Effects of LX4211, a Dual SGLT1/SGLT2 Inhibitor, Plus Sitagliptin on Postprandial Active GLP-1 and Glycemic Control in Type 2 Diabetes

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ABSTRACT

Background: Combination therapy is required to provide adequate glycemic control in many patients with type 2 diabetes mellitus (T2DM). Because sodium-dependent glucose transporter (SGLT)-1 inhibition results in an increased release of glucagon-like peptide (GLP)-1, and because dipeptidyl peptidase (DPP)-4 inhibitors prevent its inactivation, the 2 mechanisms together provide an intriguing potential combination therapy.

Objectives: This combination was explored in preclinical models and then tested in patients with T2DM to compare the effects of single-dose LX4211 400 mg and sitagliptin 100 mg, administered as monotherapy or in combination, on GLP-1, peptide tyrosine tyrosine (PYY), gastric inhibitory peptide (GIP), glucose, and insulin.

Methods: Preclinical: Obese male C57BL6J mice were assigned to 1 of 4 treatment groups: LX4211 60 mg/kg, sitagliptin 30 mg/kg, LX4211/sitagliptin, or inactive vehicle. Clinical: This 3-treatment, 3-crossover, randomized, open-label study was conducted at a single center. Patients on metformin monotherapy were washed out from metformin and were randomly assigned to receive sequences of single-dose LX4211, sitagliptin, or the combination. In both studies, blood was collected for the analysis of pharmacodynamic variables (GLP-1, PYY, GIP, glucose, and insulin). In the clinical study, urine was collected to assess urinary glucose excretion.

Results: Preclinical: 120 mice were treated and assessed (5/time point/treatment group). With repeat daily dosing, the combination was associated with apparently synergistic increases in active GLP-1 relative to monotherapy with either agent; this finding was supported by findings from an additional 14-day repeated-dose experiment. Clinical: 18 patients were enrolled and treated (mean age, 49 years; 56% male; 89% white). The LX4211 + sitagliptin combination was associated with significantly increased active GLP-1, total GLP-1, and total PYY; with a significant reduction in total GIP; and with a significantly improved blood glucose level, with less insulin, compared with sitagliptin monotherapy. LX4211 was associated with a significant increase in total GLP-1 and PYY and a reduced total GIP, likely due to a reduction in SGLT1-mediated intestinal glucose absorption, whereas sitagliptin was associated with suppression of all 3 peptides relative to baseline. All treatments were well tolerated, with no evidence of diarrhea with LX4211 treatment.

Conclusions: The findings from the preclinical studies suggest that the LX4211 + sitagliptin combination produced synergistic increases in active GLP-1 after a meal challenge containing glucose. These initial clinical results also suggest that a LX4211 + DPP-4 inhibitor combination may provide an option in patients with T2DM. The potential long-term clinical benefits of such combination treatment need to be confirmed in large clinical trials. ClinicalTrials.gov identifier: NCT01441232. (Clin Ther. 2013;35:273–285) © 2013 Elsevier HS Journals, Inc. All rights reserved.
Key words: adult, diabetes, dipeptidyl peptidase-4, GIP, GLP-1, glucose, glycemic control, human, insulin, PYY, SGLT1, SGLT2, sitagliptin, urinary glucose excretion.

INTRODUCTION
Type 2 diabetes mellitus (T2DM) is a disease characterized by increased blood glucose levels that result in both microvascular and macrovascular complications.1–6 Microvascular complications include nephropathy, retinopathy, and neuropathy, whereas macrovascular complications include heart disease, stroke, and peripheral vascular disease. Diabetes is a growing health concern in the United States and worldwide. In 2009, there were an estimated 24 million individuals with T2DM in the United States, and that number is forecast to roughly double by 2034.7 The worldwide incidence of T2DM is now estimated at 346 million.8 This surge in T2DM has been correlated with an increase in obesity.9–11 The economic burden of diabetes is enormous, with 2009 US spending on diabetes and related costs estimated at ~$100 billion; that figure is expected to triple by 2030.7

Treatments that help to control blood glucose have been demonstrated to decrease the microvascular complications of diabetes.1–6 Metformin is standard first-line therapy; however, findings from studies suggest that only 50% of patients are well controlled by monotherapy at 3 years into treatment, and only 25% at 9 years.12 Thus, combination therapy is required and becomes increasingly important as the disease progresses.13–16

LX4211 is a dual inhibitor of sodium dependent glucose transporters (SGLT) 1 and 2. SGLT1 is the major glucose/galactose transporter of the gastrointestinal (GI) tract, involved in the uptake of glucose from the diet.17 Study findings suggest that inhibiting SGLT1 results in reduced glucose absorption, which stimulates an increased release of beneficial peptides such as glucagon-like peptide (GLP)-1 and peptide tyrosine tyrosine (PYY) from the GI tract, commencing 1 hour after meals, with sustained effects throughout the day.18 This GI response is likely the result of a triggering of the natural homeostatic mechanism that senses increased nutrient levels resulting from meals containing excessive carbohydrates, fats, or other nutrients.19 Glucose in the distal small intestine and short chain fatty acids (SCFAs)—the bacterial fermentation products of glucose in the colon—are sensed by L cells and trigger their release of GLP-1, PYY, and other beneficial peptides (ie, oxyntomodulin). This mechanism of SGLT1 inhibition is supported by the report of a highly selective SGLT1 inhibitor that increased glucose levels in the distal small intestine and cecum and increased GLP-1 and PYY in rats.20 Similar GI responses are triggered by roux-en-Y gastric bypass surgery20–24 and the ingestion of dietary-resistant starch.25–27 In addition to inhibiting SGLT1, LX4211 also inhibits SGLT2, thereby reducing renal glucose reabsorption and enhancing urinary glucose excretion (UGE). In a Phase 2 clinical trial, LX4211 was well tolerated and provided significant improvement in glycemic control over 4 weeks of dosing in patients with T2DM.18 Importantly, LX4211 also lowered triglycerides and produced trends in reductions of both body weight and blood pressure.

Sitagliptin inhibits dipeptidyl peptidase (DPP)-4, resulting in incretin-mimetic effects.28,29 Active (α) GLP-1 and gastric inhibitory peptide (GIP) are released by the GI tract in response to meals but have short half-lives due to inactivation by the DPP-4 enzyme.30–32 Sitagliptin and other DPP-4 inhibitors thus increase levels of αGLP-1 and αGIP. αGLP-1 and αGIP enhance glucose-dependent insulin release from pancreatic β cells. GLP-1 has additional activities such as delaying gastric emptying, appetite suppression, and inhibition of glucagon release. In contrast, αGIP enhances glucagon secretion and induces adiposity.30–32 The net effect of sitagliptin treatment is enhanced glycemic control, with no significant effect on body weight.33

Theoretically, LX4211 in combination with a DPP-4 inhibitor such as sitagliptin might provide enhanced benefit in patients with T2DM because they act through complementary mechanisms. LX4211 has 2 insulin-independent mechanisms of action—(1) SGLT2-dependent reductions in renal glucose reabsorption and (2) SGLT1-mediated reduced intestinal glucose absorption—whereas sitagliptin enhances glucose-dependent insulin release. Additionally, LX4211 might enhance the incretin effects of sitagliptin because LX4211 increases GLP-1 release after meals and sitagliptin inhibits αGLP-1 inactivation.

The present studies examined whether the LX4211 + sitagliptin combination results in increases in αGLP-1 levels, relative to either monotherapy, in mice, and whether the combination enhances the GI peptide pro-
file and provides improved glycemic control over sitagliptin monotherapy in patients with T2DM.

MATERIALS AND METHODS

Preclinical Pharmacology In Mice

Study Design

General methods for mouse care have been described.34 Both the single-dose and 14-day studies were carried out using obese male C57BL6J mice fed a high-fat diet containing 45% kcal from fat (D12451, Research Diets Inc, New Brunswick, New Jersey) since weaning and were at least 24 weeks of age at the time of study. Randomization was based on body weight, to 1 of 4 treatment groups: LX4211 60 mg/kg monotherapy, sitagliptin 30 mg/kg monotherapy, the combination of LX4211 + sitagliptin, or inactive vehicle. Treatment was administered in the morning by oral gavage. On the day of meal challenge, the mice received 25 mL/kg of low-fat diet (10% kcal as fat; D12450B, Research Diets) suspended in dextrose water (9.2 g/kg glucose, 2.5 g/kg protein, 0.6 g/kg fat) by oral gavage 30 minutes after active or inactive drug administration.

Assessments

Blood was collected from the mice before and at 0.5, 1, 2, 3, and 6 hours after meal challenge to measure circulating levels of aGLP-1 by ELISA (GLP-1 Active ELISA Kit, Millipore Corporation, St. Charles, Missouri). Blood collection and handling, and all aspects of the assay protocol, were performed as recommended by Millipore. Because 500 μL of blood is required for a single aGLP-1 assessment, multiple measurements in the same mouse were not feasible; instead, independent groups of mice (n = 5) were assigned to each time point in each treatment arm.

Statistical Analysis

All preclinical pharmacology data are presented as mean (SEM). The 0–6-hour time-course data for aGLP-1 levels in each treatment group were converted to AUC values by trapezoidal summation using Prism version 4.03 (GraphPad Software, Inc, San Diego, California). The effect of LX4211 and/or sitagliptin on aGLP-1 levels was analyzed by 2-way ANOVA also using Prism version 4.03. When a significant interaction was demonstrated for the combination of LX4211 + sitagliptin, the effect of these compounds individually on aGLP-1 levels was assessed by ANOVA followed by Bonferroni testing; these analyses included data from all of the mice except those that received both compounds.

Clinical Study In Patients With Type 2 Diabetes

Study Design

This clinical study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the institutional review board with jurisdiction over the site (IntegReview Ethical Review Board, Austin, Texas), and all patients provided written informed consent before being enrolled into the study. The 3-treatment, 3-crossover, randomized, open-label study was conducted at a single center (Phase I Center, ICON Development Solutions plc, San Antonio, Texas) between October 4, 2011, and November 22, 2011, and included patients 28 to 65 years of age diagnosed with T2DM at least 3 months before screening and currently taking metformin monotherapy. At screening, all patients were required to have a hemoglobin A1c value of 6.5% to 10.5%, C-peptide ≥1 ng/mL, and a body mass index <45 kg/m². Patients were screened for adequate renal function and were excluded if they had an estimated glomerular filtration rate <60 mL/min/1.73 m². Patients’ demographic characteristics are shown in Supplemental Table I in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010 http://www.blogger.com/blogger.g?blogID=818251595177931289 - _msocom_1, and a complete list of inclusion and exclusion criteria is included in Supplemental Appendix in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010.

Metformin washout, which included daily glucometer assessments and instructions on dietary guidelines and restrictions, was performed over a 14-day period starting on day –15. Patients were instructed to return to the clinic if their fasting plasma glucose (FPG) exceeded 280 mg/dL on 2 consecutive days and were to be discontinued from the study if they had laboratory confirmation of FPG >280 mg/dL. Patients completing metformin washout entered the inpatient facility beginning 2 days prior to randomization (day –2) for verification of eligibility and diet stabilization. Baseline parameters, obtained on day –1, included glucose, insulin, aGLP-1, total (t) GLP-1, tGIP, tPYY, ghrelin, and leptin.

A randomization schedule was generated before the first dosing period by ICON Development Solutions, the contract research organization that performed data
management for the study. Eighteen patients were randomly assigned among 3 blocks of 6 treatment sequences to receive LX4211 400 mg, sitagliptin 100 mg, or the combination, at 8 AM, 1 hour before the morning meal. The treatment block and treatment sequence structures were generated in accordance with a $3 \times 3$ Latin square design balanced for first-order carryover effects. No formal sample size calculation was made, but the number of required patients was consistent with those in other trials with similar objectives and design. The LX4211 dose was selected because it produced maximal hemoglobin A1c reduction in a Phase 2b dose-ranging study.\textsuperscript{35} The maximum dose of sitagliptin was chosen to determine whether the LX4211 combination produces any additional benefit.

Each patient received a single dose of LX4211, a single dose of sitagliptin, and the combination dose over the course of the study, in 3 successive treatment periods (doses on days 1, 8, and 15) separated by 7-day washout, during which patients were fed a low–glycemic index diet of 2200 kcal/d, with caloric intake on any given day consisting of 38% to 51% carbohydrate, 17% to 22% protein, and 35% to 41% fat. Three identical meals were provided at baseline (day −1), and on each of the 3 dosing days (days 1, 8, and 15), with breakfast 1 hour after dosing, lunch at 4 hours after dosing, dinner at 8 hours after dosing, and a snack at 12 hours after dosing. All meals were 0.5 hour in duration, with instructions on completing the meal provided. Meal compositions were 493 total kcal for breakfast (52% carbohydrate, 14% protein, and 34% fat), 772 total kcal for lunch (47% carbohydrate, 23% protein, and 30% fat), 833 total kcal for dinner (44% carbohydrate, 21% protein, and 35% fat), and a 135-kcal snack that was given after all pharmacodynamic measurement samples were obtained for the day. The inpatient active-treatment period included a final follow-up and discharge 2 days after the final dosing day. Methods used to generate and implement the random allocation sequence are described in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010.

The end points were measured at baseline (day −1) and on each of the 3 days of dosing. Plasma glucose, insulin, GLP-1, and PYY measures were obtained at multiple time points throughout the day (Supplemental Table II in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010) to capture the effects on these parameters after each meal. Plasma GIP, leptin, and ghrelin measures were taken at time points to examine breakfast responses to minimize blood draws. Total and active GLP-1, tPYY, tGIP, ghrelin, and leptin were measured by Pacific Biomarkers (Seattle, Washington). Comparisons of active and total GLP-1, tPYY, glucose, and insulin were based on total AUCs 0 to 13 hours after dosing. Comparisons of tGIP, leptin, and ghrelin were based on total AUC 0 to 4 hours.

Tolerability assessments included physical examination (including weight and vital signs), ECG, clinical laboratory assessment, and adverse-events (AE) assessments; AEs were coded and listed by organ system and preferred terms based on the Medical Dictionary for Regulatory Activities (MedDRA) version 12.0.

Continuous variables were summarized by the number of patients with nonmissing data, mean (SD), or median (range). Categorical variables were summarized as number (%). All tests of treatment effects were 2-sided at the 0.05 level of significance, with no adjustments made for multiple comparisons. SAS version 9.1.3 (SAS Institute Inc., Cary, North Carolina) was used to make all statistical comparisons and to summarize the data descriptively.

Imputation or any other data-assignment rule was not used to substitute values for missing observations. All data analyses and summaries were based on observed cases. The lone exception was for the pharmacodynamic variables, whereby values reported below the lower limit of quantification were assigned a value.
of LLOQ/2. For all calculations of changes from baseline, *baseline* was defined as day −1 unless noted otherwise.

Analysis of a few pharmacodynamic measures was based on single data point values taken on days −1, 1, 8, and 15. These measures included UGE and FPG. Other measures required a more complex modeling of daily values, and to fully capture the effect of treatments on the extent of changes in these variables, different derivations of AUC were calculated. AUC values were calculated for GLP-1 (total and active), PPG, insulin, PYY, and GIP. The AUC measures were derived using all time points collected within a study day (days −1, 1, 8, and 15) but varied as to how they were calculated. The total AUC (ie, AUC0−last) reflects the summation of subareas from time 0 (predose value) to the last value measured on a day. Thus, the total area was the AUC measured down to a metabolic level of “zero.” The net incremental AUCs were estimated by subtracting the area below the predose level from the area above the predose level. The linear trapezoidal algorithm was used to derive all AUC estimates.

A Latin square design balanced for first-order carryover effects was implemented to allow the effects of treatment and period (time-related effects) to be evaluated independently and efficiently. A generalized linear mixed model (GLMM) was parameterized to reflect these design restrictions and permitted unbiased statistical testing/estimation of the desired treatment comparisons.

Analysis of the tGLP-1 data using AUC0−last was subjected to a period effect (*P* < 0.001). However, using net incremental AUC data, the statistical model showed no significant period (*P* = 0.79) or interaction (*P* = 0.40) effects. This was due, in part, to the subtraction of the period effect from these measures by adjusting for time 0 on each study day.

Interpretation of the tPYY and tGIP AUC0−last data was influenced by the presence of a treatment-by-period interaction effect (*P* < 0.10); tPYY results were also affected by a significant period effect (*P* < 0.001). As such, analysis of tPYY and tGIP AUC0−last data was based on a GLMM with an added term for first-order carryover effects. Use of this adjusted GLMM for the tPYY data allowed for a more clear-cut interpretation of between-treatment effects using the AUC0−last values across all periods. Analysis of the tGIP AUC0−last data using the first-order carryover effect–adjusted GLMM provided a nonsignificant period effect (*P* = 0.74) as well as a remedy for the interaction effect.

**RESULTS**

**Preclinical Pharmacology In Mice**

In initial studies, mice dosed with either LX4211 or sitagliptin monotherapy exhibited increased aGLP-1 levels relative to vehicle at 1 hour after meal challenge, and the combination of LX4211 + sitagliptin was associated with a further additive, but not synergistic, increase in aGLP-1. However, at 2 hours after meal challenge, LX4211 + sitagliptin was associated with a clear synergistic increase in aGLP-1 (Supplemental Figure 2 in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010). In a confirmatory study, single-dose LX4211 or sitagliptin monotherapy was associated with a significantly increased AUC for aGLP-1 levels measured between 0 and 6 hours after meal challenge, and the LX4211 + sitagliptin combination was associated with a further, significant, apparently synergistic increase in aGLP-1 AUC (Figure 1A).

On multidose testing (14 days of once-daily dosing) of LX4211 or sitagliptin monotherapy, aGLP-1 levels measured between 0 and 6 hours after meal challenge were increased relative to those with vehicle (Figure 1B), and the LX4211 + sitagliptin combination was associated with a significant, apparently synergistic increase in aGLP-1 compared with that with either agent alone. With repeated daily dosing, the combination appeared to produce greater increases in aGLP-1 than were found in the single-dose studies, an effect that was supported by findings from an additional 14-day repeated-dosing experiment (Supplemental Figure 3 in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010). These findings suggest that the LX4211 + sitagliptin combination produces synergistic increases in aGLP-1 after a meal challenge containing glucose.

**Clinical Studies In Patients With Type 2 Diabetes**

In the 18 T2DM patients enrolled (Supplemental Figure 4 in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010), LX4211 monotherapy was associated with significantly increased 24-hour UGE, as expected based on its inhibition of SGLT2 in the kidney (Figure 2 and Table I), and the LX4211 + sitagliptin combination was associated with a similar increase. As anticipated based on its mechanism of ac-
sitagliptin monotherapy was not associated with increased UGE.

On assessment of GI peptides, glucose, insulin, ghrelin, and leptin, combination treatment was associated with a significant increase in aGLP-1 over that resulting from either monotherapy (Figure 3A and Table I). This increase in aGLP-1 appeared to have occurred in a synergistic manner with the combination as was also observed in mice. tGLP-1 and tPYY, with combination treatment, were significantly increased relative to those resulting from sitagliptin monotherapy and were significantly reduced relative to those resulting from LX4211 monotherapy (Figure 3B and 3C and Table I). tGIP was significantly reduced with the combination treatment relative to either monotherapy (Figure 3D and Table I). There were no significant differences in ghrelin or leptin levels with combination therapy relative to either monotherapy (data not shown). Taken together, LX4211 + sitagliptin treatment was associated with increased aGLP-1, tGLP-1, and tPYY and decreased tGIP compared with sitagliptin monotherapy.

There was evidence of a period effect in this study; however, the presence of a period effect did not influence the interpretation of between-treatment differences. The period effect may have impacted the interpretation of within-treatment effects, and to this point, further statistical analyses were performed to compare the results versus baseline (Figure 3). Analytic findings derived using the net incremental AUC data on tGLP-1 are presented in Supplemental Table III in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010. These data suggest that LX4211 increased the tGLP-1 level from baseline (change in least squares mean [LSM], 24.63 pmol · h/L; \( P < 0.001 \)), whereas sitagliptin had an opposing effect, with a reduction from baseline of nearly the same magnitude as the increase seen with LX4211 (change in LSM, \(-23.78 \) pmol · h/L; \( P < 0.001 \)). The comparison of the combination treatment to sitagliptin monotherapy further underscored the apparent effect of LX4211, whereby the difference in LSMs demonstrated an increase of \(23.21 \) pmol · h/L (\( P = 0.005 \)), possibly attributable to LX4211.

Figure 1. Mean (SEM) active glucagon-like peptide (aGLP)-1 AUCs with meal challenge after the administration of a single dose (A) or multiple daily doses (B) of LX4211, sitagliptin, LX4211 + sitagliptin, or inactive vehicle by oral gavage in mice (n = 5 per group). In A and B, \( P < 0.05 \) for LX4211 and sitagliptin monotherapies versus vehicle; \( P < 0.005 \) for LX4211 versus sitagliptin (2-way ANOVA).

Figure 2. Mean (SD) 24-hour urinary glucose excretion (UGE) with LX4211, sitagliptin, and their combination in patients with type 2 diabetes mellitus. *\( P < 0.001 \) versus LX4211 + sitagliptin.
The tPYY and tGIP data resulting from the adjusted GLMM are presented in Supplemental Tables IV and V, respectively, in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010. LX4211 was associated with an increase from baseline in LSM tGIP AUC\textsubscript{0–last} of 61.67 pmol·h/L (P < 0.001). In contrast, sitagliptin was associated with a reduced tPYY AUC\textsubscript{0–last} over time, by 134.72 pmol·h/L (P < 0.001). Comparison of the combination treatment to sitagliptin showed a difference in AUC\textsubscript{0–last} LSMs of 43.61 pmol·h/L (P = 0.014). This difference suggests a positive effect of LX4211 (ie, increasing tPYY levels) when used in the combination treatment. On analysis of day-1 data only (period 1), which was absent of any period effect, tPYY was increased with combination treatment compared with sitagliptin monotherapy, as reflected by a difference in LSMs of 55.20 pmol·h/L. All treatments were associated with significant reductions from baseline over time in tGIP AUC\textsubscript{0–last} (P < 0.001). Use of both drugs in combination appeared to have been additive in effect. The net contribution of LX4211 in the combination was a reduction of 32.24 pmol·h/L (P < 0.001).

Importantly, glycemic control was improved throughout the day with the combination therapy relative to either monotherapy (FPG, 2366.7, 2412.8, and 2209.6 mg·h/L with LX4211, sitagliptin, and the combination, respectively) and was achieved with significantly less insulin than that required with sitagliptin monotherapy, respectively) and was achieved with significantly less glucose production (metformin) and improved glucose-dependent enhanced insulin sensitivity and decreased hepatic glucose production (metformin) and improved glucose-depen-

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**Table I. Pharmacodynamic properties of LX4211 and sitagliptin administered as monotherapy and in combination in patients with type 2 diabetes (N = 18).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Monotherapy</th>
<th>Δ vs Combination*</th>
<th>Monotherapy</th>
<th>Δ vs Combination*</th>
<th>LX4211 + Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGE, g/24 h</td>
<td>92.2</td>
<td>−8.21 (−24.8 to 8.4)</td>
<td>7.5</td>
<td>76.4 (59.8 to 93.0)</td>
<td>83.9</td>
</tr>
<tr>
<td>Insulin, μM·h/mL</td>
<td>479.2</td>
<td>68.1 (2.3 to 133.9)</td>
<td>623.4</td>
<td>−76.1 (−141.9 to −10.3)</td>
<td>547.3</td>
</tr>
<tr>
<td>PPG, mg·h/L, 0–13 h</td>
<td>2366.7</td>
<td>−157.0 (−312.9 to −1.2)</td>
<td>2412.8</td>
<td>−203.2 (−359.0 to −47.3)</td>
<td>2209.6</td>
</tr>
<tr>
<td>PYY, pmol·h/L</td>
<td>403.3</td>
<td>−152.8 (−187.1 to −118.5)</td>
<td>206.9</td>
<td>43.6 (9.3 to 77.9)</td>
<td>250.5</td>
</tr>
<tr>
<td>tGLP-1, pmol·h/L</td>
<td>161.9</td>
<td>−26.1 (−38.8 to −13.3)</td>
<td>105.9</td>
<td>29.9 (17.2 to 42.6)</td>
<td>135.8</td>
</tr>
<tr>
<td>aGLP-1, pmol·h/L</td>
<td>69.2</td>
<td>98.2 (80.9 to 115.4)</td>
<td>128.8</td>
<td>38.6 (21.3 to 55.9)</td>
<td>167.4</td>
</tr>
<tr>
<td>GIP total, pmol·h/L</td>
<td>117.4</td>
<td>−45.3 (−62.7 to −29.0)</td>
<td>109.2</td>
<td>−32.2 (−49.1 to −15.4)</td>
<td>78.4</td>
</tr>
</tbody>
</table>

GIP = gastric inhibitory peptide; GLP = glucagon-like peptide; PPG = postprandial glucose; PYY = peptide tyrosine tyrosine; UGE = urinary glucose excretion.

*Estimated difference (95% CI) between combination and single-dose drug adjusted means.

†P < 0.001 versus combination.

‡P < 0.01 versus combination.

The combination provides complementary mechanisms of enhanced insulin sensitivity and decreased hepatic glucose production (metformin) and improved glucose-depen-
dent insulin release and reduction in glucagon (sitagliptin), mediated in part through the common pathway of enhanced GLP-1 activity, with metformin enhancing the release of GLP-1 from the GI tract and sitagliptin inhibiting its inactivation.41 Future combinations may be valuable for diabetes treatment, especially if they provide enhanced glycemic control in addition to cardiovascular and metabolic benefits such as weight loss. Weight loss is of particular importance given the correlation between obesity and diabetes.42–45

LX4211 + sitagliptin could theoretically provide both complementary and synergistic benefits. The mechanism of action of LX4211 includes 2 insulin-independent benefits, enhanced UGE, and reduced glucose absorption by the intestine, which could augment the insulin-dependent mechanism of enhanced glucose-dependent insulin release produced by DPP-4 inhibition. In addition, the mechanisms of both LX4211 and sitagliptin converge to act on GI peptide mechanisms, which might provide an additive benefit. LX4211 enhances postprandial release of aGLP-1 as sitagliptin prevents its inactivation, and LX4211 also enhances the secretion of tPYY.

The authors have previously reported that LX4211 triggers increased GLP-1 and PYY release from the GI tract after meals.18 This finding appears to have been the result of reduced glucose absorption, which allows glucose to reach the distal end of the small intestine and colon.20 Glucose in the distal end of the small intestine is sensed by L cells and triggers their release of GLP-1, PYY, and other beneficial peptides.19 Once the glucose reaches the colon, it is fermented by bacteria into SC-
FAs, which can also be sensed by L cells, thus stimulating additional secretion of GLP-1, PYY, and other peptides. SCFAs (e.g., acetate, propionate, butyrate) are rapidly absorbed by the colon and have been shown to decrease ghrelin release and increase leptin release. In the present study, it is likely that LX4211 + sitagliptin produced enhanced effects with respect to GI peptides relative to sitagliptin monotherapy, through mechanisms similar to those observed with LX4211 monotherapy.

LX4211 + sitagliptin was associated with increased aGLP-1, tGLP-1, and tPYY relative to sitagliptin monotherapy. GLP-1 and PYY might provide multiple benefits because both are appetite suppressants, and GLP-1 has additional effects, including suppression of glucagon release, suppression of hepatic gluconeogenesis, and stimulation of metabolic rate. The activity of the combination on GLP-1 and PYY may contribute to enhanced glycemic control as well as other metabolic benefits for patients. In mice, repeated dosing with LX4211 + sitagliptin was associated with increases in aGLP-1 greater than those achieved with single dosing. Based on these multiple-dose pharmacology results in mice, single-dose studies in patients may underestimate the full effect of long-term combination therapy on aGLP-1.

The pharmacodynamic properties of SGLT1 inhibition, with respect to GLP-1 release at varying times relative to glucose exposure/meals, have emerged from preclinical studies conducted elsewhere in mice and in recent clinical trials of LX4211 conducted by the authors. The findings from 2 separate preclinical studies suggest that SGLT1 activity is required for GI release of GLP-1 shortly after exposure to glucose. In 1 study, coadministration (by injection into the upper small intestine) of glucose and phloridzin, an inhibitor of SGLT1, was associated with suppression of glucose-dependent GLP-1 release from the upper small intestine at 5 minutes postinjection. In a

![Figure 4](image)

**Figure 4.** Mean (SD) plasma concentrations of glucose (A) and insulin (B) in patients with type 2 diabetes mellitus after single-dose administration of LX4211, sitagliptin, or LX4211 + sitagliptin. Statistical comparisons are based on total AUCs. *P < 0.05 versus combination.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LX4211</th>
<th>Sitagliptin</th>
<th>LX4211 + Sitagliptin</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with ≥1 TEAE</td>
<td>1 (5.6)</td>
<td>2 (11.1)</td>
<td>4 (22.2)</td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>Gastrointestinal disorder</td>
<td>0</td>
<td>1 (5.6)</td>
<td>2 (11.1)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>Constipation</td>
<td>0</td>
<td>1 (5.6)</td>
<td>2 (11.1)</td>
<td>3 (16.7)</td>
</tr>
</tbody>
</table>

Table II. Treatment-emergent adverse events (TEAEs) of the gastrointestinal system organ class with LX4211 and sitagliptin administered as monotherapy and in combination in patients with type 2 diabetes (N = 18). Data are number (%) of patients.
separate study, SGLT1 knockout mice exhibited a reduced GLP-1 response relative to that in wild-type mice at 5 minutes after an oral glucose challenge. \(^{51}\) These near-term measures are in contrast to longer-term increases in GLP-1 responses observed in humans treated with LX4211\(^{18}\) and have also been observed in rats with a selective SGLT1 inhibitor. \(^{20}\) The authors have also observed a transient inhibition of aGLP-1 release after LX4211 treatment in mice (data not shown), which was followed by a sustained increase of GLP-1 levels over time. Together, these data suggest that SGLT1 activity may be required for the transient early aGLP-1 release within minutes after a meal but not the more sustained GLP-1 release over time.

Findings from previous studies suggest that while sitagliptin increases postprandial aGLP-1 and aGIP, it suppresses tGIP, tGIP, and tPYY levels, a reflection of all GLP-1, GIP, and PYY released from the GI tract. \(^{41,52}\) This decrease in GI peptide release has been hypothesized to be due to a negative-feedback loop possibly fueled by a pharmacologically induced increase in aGLP-1, the result of DPP-4 inhibition. Suppression of both GLP-1 and PYY release may explain the weight neutrality with sitagliptin treatment. \(^{52}\) Postprandial GLP-1 and PYY increases with the LX4211 + sitagliptin combination relative to sitagliptin monotherapy, in the context of an overall reduction in caloric load associated with LX4211-mediated UGE and reduced intestinal glucose absorption, may produce weight loss and its associated benefits—a testable hypothesis in a longer-term trial.

Although the focus is often placed on the absolute blood concentrations of GLP-1 and PYY, it should be emphasized that the release of these peptides from their normal site may be important for obtaining optimal benefits. \(^{53-59}\) The GI tract is innervated by afferent vagal nerves that express both GLP-1 and PYY receptors. The vagus nerve signals directly from the GI tract to the brain, and it has been demonstrated in animals that the full benefits of GLP-1 and PYY are lost when the vagus nerve is cut in the abdomen. Similarly, it has been reported that the full effects of GLP-1 on postprandial glucose, gastric emptying, and appetite are lost in vagotomized human subjects. \(^{60}\) Thus, the release of these peptides from the GI tract may provide enhanced benefits at lower blood concentrations than when GLP-1 analogues are delivered parenterally.

In this current clinical study, tGIP, leptin, and ghrelin were measured after LX4211 treatment. Combination treatment was associated with a significant reduction in tGIP relative to that resulting from either monotherapy. K cells secrete GIP, are found primarily in the upper small intestine, and respond to glucose and fats in the diet. \(^{30}\) In particular, the transport of glucose seems important for GIP release. \(^{50,51,61}\) This hypothesis is supported by findings from preclinical studies, which have suggested that pharmacologic inhibition of sodium-dependent glucose transport in the GI tract reduces postprandial GIP release. This finding may explain the reduction in GIP release after breakfast with SGLT1 inhibition. The apparent enhanced effect of LX4211 + sitagliptin on GIP reduction is consistent with a feedback-inhibition effect on tGIP observed with sitagliptin treatment. \(^{41}\) In the context of diabetes, GIP has beneficial effects, such as enhancing glucose-dependent insulin release, as well as potential negative effects (eg, increasing glucagon, increasing peripheral adiposity). \(^{30-32}\) GIP-receptor knockout mice are resistant to diet-induced obesity and have reduced adipocyte mass and obese/obese (ob/ob); GIP-receptor/ob/ob double-knockouts exhibit less weight gain and adiposity and improved glucose tolerance and insulin sensitivity relative to ob/ob knockout mice. \(^{62}\) The net effect of GIP reduction after pharmacologic inhibition of SGLT1 remains to be determined.

Ghrelin and leptin concentrations were not significantly changed after breakfast with combination treatment relative to either monotherapy. It may be that SGLT1 inhibition does not increase systemic SCFAs sufficiently to trigger effects on these peptides. Alternatively, the effects of SCFAs might not yet be present at breakfast, or longer-term treatment may be required. Potential leptin and ghrelin effects warrant further exploration in additional clinical trials of LX4211.

Perhaps the most important result of the present study was the net effect of the LX4211 + sitagliptin combination treatment on glucose and insulin. The combination was associated with significant reductions in blood glucose levels throughout the day relative to that resulting from either monotherapy. Insulin level was significantly reduced relative to that resulting from sitagliptin monotherapy and significantly increased relative to that resulting from LX4211 monotherapy. The net effect of the combination treatment on glycemic control was apparently enhanced, with a lower insulin concentration relative to that resulting from sitagliptin monotherapy. This reduction in insulin requirements with combination dosing was likely
the result of the insulin-independent activity of LX4211 on a reduction in overall glucose load. Notably, LX4211 and the LX4211 + sitagliptin combination were well tolerated, with no notable GI AEs reported in this study. Although theoretical concerns over the potential for SGLT1 inhibition to cause diarrhea have led most drug-development efforts in the industry to have been focused on selective SGLT2 inhibitors, the present study provides additional support for the favorable GI safety profile observed with LX4211 in multiple studies in both healthy subjects and patients with T2DM.

CONCLUSIONS

LX4211, a first-in-class dual SGLT1/SGLT2 inhibitor, provides a potential option for T2DM. The delayed intestinal glucose absorption and increased postprandial GLP-1 and PYY release may provide differentiation from, and the potential for producing enhanced benefits over, selective SGLT2 inhibitors. The findings from this study further this differentiation by suggesting that combinations of LX4211 with DPP-4 inhibitors provide additive or synergistic incretin benefits that would not be expected with a selective SGLT2 inhibitor. This study also provides an important benchmark for the assessment of the novel mechanism of action of LX4211 compared with, and in combination with, an established agent and mechanism used extensively in the treatment of T2DM. These initial results suggest that a LX4211 + DPP-4 inhibitor combination has the potential not only to enhance glycemic control but also to provide additional improvements in metabolic and cardiovascular parameters observed to date with LX4211 but not with DPP-4 inhibitors. The potential long-term clinical benefits of such combination treatment need to be confirmed in large clinical trials.

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The authors thank Kristi A. Boehm for her writing and editing assistance, Johanna Bronner for ensuring the integrity of the database, Gui-lan Ye for monitoring safety, and Kevin Rando for study monitoring.

Dr. Zambrowicz, Dr. Ogbaa, Mr. Frazier, Mr. Banks, Ms. Turnage, Dr. Freiman, Dr. Ruff, and Dr. Sands contributed to the clinical study design; Dr. Ding, Ms. Smith, and Dr. Powell contributed to the preclinical study design. All of the authors contributed to the writing and final approval of the manuscript.

CONFLICTS OF INTEREST

Lexicon Pharmaceuticals, Inc. provided the funding for these studies.

At the time this work was conducted, all of the authors, with the exception of D. Ruff, were employees of, and owned stock options in, Lexicon Pharmaceuticals. The authors have indicated that they have no other conflicts of interest with regard to the content of this article.

SUPPLEMENTAL MATERIAL

Supplemental files accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.01.010.

REFERENCES

Clinical Therapeutics


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Supplemental Appendix

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

Subjects must have met all of the following criteria to be considered eligible to participate in the study:

1. Adults ≥18 to ≤65 years of age at the time of Screening:
   a. Females must have been of non-childbearing potential, surgically sterile (documented hysterectomy, tubal ligation, or bilateral salpingo-oophorectomy) or postmenopausal (defined as 12 months of spontaneous amenorrhea). If necessary, follicular-stimulating hormone results ≥35 IU/L at Screening were confirmatory in the absence of a clear postmenopausal history.
   b. Males must have agreed to use an adequate method of contraception during the study and for 30 days after the Discharge visit. Adequate methods of contraception for subjects or partner included the following: condom with spermicidal gel, diaphragm with spermicidal gel, coil (intrauterine device), surgical sterilization, vasectomy, oral contraceptive pill, depo-progesterone injections, progesterone implant, NuvaRing®, Ortho Evra®, and abstinence. If a subject was not sexually active but became active, he or her partner was to have used medically accepted forms of contraception.
2. History of T2DM for at least 3 months prior to Screening with the following laboratory values:
   a. HbA1c value of ≥6.5% to ≤10.5%
   b. C-peptide ≥1.0 ng/mL
3. Body mass index (BMI) ≤45 kg/m² at Screening and planned Day -2
4. Willing and able to perform self-monitoring of blood glucose
5. Willing and able to provide written informed consent

Exclusion Criteria:

Subject who met any of the following criteria were to be excluded from participating in the study:

1. History of any of the following: type 1 diabetes mellitus, diabetic ketoacidosis, hyperosmolar non-ketotic syndrome, or diabetes resulting from pancreatic disorder or secondary diabetes (eg, from acromegaly, Cushing’s disease, etc)
2. Current use of any blood glucose lowering agent other than metformin
3. Exposure to metformin within 14 days prior to planned Day -1
4. History of renal disease, or clinically significant abnormal kidney function tests at Screening or planned Day -2, including glomerular filtration rate <60 mL/min as calculated using the Cockcroft-Gault equation
5. Presence of active hepatic disease or clinically significant abnormal liver function tests at Screening or planned Day -2 (aspartate transaminase [AST] or alanine transaminase [ALT] >2.5 times the upper limit of normal)
6. History of myocardial infarction, severe/unstable angina, or coronary revascularization procedure within 6 months prior to planned Day -2
7. History of clinically significant cardiac arrhythmias (eg, supraventricular tachycardia, ventricular tachycardia, or atrial fibrillation) within 1 year prior to planned Day -2
8. Congestive heart failure and/or New York Heart Association Class III or IV symptoms of heart failure
9. Subjects with uncontrolled Stage III hypertension (defined as systolic BP >180 mm Hg or diastolic BP >110 mm Hg)
10. History of 2 or more emergency room visits, doctors’ visits, or hospitalizations due to hypoglycemia within the 6 months prior to planned Day -2, or has a current diagnosis of hypoglycemia unawareness
11. History of alcohol or drug abuse (using Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria) within 12 months prior to Screening
12. History of bowel resection >20 cm, any malabsorptive disorder, severe gastroparesis, any GI procedure for the purpose of weight loss (including LAPBAND™), which would slow gastric emptying
13. History of human immunodeficiency virus or hepatitis C virus antibody
14. History of major surgery within 3 months prior to planned Day -2 or was planning any surgery during the course of the study
15. History of any active infection within 2 weeks prior to planned Day -2
16. History of pancreatitis, including hemorrhagic or necrotizing pancreatitis
17. History of any malignancy within the last 5 years (except benign skin cancer, including basal and squamous cell carcinoma of skin and carcinoma in situ of uterine cervix), which could have affected the diagnosis of T2DM or assessment of LX4211 or sitagliptin
18. History of any serious adverse reaction or hypersensitivity to an SGLT inhibitor or any inactive component of LX4211, (ie, microcrystalline cellulose, croscarmellose sodium [disintegrant], talc, silicone dioxide, and magnesium stearate [non-bovine]), unless reaction was deemed irrelevant to the study by the Investigator and Lexicon
19. History of any serious adverse reaction or hypersensitivity to sitagliptin or any inactive component of sitagliptin, (ie, microcrystalline cellulose, anhydrous dibasic calcium phosphate, croscarmellose sodium, magnesium stearate, sodium stearyl fumarate, polyvinyl alcohol, polyethylene glycol, talc, titanium dioxide, red iron oxide, and yellow iron oxide), unless reaction was deemed irrelevant to the study by the Investigator and Lexicon
20. The presence of clinically significant physical, laboratory, or ECG findings or any concurrent condition at Screening that, in the opinion of the Investigator and/or Lexicon might have interfered with any aspect of safety, study conduct, or interpretation of results
21. Triglycerides >1000 mg/dL at Screening or planned Day -2
22. Positive urine pregnancy test at Screening or serum pregnancy at planned Day -2 (females only)
23. Positive urine screen for drugs of abuse at Screening or planned Day -2
24. Positive breath test for alcohol at Screening or planned Day -2
25. Donation or loss of >400 mL of blood or blood product within 8 weeks prior to planned Day -2
26. Receipt of any protein or antibody-based therapeutic agents (eg, growth hormones or monoclonal antibodies) within 3 months prior to planned Day-2. Note: Influenza vaccine was allowed if administered >14 days prior to Day -2
27. Unwillingness to discontinue use of cigarettes or any tobacco product for the duration of study participation, beginning on Day -2
28. Use of corticosteroids (eg, prednisone) within 2 weeks prior to planned Day 1 (day of 1st dose of study medication). Note: Ocular, topical, or inhaled steroid preparations were permitted
29. Use of digoxin or warfarin within 2 weeks prior to Screening
30. Need for dietary restrictions, unless the restrictions were approved by the Investigator and Lexicon
31. Use of any other investigational agent or treatment within 30 days prior to planned Day -2
32. Inability or difficulty swallowing whole tablets
33. Unwilling or unable to communicate or cooperate with the Investigator for any reason
### Supplemental Table I. Baseline demographic and clinical characteristics of the population in this study of LX4211, sitagliptin, or both in patients with type 2 diabetes mellitus (N = 18).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
</tr>
<tr>
<td>Age, median (range), y</td>
<td>49.0 (28–65)</td>
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<tr>
<td>Sex, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (55.6)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>Race, no. (%)</td>
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<tr>
<td>Black or African American</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>White</td>
<td>16 (88.9)</td>
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<tr>
<td>Hispanic ethnicity, no. (%)</td>
<td>14 (77.8)</td>
</tr>
<tr>
<td><strong>Clinical, mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>169.05 (9.3)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>90.26 (12.3)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.69 (4.5)</td>
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<tr>
<td>C-Peptide, ng/mL</td>
<td>2.965 (1.088)</td>
</tr>
<tr>
<td>FPG, mg/dL</td>
<td>182.9 (55.42)</td>
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<tr>
<td>HbA₁c, %</td>
<td>8.028 (1.120)</td>
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<tr>
<td>Insulin, μU/mL</td>
<td>51.46 (26.96)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>162.89 (59.28)</td>
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<td>24-hr UGE, g/d</td>
<td>24.07 (35.27)</td>
</tr>
<tr>
<td>GIP, pmol/L</td>
<td>46.32 (16.46)</td>
</tr>
</tbody>
</table>

BMI = body mass index; FPG = fasting plasma glucose; GIP = gastric inhibitory peptide; Hb = hemoglobin; UGE = urinary glucose excretion.
Supplemental Figure 1. Study schema.

Number of Subjects per Sequence
<table>
<thead>
<tr>
<th></th>
<th>Period 1 Treatment</th>
<th>Period 2 Treatment</th>
<th>Period 3 Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td>400 mg LX4211</td>
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<td></td>
</tr>
<tr>
<td>Treatment B</td>
<td></td>
<td>100 mg sitagliptin</td>
<td></td>
</tr>
<tr>
<td>Treatment C</td>
<td>400 mg LX4211 + 100 mg sitagliptin, concurrently</td>
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</table>

Supplemental Table II. Timing of all pharmacodynamic measures relative to administration times of LX4211, sitagliptin, or both on study days −1 (baseline), 1, 8, and 15 in patients with type 2 diabetes mellitus (N = 18).*

<table>
<thead>
<tr>
<th>Time Point/ Parameter</th>
<th>Predose</th>
<th>0†</th>
<th>0.5</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>5.5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>9.5</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>&gt;13</th>
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</thead>
<tbody>
<tr>
<td>Meal¹</td>
<td>B</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
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<td>X</td>
<td></td>
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<tr>
<td>GLP-1, glucose, insulin,</td>
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<tr>
<td>GIP, and leptin (1-8.5 mL P800 tube)</td>
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<tr>
<td>Ghrelin (1-2 mL EDTA tube)</td>
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<td>GLP-1, glucose, insulin,</td>
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<tr>
<td>and PYY (2 mL P800 tube)</td>
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</tr>
</tbody>
</table>

B = breakfast; D = dinner; GIP = gastric inhibitory peptide; GLP = glucagon-like peptide; L = lunch; PYY = peptide tyrosine tyrosine; S = snack.

*All dosing and sampling times are based on meal times (sample times are ±3 minutes).

¹With the exception of day −1, study drugs (LX4211, sitagliptin, or both) were administered at 0 hour on each dosing day.

²Patients were given 30 minutes to complete each meal.
Supplemental Figure 2. Increases from baseline in active glucagon-like peptide (aGLP)-1 concentrations at (A) 1 hour and (B) 2 hours after meal challenge in mice administered single-dose LX4211, sitagliptin, LX4211 + sitagliptin, or inactive vehicle (n = 5 per group). *P = 0.0208; †P = 0.116; ‡P = 0.0006 (2-way ANOVA).

Supplemental Figure 3. Active glucagon-like peptide (aGLP)-1 concentrations after meal challenge in mice administered multiple-dose (14-day) LX4211, sitagliptin, LX4211 + sitagliptin, or inactive vehicle (n = 5 per group). *P < 0.0001; †P = 0.011; ‡P = 0.0003 (2-way ANOVA).
Supplemental Figure 4. Study flow.

Supplemental Table III. Changes from baseline in total glucagon-like peptide 1 with LX4211, sitagliptin, or both in patients with type 2 diabetes mellitus (adjusted generalized linear mixed model) (N = 18). Values are least squares mean (95% CI) (pmol · h/L).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LX4211 Value (95% CI)</th>
<th>P</th>
<th>Sitagliptin Value (95% CI)</th>
<th>P</th>
<th>LX4211 + Sitagliptin Value (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change vs baseline</td>
<td>+24.63 (12.20 to 37.06)</td>
<td>&lt;0.001</td>
<td>-23.78 (-36.20 to -11.35)</td>
<td>&lt;0.001</td>
<td>-0.56 (-12.99 to 11.87)</td>
<td>0.93</td>
</tr>
<tr>
<td>Difference vs combination</td>
<td>-25.19 (-40.70 to -9.69)</td>
<td>0.002</td>
<td>+23.21 (7.71 to 38.71)</td>
<td>0.005</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Supplemental Table IV. Changes from baseline in total peptide tyrosine tyrosine with LX4211, sitagliptin, or both in patients with type 2 diabetes mellitus (adjusted generalized linear mixed model) (N = 18). Values are least squares mean (95% CI) (pmol · h/L).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LX4211</th>
<th>Sitagliptin</th>
<th>LX4211 + Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>P</td>
<td>Value</td>
<td>P</td>
</tr>
<tr>
<td>Change vs baseline</td>
<td>+61.67 (38.24 to 85.10)</td>
<td>&lt;0.001</td>
<td>-134.72 (−158.14 to −111.29)</td>
</tr>
<tr>
<td>Difference vs combination</td>
<td>-152.78 (−187.07 to −118.49)</td>
<td>&lt;0.001</td>
<td>+43.61 (9.32 to 77.90)</td>
</tr>
</tbody>
</table>

Supplemental Table V. Changes from baseline in total gastric inhibitory peptide with LX4211, sitagliptin, or both in patients with type 2 diabetes mellitus (adjusted generalized linear mixed model) (N = 18). Values are least squares mean (95% CI) (pmol · h/L).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LX4211</th>
<th>Sitagliptin</th>
<th>LX4211 + Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>P</td>
<td>Value</td>
<td>P</td>
</tr>
<tr>
<td>Change vs baseline</td>
<td>-55.16 (−66.68 to −43.65)</td>
<td>&lt;0.001</td>
<td>-68.78 (−80.29 to −57.26)</td>
</tr>
<tr>
<td>Difference vs combination</td>
<td>-45.85 (−62.71 to −29.00)</td>
<td>&lt;0.001</td>
<td>-32.24 (−49.10 to −15.38)</td>
</tr>
</tbody>
</table>