nature portfolio

Peer Review File

Enabling next-generation engineered TCR-T therapies based on high-throughput TCR discovery from diagnostic tumor biopsies

Corresponding Author: Dr Gavin Bendle

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 thank the authors for thier comments. Two points remain that I think are worth further clarification:

1) I still think the authors are somewhat understating the progress in the field (sorry for omitting a key reference before - "Rapid construction of antitumor T-cell receptor vectors from frozen tumors for engineered T-cell therapy" by Tsuji et al - which bears similarities in producing TCR repertoires from biopsy samples). This work still is well done and I don't think a paper has quite lined up this array of approaches before, but each piece remains more precedented in literature than I think comes through by reading the intro/discussion.

2) The language added in lines 326-331 is still not quite responsive to my point - while it is reasonable to assume the most disease-relevant (and clinically relevant TCRs will be in the top of the list of clonotypes), the data shown in Extended data figures 4c/7a further underscore the notion that the distribution of TCR rank vs. frequency is essentially continuous. Since the processing pipeline in this manuscript is by its nature not quantitative, this means it is likely that sometimes a TCR that I s'ground truth' the 30th most abundant in a sample will be present as the 20th most identified TCR alpha chain but only the 60th most abundant TCR beta (due to sequencing/primer amplification bias, etc). This is a reasonable and understandable limitation, but I think should be more clearly laid out for readers who may be interested in studying TCRs but are not as well versed in some of the quirks of collecting this type of data.

Reviewer #3

(Remarks to the Author)
I am completely satisfied with the additional work done by the authors and the clarifications in the text, all points raised were addressed entirely and appropriately.

Reviewer #4

(Remarks to the Author)
To the editor,

Thank you for the opportunity to review the manuscript titled “Enabling next-generation engineered TCR-T therapies based on high-throughput TCR discovery from diagnostic tumor biopsies”, as well as the authors’ responses to the prior reviewers’ comments.

In this submission, the authors describe a discovery platform designed to screen tumor-infiltrating lymphocytes (TILs) to identify T cell receptors (TCRs) specific to tumor antigens and neoantigens. This method uses patient-derived tumor samples, including suboptimal material, to find effective sequences which could then be included in an immunotherapeutic product. The platform entails co-culturing engineered cell lines expressing either a TCR library or an antigen library and sorting activated T cells based on a functional readout. The authors first evaluate the sensitivity of their method with TCRs of known specificities, and thereafter apply it to an array of tumor samples. They identify promising hits from shuffled TCR

chains and validate the overall effectiveness of the approach via low-throughput confirmatory screens.

I find this research manuscript to be of high quality and the scale of the work accomplished to be impressive. While I concur with Reviewer #1 that it represents an “evolution” with respect to its technical advancements, I would say that the “library-on-library” aspect is quite powerful and distinguishes it from most work in this field. The method is well-described, both in text and graphically, and the insights that follow from it are clear. It is apparent that the manuscript was much improved following the initial round of review. In particular, the addition of Extended Figure 7b was essential as it answers a question that I also shared with all the reviewers. In general, I felt that all points raised were suitably addressed by the authors.

I have but a few minor points of my own that require clarification/edits:

1. The platform relies on the use of the Jurkat cell line, as well as CD69 positivity as a readout. This combination grants them the sensitivity they seek, but CD69 expression is a low bar for T cell activation, especially in this context. In the discussion, I would like the authors to briefly comment on the expected translatability of their results and the likelihood of false positives that will necessitate additional screening of their top TCRs in primary T cells.

2. In lines 336-337, the authors note that their method may be used for enhancing TCR functionality. This not supported by their work, which finds rare hits within a largely nonfunctional pool, as opposed to finding incrementally stronger responders within a largely functional pool. I would remove this statement, while retaining the part about improving specificity since this would resemble more their workflow to date.

3. The text refers to 48 tandem minigenes (TMGs) on lines 189 and 193, but Extended Figure 4 shows 49 TMGs and the legend does not refer to the last one. Was this 49th just a placeholder? If so, perhaps better to leave that cell empty to avoid confusion.

4. The sentence on lines 334-336 appears to have a typo. I imagine it should say “shared tumor antigens” and not “shared tumor antigen-specific TCRs”.

Notwithstanding these points, the authors’ claims are overall credible, and the research described is both sound and novel. The prior reviewers’ comments were adequately addressed, and only the discussion needs to be amended. Provided that the above issues are resolved, I would recommend to the editor that this work be published in Nature Communications as it would certainly benefit the field of T cell immunotherapy by providing a blueprint for the discovery of potentially effective TCRs.

1 REVIEWER #1

Remarks to the Author: I thank the authors for thier comments. Two points remain that I think are worth further clarification:
Point 1) I still think the authors are somewhat understating the progress in the field (sorry for omitting a key reference before - "Rapid construction of antitumor T-cell receptor vectors from frozen tumors for engineered T-cell therapy" by Tsuji et al - which bears similarities in producing TCR repertoires from biopsy samples). This work still is well done and I don't think a paper has quite lined up this array of approaches before, but each piece remains more precedented in literature than I think comes through by reading the intro/discussion.

We thank the reviewer for pointing out this additional reference. We have included this reference and removed the text ‘, as no such technology is available to date’ (lines 84-85) to better reflect the status prior to our work.

Point 2) The language added in lines 326-331 is still not quite responsive to my point - while it is reasonable to assume the most disease-relevant (and clinically relevant TCRs will be in the top of the list of clonotypes), the data shown in Extended data figures 4c/7a further underscore the notion that the distribution of TCR rank vs. frequency is essentially continuous. Since the processing pipeline in this manuscript is by its nature not quantitative, this means it is likely that sometimes a TCR that I s'ground truth' the 30th most abundant in a sample will be present as the 20th most identified TCR alpha chain but only the 60th most abundant TCR beta (due to sequencing/primer amplification bias, etc). This is a reasonable and understandable limitation, but I think should be more clearly laid out for readers who may be interested in studying TCRs but are not as well versed in some of the quirks of collecting this type of data.

We agree with the reviewer that this is a limitation of the platform. We have now included a more detailed description of this specific limitation (lines 331-332), adding ‘due to the potential amplification bias during bulk TCR identification and due to the use of combinatorial TCR libraries’.

2 REVIEWER #3:

Remarks to the Author: I am completely satisfied with the additional work done by the authors and the clarifications in the text, all points raised were addressed entirely and appropriately.

We thank reviewer #3 for feedback during both rounds of review, and for the support for publication of our manuscript.

3 REVIEWER #4:

Remarks to the Author: In this submission, the authors describe a discovery platform designed to screen tumor-infiltrating lymphocytes (TILs) to identify T cell receptors (TCRs) specific to tumor antigens and neoantigens. This method uses patient-derived tumor samples, including suboptimal material, to find effective sequences which could then be included in an immunotherapeutic product. The platform entails co-culturing engineered cell lines expressing either a TCR library or an antigen library and sorting activated T cells based on a functional readout. The authors first evaluate the sensitivity of their method with TCRs of known specificities, and thereafter apply it to an array of tumor samples. They identify promising hits from shuffled TCR chains and validate the overall effectiveness of the approach via low-throughput confirmatory screens.

I find this research manuscript to be of high quality and the scale of the work accomplished to be impressive. While I concur with Reviewer #1 that it represents an "evolution" with respect to its technical advancements, I would say that the "library-on-library" aspect is quite powerful and distinguishes it from most work in this field. The method is well-described, both in text and graphically, and the insights that follow from it are clear. It is apparent that the manuscript was much improved following the initial round of review. In particular, the addition of Extended Figure 7b was essential as it answers a question that I also shared with all the reviewers. In general, I felt that all points raised were suitably addressed by the authors.

> We thank the reviewer for the review, for acknowledging the impact of the manuscript and for agreeing that raised points were appropriately addressed.

I have but a few minor points of my own that require clarification/edits:

Minor point 1. The platform relies on the use of the Jurkat cell line, as well as CD69 positivity as a readout. This combination grants them the sensitivity they seek, but CD69 expression is a low bar for T cell activation, especially in this context. In the discussion, I would like the authors to briefly comment on the expected translatability of their results and the likelihood of false positives that will necessitate additional screening of their top TCRs in primary T cells.

> We thank the reviewer for raising this point. We agree that generally there are differences in sensitivity between Jurkats and primary T cells; however, generally Jurkat cells are less sensitive. There is a small chance that we may fail to identify TCRs with low functional avidity using Jurkats in our TCR discovery platform. However, such TCRs will likely not be selected for inclusion in TCR-T product manufacture, which is the main use-case for which this platform is developed. Thus, we believe that the use of Jurkat cells has no effect on identification of high-avidity TCRs (and has a number of advantages over using primary T cells, including reproducibility and ease of cultivation).
>
> Regarding CD69 being a low bar for T cell activation, we agree that this marker can have a relatively high background labeling depending on tissue culture conditions. We would like to stress that this does not mean that sensitivity of TCR identification using our platform is also affected. We identify TCRs based on a relative enrichment of an individual TCR in 'activated T cell samples' relative to 'non-activated T cell samples'. As long as the majority of cells expressing an individual TCR are

represented in the activated sample, that TCR will be enriched relative to other TCRs even if there is some (aspecific) background.

Minor point 2. In lines 336-337, the authors note that their method may be used for enhancing TCR functionality. This not supported by their work, which finds rare hits within a largely nonfunctional pool, as opposed to finding incrementally stronger responders within a largely functional pool. I would remove this statement, while retaining the part about improving specificity since this would resemble more their workflow to date.

We thank the reviewer for raising this point, and agree that this specific point is not further supported in the manuscript. Given the objection of the reviewer, we have decided to remove this statement.

3. The text refers to 48 tandem minigenes (TMGs) on lines 189 and 193, but Extended Figure 4 shows 49 TMGs and the legend does not refer to the last one. Was this 49th just a placeholder? If so, perhaps better to leave that cell empty to avoid confusion.

We thank the reviewer for raising this. The 49th B cell line is a positive control (indicated as '+' in Extended / Supplementary Figure 4a), which expresses a mutated CDK4 epitope. This has been described in the Extended / Supplementary Figure 4 legends.

4. The sentence on lines 334-336 appears to have a typo. I imagine it should say "shared tumor antigens" and not "shared tumor antigen-specific TCRs".

We thank the reviewer for pointing this out; this was indeed a mistake and has been corrected in the final submission.

Notwithstanding these points, the authors' claims are overall credible, and the research described is both sound and novel. The prior reviewers' comments were adequately addressed, and only the discussion needs to be amended. Provided that the above issues are resolved, I would recommend to the editor that this work be published in Nature Communications as it would certainly benefit the field of T cell immunotherapy by providing a blueprint for the discovery of potentially effective TCRs.

We thank the reviewer for support for publication of this manuscript.