Author's response to reviews

Title: Safety, Pharmacokinetics and Pharmacodynamics of Remogliflozin Etabonate, a Novel SGLT2 Inhibitor, and Metformin when Co-administered in Subjects with Type 2 Diabetes Mellitus

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Author's response to reviews: see over
Response to Reviewer Comments

Title: Safety, Pharmacokinetics and Pharmacodynamics of Remogliflozin Etabonate, a Novel SGLT2 Inhibitor, and Metformin when Co-administered in Subjects with Type 2 Diabetes Mellitus

Version: 4 Date: 11 February 2013
Reviewer: Mark S Fineman

Reviewer's report:
The authors have satisfactorily addressed all of the points raised in the original manuscript review.

Major Compulsory Revisions: None

Minor Essential Revisions: None

Discretionary Revisions
1. Add information to the methods section on how metformin was measured. Thank you for pointing this out; the following text has been added to the manuscript:

The concentrations of metformin in plasma were determined by HPLC-MS/MS using a [\(^{2}H_6\)]-metformin isotopically labelled internal standard. Plasma proteins from a 50 mL plasma aliquot were precipitated using acetonitrile containing the internal standard (200 ng mL\(^{-1}\)). Samples were vortex mixed then centrifuged. The resulting supernatant was transferred and mixed with 200 mL of HFBA buffer (water containing 10 mM ammonium acetate and 0.26% (v/v) of heptofluorobutyric acid) prior to injection. HPLC was performed on a Shimadzu LC-10A HPLC system. Chromatography was performed on a MAC-MOD Ace 3 C18, 4.6 x 50 mm column at a flow rate of 1.0 mL min\(^{-1}\). An isocratic mobile phase elution with 82:18 (v/v) HFBA buffer: Acetonitrile was used. Samples were analysed in positive ion mode by Turbo Ionspray LC/MS/MS with a PE/Sciex API 3000. The calibration range was 20 to 5000 ng mL\(^{-1}\). Performance of the method was assessed during a 3 day validation study using quality control samples at 5 concentrations 20, 80, 500, 4000 and 5000 ng mL\(^{-1}\). The average within-run precision [coefficient of variation (CV %)] was <9.6% and the between-run precision CV% was < 4.7%. Similar assay performance was observed during study sample analyses.

2. Discussion: Text says: “Mean lactate concentrations showed an increase or increasing trend during the three day MET BID treatment period. In contrast, the mean lactate concentrations decreased during RE BID and MET+RE BID periods”. Although it is clear that lactate increases in the met alone treatment, lactate does not decrease in the RE treatment arm. Would change to “In contrast, the mean lactate concentrations are unchanged or decreased slightly during RE BID and MET+RE BID periods.” Thank you for pointing this out; the change has been made to the text.

Version: 4 Date: 25 January 2013
Reviewer: David Boulton
Reviewer's report:
The manuscript is acceptable.

**Reviewer's report**  
**Version:** 4  
**Date:** 6 February 2013  
**Reviewer:** Yutaka Seino

**Reviewer's report:**  
The manuscript entitled "Safety, Pharmacokinetics an Pharmacodynamics of Remogliflozin Etabonate, a Novel SGLT2 Inhibitor, and Metformin when Co-administered in Subjects with Type 2 DM" by Hussey et al is now revised substantially. However, the authors still need to address the issue #1, which I raised in the previous communication (i.e. definition of nephrotic proteinuria). The authors should cite appropriate references to validate their cut-off value, >2.5g/gCre.

Thank you; a reference has been added for the nephrotic-range proteinuria cut off of 3g protein / g Crn in urine samples. The 2.5g/g Crn cut off was used instead of 3 g/g Crn to be more conservative.