Author’s response to reviews

Title: Investigating the effects of Pirfenidone on TGFbeta1-stimulated Non-SMAD signaling pathways in Dupuytren's disease-derived fibroblasts

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Author’s response to reviews:

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To,
The Editor,
BMC Musculoskeletal Disorders

Dear Editor,

Thanks very much for your comments on our manuscript entitled “Pirfenidone inhibits TGFbeta1-stimulated Non-SMAD signaling pathways in Dupuytren's disease-derived fibroblasts” (BMSD-D-18-01179R1) which will help in improving the manuscript substantially and to abide to the journal’s submission guidelines.

Our responses are below the Editor’s comments and are italicized.
Editor Comments:

1) Please ensure that the section headings in the Abstract and the main text are the same as those outlined in our Submission Guidelines (https://na01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fbmcmusculoskeletdisord.biomedcentral.com%2Fsubmission-guidelines%2Fpreparing-your-manuscript%2Fresearch-article%23preparing%2Byour%2Bmanuscript&amp;data=02%7C01%7Clas238%40pitt.edu%7C499717e78d65405bf42408d64fa32367%7C9ef9f489e0a04eeb87cc3a526112fd0d%7C1%7C0%7C636783959430777167&amp;sdata=jA%2Bye4bcoYHIEB0icG27s6uFksmYXN%2FHjs7ZQn324g8%3D&amp;reserved=0).

We have made the necessary changes as specified in the Submission Guidelines.

2) Please remove the figure titles embedded within the figures and re-upload the corrected versions. Please upload each Figure individually, as separate files.

The corrected versions of the figures are uploaded.

3) Please provide a labelled ladder for all Western Blots.

We have provided the labeled ladder for all Western Blots.

4) Please state whether error bars are SEM or SD in the figure legends and in the methods.

The error bars shown are from the SEM values and it is mentioned in the figure legends and in the methods.

5) Please explain what the abbreviation “Ntx” in the figures refers to.

Ntx refers to “no treatment” and this is mentioned in the figure legends.

6) We note that you have used t-tests to compare results. However, you have two groups (CT cells and DD cells) and four treatments in each. Therefore, it would appear that a two-way repeated measures ANOVA followed by post-hoc multiple comparisons would be the most appropriate test (provided that all assumptions for ANOVA are met).
As suggested by the editor we have used ANOVA to compare the results. We have used GraphPad Prism Version 8 and performed one-way ANOVA followed by post-hoc comparisons using Dunnett’s and Sidak’s post-hoc multiple comparisons. We feel that one way ANOVA analysis was most appropriate for our data sets.

7) Ensure that all abbreviations are included in the List of Abbreviations.

We have made sure that all abbreviations are included in the List of Abbreviations.

8) Please include the heading “Declarations” at the start of this section.

The heading “Declarations” is included.

9) Please move the List of Abbreviations to after the Conclusions and before the Declarations.

We have made changes as suggested.

10) Please describe the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.

We have made a statement regarding the role of the funding body in the manuscript.

11) Please note that listed author contributions of SK and MEB does not automatically qualify them for authorship. Reviewing and providing comments on the manuscript is not sufficient to qualify for authorship.

We ask that you ensure that author contributions are in line with the ICMJE guidelines (https://na01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.icmje.org%2Frecommendations%2Fbrowse%2Froles-and-responsibilities%2Fdefining-the-role-of-authors-and-contributors.html&amp;data=02%7C01%7Cclas238%40pitt.edu%7C499717e78d65405bf42408d64fa32367%7C9ef9f489e0a04eeb87cc3a526112fd0d%7C1%7C0%7C636783959430777167&amp;amp;sdata=wVN1%2Boaxl0MdM9VNm2eCkye5SpahFVwwM2ZhKziBcZw%3D&amp;amp;reserved =0), and that all listed authors have performed all four points specified below.
An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. According to the ICMJE guidelines, to qualify as an author one should have performed all 4 of the following points:

A. Made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data;

B. Been involved in drafting the manuscript or revising it critically for important intellectual content;

C. Given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; and

D. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

We have revised the statements regarding SK’s and MEB’s author contributions to qualify them as the author of the manuscript. As a senior author of the manuscript, I can assure that they provided enough insights, suggestions and was involved in the discussions regarding the project, which qualify them as an author of the manuscript.

I hope we have responded satisfactorily to the Editor’s comments. Please do not hesitate to contact me if further details are required.

Thanking you,

Sincerely yours,
Thanks for the recent note regarding our recent manuscript submission titled “Pirfenidone inhibits TGF-β1- stimulated Non-SMAD signaling pathways in Dupuytren’s disease-derived fibroblasts” (Manuscript no. BMSD-D-18-01179R1). Firstly, we have changed the title of the manuscript to “Investigating the effects of Pirfenidone on TGF-β1- stimulated Non-SMAD signaling pathways in Dupuytren’s disease-derived fibroblasts”. We are thankful for the comments that we have received from both the Reviewers, which has immensely helped in improving the quality of the manuscript.

We have made every effort to clarify the queries raised by the reviewer with our response being italicized following the reviewer’s comments.
Reviewer #1:

Major revision:

Overview: This submission addresses the effects of Pirfenidone on TGF-β1-stimulated, non-canonical signaling pathways in primary fibroblasts derived from fibrotic (Dupuytren disease) and phenotypically normal palmar fascia as controls. The findings indicate that, in the presence or absence of TGF-β1 treatment, Pirfenidone decreases the phosphorylation (and by extension, the activation) of several TGF-β1-stimulated non-canonical signaling pathways in both DD and CT fibroblasts. These findings could have relevance to the potential for using Pirfenidone as a novel treatment or adjunct treatment for patients with Dupuytren disease-associated palmar digital contractures.

We are truly appreciative of this reviewer for providing a positive note for the findings in the manuscript.

Major typos/edits required: In the results section, the authors make several claims that are not clearly substantiated by the data. A non-significant trend cannot be considered equivalent to a statistically significant difference if the study is appropriately powered. This reviewer wondered if the authors were confusing the effects of PFD and TGF-β1 treatments within groups (CT, DD), which are often significant, with between group comparisons (CT vs DD), which do not appear to have been assessed in most, if not all, figures. All of these interpretations and statistical analyses need to be clarified and/or corrected in the manuscript. Specifically, they claim to show that "the basal phosphorylation levels of AKT, ERK1/2, and MLC were elevated in DD-derived fibroblasts compared to CT-derived fibroblasts", yet the statistics for the densitometry in the figures pertaining to these data (Figs 1, 2 and 4 respectively) do not indicate any statistically significant differences between the no treatment (Ntx) readings for CT and DD fibroblasts. The authors claim that "TGF-β1 further increased the phosphorylation levels of the above proteins in both CT- and DD-derived fibroblasts", yet the statistics for the densitometry in the corresponding figures do not indicate any statistically significant differences between the no treatment (Ntx) readings and the TGF-β1 treatments for CT and DD fibroblasts. The authors claim that Pirfenidone (PFD) "inhibited both basal and TGF-β1-induced phosphorylation of AKT, ERK1/2, and p38 in both CT- and DD derived fibroblasts". In contrast, their data show no indication that PFD treatment altered ERK1/2 phosphorylation in basal DD fibroblasts, nor that p38 phosphorylation was altered by PFD treatment alone in CT or DD fibroblasts under basal conditions.
We have substantially revised the manuscript in accordance with the reviewer’s comments and recommendations. We have improved the way we have performed the statistical analyses. Since the Editor of the manuscript suggested using ANOVA, we have used GraphPad PRISM version 8 and performed statistical significance using one-way ANOVA and post-hoc comparisons which we find it more appropriate to compare the values between the treatment conditions within the group. We also understand that this kind of analyses is much more stringent and fit our study. We have set the “no treatment” as 1 for CT- and DD-derived fibroblasts and then compared it to other treatment conditions. This way the comparison is within treatment conditions in CT- and the same for DD- cells and not between CT- and DD-cells. Since it might be repetitive to write the entire results in this section, we request the reviewer to read the revised manuscript (with track changes) and provide his comments. We believe that we have not overstated the results and we have presented it as is depending on the statistical significance that we obtained from our data sets.

Minor typos/edits: There is substantial pixilation in the images of the immunoblots in Fig 2, whereas the other immunoblotting images are of relatively good quality. The authors are requested to provide better quality images in Fig. 2 if that is possible. The lines in the densitometry graphic indicating statistical significance between treatments were discontinuous in the version of Fig 4 I received, making it difficult to discern which treatments were being compared. Please edit this figure accordingly.

We have made every effort to provide good quality images for Western Blots. The densitometry graphs are redone since the statistical analyses and the way the comparison between the conditions has been modified. We hope the clarity and quality of the images are better than the previous version.

Reviewer # 2

Minor Revisions.

This is a nice paper, investigating the effect of Pirfenidone on diseased fibroblasts. The methodology is straightforward, and well executed. Appropriate controls were carried out. Since the hallmark of fibrosis is the unwanted accumulation of collagen type I, it would be informative the know what the effect of Pirfenidone is on the gene expression level of this collagen type.
Our thanks to the reviewer for his positive comments and supporting the research work carried out. We have previously published our findings on the effect of Pirfenidone on type I and type III collagens. We reported that Pirfenidone inhibited TGF-beta1 induced type I and type III collagen gene expression in Dupuytren’s-derived fibroblasts. Please see the reference below.


Furthermore, what is missing is a comparison of your findings with Pirfenidone with that of others. Quite some papers have been published on this topic, but none are mentioned, such as:


Thanks to the Reviewer for collecting information on the findings from other authors about Pirfenidone related to fibrosis. We now incorporate these references with a short description on the findings from these studies in the “Discussion” section of the manuscript.

We again thank the Reviewers for their time and valuable comments, and we hope that we have addressed their concerns satisfactorily.
Very truly yours,

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