

A randomized phase 2 clinical trial to treat moderate-to-severe plaque psoriasis patients with high-induction dosing of risankizumab

Corresponding Author: Dr Andrew Blauvelt

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)

An excellent work, well written, of great importance for the scientific community. The authors have addressed a hot topic of the moment, with important implications not only for the health of patients but also with important implications for pharmacoeconomics. In my opinion, the work can be accepted in its current form.

Reviewer #2

(Remarks to the Author)

This is a well-conducted and clinically relevant study addressing long-term remission and immunologic correlates in patients with moderate-to-severe plaque psoriasis treated with risankizumab using an intensified induction regimen. The integration of clinical and transcriptomic data adds considerable value to the field. The work is well discussed.

However, I have a few concerns and suggestions for improvement:

Patient matching: The two treatment groups do not appear to be fully matched for baseline characteristics, which may introduce confounding effects. Although randomization is mentioned, imbalances are still possible, particularly given the relatively small sample sizes in some analyses. I would suggest acknowledging this as a limitation and clarifying whether any adjustment was performed in the analyses.

Control group: It would have been interesting to have a control group receiving standard dosage of risankizumab (150 mg) in order to see any difference, in particular given the high persistence of treatment response in the IMMHance study.

Terminology regarding adverse events: The manuscript refers to serious/severe treatment-emergent adverse events (TEAEs) somewhat interchangeably. However, serious and severe are not synonymous in the context of AE reporting: serious refers to outcomes (hospitalization, death, etc.), while severe refers to intensity. The terminology should be revised for accuracy and consistency.

Safety analyses: Blood tests were performed at weeks 4,8,22,46, so basically they were not done for more than one year up to week 100. I think that is unusual. It would have been useful to provide more long term safety data, since the last visit was week 100

Methods placement: It is unusual for the Methods section to appear after the Discussion. For clarity and to align with the standard structure of Nature Communications, the Methods should be moved before the Discussion, unless a specific journal style requires otherwise (which is not typical for this journal).

Additional minor points:

- A CONSORT diagram would help clarify patient flow and the number of subjects included in each analysis.
- It would be helpful to provide more details on the RNA-Seq analysis pipeline (QC steps, normalization methods, multiple testing corrections).
- In the abstract please define all abbreviations (IL, PASI, etc)

- page 5 lines 93-97: this last sentence is not clear, I would suggest to rewrite it for clarity
- page 5 lines 111-113: "one patient received one dose (of which dosage?) and one patient received two doses (of which dosage?)"
- Recent real-world studies have highlighted a super responder phenotype among patients treated with risankizumab, characterized by rapid and sustained complete skin clearance. For example, the IL PSO study (JEADV doi:10.1111/jdv.19464) identified such profiles in patients who had a short disease duration. I encourage the authors to briefly discuss these findings to help contextualize their results within the broader treatment landscape. I would also compare this findings to the GUIDE study on guselkumab
- Page 16 line 351-352: the Authors refer to recent studies on tildrakizumab that are not cited among the references.
- Table 1: disease duration is presented in years? Please specify

Reviewer #3

(Remarks to the Author)

While the obtained results are interesting, novel, and offer valuable insights, they tend to be somewhat descriptive, supporting previous data showing the role of Trms in long-term remission. Although I like the concept that reducing Trm cells in lesional skin could be a key issue to prevent relapse, it is not clear why the authors used higher-than-approved initial dosing of risankizumab. Especially, that using 600 mg risankizumab was not better than 300 mg risankizumab. The conclusion that higher-than-approved initial dosing of risankizumab „represents a potential novel treatment strategy for patients with psoriasis” is a bit early, considering the low number of enrolled patients.

Other Comments:

1. Low number of enrolled patients. Lesional skin biopsies could be taken only 13 of the 16 patients at Week 52. Only 6 patients completed all study visits, the 12 remaining evaluable patients withdrew consent to seek other treatment prior to Week 100. What was the reason for withdrawal of the consent?
2. In the second phase of the study, „Two of the six patients who completed the study (i.e., completed the Week 100 visit) demonstrated completely clear skin (PASI 100 of 11.1%; n=2; [95% CI 1.4, 34.7]) at Week 100, 84 weeks following their last dose of risankizumab.” How was this 11.1% calculated? Anyway, the calculation of percentages in such a low number of patients is inaccurate,
 1. The nonlesional samples: What was the distance from the lesions? Were these areas „never-lesional”?
 2. Generation of single-cell suspensions for scRNA-seq: How were the bi-sectioned samples prepared? Horizontal sectioning separating epidermis and dermis, or simply the whole sample was cut into parts, both containing epidermis and dermis?
 3. Dermal and epidermal cells were combined in a 2:1 ratio. Why the 2:1 ratio?
 4. It would be good to know whether there were any novel findings using risankizumab that were previously not described with other IL23 inhibitors.

Minor suggestion, that could be considered:

1. The use of qPCR to confirm differential expression levels of key DEGs could be useful. This could confirm that computationally identified gene expression changes reflect true biological differences.
2. The bioinformatic analysis could be improved.

To reduce algorithm-dependent biases multiple methods might be used to help identify consistent patterns. Consistent findings across different analytical algorithms would indicate that results are stable, reliable, and not artifacts of a specific method. If multiple algorithms detect the same biological patterns, it would strengthen confidence that these are genuine, method independent signals. Therefore, for trajectory inference, application of Monocle (v2/v3), Slingshot, PAGA and/or SCORPIUS algorithms could be used.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I have reviewed the revised version of the manuscript entitled "Long-term remissions and marked decreases in resident memory T cells following high induction dosing with risankizumab in randomized patients with moderate-to-severe plaque psoriasis".

As noted in my previous review, this is an excellent work, well written and highly relevant for the scientific community. The authors address a timely and important topic with implications not only for patient health but also for pharmacoeconomic strategies.

In this revised version, the authors have responded thoroughly and thoughtfully to the comments raised by the other reviewers. Where possible, they have integrated the requested changes, improving both the clarity and completeness of the manuscript.

I believe the authors have made a genuine effort to strengthen the paper, and I find the revised version to be suitable for publication.

Reviewer #2

(Remarks to the Author)

Thank you for providing the revised version of this interesting manuscript.
I have no further queries for this work.

Reviewer #3

(Remarks to the Author)

The authors adequately addressed all my comments. I have learned a lot, even from the answers to my questions.
Congratulations on this excellent paper!

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:

An excellent work, well written, of great importance for the scientific community. The authors have addressed a hot topic of the moment, with important implications not only for the health of patients, but also with important implications for pharmacoeconomics. In my opinion, the work can be accepted in its current form.

RESPONSE: We thank the reviewer for their interest, excitement, and supportive comments.

Reviewer #2:

This is a well-conducted and clinically relevant study addressing long-term remission and immunologic correlates in patients with moderate-to-severe plaque psoriasis treated with risankizumab using an intensified induction regimen. The integration of clinical and transcriptomic data adds considerable value to the field. The work is well discussed.

However, I have a few concerns and suggestions for improvement:

Patient matching: The two treatment groups do not appear to be fully matched for baseline characteristics, which may introduce confounding effects. Although randomization is mentioned, imbalances are still possible, particularly given the relatively small sample sizes in some analyses. I would suggest acknowledging this as a limitation and clarifying whether any adjustment was performed in the analyses.

RESPONSE: We thank the reviewer for their supportive comments.

As pointed out, baseline characteristics of the patient groups were not fully matched due to the relatively small sample size of the study. No adjustments were made for confounding effects, also due to the relatively small sample size. A sentence has been added regarding this limitation on pages 18 to 19 (lines 395-397).

Control group: It would have been interesting to have a control group receiving standard dosage of risankizumab (150 mg) in order to see any difference, in particular given the high persistence of treatment response in the IMMhance study.

RESPONSE: We thank the reviewer for their comment and agree that a broader range of doses, including a 150 mg control group, would have provided more

clarity to our data. Nevertheless, we believe the large amount of historical data using the 150 mg standard dose (including withdrawal from this dose) provides some context to both our induction and withdrawal data, which we highlight in the Discussion.

Terminology regarding adverse events: The manuscript refers to serious/severe treatment-emergent adverse events (TEAEs) somewhat interchangeably. However, serious and severe are not synonymous in the context of AE reporting: serious refers to outcomes (hospitalization, death, etc.), while severe refers to intensity. The terminology should be revised for accuracy and consistency.

RESPONSE: We thank the reviewer for their comment and have clarified the safety statement on page 9 (lines 180-182).

Safety analyses: Blood tests were performed at weeks 4,8,22,46, so basically, they were not done for more than one year up to week 100. I think that is unusual. It would have been useful to provide more long term safety data, since the last visit was week 100.

RESPONSE: We thank the reviewer for their comments. Given that the half-life of risankizumab is approximately 4 weeks, it is unlikely that any significant levels of drug were present in the body at Week 52 (36 weeks/9 half-lives after the last dose) or beyond. Because of this, we believed it was unnecessary to continue monitoring blood tests. However, safety events via patient history were collected from Week 52 to Week 100. No new safety concerns were revealed during the second year of the study.

Methods placement: It is unusual for the Methods section to appear after the Discussion. For clarity and to align with the standard structure of Nature Communications, the Methods should be moved before the Discussion, unless a specific journal style requires otherwise (which is not typical for this journal).

RESPONSE: We thank the review for their comment, but this is the format style of Nature Communications.

Additional minor points:

A CONSORT diagram would help clarify patient flow and the number of subjects included in each analysis.

RESPONSE: We thank the reviewer for their comment and have added clarifying patient disposition information on pages 6 and 7 (lines 116 to 127), but we believe study numbers were too small to warrant a full CONSORT diagram.

It would be helpful to provide more details on the RNA-Seq analysis pipeline (QC steps, normalization methods, multiple testing corrections).

RESPONSE: We thank the reviewer for their comment and have provided additional methodology text on pages 23 (line 486).

In the abstract, please define all abbreviations (IL, PASI, etc)

RESPONSE: As suggested, we have defined all abbreviations used in the Abstract on page 4.

Page 5 lines 93-97: this last sentence is not clear, I would suggest to re-write it for clarity.

RESPONSE: As suggested, we have clarified this sentence on page 6 (lines 99 to 102).

Page 5 lines 111-113: "one patient received one dose (of which dosage?) and one patient received two dose (of which dosage?)"

RESPONSE: We have clarified the dosages of the two patients who did not complete dosing on pages 6 and 7 (lines 116 to 127)

Recent real-world studies have highlighted a super responder phenotype among patients treated with risankizumab, characterized by rapid and sustained complete skin clearance. For example, the IL PSO study (JEADV doi:10.1111/jdv.19464) identified such profiles in patients who had a short disease duration. I encourage the authors to briefly discuss these findings to help contextualize their results within the broader treatment landscape. I would also compare this findings to the GUIDE study on guselkumab.

RESPONSE: Thank you for mentioning this very interesting point. As suggested, we have added these two references (and one other) and have modified the Discussion on page 16 (lines 340 to 348).

Page 16 line 351-352: the Authors refer to recent studies on tildrakizumab that are not cited among the references.

RESPONSE: Thank you for pointing this out. We have now added the appropriate new reference on page 18 (line 376).

Table 1: disease duration is presented in years? Please specify.

RESPONSE: Thank you for pointing this out. We have clarified that the disease duration is in years in Table 1 on page 30.

Reviewer #3:

While the obtained results are interesting, novel, and offer valuable insights, they tend to be somewhat descriptive, supporting previous data showing the role of Trms in long-term remission. Although I like the concept that reducing Trm cells in lesional skin could be a key issue to prevent relapse, it is not clear why the authors used higher-than-approved initial dosing of risankizumab. Especially, that using 600 mg rizankizumab was not better than 300 mg rizankizumab. The conclusion that higher-than-approved initial dosing of risankizumab „represents a potential novel treatment strategy for patients with psoriasis” is a bit early, considering the low number of enrolled patients.

RESPONSE: We thank the reviewer for saying that our findings are interesting, novel, and offer valuable insights.

Higher-than-approved doses of risankizumab were used in the phase 1 study of risankizumab (Krueger et al, ref #7). These doses led to long-term clearance of psoriasis, longer than what was reported in IMMhance, the randomized withdrawal study of risankizumab at standard doses (Blauvelt et al, ref #6). We wanted to build upon the risankizumab phase 1 results and provide mechanistic data to help explain long-term remissions. As stated in the Discussion, we believe the only reason that efficacy in the 300 mg and 600 mg groups was not different was due to imbalances in baseline disease characteristics. Given our efficacy results are so markedly different than efficacy with standard doses, we stand by our statement that higher-than-approved initial dosing of risankizumab may represent a novel treatment strategy for patients with psoriasis.

Other Comments:

Low number of enrolled patients. Lesional skin biopsies could be taken only 13 of the 16 patients at Week 52. Only 6 patients completed all study visits, the 12 remaining

evaluable patients withdrew consent to seek other treatment prior to Week 100. What was the reason for withdrawal of the consent?

RESPONSE: We have further clarified the reason for patient discontinuation on page 6 and 7 (lines 116 to 127).

In the second phase of the study, two of the six patients who completed the study (i.e., completed the Week 100 visit) demonstrated completely clear skin (PASI 100 of 11.1%; n=2; [95% CI 1.4, 34.7]) at Week 100, 84 weeks following their last dose of risankizumab.” How was this 11.1% calculated? Anyway, the calculation of percentages in such a low number of patients is inaccurate.

RESPONSE: We thank the reviewer for their question and can elaborate on the calculations. Throughout the manuscript, results are reported using modified non-responder imputation, where patients who discontinued risankizumab due to reasons other than lack of efficacy intolerance were excluded from efficacy analyses. Of the 20 patients originally enrolled in the study, two were excluded from all efficacy analyses due to not receiving the complete dosing schedule, leaving 18 patients for all efficacy analyses. At Week 100, 2 of the 18 patients that completed the study achieved PASI 100, providing the 11.1% calculation.

While the authors acknowledge that percentages for low Ns can have drawbacks, we believe that they are an easy way to report our results to readers. Throughout the manuscript, where applicable, the authors have provided both the n and N of results for further interpretation.

The nonlesional samples: What was the distance from the lesions? Were these areas „never-lesional”?

RESPONSE: Biopsies of non-lesional skin were performed 2-3 cm from the edge of lesions used for lesional biopsies. We did not determine whether this skin was “never lesional.”

Generation of single-cell suspensions for scRNA-seq: How were the bisected samples prepared? Horizontal sectioning separating epidermis and dermis, or simply the whole sample was cut into parts, both containing epidermis and dermis?

RESPONSE: Thank you for pointing that out. The bisection was vertically from the epidermis down to the dermis. The epidermal to dermal separation was done

using enzymatic digestion and then each part was digested. We have added these details on page 24.

Dermal and epidermal cells were combined in a 2:1 ratio. Why the 2:1 ratio?

RESPONSE: Thank you for pointing that out. The majority of cells in the skin biopsies were derived from the epidermis, which is >95% keratinocytes. If we were to mix the cells isolated from the digestion at a 1:1 ratio, close to 50% of the cell-types we would have in the dataset would be keratinocytes. To avoid this, and to enrich for cell populations in the dermis, we mixed these at 2:1 ratio (dermis:epidermis). We have clarified this on page 24.

It would be good to know whether there were any novel findings using risankizumab that were previously not described with other IL-23 inhibitors. Minor suggestion, that could be considered.

RESPONSE: Thank you for pointing that out. Yes, we believe our findings here are novel, including marked shifts in treatment response with high-dose risankizumab, prolonged remission, the presence of two TRM subsets in psoriatic skin (TRM1 and TRM17), and dose-associated decrease in the frequency of TRM17s (in contrast to TRM1s). These are novel findings that to our knowledge have not previously been reported.

The use of qPCR to confirm differential expression levels of key DEGs could be useful. This could confirm that computationally identified gene expression changes reflect true biological differences.

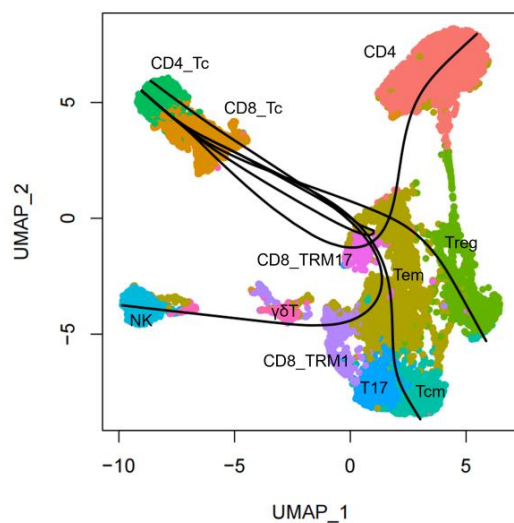
RESPONSE: Thank you for this suggestion. We would like to point out that such qPCR validation would be at the bulk level, whereas the strength of our data is that it provides details down to a single-cell level in regard to biologic mechanisms. Furthermore, this “validation” would be on the same samples as we did the single-cell sequencing on and therefore not a true validation. While we would have been happy to add this to our manuscript, we unfortunately do not have any cDNA or RNA left for this validation and therefore are unable to add this to our revised manuscript.

The bioinformatic analysis could be improved. To reduce algorithm-dependent biases multiple methods might be used to help identify consistent patterns. Consistent findings across different analytical algorithms would indicate that results are stable, reliable, and not artifacts of a specific method. If multiple algorithms detect the same biological

patterns, it would strengthen confidence that these are genuine, method independent signals. Therefore, for trajectory inference, application of Monocle (v2/v3), Slingshot, PAGA and/or SCORPIUS algorithms could be used.

RESPONSE: Thank you for your comment. We do believe our approach and analytics as outlined in our manuscript are robust and align with both clinical responses seen in our patient cohort as well as molecular data that has been published on biology of TRM1/TRM17 (see work from Laura McKay's group: see PMID:38033053). We respectfully disagree that applying multiple algorithms to detect the same biologic patterns would necessarily strengthen our data, as these tools have heterogeneous outputs making such "consensus" approach nontrivial and may give conflicting results. As suggested, we conducted the slingshot analysis on global T cells and demonstrated that TRM1 and TRM17 cells belong to two distinct trajectories derived from CD8_Tc cells (new Supplementary Figure 4d) (see below), consistent with our monocle analysis. We added these results in our revised manuscript on page 11 and Supplementary Figure 4 (line 227 to 233). Also, with these tools, there is no universal benchmark and many of them have shared assumptions and overlapping databases. These issues may give rise to circular validation. Instead, we have opted to provide different and independent forms of validation using different technologies such as spatial-sequencing and other types of data that we believe strengthens our manuscript.

d
lineage 1: CD4_Tc CD8_Tc Tem CD8_TRM1 γδT NK
lineage 2: CD4_Tc CD8_Tc Tem T17 Tcm
lineage 3: CD4_Tc CD8_Tc Tem Treg
lineage 4: CD4_Tc CD8_Tc Tem CD8_TRM17



e

