advantages. Nanomedicine enables more

accurate delivery to the target tissues. Due to its modular flexibility, anti-cancer medications can exist in various forms enabling more effective transportation and absorption.

e. Page 10 line 41: An an acidic -> As an acidic
Reply: The sentence was corrected to be clearer.
Text: Acidic tumor environment is generally T cell inhibitory, and a pH-sensitive signaling pathway has recently been suggested as a candidate mechanism to balance localized activation of T cells in the tumor microenvironment while avoiding unwanted systemic immune responses

f. Page 10 line 46: TAFs -> CAFs?
Reply: I apologize for the mistake.
Text: TAFs were changed to CAFs

Reviewer C

Lim et al. should be commended for their efforts to summarize recent progress and current understanding of tumor immune microenvironment (TIME) in Non-Small Cell Lung Cancer (NSCLC). They listed major components (cell types) and potential targets for developing better therapeutics. There are a few points that should be further addressed:
1. List cell origin and function of each cytokine and chemokine in TIME: The authors summarized cytokines and chemokines in Table 2. However, the cell type secreting these cytokines/chemokines and their function were not listed. We would suggest the author add this missing information.
Reply: Thank you for your comment. We have edited the table with description of the each cyto- and chemokine with sources and roles. For some cytokines, both immunosuppresive and immunogenice roles are present, and we took it into account. The table is meant to provide schematic view of TME-related cytokines and chemokines, so detailed description was limited. However, we added over 30 new citations to not miss out important role. We hope this correction helped elevating the quality of the manuscript.

2. Systemically summarize immune cell types: The author listed cytotoxic T cells, B cells, regulatory T cells (Tregs), Resident memory T (Trm) CD8 T cells, B cells, dendritic cells, TAM, MDSC, and NK cells. Immune cells can be divided as lymphoid vs. myeloid. Among lymphoid populations, there are T cells and B cells. T cells are CD3 positive and further divided based on their cell surface markers: CD8 T cells (also called cytotoxic CD8 T cells) and CD4 T cells (T helper cells). CD8 T cells are further divided into different categories based on their cytokine profiles. Treg cells are among one the CD4 T cell subsets. CD8 Trm cells are not a separate lineage but a CD8 T cell population with different functions. Therefore, these T cell subsets should always be discussed together. Vise versa, the myeloid cells, TAM, MDSC, and

DCs, should be addressed separately. Therefore, we suggest the authors reorganize Table 3 and add more specific markers to define each cell population (eg. Tregs are CD3+CD4+Foxp3+CD35+).

Reply: We appreciate your helpful comment. Following your suggestions, we have categorized the immune cells into myeloid and lymphoid groups. We have specified the immune cell populations in order to illustrate them in more organized ways. Most of the study results mentioned are about non-small cell lung cancer, so we changed the table title to "*Key immune cells related to pro-immunogenic/immunosuppressive roles in the tumor immune microenvironment in non-small cell lung cancer*"

According to your opinions we have added more specific CD markers to each immune cell population mentioned (for example, Tregs and CD8 cytotoxic Tcell). We hope this correction met your standards. Please let us know if more corrections are necessary.

3. Add a table to summarize factors for a good TIME: we should suggest the authors add a table to summarize better factors indicating favorable TIME, which were described on Page 6.

Reply: We have added a brief summary of the content described on Page 6 and presented it as Table 4. Thank you for your comment.

Reviewer D

The authors have compiled a review on the tumor immune microenvironment (TIME) in lung cancer, and postulate that heterogeneity, at both spatial and temporal level, in the tumor immune microenvironment is the current bottleneck for improving lung cancer care. I have major concerns on this manuscript; which I would suggest to seriously take into consideration.

1. The review lacks a clear focus or underlying model that can be brought across; intra-tumoral heterogeneity (at several levels, e.g. genetic, metabolic) is since long considered as one of the main obstacles for effective treatment. Throughout the review, on all the mentioned components of the TIME, the authors fail to pinpoint how heterogeneity plays a role for each component and how to overcome. It would be very helpful to start with an hypothesis or model that clearly indicates how the authors see heterogeneity as obstacle in the context of immune therapy or TIME. Without such original conceptual framework, paragraphs like page 6 line 9-14 cannot be put in context.

Reply: We fully agree with your concern that our manuscript did not show fully how "the heterogeneity" of TIME may be a target of the future treatment. Reviewer B has provided similar query, so we made some changes in the revised version. Reading our manuscript again, we felt that there are some unnecessary subsections, we combined them where we can.

2. The authors touch upon a broad range of components of the TIME, treatments

and trials, but discuss the literature only very superficially. For example, page 5 line 47-49; 'can occur', 'various anti-cancer treatments', 'can influence', 'various treatments', 'several subclones', 'various forms', 'can occur'. Or page 5 line24 'and other associated molecules' etcetera.

Reply: The following sentence was added to page 6, line 6-8 describe the sentence in details.

-The analysis of peripheral blood samples of patients who underwent paclitaxel treatment showed that the inhibitory function of Treg was reduced, while the levels of IFN-γ and IL-2 were increased after paclitaxel treatment (61).

Also for Page 5 line 24, "*such as lipopolysaccharide"* was added to the segment *"other associated molecules"*

As was mentioned before the Page 5 line 47-49 were paraphrased as a one sentence and was combined with the following paragraph.

For sentences Page 5 line 47-page 6 line 2, we do

3. The title claims impact on patient management, but the review doesn't provide concrete and practical tips or tricks, nor does it provide guidance in treatment choices for lung cancer patients. I would remove the claim from the title and abstract and clearly state that this is a summary of current literature.

Reply: We agree that the title does not correlate with the contents of the review, Title was changed to *"Current literatures on the tumor immune micro-environment, its heterogeneity and future perspectives in treatment of advanced non-small cell lung cancer"*

Sentence such as *"The heterogeneity of TIME can be spatial and temporal and significantly influence efficacy of anti-cancer treatment modalities, especially ICIs."* Was removed from the abstract. Overall, abstract was corrected so that it does not create unnecessary confusion.

4. Be more precise, e.g. page 3 line 4 'fibroblasts' in the following sentence line 6 'carcinoma associated fibroblasts'; or page 6 line 20 'despite the tumor-killing immune responses' probably means 'despite the presence of tumor-antigen-specific CD8+ T-cell populations'; or page 6 line 32 'immune-infiltrating Treg' should probably be 'tumor-infiltrating Tregs' etcetera.

Reply: We appreciate your comment on the regarding sentences. We tried to be more precise in the revised version. We corrected the sentences following your advice.

Page 3 line 4-6: "fibroblasts" (line 4) were deleted, as it seems redundant, and the two consecutive sentences were combined to one sentence.

Page 3 line 20: The sentence was corrected accordingly.

Page 3 line 32: The sentence was corrected accordingly.