

Spatially mapping the tumour immune microenvironments of non-small cell lung cancer

Corresponding Author: Dr Logan Walsh

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I find the revised manuscript to be strengthened with additional analyses, clarifications and responses to my previous comments, emphasis on well-powered analyses of myeloid cells and their association with DFS, and longer DFS follow-up of the patient cohort. The rigor of the analysis was improved by additional clarifications and discussion of limitations of prior studies in the field that are addressed here, the statistical analyses (correction of p values), a discussion of how this study fits in the framework of the field, and potentially interesting new associations without overstating.

I therefore find this study to be meaningful addition to the current literature and support its publication.

Benjamin Izar

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

Review of Desharnais and Sorin et al. Reviewer 2 responses in revision.

The core criticism from Reviewer 2 seemed to be focused on statistical / correlative conclusions made in the original manuscript that were not grounded in NSCLC biology (cell types / activation states / interactions). In the authors defense, while Reviewer 2 leveled these criticisms, their recommended resolutions were vague. In response to this, the authors appear to have dug into the underlying data more – pulling out the expression of CD163 on macrophage driven CNs associated with LUAD survival. They also pull out 'functional markers' via endothelia proliferation which seems to also be specifically associated with LUAD (vs LUSC) survival. Given this, and other minor fixes, it seems the authors have made an effort to respond to all of reviewer 2 comments. Still, these responses – while present in the PBP and figures – don't appear to be well integrated into the text (see #3 below)

Comments from this reviewer regarding them manuscript:

We believe the manuscript could further benefit from some additional clarification and additions that should not be prohibitive for the authors to add:

1) The resource nature of this study is evident – but the authors have only provided a vague data sharing statement with no actual repositories or detailed outline for sharing (this would have been helpful to include with the original review). Without all of this in the repository the value of this study is significantly reduced. The authors at a minimum should include in the repository:

- a. Processed TIFF images with cell segmentation masks
 - b. Clinical meta data / patient table to capture figure 1b plus survival data in KM plots
 - c. Cell feature table with cluster/population labels, CN labels, XY/FOV ids
- 2) More context in the introduction and discussion to differentiate the labs previous spatial proteomics NSCLC paper in Nature 2023 – currently this is glossed over – probably an addition to intro pointing out complementarity would help.
- 3) While there is some mention in the discussion – the abstract / introduction of the paper is somewhat lacking a tangible description of any actual biological insights – instead they read as more of a methodological overview of the study – capturing some take home messages here about what biology of LUAD/LUSC was revealed would improve the papers interpretability.

(Remarks on code availability)

the code is generic and associated with older studies from the lab - no code specific to this study was provided

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have previously addressed my comments. I support publication of this work.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

We appreciate the further improvements the authors have made to the text of the manuscript contextualizing their previous studies - this offers significant improvement. We were unable to access the linked repository data for further review despite significant troubleshooting efforts. We were told by our own IT help desk that this link is still access controlled. Still, the description of the author's additionally provided data seems sufficient.

For future reference - since the authors seem interested in using a Zenodo for sharing - it has an "off-line" link feature specifically for paper review; this way the reviewers can actually see the repository to be published - the repository can be modified following review - and then the repository and resulting doi can be formalized upon acceptance. I would encourage the authors to become familiar with this for use in the future

(Remarks on code availability)

The code seems to match what is described and has been used previously - there was no means to run the code on any relevant data

Open Access This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

I find the revised manuscript to be strengthened with additional analyses, clarifications and responses to my previous comments, emphasis on well-powered analyses of myeloid cells and their association with DFS, and longer DFS follow-up of the patient cohort. The rigor of the analysis was improved by additional clarifications and discussion of limitations of prior studies in the field that are addressed here, the statistical analyses (correction of p values), a discussion of how this study fits in the framework of the field, and potentially interesting new associations without overstating.

I therefore find this study to be meaningful addition to the current literature and support its publication.

[We thank reviewer 1 for their supportive comments and their insightful feedback.](#)

Reviewer #3 (mediator for unavailable R#2, Remarks to the Author):

Review of Desharnais and Sorin et al. Reviewer 2 responses in revision.

The core criticism from Reviewer 2 seemed to be focused on statistical / correlative conclusions made in the original manuscript that were not grounded in NSCLC biology (cell types / activation states / interactions). In the authors defense, while Reviewer 2 leveled these criticisms, their recommended resolutions were vague. In response to this, the authors appear to have dug into the underlying data more – pulling out the expression of CD163 on macrophage driven CNs associated with LUAD survival. They also pull out ‘functional markers’ via endothelia proliferation which seems to also be specifically associated with LUAD (vs LUSC) survival. Given this, and other minor fixes, it seems the authors have made an effort to respond to all of reviewer 2 comments. Still, these responses – while present in the PBP and figures – don’t appear to be well integrated into the text (see #3 below)

[We sincerely thank the reviewer for their supportive comments and insightful feedback. We fully agree with all the points raised and have incorporated the suggested changes accordingly. Please find detailed responses to each point below.](#)

Comments from this reviewer regarding them manuscript:

We believe the manuscript could further benefit from some additional clarification and additions that should not be prohibitive for the authors to add:

- 1) The resource nature of this study is evident – but the authors have only provided a vague data sharing statement with no actual repositories or detailed outline for sharing (this would have been helpful to include with the original review). Without all of this in the repository the value of this study is significantly reduced. The authors at a minimum should include in the repository:
 - a. Processed TIFF images with cell segmentation masks
 - b. Clinical meta data / patient table to capture figure 1b plus survival data in KM plots
 - c. Cell feature table with cluster/population labels, CN labels, XY/FOV ids

[We have uploaded a clinical metadata table that includes the clinical information presented in Figure 1B, frequency of cell types, and information on disease-free and overall survival. All processed TIFF images,](#)

cell segmentation masks, and the cell features table will be made publicly available on Zenodo upon acceptance of the article. In the meantime, the reviewer can access this information at the following link:

https://mcgill.sharepoint.com/sites/QuailWalshLabs_Group/External%20Data%20Share/Forms/AllItems.aspx?id=%2Fsites%2FQuailWalshLabs%5FGroup%2FExternal%20Data%20Share%2FSQCCADK&p=true&ga=1&LOF=1

2) More context in the introduction and discussion to differentiate the labs previous spatial proteomics NSCLC paper in *Nature* 2023 – currently this is glossed over – probably an addition to intro pointing out complementarity would help.

We appreciate the feedback regarding the addition of context to the introduction and discussion sections and agree that the original version lacked sufficient context. To address this, we have added additional text to highlight the findings of our *Nature* 2023 paper and to distinguish them from the current study.

We have uploaded a PDF comparing the text from the original submission to the updated version, allowing the reviewer to easily identify where changes and additions have been made

3) While there is some mention in the discussion – the abstract / introduction of the paper is somewhat lacking a tangible description of any actual biological insights – instead they read as more of a methodological overview of the study – capturing some take home messages here about what biology of LUAD/LUSC was revealed would improve the papers interpretability.

We completely agree with the reviewer and have rewritten the abstract and added substantial text to the introduction. We sincerely thank the reviewer for these suggestions, which we believe significantly strengthen the narrative and highlight the overall impact of the study.

Reviewer #3 (Remarks on code availability):

the code is generic and associated with older studies from the lab - no code specific to this study was provided

The code used in this study is identical to that used in our *Nature* 2023 paper. The GitHub repositories we provided are well-documented, including README files as requested by the reviewer, which clearly explain how to run each repository. Additionally, we have added a new section in the GitHub repository with the pairwise interaction analysis code specific to this study. We have also included the updated lineage assignment code used in this study. We hope these updates will be helpful and appreciate the reviewer's feedback regarding our code.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have previously addressed my comments. I support publication of this work.

We thank Reviewer 1 for their supportive comments and insightful feedback.

Reviewer #3 (Remarks to the Author):

We appreciate the further improvements the authors have made to the text of the manuscript contextualizing their previous studies - this offers significant improvement. We were unable to access the linked repository data for further review despite significant troubleshooting efforts. We were told by our own IT help desk that this link is still access controlled. Still, the description of the author's additionally provided data seems sufficient.

For future reference - since the authors seem interested in using a Zenodo for sharing - it has an "off-line" link feature specifically for paper review; this way the reviewers can actually see the repository to be published - the repository can be modified following review - and then the repository and resulting doi can be formalized upon acceptance. I would encourage the authors to become familiar with this for use in the future

We thank Reviewer 3 for their insightful feedback and apologize for the issues in accessing the data on Zenodo. We will use the offline features as suggested in the future. All data is now publicly available on Zenodo as requested.

Reviewer #3 (Remarks on code availability):

The code seems to match what is described and has been used previously - there was no means to run the code on any relevant data

We thank Reviewer 3 for their insightful feedback.