Author Response

Comments from reviewers

Reviewer1:

Comment 1: Please, explain in details how were the drugs up-titrated for treatment-naive patients. Was selexipag and riociguat up-titrated in the same time period? Was this direct up-front combination? Or was it sequential combination therapy in a short time period?

Response 1: We appreciate reviewer's valuable comments. In most patients, macitentan 10 mg was initiated at first. One or 2 week later, riociguat 3.0 mg was also initiated. The dose of riociguat was up-titrated by 1.5 mg every 2 weeks with attention to adverse events. The timing of initiation of selexipag depended on patients. In 36% of treatment naïve patients, selexipag was initiated after riociguat was up-titrated to maximum tolerated dose, whereas selexipag was started within 2 weeks from initiation of macitentan in the others. Selexipag was increased by 0.4 mg every 2 weeks to the maximum tolerated dose with attention to adverse events. We added the details of drug up-titration in the Results section (page 10, line 26-29~page 11, line 1~3 in the marked-up version).

The median time from initiation of the first PAH drug to the start of the third PAH drug in treatment naïve patients was 24 days (interquartile range, 12-47 days). Except for one patient, these three types of vasodilator were initiated within 3 months. Although we defined upfront combination therapy as initiation of triple combination therapy within 3 months, the definition of upfront combination has not been clarified in the current guideline. Therefore, we removed the definition of upfront combination therapy, and just described the duration of up-titration and way of up-titration (page 5, line 15-16; page 10, line 23-26; page 15, line 17-19 in the marked-up version). We also added this information regarding the initiation of drugs into abstract (page 2, line 15-17).

Comment 2: Was the thermodilution also used for cardiac output measurement? The authors described that indirect Fick was used to calculate it, which is imprecise method of analysis according to current standards.

Response 2: We appreciate the reviewer's comment. In the ESC/ERS Guidelines 2015, the indirect Fick using estimated oxygen consumption is not recommended. However, the direct Fick method is practically difficult to measure routinely in Japanese hospitals and misestimation of cardiac output with thermodilution method is reported especially in the patients who present severe tricuspid regurgitation or low cardiac output. From these points of view, the indirect Fick methods has been often adopted in Japanese practical situations and this was mentioned in the Japanese Pulmonary Hypertension Guideline (K. Fukuda et al. Circ J 2019; 83: 842–945). In the present study, it was difficult to perform the analysis of cardiac output base on thermodilution method or direct Fick method, because there were a lot of missing values of cardiac output measured by them. Thus, the analysis based on the indirect Fick method was adopted. However, as you pointed out, this was an important study limitation. Therefore, we added the following sentence in the study limitation in Discussion section (page 15, line 19-22 in the marked-up version). "Indirect Fick method was adopted as the measurement of CO. This was not recommended in the ESC/ERS Guidelines 2015, but

was admitted to use in the practical situation in the Japanese PAH guideline. As the results could be affected by the measurement methods of CO, further validation would be warranted."

Comment 3: Please explain the results of tables 2 and 3. Were the parameters analysed for the same equal number of subjects at the baseline and follow-up?

Response 3: The number of subjects at baseline and follow-up was the same equal number in each parameter. The number of patients analyzed in table 2 was 18 in hemodynamics; 20 in echocardiography. The number of patients analyzed in table 3 was 10 in hemodynamics, 9 in echocardiography. We added these annotations in Table 2 and Table 3. (page 25, line 2-4 and page, 27 line 2-4 in the marked-up version)

Comment 4: In my opinion the Authors can not present and title table 2 "Changes in clinical parameters among all enrolled patients" when the RHC data were collected only for 18 patients and ECHO parameters for 20 subjects (but the same equal ones?).

Response 4: We appreciate reviewer's recommendation. We corrected the title of table 2 as "Changes in clinical parameters" (page 25, line 1 in the marked-up version). As reviewer pointed out, the RHC data were analyzed only for 18 patients and ECHO parameters for only 20 patients. We added the annotation in Table 2 and Table 3. (page 25, line 2-4 and page 27 line 2-4 in the marked-up version)

Comment 5: In my opinion, there should be presented the the results of paired analysis for specific clinical parameters, and that would reflect a real improvement (those who had not completed follow up in a single parameter should be excluded from the analysis).

Response 5: Thank you for your comment about statistical analysis. In order to compare the parameters at baseline to follow-up, we adopted Wilcoxon's signed rank test which was alternative methods of paired Student's t-test in the case of non-parametric data. The cases which had a missing value either at baseline or follow-up were excluded from each analysis. As the information about statistical method was insufficient, we added the detail in the Method section (page 7, line 13-17 in the marked-up version).

Comment 6: Please explain the supplementary table 1 and one patient who reached maximum tolerated dose of 0.0 mg of macitentan? The doses for riociguat and selexipag are unclear in the table.

Response 6: Thank you for your kind comments. The topic of maximum tolerated dose was written in supplemental table 2. In the case in which maximum tolerated dose was 0.0 mg, the triple oral

combination therapy including macitentan 10mg was initiated, but macitentan was discontinued due to myelosuppression. Therefore, macitentan could not be tolerated for that patient. We deleted the first row of Supplemental Table 2 and added the annotation about the above-mentioned patient. We also corrected the position of drug names, because it was difficult to understand the dose of riociguat and macitentan (Supplemental Table 2 in Supplemental Table-R1).

Comment 7: There is a mismatch between main text and supplementary table 2 regarding the number of treatment naive patients. What was the real number of treatment naive patients?

Response 7: We would like to explain supplemental table 1, because the number of treatment naive patients was written in supplemental table 1. In this analysis, we conducted subgroup analysis regarding the changes of clinical parameters in the patients who were treatment naive, and treated with only macitentan, riociguat and selexipag for PAH. Therefore, the number of treatment naive patients at baseline was 17, but the number of patients who were treatment naive at baseline and treated only with macitentan, riociguat, and selexipag were 11. The rest of 6 patients were excluded from this subgroup analysis, because they were treatment naive at baseline, but treated with other vasodilators before the triple oral combination therapy. In 5 out of these 6 patients, oral medication was switched from beraprost to selexipag, and in one patient, oral medication was switched from ambrisentan and tadalafil to macitentan and riociguat. We added the explanation about the patients included in the subgroup analysis in the Methods and the Results sections (page 7, line 6-7 in the marked-up version; page 10, line 19-23).

Comment 8. I suggest to remove supplementary figure 1.

Response 8: Thank you for your recommendation. We removed supplementary figure 1 (page 5, line 20-21; page 9, line 3 in the marked-up version. Supplemental figure legend-R1).

Reviewer2:

Comment 1: explanation of the choice of risk assessment as "hemodynamic risk assessment"; while I personally have no objection to this, I would have used the more standard European risk assessment (on which guidelines are created) or just only mention and report REVEAL risk score if this is the program's usual practice. For the sake of consistency with the PH community I would consider reanalyzing the date using the European risk score. (see also below)

Response 1: We appreciate the valuable suggestions from the reviewer. As you pointed out, risk assessment based on only hemodynamics could not be standard. We corrected the way of analysis and just only performed risk analysis based on REVEAL risk score, as you recommended. We also reanalyzed the data with updated three-category REVEAL 2.0 risk score. As a result, one patient changed from intermediate-risk group to high risk group, but the others remained the same. The prostacyclin infusion free rate was almost similar to that before the change; 92.9% in low-/intermediate-risk patients and 55.0% in high-risk patients. We corrected sentences in the Methods

and the Results sections, Table 1, Supplemental Table 1, and Figure 3 (page 2, line 25-26; page 6, line 22-29~page 7, line 1-3; page 10, line 2-6, 9-16; page 19 line 11-16; page 21, line 17-18; page 23, Table 1; page 23, line 3-4~page 24, line 1-5 in the marked-up version. Supplemental Table 1 and the annotation in the supplemental table-R1. Figure 3 in the Figure3-R1).

Comment 2: Taking the limitation above, they classify 42% patients at high risk (not sure if this is the same high risk that guidelines refer to) and 3.8% of patient are in WHO IV. In this situation guidelines recommend initiation of combination therapy that includes parenteral prostacyclin. It would be very important to explain why guidelines were not followed. More importantly, the message that transpires is that we can avoid parenteral prostacyclins in 50% of patients at high risk and obtain good results. This might be true, but a single center retrospective case series is hardly convincing evidence. I would be very careful with sending out this message to the practicing physician, I would dial down the strength of the conclusion and say something like "observation that requires further investigation etc"

Response 2: Thank you for your important comments and recommendation. Although current guidelines recommend initial combination therapy including prostacyclin infusion, patients in this study did not present decompensated heart failure, and the urgent initiation of prostacyclin agency was not necessary. Therefore, the triple oral combination therapy was initiated firstly and its efficacy was reassessed within 6 months. We added the explanation to Discussion section (page 13, line 26-28). We also added the sentence that reduces the strength of the conclusion (page 3, line 1-2; page 16, line 1-2).

Comment 3: Would also offer explanation for a few different than standard of care choices: CO is only calculated by assumed O2 consumption, parenteral prostacyclins were added to a triple regimen that includes selexipag (as opposed to discontinuing the prostacyclin receptor agonist); would be fair for the reader to also understand why patients on other combinations were switched to maci, rio, selexipag at the time of enrollment.

Response 3: We appreciate reviewer’s valuable comments. In this study, we could not calculate the CO based on the thermodilution method and the direct Fick methods because of missing values. Although the indirect Fick methods were often adopted in Japanese practical situations and this was mentioned in the Japanese Pulmonary Hypertension Guideline (K. Fukuda et al. Circ J 2019; 83: 842–945), this was an important study limitation. Therefore, we added the study limitation in the Discussion section (page 15, line 19-22 in the marked-up version).

In 5 of 6 patients who needed prostacyclin infusion during the triple oral combination therapy, selexipag was continued when prostacyclin was initiated. The past study reported that selexipag less tended to cause desensitization than prostacyclin infusion (Gatfield J et al. J Pharmacol Exp Ther. 2017;362:186–199). We continued selexipag in the hope that it would reduce the maintenance dose of the prostacyclin for infusion. We added the further explanation in the Result section (page 9, line 23-24 in the marked-up version).

When the improvement of hemodynamic was insufficient with baseline medication, we switched the medication to the combination of macitentan, riociguat, and selexipag. We added the further method in revised manuscript (page 5, line 19-20 in the marked-up version).

Comment 4: would mention the significance of the findings of the current trial in the light of the recent data of the TRITON study a multicenter randomized trial that looks at the effect of upfront triple combination in PAH.

Response 4: Thank you for your valuable comment. We compared the results in this study and those of TRITON study provided in the ESC congress (reference 18). The improvement of PVR in the TRITON study was 54% in triple oral combination therapy group with the follow-up period of 26 weeks. The improvement of 66% in PVR in the treatment naive patients of the present study was greater than that of TRITON study, although study design was largely different. The degree of improvement of 6MWD was also greater in the treatment naive patient of present study than in the triple combination therapy group of TRITON study (72m vs 55m). Our findings provide the important aspect of triple oral combination therapy as a real-world data. We added these discussions in the Discussion section. (page 12, line 26-29~page 13, line 1-3)

With respect to prognosis in the TRITON study, only 2 of 123 patients died and 41% reduction in the risk of PAH-related hospitalization and all-cause death was observed in the triple oral combination therapy group. The low rate of mortality and hospitalization for heart failure in the present study was consistent with TRITON study. These findings in this study could verify the efficacy for preventing disease progression of triple oral combination therapy observed in the randomized controlled study. We added these discussions in the revised manuscript (page13, line 10-14 in the marked-up version). We also modified the beginning of Discussion and Reference (page 12, line 9, 12-15; page 19, line 20-23 in the marked-up version).

Comment 5: page 12, second paragraph suggest to change "treatment reactivity" to "treatment response

Response 5: Thank you for your recommendation. We corrected the sentence in the Discussion section (page 13, line 21 in the marked-up version).

Reviewer: 3

Comment 1: Mizuki et al studied 26 patients who were administered triple oral combination therapy with macitentan, riociguat, and selexipag for the treatment of PAH. Two-thirds were treatment naïve while the rest were under some PAH medications at baseline. In the 18 patients who had available hemodynamic data, during a median follow-up period of 441 days, mean pulmonary arterial pressure, pulmonary vascular resistance, and cardiac output improved by 29%, 65%, and 82%, respectively. Three-year survival rate and prostacyclin infusion-free rate since administration of all 3 drugs was 93.3% and 74.6% respectively. This was a retrospective study and this bears significant limitations when studying the safety and efficacy of PAH targeted drug therapies. I believe that the

most important finding of the study that should be mostly expressed is the subgroup analysis in treatment-naïve patients. These patients experienced a 29% decrease in the mean PAP, a 66% decrease in the PVR and a 90 increased in CO from baseline to follow-up (median 293 days). These findings should be discussed in the light of the results of the TRITON trial the only trial that has used a similar upfront triple combination (macitentan, tadalafil and selexipag).

Response 1: Thank you for your valuable comment. We compared the results in this study and those of TRITON study provided in the ESC congress (reference 18). The improvement of PVR in the TRITON study was 54% in triple oral combination therapy group with the follow-up period of 26 weeks. The improvement of 66% in PVR in the treatment naive patients of the present study was greater than that of TRITON study, although study design was largely different. The degree of improvement of 6MWD was also greater in the treatment naive patient of present study than in the triple combination therapy group of TRITON study (72m vs 55m). Our findings provide the important aspect of triple oral combination therapy as a real-world data. We added these discussions in the Discussion section (page 12, line 26-29~page 13, line 1-3).

With respect to prognosis in the TRITON study, only 2 patients died and 41% reduction in the risk of PAH-related hospitalization and all-cause death was observed in the triple oral combination therapy group. The low rate of mortality and hospitalization for heart failure in the present study was consistent with TRITON study. These findings in this study could verify the efficacy for preventing disease progression of triple oral combination therapy observed in the randomized controlled study. We added these discussions in the revised manuscript (page13, line 10-14 in the marked-up version). We also modified the beginning of Discussion and Reference (page 12, line 9, 12-15; page 19, line 20-23 in the marked-up version).

Comment 2: It is also strange that prostacyclin infusion was initiated in 31.8% of patients within 7 months, and no patients underwent prostacyclin infusion after 7 months. This points towards a bias of an initial high risk population of patients in which initial triple oral combination treatment was chosen.

Response 2: Thank you for your comment. As you pointed out, the selection of high-risk population would be also the limitation in this study. We added this limitation in the Discussion section (page 15, line 13-16 in the marked-up version).

Comment 3: It is important for the authors to delineate in abstract how many of treatment naïve patients the oral combination was upfront (n=10), in how many it was sequential (n=1) and how many patients were taking some PAH medications at baseline.

Response 3: We appreciate reviewer's recommendation. We added the information into the abstract and adjusted the number of characters (page 2, line 2-4, 6-7, 13-17, 20-21 in the marked-up version). According to another author's recommendation, we removed the definition of upfront combination therapy and instead described the median time from initiation of the first PAH drug to

the start of the triple oral combination therapy: 24 days (interquartile range, 12-47 days) (page 5, line 15-16; page 10, line 23-26; page 15, line 17-19 in the marked-up version).

Comment 4: Authors state that “For patients who had already received other PAH-specific vasodilators at the time of enrollment, these drugs targeting the endothelin pathway, nitric oxide pathway, and prostacyclin pathway were switched to macitentan, riociguat, and selexipag, respectively”. This method has important flaws since patients are not treatment naïve and the comparison of hemodynamic and other parameters is from a baseline state in which the patient already received another vasodilation therapy.

Response 4: We agree with your assessment. This study included patients who were not treatment naive at baseline, which was one of the major limitations in this study. We added the limitation in the Discussion section (page 15, line 12).

Comment 5: Page 6, Did authors use the original REVEAL risk score or the updated three-category REVEAL 2.0 score?

Page 6, In the risk assessment based on guideline hemodynamics, the authors did not include SVO2.

Response 5: Thank you for providing these insights. We adopted original REVEAL risk score at first. However, we reanalyzed the data with updated three category REVEAL 2.0 risk score because it has greater discrimination with respect to clinical worsening and long-term mortality. As a result, one patient changed from intermediate-risk group to high risk group, but the others remained the same. The prostacyclin infusion free rate was almost similar to that before the change; 92.9% in low-/intermediate-risk patients and 55.0% in high-risk patients.

Regarding the risk assessment based on guideline hemodynamics, we did not include SvO2. Another reviewer recommended that we should focus the risk assessment on REVEAL risk score as the risk assessment based on guideline hemodynamics was not common method. We reconsidered again and decided to adopt only REVEAL 2.0 risk score as the risk assessment in this study. We corrected sentence in the Methods and Results section, Table 1, supplementary table 1, and Figure 3 (page 2, line 25-26; page 6, line 22-29~page 7, line 1-3; page 10, line 2-6, 9-15; page 19 line 10-16; page 21, line 17-18; page 23, Table 1; page 23, line 3-4~page 24, line 1-5 in the marked-up version. Supplemental Table 1 and the annotation in the supplemental table-R1. Figure 3 in the Figure3-R1).

Comment 6: Page 6, “The observation period was terminated when patients died, underwent prostacyclin infusion, or stopped taking macitentan, riociguat, or selexipag for any reason”. Did the authors perform the analysis of survival without taking onto account these patients?

Response 6: Thank you for your valuable comments. The overall survival rate was 92.3% with median follow up period of 33 (24–43) months. We added this result to the Result section (page 9, line 17-18 in the marked-up version).

Comment 7: Page 8, “Most patients were treatment-naïve (65.4%), and others were taking some PAH medications at baseline.”

Page 9, “intravenous infusion of epoprostenol was added to the triple oral combination therapy in another patient, and a subcutaneous infusion of treprostinil was added to the triple oral combination therapy in the remaining 4 patients”. Why did the authors continue to administer oral selexipag in these patients?

Response 7: Thank you for your comment. In 5 of 6 patients who needed prostacyclin infusion during the triple oral combination therapy, selexipag was continued when prostacyclin was initiated. The past study reported that selexipag less tended to cause desensitization than prostacyclin infusion (Gatfield J et al. J Pharmacol Exp Ther. 2017;362:186–199.). We continued selexipag in the hope that it would reduce the maintenance dose of the prostacyclin for infusion. We added the further explanation in the Result section (page 9, line 23-24 in the marked-up version).

Comment 8: Page 10, please provide follow-up measurements of all hemodynamic variables apart from mPAP in the low/intermediate and high risk group.

Response 8: Thank you for providing pivotal insight. We added all the hemodynamic measurements at baseline and follow-up in low/intermediate and high-risk groups as the Supplemental Table 3 in the supplemental table-R1. We also added the description in the Results section (page 10, line 6-8 in the marked-up version).

Comment 9: Page 10, line 20-22 “Mean PAP at follow-up in low/intermediate risk patient was 34 (26– 21). Do you mean high risk here?

Response 9: We appreciate reviewer’s valuable question. As a result of reanalysis with REVEAL2.0 risk score, the mean PAP at follow-up in low-/intermediate- risk patients was 35 (28-40) mmHg (shown in Supplemental Table 3). From the perspective of hemodynamic assessments based on current ESC guideline, the hemodynamic at follow-up in low-/intermediate-group correspond with Low risk. When we also calculated the three-category REVEAL2.0 risk score in each group at the time of RHC follow-up, all of low-risk patients at baseline and 89% of high-risk patients at baseline were categorized into low-/intermediate-risk group. However, we did not include this analysis in the manuscript because this analysis is limited to those who underwent hemodynamic follow-up.

Comment 10: Page 10, line 22-26 “Among 5 patients who were classified as high-risk based on the guideline and needed prostacyclin infusion, 3 patients had BMPR2 mutations and 1 patient had a RNF213 25 mutation”. This is a repetition!

Response 10: We appreciate reviewer's comment. We deleted the sentence. (page 10, line 14-16 in the marked-up version)

Comment 11: Discussion, page 11 "Most of the low-/intermediate-risk patients and around half of the high-risk patients could be treated sufficiently with this triple oral combination therapy." This depends always on the studied population and also the follow-up period and cannot be a generalized conclusion.

Response 11: We agree with your assessment. It would be difficult to generalize the results in this study and it need further studies to validate our findings. We incorporated these sentences in the Discussion section (page 12, line 6 in the marked-up version), Abstract, and Conclusion section (page 3, line 1-2; page 16, line 1-2 in the marked-up version).

Comment 12: In general, although the idea behind the paper has many advantages, the authors use a mix of findings in the Discussion, such as risk assessment which is not the main research question of the present study. For instance, discussing the prognostic significance of the mPAP and cardiac index, as well as echo parameters and BNP is out of the scope if this work.

Response 12: Thank you for your comment. We reconsider the discussion and excluded and modified the description which are irrelevant to main research question (page 13, line 4-6, 14-20; page 14, line 8-15 in the marked-up version).

Comment 13: Do the authors imply in the Discussion that Eisenmenger patients should be treated more aggressively with parenteral prostanoids? If yes, this is not correct.

Response 13: We did not mean to imply the use of parenteral proteinoids in Eisenmenger patients, although this sentence was so confusing. We modified the sentence to avoid misunderstanding (page 14, line 2-7 in the marked-up version).

Comment 14: In terms of safety, please parallel your findings with the GRIPHON trials in which patients who received triple therapy had the highest frequency of side effects.

Response 14: We appreciate reviewer's important insight. In the subgroup analysis of GRIPHON study which investigated the effectiveness of selexipag for the patients of PAH, a triple oral combination therapy with selexipag, ERA, and PDE5 inhibitor was discontinued in 19.0% of patients due to adverse events, while the rate of discontinuation was 15% in our study. Serious adverse events did not occur in the present study, whereas 44.7% of patients presented serious adverse events in the triple combination therapy group in the GRIPHON study. We incorporated these discussions in the revised manuscript (page 14, line 18-25 in the marked-up version).

Specific comments

Comment 15: Page 4, revise to “Pulmonary arterial hypertension (PAH) is a lethal disease characterized by elevated pulmonary arterial pressure (PAP) due to remodeling of the pulmonary arterial bed and right sided-heart failure”.

Response 15: We appreciate reviewer’s recommendation. We revised the sentence in the Introduction section (page 4, line 3-4 in the marked-up version).

Comment 16: Page 4, line 38: Add the mechanism of action of selexipag

Response 16: Thank you for your comment. We incorporated the pharmacological activity of selexipag in the Introduction section (page 4, line 19 in the marked-up version).

Comment 17: Page 4, line 50-52: “which oral vasodilators are most appropriate for combination in such patients remain poorly documented to date”. Please revise or omit since this is a single-arm study and you do not compare oral combinations in order to find the most efficient.

Response 17: We agree with you and omitted this sentence (page 4, line 24-25 in the marked-up version).

Comment 18: Page 5. How did authors acquire written informed consent from patients, since this was a retrospective study?

Response 18: We obtained the informed consent about the usage of clinical data for medical research when patients were hospitalized. We added this information in the Methods section (page 5, line 5 in the marked-up version).

Comment 19: Page 5, calculation of cardiac index with the use of the Fick technique with a stable oxygen consumption is not supported by the PH Guidelines. This is a limitation of the study, although comparisons between baseline and follow-up hemodymanics were made using the same technique.

Response 19: I appreciate reviewer’s valuable comment. In this study, we could not calculate the cardiac output based on the thermodilution method and the direct Fick methods because of missing values. Although the indirect Fick methods were often adopted in Japanese practical situations and this was mentioned in the Japanese Pulmonary Hypertension Guideline (K. Fukuda et al. Circ J 2019;

83: 842–945), this was an important study limitation. Therefore, we added the study limitation in the Discussion section (page 15, line 19-22 in the marked-up version).