THE ROYAL SOCIETY PUBLISHING

ROYAL SOCIETY OPEN SCIENCE

Novel elucidation and treatment of pancreatic chronic graft-versus-host disease in mice

Shin Mukai, Yoko Ogawa, Fumihiko Urano, Yutaka Kawakami and Kazuo Tsubota

Article citation details
R. Soc. open sci. **5**: 181067.
http://dx.doi.org/10.1098/rsos.181067

Review timeline
Original submission: 7 May 2017
Revised submission: 30 June 2018
Final acceptance: 20 September 2018

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Note: This manuscript was transferred from another Royal Society journal without peer review.

Review History

RSOS-170456.R0 (Original submission)

Review form: Reviewer 1 (Michaela Lucas)

Is the manuscript scientifically sound in its present form?
No

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
No

Is it clear how to access all supporting data?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
Yes

Recommendation?
Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
In their paper, Ogawa et al. investigate the pathological changes occurring in the pancreas of a murine model of chronic graft-versus host disease by histopathology, immunohistochemistry, electron microscopy and western blotting. To my knowledge this is the first study to do so in such detail. Furthermore, the authors show that they are able to reverse some of the pathological changes found in their model by administrating 4-phenylbutyric acid (PBA). Although the described positive effect of PBA on the cGVHD changes in the pancreas in particular are novel, the authors have another paper currently being accepted (but not available for me to read), looking at the overall beneficial aspects of PBA in cGVHD. Of note, PBA is FDA approved for human use with a relatively good safety profile, thus the finding of this study are potentially clinically translatable.

Despite these novel aspects, there are some major concerns with this study.

1. The authors do not discuss the current human literature of cGVHD of the pancreas, e.g. they do not distinguish between exocrine and endocrine failure of the pancreas.
2. The authors do not discuss the short-falls of the murine model, compared to human cGVHD, nor do they describe any clinical signs in their mice that are consistent with chronic pancreatitis, e.g. did these mice develop diarrhoea, steatorrhoea or alteration of their blood glucose? Therefore, the diagnosis of cGVHD is based on the histopathological, immunohistopathological and EM analysis of the authors alone.
3. The presentation of the data is at times not convincing. The inflammatory infiltrates on the HE staining are not clearly visible due to the size of the figure 1. Have the authors quantified the infiltrate to allow for a more objective analysis?
4. The densitometric comparison of Figure 2 and 4 is not convincing, the authors should plot the raw data.
What do the authors mean with data from two similar experiments are shown?
5. All p-values are given as <0.05, the exact p-values should be stated. What is the meaning of * versus ** versus ***?
6. Figure 5, the photographs of the histopathological findings are too small to evaluate.
7. Figure 3a and Figure 6a, show blue and green staining (not red), please correct.
8. Most of the results are given in the figure legends but not in the result section, this should be changed.
9. Better anatomical descriptions of the histopathological and EM findings should be provided.
10. The English of the manuscript should be reviewed as it is verbose at times, e.g. “Once we gleaned these outcomes of medical importance” ; “we endeavored’’; “this fact urged us to carry out groundbreaking research”;
11. Figure 7 should be omitted and instead can be discussed in the text, as it is speculative and the study has not been designed to confirm the suggestions made in the figure.

Decision letter (RSOS-170456.R0)

14-Sep-2017

Dear Dr Ogawa:

Manuscript ID RSOS-170456 entitled "Novel Elucidation and Treatment of Pancreatic Chronic Graft-Versus-Host Disease in Mice" which you submitted to Royal Society Open Science, has been reviewed. The comments from reviewers are included at the bottom of this letter.

In view of the criticisms of the reviewers, the manuscript has been rejected in its current form. However, a new manuscript may be submitted which takes into consideration these comments.

Please note that resubmitting your manuscript does not guarantee eventual acceptance, and that your resubmission will be subject to peer review before a decision is made.

You will be unable to make your revisions on the originally submitted version of your manuscript. Instead, revise your manuscript and upload the files via your author centre.

Once you have revised your manuscript, go to https://mc.manuscriptcentral.com/rsos and login to your Author Center. Click on "Manuscripts with Decisions," and then click on "Create a Resubmission" located next to the manuscript number. Then, follow the steps for resubmitting your manuscript.

Your resubmitted manuscript should be submitted by 14-Mar-2018. If you are unable to submit by this date please contact the Editorial Office.

We look forward to receiving your resubmission.

Sincerely,
Alice Power
Editorial Coordinator
Royal Society Open Science

on behalf of
Andrew Dunn, Royal Society Open Science
openscience@royalsociety.org

Associate Editor Comments to Author:

The authors must engage in the additional experiments recommended by the reviewer that support the relevance of their mouse model of cGVH disease to the human condition, and differentiate between pancreatic exocrine and endocrine abnormalities. They should also address the reviewer's comments on about presentation of experiments.

Reviewers' Comments to Author:
Reviewer: 1

Comments to the Author(s)
In their paper, Ogawa et al. investigate the pathological changes occurring in the pancreas of a murine model of chronic graft-versus host disease by histopathology, immunohistochemistry,

electron microscopy and western blotting. To my knowledge this is the first study to do so in such detail. Furthermore, the authors show that they are able to reverse some of the pathological changes found in their model by administrating 4-phenylbutyric acid (PBA). Although the described positive effect of PBA on the cGVHD changes in the pancreas in particular are novel, the authors have another paper currently being accepted (but not available for me to read), looking at the overall beneficial aspects of PBA in cGVHD. Of note, PBA is FDA approved for human use with a relatively good safety profile, thus the finding of this study are potentially clinically translatable.

Despite these novel aspects, there are some major concerns with this study.

1. The authors do not discuss the current human literature of cGVHD of the pancreas, e.g. they do not distinguish between exocrine and endocrine failure of the pancreas.
2. The authors do not discuss the short-falls of the murine model, compared to human cGVHD, nor do they describe any clinical signs in their mice that are consistent with chronic pancreatitis, e.g. did these mice develop diarrhoea, steatorrhoea or alteration of their blood glucose? Therefore, the diagnosis of cGVHD is based on the histopathological, immunohistopathological and EM analysis of the authors alone.
3. The presentation of the data is at times not convincing. The inflammatory infiltrates on the HE staining are not clearly visible due to the size of the figure 1. Have the authors quantified the infiltrate to allow for a more objective analysis?
4. The densitometric comparison of Figure 2 and 4 is not convincing, the authors should plot the raw data.
What do the authors mean with data from two similar experiments are shown?
5. All p-values are given as <0.05, the exact p-values should be stated. What is the meaning of * versus ** versus ***?
6. Figure 5, the photographs of the histopathological findings are too small to evaluate.
7. Figure 3a and Figure 6a, show blue and green staining (not red), please correct.
8. Most of the results are given in the figure legends but not in the result section, this should be changed.
9. Better anatomical descriptions of the histopathological and EM findings should be provided.
10. The English of the manuscript should be reviewed as it is verbose at times, e.g. “Once we gleaned these outcomes of medical importance” ; “we endeavored’’; “this fact urged us to carry out groundbreaking research”;
11. Figure 7 should be omitted and instead can be discussed in the text, as it is speculative and the study has not been designed to confirm the suggestions made in the figure.

Author's Response to Decision Letter for (RSOS-170456.R0)

See Appendix A.

RSOS-181067.R0

Review form: Reviewer 1 (Michaela Lucas)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept as is

Comments to the Author(s)
Thank you for addressing all comments. The manuscript has improved significantly, no further comments.

Decision letter (RSOS-181067.R0)

20-Sep-2018

Dear Dr Ogawa,

I am pleased to inform you that your manuscript entitled "Novel Elucidation and Treatment of Pancreatic Chronic Graft-Versus-Host Disease in Mice" is now accepted for publication in Royal Society Open Science.

You can expect to receive a proof of your article in the near future. Please contact the editorial office (openscience_proofs@royalsociety.org and openscience@royalsociety.org) to let us know if you are likely to be away from e-mail contact. Due to rapid publication and an extremely tight schedule, if comments are not received, your paper may experience a delay in publication.

Royal Society Open Science operates under a continuous publication model (http://bit.ly/cpFAQ). Your article will be published straight into the next open issue and this will be the final version of the paper. As such, it can be cited immediately by other researchers. As the issue version of your paper will be the only version to be published I would advise you to check your proofs thoroughly as changes cannot be made once the paper is published.

You have the opportunity to archive your accepted, unbranded manuscript, but access to the full text must be embargoed until publication.

Articles are normally press released. For this to be effective we set an embargo on news coverage corresponding to the publication date of the article. We request that news media and the authors do not publish stories ahead of this embargo (when final version of the article is available).

On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,
Andrew Dunn
Senior Publishing Editor
Royal Society Open Science
openscience@royalsociety.org

on behalf of Professor Douglas Fearon
openscience@royalsociety.org

Associate Editor Comments to Author (Professor Douglas Fearon):
Associate Editor
Comments to the Author:
None

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
Thank you for addressing all comments. The manuscript has improved significantly, no further comments.

Appendix A

Point-by-point responses

With sincere thanks for the time and energy you invested in reviewing our manuscript, we would like to respond to your comments in a point-by-point manner. Unfortunately, the main lead author Shin Mukai suffered from a debilitating kidney disorder. Hence, the revision of our manuscript has taken longer than expected. However, we have revised our text to the greatest extent possible.

1. The authors do not discuss the current human literature of cGVHD of the pancreas, e.g. they do not distinguish between exocrine and endocrine failure of the pancreas.

To the best of our knowledge, there are few to no articles about the pancreas affected by cGVHD. Our new-found knowledge in this study is as follows:

1) When allogeneic BMT (allo-BMT) recipient mice are untreated or treated with a solvent vehicle, severe inflammation and fibrosis around pancreatic ducts (exocrine glands) were observed. In addition, the size of their pancreatic islets (endocrine glands) was reduced. These phenomena did not occur in syngeneic BMT (syn-BMT) recipients or allo-BMT recipients treated with PBA.

Accordingly, we placed the following statements in the main article.

Page 11, Line 249

As indicated by HE and Mallory's staining, tissues around ducts in the pancreas collected from allogeneic BMT recipients was excessively inflamed in association with mononuclear cell infiltration and fibrotic in concert with extracellular matrix accumulation. (**Figure 1a, b, Supplementary Figures 1, 2**) Conversely, when mice underwent syngeneic BMT, their pancreas seemed to be virtually normal. (**Figure 1a, b; Supplementary Figures 1, 2**) This finding indicated that pancreatic exocrine failure could be induced by

cGVHD.

Page 11, Line 259

In addition, the size of pancreatic islets was greatly reduced in the GVHD-affected mice in contrast to that in their syngeneic control counterparts. (Figure 1e, Supplementary Figure 4). Presumably owing to the dysfunctional exocrine and endocrine glands,[26-28] the blood glucose levels in the cGVHD-affected mice was substantially greater than those in the syngeneic control subjects. (P = 0.000029) (**Figure 1f**)

Page 14, Line 325

The HE and Mallory pictures suggested that areas around pancreatic ducts in PBA-medicated pancreas was less inflamed and fibrotic compared with its vehicle-medicated partner. (**Figure 5a**, **b, Supplementary Figures 11, 12**).

Page 14, Line 332

Furthermore, pancreatic islets in the PBA-treated allo-BMT recipient mice were larger than those in their vehicle-treated counterparts. (Figure 5e, Supplementary Figure 15) Presumably, as a result of the protection of exocrine and endocrine glands, the blood glucose levels in the PBA-medicated allo-BMT recipients were normal in contrast to those in their vehicle-medicated equivalents. (P = 2.2 x 10-7) (**Figure 5f**)

Page 16, Line 372

In particular, severe inflammation and fibrosis around pancreatic ducts were induced, and pancreatic islets were shrunk. Previous reports indicated that exocrine failure as well as endocrine failure in the pancreas could result in diabetes.[26-28] Hence, it is conceivable that the exocrine and endocrine failure in cGVHD-affected pancreas resulted in diabetes-like symptoms and thereby the blood glucose levels in mice with cGVHD were augmented.

2. The authors do not discuss the short-falls of the murine model, compared to human cGVHD, nor do they describe any clinical signs in their mice that are

consistent with chronic pancreatitis, e.g. did these mice develop diarrhoea, steatorrhoea or alteration of their blood glucose? Therefore, the diagnosis of cGVHD is based on the histopathological, immunohistopathological and EM analysis of the authors alone.

With regard to the dependability of the mouse model used in this study, we placed the following sentences in the Discussion section.

Page 16, Line 364

As demonstrated by our previous investigation, the pathogenic processes of lacrimal gland cGVHD in this murine model were similar to those in patients with cGVHD [3, 33-35]. As with the human lacrimal glands affected by cGVHD, abnormal inflammation and fibrosis were observed in their murine counterparts. In addition, previous reports demonstrated that inflammation and fibrosis were induced systemically in this mouse model [3, 34, 36]. Therefore, we examined the development of pancreatic cGVHD utilizing this murine model.

In response to your comment on clinical signs, we solicited the Japanese company, Sanritsu Zelkova (Kanagawa, Japan), to measure blood glucose levels. The results indicated that the blood glucose levels in untreated all-BMT recipients and BMT-recipients treated with the solvent vehicle were significantly higher than those in syn-BMT recipients and allo-BMT recipients treated with PBA. Accordingly, we placed the following statement in the main text and showed the results in **Figures 1f, 5f**.

Page 10, Line 238

(f) Measurement of blood glucose levels

Blood glucose levels were measured by the Japanese company, Sanritsu Zelkova (Kanagawa, Japan). The measurement was conducted with the use of a kit (27E1X80166000006, LSI Medience, Japan).

3. The presentation of the data is at times not convincing. The inflammatory infiltrates on the HE staining are not clearly visible due to the size of the figure 1. Have the authors quantified the infiltrate to allow for a more objective analysis?

The enlarged version of Figure 1 has been shown in **Supplementary Figures 1, 2, 3, 4, 7.**

Furthermore, we carried out immunostaining for $CD45^+$ cells and determined the number of immune cell infiltrates. Accordingly, the following sentences have been placed in the Method section.

Page 7 Line 156

As for the counting of $CD45^+$ cells, five areas of each tissue section were randomly photographed under 200× magnification, and the number of $CD45^+$ cells in the individual images was subsequently determined.

4. The densitometric comparison of Figure 2 and 4 is not convincing, the authors should plot the raw data.

The raw data and instructions on how to see the data have been provided.

What do the authors mean with data from two similar experiments are shown?

We replaced the sentence with 'Results are representative of 2 independently performed experiments with similar results.'

5. All p-values are given as <0.05, the exact p-values should be stated. What is the meaning of * versus ** versus ***?

The exact P-values have been stated in the figures.

6. Figure 5, the photographs of the histopathological findings are too small to evaluate.

Their enlarged versions have been shown in **Supplementary Figures 11, 12, 13, 15, 18**.

7. Figure 3a and Figure 6a, show blue and green staining (not red), please correct.

According to your suggestion, the mistakes have been corrected.

8. Most of the results are given in the figure legends but not in the result section, this should be changed.

As with some other research articles, P-values have been placed where applicable in the Results section.

Page 11, Line 256

Immunostaining for the generic leukocyte marker CD45 also revealed that abnormal immune cell migration was observed in the cGVHD-disordered pancreas by contrast with its syngeneic control equivalent. ($P = 0.00038$) (**Figure 1c, d, Supplementary Figure 3**)

Page 11, Line 261

Presumably owing to the dysfunctional exocrine and endocrine glands,[26-28] the blood glucose levels in the cGVHD-affected mice was substantially greater than those in the syngeneic control subjects. ($P = 0.000029$) (**Figure 1f**)

Page 11, Line 266

Our data indicated that these two markers were expressed at higher level in the cGVHD-affected pancreas compared with its syngeneic control partner. (IL-6: $P = 6.9 \times 10^{-5}$, CTGF: $P = 1.2 \times 10^{-4}$) (**Supplementary Figures 5, 6**)

Page 12, Line 280

As judged by immunoblot analysis, the ER stress markers in the cGVHD-affected pancreas were expressed at higher level than its syngeneic control partner. (GRP78: $P = 1.3 \times 10^{-5}$, CHOP: $P = 2.0 \times 10^{-4}$, p-PERK: $P = 6.0 \times 10^{-6}$, p-eIF2α: $P = 2.4 \times 10^{-4}$, p-IRE1α: $P = 0.0012$) (**Figure 2a, b, Supplementary Figure 8a-e**)

Page 12, Line 284

As a consequence of the activation of ER stress signalling pathways, the following two inflammation-related molecules were elevated and/or activated in the cGVHD-disordered pancreas in contrast to its syngeneic control counterpart: NF-κB and TXNIP. (NF-κB: $P = 3.9 \times 10^{-7}$, TXNIP: $P = 4.9 \times 10^{-6}$) (**Figure 2a, b, Supplementary Figure 8f, g**)

Page 13, Line 299

As shown by immunohistochemical and immunoblot assays, the expression of the EMT indicator E-cadherin in the cGVHD-affected pancreas was decreased in contrast to that in its syngeneic control equivalent. ($P = 0.0016$) (**Figure 3a-c, Supplementary Figure 9a**)

Page 13, Line 302

Moreover, immunoblot analysis indicated that the EMT markers α-smooth muscle actin (α-SMA) and Snail in the cGVHD-affected pancreas were expressed at higher level than those in their syngeneic control partners. (α-SMA: $P = 0.0084$, Snail: $P = 0.022$) (**Figure 3b, c, Supplementary Figure 9b, c**)

Page 13, Line 312

As judged by immunoblot analysis, the pancreas collected from the PBA-treated mice displayed the lower protein levels of GRP78, CHOP, p-PERK, p-eIF2α, and p-IRE1α compared with that collected from their vehicle-treated counterparts. (GRP78: $P = 3.0 \times 10^{-8}$, CHOP: $P = 5.7 \times 10^{-7}$, p-PERK: $P = 4.4 \times 10^{-7}$, p-eIF2α: $P = 1.3 \times 10^{-5}$, p-IRE1α: $P = 5.4 \times 10^{-4}$) (**Figure 4a, b, Supplementary Figure 10a-e**)

Page 14, Line 317

As a consequence, the proinflammatory molecules NF-κB and TXNIP, which are in the downstream of ER stress signalling pathways, were repressed in the pancreas treated with PBA in comparison to its vehicle-treated equivalent.

(NF-κB: P = 1.2 x 10^{-5}, TXNIP: P = 1.9 x 10^{-4}) (**Figure 4a, b, Supplementary Figure 10f, g**)

Page 14, Line 327

In addition, immunohistochemical analysis showed (1) that the number of immune cells in the PBA-injected pancreas was vastly lower than that in its vehicle-injected equivalent (P = 0.005) (**Figure 5c, d, Supplementary Figure 13**)

Page 14, Line 334

Presumably, as a result of the protection of exocrine and endocrine glands, the blood glucose levels in the PBA-medicated allo-BMT recipients were normal in contrast to those in their vehicle-medicated equivalents. (P = 2.2 x 10^{-7}) (**Figure 5f**)

Page 14, Line 337

Immunoblot analysis of IL-6 and CTGF also indicated that systemic injection of PBA suppressed cGVHD-elicited inflammation and fibrosis in contrast to the solvent-vehicle. (IL-6: P = 6.9 x 10^{-7}, CTGF: P = 1.6 x10^{-8}) (**Supplementary Figures 16, 17**)

Page 15, Line 349

We subsequently investigated whether PBA could suppress EMT, which were conceivably associated with excessive fibrosis in the pancreas affected by cGVHD. Immunohistochemistry and immunoblot analysis revealed that E-cadherin was retained in the PBA-treated pancreas in contrast to its vehicle-treated counterpart. (P = 7.4 x 10^{-4}) (**Figure 6a-c, Supplementary Figure 19a**)

Page 15, Line 353

In addition, the protein level of α-SMA and snail in the PBA-medicated

pancreas seemed to be normal, whereas α-SMA and Snail was overexpressed in its vehicle-medicated partner. (α-SMA: P = 0.0019 and Snail: P = 0.0082) (**Figure 6b, c, Supplementary Figure 19b, c**)

9. Better anatomical descriptions of the histopathological and EM findings should be provided.

The following statements regarding the histopathological and EM findings have been added to the main article.

Page 11, Line 249

As indicated by HE and Mallory's staining, tissues around ducts in the pancreas collected from allogeneic BMT recipients was excessively inflamed in association with mononuclear cell infiltration and fibrotic in concert with extracellular matrix accumulation. (**Figure 1a, b, Supplementary Figures 1, 2**) Conversely, when mice underwent syngeneic BMT, their pancreas seemed to be virtually normal. (**Figure 1a, b; Supplementary Figures 1, 2**) This finding indicated that pancreatic exocrine failure could be induced by cGVHD.

Page 11, Line 259

In addition, the size of pancreatic islets was greatly reduced in the GVHD-affected mice in contrast to that in their syngeneic control counterparts. (Figure 1e, Supplementary Figure 4). Presumably owing to the dysfunctional exocrine and endocrine glands,[26-28] the blood glucose levels in the cGVHD-affected mice was substantially greater than those in the syngeneic control subjects. (P = 0.000029) (**Figure 1f**)

Page 12, Line 270

The electron micrographs of cGVHD-affected pancreatic epithelia suggested (1) that the ER was abnormally expanded and distorted owing to aberrant accumulation of proteins and (2) that the walls of blood vessels in the stroma were extremely thin in association with multiple fenestrations and (3) that the cristae of mitochondria in acinar cells were distorted and damaged and (4) that

the mitochondria were expanded. (**Figure 1g, Supplementary Figure 7**)

Page 14, Line 325

The HE and Mallory pictures suggested that areas around pancreatic ducts in PBA-medicated pancreas was less inflamed and fibrotic compared with its vehicle-medicated partner. (**Figure 5a**, **b, Supplementary Figures 11, 12**).

Page 14, Line 332

Furthermore, pancreatic islets in the PBA-treated allo-BMT recipient mice were larger than those in their vehicle-treated counterparts. (Figure 5e, Supplementary Figure 15) Presumably, as a result of the protection of exocrine and endocrine glands, the blood glucose levels in the PBA-medicated allo-BMT recipients were normal in contrast to those in their vehicle-medicated equivalents. ($P = 2.2 \times 10^{-7}$) (**Figure 5f**)

Page 14, Line 340

Moreover, judging from electron micrographic analysis, pancreatic epithelia treated with PBA had almost normal structure of ER, blood vessels and mitochondria. (**Figure 5g, Supplementary Figure 18**) In contrast, in vehicle-treated pancreatic epithelia, the ER appeared to be expanded due to abnormal accumulation of proteins, the wall of a capillary was extremely thin, and mitochondria seemed to be damaged and lose their cristae. (**Figure 5g, Supplementary Figure 18**)

Page 16, Line 372

In particular, severe inflammation and fibrosis around pancreatic ducts were induced, and pancreatic islets were shrunk. Previous reports indicated that exocrine failure as well as endocrine failure in the pancreas could result in diabetes.[26-28] Hence, it is conceivable that the exocrine and endocrine failure in cGVHD-affected pancreas resulted in diabetes-like symptoms and thereby the blood glucose levels in mice with cGVHD were augmented.

Page 16, Line 384

Furthermore, our electron microscopic examination into pancreas disordered by cGVHD suggested (1) that the ER in the cGVHD-affected epithelia was expanded owing to accumulation of proteins, (2) the blood vessels were seriously demolished and (3) mitochondria in the epithelia were severely damaged.

Page 18, Line 417

Especially, PBA treatment subdued inflammation and fibrosis around pancreatic ducts, prevented pancreatic islets from shrinking and retained normal blood glucose levels in all-BMT recipient mice.

Page 17, Line 419

Electron microscopic assays also underpinned our claim by showing that PBA kept the ER, blood vessels and mitochondria in pancreatic epithelia virtually intact.

10. The English of the manuscript should be reviewed as it is verbose at times, e.g. “Once we gleaned these outcomes of medical importance” ; “we endeavored”; “this fact urged us to carry out groundbreaking research”;

As suggested, we have modified the following sentences in order to make them more succinct and less self-congratulatory.

Page 17, Line 409

Once we gleaned these outcomes of medical importance, we subsequently endeavored to treat pancreatic cGVHD.

Revised Sentence) With these outcomes, we subsequently attempted to treat pancreatic cGVHD.

Page 3, Line 80

This fact urged us to carry out ground-breaking research on cGVHD in the

pancreas.

Revised Sentence) This fact urged us to gain more insights into cGVHD in the pancreas.

11. Figure 7 should be omitted and instead can be discussed in the text, as it is speculative and the study has not been designed to confirm the suggestions made in the figure.

We have removed Figure 7.