Author’s response to reviews

Title: First Human Dose-Escalation Study with Remogliflozin Etabonate, a Selective Inhibitor of the Sodium-Glucose Transporter 2 (SGLT2), in Healthy Subjects and in Subjects with Type 2 Diabetes Mellitus

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Author’s response to reviews: see over
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Title: First Human Dose-Escalation Study with Remogliflozin Etabonate, a Selective Inhibitor of the Sodium-Glucose Transporter 2 (SGLT2), in Healthy Subjects and in Subjects with Type 2 Diabetes Mellitus

Responses to Reviewer 1:

Minor essential revisions:
1. In page 4/ line 100, the authors stated “Because SGLT2 inhibitors work by an insulin independent mechanism, this class of compounds may be of benefit in patients whose pancreatic function is diminished.” This sentence must be deleted or revised because it is insulin, not SGLT-2 inhibitors, that should be primarily used for insulin-dependent patients with diabetes. Thank you for pointing out the lack of clarity in this sentence. We were trying to convey that SGLT-2 inhibition can provide additive effect in glucose lowering because of its insulin independent mechanism, not that it should replace insulin for insulin dependent diabetics. The text has been revised as follows:

   Because SGLT2 inhibitors work by an insulin-independent mechanism, this class of compounds may be of benefit as adjunctive therapy in patients whose pancreatic function is diminished or in patients who have insulin resistance.

2. In page 5/ line 132-, the authors should provide demographic data for healthy volunteers and T2DM patients in a table including presence or absence of comorbidity such as hypertension and dyslipidemia. In order to avoid duplicating demographic information that already appears in the text (current lines 142-161), the authors have chosen not to repeat it in a table. However, to confirm, the healthy volunteers were, of course, required to be healthy, and the T2DM subjects were required to be healthy other than their diagnosis of T2DM.

3. In page 7/ line 177-, the authors should provide proportion of carbohydrate, protein, and fat in meals served in hospitals. While in the unit, subjects were given standardized meals that were consistent in the amount of sodium, caloric content, and percentage of fat, carbohydrate, and protein. Unfortunately, it was not until we performed subsequent studies that we began recording details of the nutritional content of the diet.

4. In page 7/ line 182, the authors used 50g glucose-containing liquid rather than 75g. They should state a reason for this change. There is some variability in the amount of glucose that may be administered in an oral glucose tolerance test. Since this glucose challenge was not performed to diagnose glucose intolerance / insulin resistance, a lower glucose amount was thought to be more appropriate for the T2DM subjects. The text has been revised:

   Fifteen 15 minutes after dosing in each treatment period in Part B, a fasting OGTT was performed using 50 g glucose administered as 50 g Glucola™. A 50 g glucose load was chosen since the OGTT was being performed in subjects already known to have diabetes.
5. In page 8/line 196, “rpm” for centrifugation should be converted into “g” as “rpm” is not universal among various centrifuges. The analytical methods section has been greatly condensed and now refers the reader to the following recently published article for further details:


6. In page 10/line 261-, does their intact GLP-1 assay analyze extracted plasma? Recent studies indicate necessity of plasma extraction due to the existence of interference for intact GLP-1 assay that can be removed by extraction of plasma (Journal of Diabetes Investigation 3(1); 70-79, 2012; Best Practice & Research Clinical Endocrinology & Metabolism 23(4); 425-432, 2009). Since the analyses were conducted several years prior to the publication of the articles cited above, it is likely that the method did not include an extraction step; however, it is not possible to confirm given the amount of time that has elapsed since the study and analyses were performed.

7. In page 12/line 307-, the authors stated “Two of the diabetic subjects had increased urine beta-2 microglobulin concentrations, one of the exploratory biomarkers measured as a potential early indicator of renal toxicity.” They should discuss possible mechanisms why SGLT-2 inhibitors cause increase in urine beta-2 microglobulin. The text (below) in the revised draft more fully explains the 2 instances of increased B2M:

Urine beta-2 microglobulin levels, an exploratory biomarker that was measured as a potential early indicator of renal toxicity, were within the normal range at both baseline and after treatment for all subjects except for two subjects with diabetes. One of these subjects had normal beta-2 microglobulin values at 1 and 4 days after dosing with 500 mg remogliflozin etabonate. However, 11 days after dosing, this subject returned to the clinic for the Day -2 visit of the 3rd treatment period. At this time, the subject’s beta-2 microglobulin levels were elevated to 2.5 µg/mL. The values returned to normal (<0.3 µg/mL) within 2 days. The investigator attributed the elevated pre-placebo levels to other concomitant disease. A second subject, however, did have what was considered by the investigator to be a drug-related elevation of beta-2 microglobulin of 1.62 µg/mL on day 1 after dosing with 500 mg remogliflozin etabonate. The value returned to normal levels within 4 days. No associated changes in serum creatinine and urea or urine microalbumin were observed.

There did not seem to be a clear link between SGLT2 inhibition and increase in B2M, particularly since there were no elevations in other more traditional markers of renal dysfunction.

8. Tables 2 and 3 should be combined such that they will be easily compared to table 4. We agree that combining Tables 2 & 3 would be ideal and would make the comparison to Table 4 much easier; however, it becomes very difficult to present since there are so many dose levels to be combined. Also combining the tables makes the footnotes become very complex and confusing to the reader. Because Table 4 data had fewer dose levels, it was easier to present clearly.

9. In page 14/line 397, the authors stated “There were no differences in mean Tmax or T 1/2 values of remogliflozin or GSK279782 between patients and healthy subjects.” However, Tmax or T 1/2 values of remogliflozin or GSK279782 appear to differ not statistically significantly but substantially. In addition,
Tmax or T 1/2 values of Remogliflozin are also substantially different between healthy subjects and patients with type 2 diabetes. Authors should describe potential mechanisms why these values show substantial difference between healthy volunteers and patients with type 2 diabetes.

The authors would like to refrain from over interpretation of the differences in PK parameters between healthy subjects and patients in this early study. The authors do not believe there are any apparent differences in T1/2 for any of the three compounds at the doses of 50mg and 500mg, which were administered to both populations. The T1/2 for RE is too limited and the data for R and GSK279782 are very similar. For Tmax, the overlap of the ranges for the two populations for doses 50mg and 500mg leaves the authors with no other conclusion but that there are no discernable differences in this study. Therefore, the authors are justified in saying that “There were no discernable differences in mean T\text{max} or T\text{1/2} values of remogliflozin or GSK279782 between patients and healthy subjects.”

10. For changes in glucose, insulin and intact GLP-1, the authors should show corresponding line graphs. The revised manuscript includes a figure (Fig 5) which shows glucose and insulin changes after glucose challenge. We did not include a figure with GLP-1 because, as noted in the manuscript, there was no evidence of a dose dependent change in GLP-1.

11. The authors should provide explanation why SGLT-2 specific inhibitor RE reduces secretion of GLP-1. Is this partially due to inhibition of SGLT1 in the gut? It is possible that there is some local effect in the gut on SGLT1. Upon review of the manuscript, we have revised the text of the GLP-1 results section as follows:

**Plasma intact GLP-1.** In T2DM subjects, the median baseline adjusted AUC\(_{(0-4)}\) for plasma GLP-1 following an OGTT was 6.36 pM*h (range, 0 to 16.6) for placebo, -8.85 pM*h (range, -46.7 to 33.4) for remogliflozin etabonate 50mg, and -1.95 pM*h (range, -11.6 to 7.0) for remogliflozin etabonate 500 mg. These data are difficult to interpret because the ranges for placebo, 50mg and 500mg groups contain zero. Although there was no evidence of a dose response, these results suggest that plasma GLP-1 could potentially be suppressed following remogliflozin etabonate administration compared to placebo.

**Responses to Reviewer 2:**

**Major Compulsory Revisions**

1. Results/discussion: “Two of the diabetic subjects had increased urine beta-2 microglobulin concentrations, one of the exploratory biomarkers measured as a potential early indicator of renal toxicity.” Please provide more detail. Were the values on the high end of normal at baseline/placebo or on any other treatments? Were there elevations compared to placebo with other subjects that feel short of the abnormal value cutoff? These results should be included and commented on in the discussion. Thank you; additional detail has been added to the text.

2. Results/Discussion: The statement “Urine excretion of electrolytes was highly variable and no treatment-related changes were observed” does not seem supported by table V. Although the results are variable, there are trends for higher values for all electrolytes at the higher doses. This is especially
true for subjects with diabetes. It also appears that the variability may be somewhat higher at the high dose for subjects with diabetes suggesting that there may be some more extreme individual cases. Please elaborate both on a mean and individual subject level. Update the discussion accordingly. Thank you for this comment. The authors have carefully reviewed the treatment groups as well as the individual data, and do not believe there is a drug related trend of increasing electrolytes. During the 500 mg treatment period, Subject #12 had high values for all 3 electrolytes (roughly 2-fold higher than other subjects), which contributed to the high standard deviations in this dosing group.

Minor Essential Revisions

3. Methods: please clarify if part B was dose-escalating or if dose sequence was randomized. Yes, Part B was randomized and dose escalating.

4. Methods: For the GLP-1 assay please clarify details of the GLP-1 assay in the manuscript. I believe it measures active GLP-1? and the kit is manufactured by Millipore. Thank you; the text has been updated to reflect that intact GLP-1 was analyzed by Pathway Diagnostics Corporation, 3003 Malibu Canyon Road, Malibu, CA, USA by ELISA (kit # EGLP-35K, EMD Millipore). The lowest level of intact GLP-1 this assay can detect is 2 pM (with a minimum plasma sample size of 0.4 mL).

5. Methods: Clarify over what time interval the oral glucose was consumed. The Glucola was consumed within ~5 minutes of being provided to the subjects at 0.25hr after administration of study drug (RE or placebo). Text has been updated.

6. Methods Line261: Linco is now Millipore. Please indicate that when referring to the DPP-4 inhibitor used. Thank you; text has been updated.

7. Results line 299: indicate how many treatments the subject in Part A completed prior to withdrawal. Text has been clarified. Subject #8 participated in all treatment related visits except for the follow up visit. Thus, safety results for the follow up visit contain data for only 9 of the 10 subjects:

In Part A, 10 subjects (8 males, 2 females, mean age of 39 years, mean BMI of 24.5 mg/kg², and mean baseline fasting plasma glucose 4.7 mmol/L [range 4.2 to 5.1 mmol/L, SD 0.29 mmol/L]) were randomized; 9 completed the study (1 subject participated in all the study visits but did not return for the follow up visit).

8. Authors should add fasting plasma glucose mean, SD, and range to the demographic section. The text has been updated to include this information.

9. Is GSK279782 equipotent to remogliflozin? Please clarify what is known about relative potency and include in the manuscript. Based on Ki values, remogliflozin and GSK279782 are equally potent in inhibiting the human SGLT2 receptor in transfected cell lines.

Discretionary Revisions

10. Results: Suggest combining Tables 2 and 3. The authors have chosen to leave the tables as currently presented.
11. Results: Suggest expanding Figure 2 to include a panel for each dose (could exclude the 20 mg dose if concentrations are mostly undetectable). This would better support comments in lines 330-336 regarding dose-related differences in profiles (monophasic vs. biphasic plasma concentration declines) and would be more informative to the reader. The following text has been removed since upon re-review the text overstates a minor, if not trivial observation consequently misleading readers. Essentially there is no important monophasic vs biphasic decline that is dose dependent. “The mean T1/2 estimates for remogliflozin appear to be longer at the 500 mg (2.57 h) and 1000 mg (2.86 h) dose levels when compared to the lower doses (1.38–1.59 h). Analysis of the data suggests this is due to the presence of modest biphasic decline in plasma concentration time profiles observed at the higher doses. In contrast, at the lower doses of 20, 50 and 150 mg, the decline in plasma concentrations are mono-exponential (Figure 2). This difference is a consequence of high assay sensitivity in combination with high plasma concentrations.” The new text is as follows: “The mean T1/2 estimates ranged from 1.38 to 2.86 h.”

12. Table V: the first column should be consistent between healthy subjects and T2DM subjects. Specifically, in the electrolyte rows of the healthy subject section, the term “(SD)” does not need to be listed as it is indicated by the note “results are expressed as mean (SD)” at the bottom of the table. Thank you for pointing out; this has been corrected.

Responses to Reviewer 3:

I have only discretionary revisions/minor essential revisions to provide some improvements in the presentation of this work:

1. General: Use past tense (e.g., line 53, line 291) Thank you; agreed.

2. Lines 45-47: Please reword for clarity -it is not clear that each subject received multiple single doses of RE. The specific doses in the healthy subjects should be identified. The number of placebo subjects/dose panel should also be stated. The text has been updated to clarify:

   All subjects received single oral doses of either RE or placebo separated by approximately 2 week intervals. In Part A, 10 subjects participated in 5 dosing periods where they received RE (20 mg, 50 mg, 150 mg, 500 mg, or 1000 mg) or placebo (4:1 active to placebo ratio per treatment period). In Part B, 6 subjects participated in 3 dose periods where they received RE (50 mg and 500 mg) or placebo (2:1 active to placebo per treatment period).

3. Line 49: Exposure to which analyte(s) was proportional to dose? Thank you for pointing this out. The dose proportionality statements have been clarified in the text.

4. Line 53: State amount of glucose excreted at the dose plateau and what period of time is being referred to (24 h post-dose). The text has been updated to indicate that in healthy subjects, approximately 200mmol of glucose was collected in the urine from 0 to 24 hours after dosing.

   All subjects showed dose-dependent increases in 24-hour UGE, which plateaued at approximately 200 to 250 mmol glucose with RE doses ≥150 mg.

6. Lines 200-234: Detail of assay methodology is excessive. Suggest shorten this section. Agreed; the methods have been greatly condensed since they can now be referenced to the Sigafoos article noted above that has recently been accepted for publication.

7. Line 244: It would also be interesting to report molecular mass-corrected exposure for GSK279782 since it is an active metabolite. The authors have decided to leave the units as currently expressed.

8. Line 261: More details about the validation of the GLP-1 assay are needed as this is not a straightforward assay. I am guessing this is intact GLP-1, as opposed to total GLP-1 and this should be stated. We measured intact GLP-1; text has been updated.

9. Line 232 -Accuracy of the methods should also be stated. Thank you; the text has been updated to reflect the lower limit of detection.

10. Line 271: Were any other urinary analytes measured (e.g. uric acid)? It seems from the results that some biomarkers of renal injury were measured. These should be stated. Urinary beta 2 microglobulin, serum creatinine, urea, and urine microalbumin were monitored to assess renal function; no changes were observed; text has been updated in Lines 301-302 of the current manuscript draft.

11. Line 272: Please justify collecting urine for only 24 h. Higher doses of RE would be expected to cause glucosuria for longer periods of time relative to lower doses, even if the UGE over 24 h were to be the same. The reviewer brings up a very valid point; however, at the time this FTIH study was designed, a 24-hour collection interval was thought to be adequate.

12. Line 300: The reason for withdrawal of consent should be stated. Subject #8 participated as required during study conduct but simply did not return for the follow up visit; text has been updated.

13. Lines 349-352: Report all of the mean slopes and 90% CIs for each parameter and each analyte. The following sentence has been added: “For remogliflozin etabonate, the mean slopes and 90% confidence intervals for AUC(0-∞) and Cmax were 1.17 (1.04, 1.30) and 1.04 (0.94, 1.14). For remogliflozin, the mean slopes and 90% confidence intervals for AUC(0-∞) and Cmax were 1.09 (1.06, 1.12) and 1.08 (1.02, 1.14). For GSK279782, the mean slopes and 90% confidence intervals for AUC(0-∞) and Cmax were 1.08 (1.05, 1.12) and 1.07 (1.01, 1.12).”

Was the LLOQ phenomenon Line 336-339) responsible for the dose proportionality analysis slightly missing linearity? The following text has been removed since upon re-review the text overstates a minor, if not trivial, observation consequently misleading readers: “The mean T1/2 estimates for remogliflozin appear to be longer at the 500 mg (2.57 h) and 1000 mg (2.86 h) dose levels when compared to the lower doses (1.38–1.59 h). Analysis of the data suggests this is due to the presence of modest biphasic decline in plasma concentration time profiles observed at the higher doses. In contrast, at the lower doses of 20, 50 and 150 mg, the decline in plasma concentrations are mono-
This difference is a consequence of high assay sensitivity in combination with high plasma concentrations.

The mean T1/2 estimates ranged from 1.38 to 2.86 h.

Which AUC is being referred to (infinity or 0-T)? The AUCs have been clarified in the pharmacokinetic text and tables.

What % of AUCinf was extrapolated? It would be helpful to also report AUC(0-T) in Tables III and IV. The percentage of AUCinfinity extrapolated was very small and to help the reader, footnotes have been added to Tables III and IV. The following footnotes have been added to Tables III and IV respectively,"The median percentage of AUC(0-∞) extrapolated for R was low ranging from 0.07% to 2.1% across all doses and for 279782 ranging from 0.29% to 4.51%", and “The median percentage of AUC(0-∞) extrapolated for remogliflozin etabonate ranged from 10% to 11% and for remogliflozin was low ranging from 0.14% to 0.42% and for GSK279782 was 0.47% to 2.0%.

14. Lines 394-398: I can’t assess whether these statements are accurate. iGLP-1 is notoriously noisy and no errors are given. What are the numbers presented? Mean change from baseline? The values presented are the median values that have been adjusted for baseline values. Text has been revised to include the ranges.

15. Line 443-445: This sentence is contradicted by Figure 4, where there is clearly not a plateauing of UGE over 24 h. I think perhaps the authors are referring to the %inhibition of glucose load excreted and if so, this should be pointed out. The %inhibition of glucose load excreted essentially takes the time course out of the assessment of UGT which is problematic since there will be substantial overlaps of concentrations between the doses and thus the level of inhibition of SGLT2 may be the same for different doses at different times. The 24 h UGT could also be subjected to a sigmoid Emax model analysis. Modeling will be the subject of a different publication.

16. Figure1: Does “chiral” mean enantiomerically pure? The molecule is chiral; however, enantiomerically pure compound was used.

17. Figure 3: Present Dose on a log scale. The most appropriate presentation is linear rather than log.

18. Figure 4: Smoothed lines should not connect the points (implies modeling) and error bars should be added (e.g., +/-SEM). Consider presenting these data in grams which is more readily understood relative to g of glucose ingested per day. The smoothed lines have been removed. All of the data has been presented in mmoles rather than grams.

19. Figure 5: Why are medians presented here? Since the number of subjects is so small, suggest show individual data in this figure. The figure has been replaced by one with individual values.