Report on the Deliberation Results

May 31, 2013
Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name] Lyxumia Subcutaneous Injection 300 μg
[Non-proprietary name] Lixisenatide (JAN*)
[Name of applicant] Sanofi K.K.
[Date of application] June 11, 2012
[Results of deliberation]
In the meeting held on May 24, 2013, the First Committee on New Drugs concluded that the product may be approved and that this result should be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The re-examination period for the product is 8 years, and the drug substance and the drug product are both classified as powerful drugs and the product is not classified as a biological product or a specified biological product.

The proposed Japanese brand name should be changed for ensuring medical safety.

**Japanese Accepted Name (modified INN)*

Review Report

May 7, 2013
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Lyxumia Subcutaneous Injection 300 μg
[Non-proprietary name]	Lixisenatide
[Name of applicant]	Sanofi K.K.
[Date of application]	June 11, 2012
[Dosage form/Strength]	Solution for injection containing 300 μg of Lixisenatide per cartridge (3 mL)
[Application classification]	Prescription drug (1) Drug with a new active ingredient

[Chemical structure]

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Ser-Lys-Lys-Lys-Lys-Lys-Lys-NH_2

Molecular formula: $C_{215}H_{347}N_{61}O_{65}S$
Molecular weight: 4858.49
Chemical name:

Lixisenatide is a synthetic analog of exendin-4 in which Pro residue at position 38 is removed and 6 Lys residues are attached at the C-terminal. Lixisenatide is a peptide consisting of 44 amino acid residues.

[Items warranting special mention]	None
[Reviewing office]	Office of New Drug I

Review Results

May 7, 2013

[Brand name] Lyxumia Subcutaneous Injection 300 μg
[Non-proprietary name] Lixisenatide
[Name of applicant] Sanofi K.K.
[Date of application] June 11, 2012
[Results of review]
Based on the submitted data, the efficacy of the product in patients with type 2 diabetes mellitus has been demonstrated and its safety is acceptable in view of its observed benefits. The occurrence of hypoglycaemia, gastrointestinal disorders, pancreatitis, and injection site reactions, the influence of antibody formation on safety and efficacy, effects on renal function, hypersensitivity reactions, cardiovascular risk, tumor development, safety in patients with renal or hepatic impairment and elderly patients (limited numbers of these patients were included in clinical studies), the safety and efficacy of the product in combination with a sulfonylurea and high-dose metformin, and the long-term safety and efficacy of the product in combination with basal insulin, etc. need to be further investigated via post-marketing surveillance.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the following indication and dosage and administration.

[Indication] Type 2 diabetes mellitus:
Lyxumia should be used only when either of the following does not provide adequate glycaemic control:
(a) diet and exercise plus sulfonylureas (with or without biguanides) or
(b) diet and exercise plus soluble prolonged-acting or intermediate-acting insulin (with or without sulfonylureas).

[Dosage and administration]
The usual adult dosage is 20 μg of Lixisenatide subcutaneously injected once daily prior to breakfast. Lixisenatide should be initiated at 10 μg once daily, which is increased to 15 μg once daily after at least 1 week and then to 20 μg once daily after at least 1 week. The dosage may be adjusted according to the patient's condition. The daily dose should not exceed 20 μg.

Review Report (1)

March 22, 2013

I. Product Submitted for Registration

[Brand name]	Lyxumia Subcutaneous Injection 300 μg
[Non-proprietary name]	Lixisenatide
[Name of applicant]	Sanofi-Aventis K.K. (a predecessor of Sanofi K.K.)
[Date of application]	June 11, 2012
[Dosage form/Strength]	Solution for injection containing 300 μg of Lixisenatide per cartridge (3 mL).
[Proposed indication]	Type 2 diabetes mellitus: Lyxumia should be used only when any of the following therapies does not provide adequate glycaemic control: (a) [redacted], (b) diet and exercise plus sulfonylureas (with or without biguanides), or (c) diet and exercise plus soluble prolonged-acting or intermediate-acting insulin (with or without sulfonylureas).
[Proposed dosage and administration]	The usual adult dosage is 20 μg of Lixisenatide subcutaneously injected once daily prior to breakfast or evening meal. Lixisenatide should be initiated at 10 μg once daily, which is increased to 15 μg once daily after at least 1 week and then to 20 μg once daily after at least 1 week. The dosage may be adjusted according to the patient's condition. The daily dose should not exceed 20 μg.

II. Summary of the Submitted Data and Outline of Review by Pharmaceuticals and Medical Devices Agency

A summary of the data submitted in the application and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries etc.

The proposed product is a solution for injection containing Lixisenatide (lixisenatide) as the active ingredient, which is a glucagon-like peptide-1 (GLP-1) receptor agonist discovered by Zealand Pharma A/S and subsequently developed by Sanofi-Aventis K.K. (a predecessor of Sanofi K.K.), which is the applicant.

GLP-1 is secreted from the gastrointestinal tract in response to ingestion of a meal, and plays an important role in postprandial glucose regulation by facilitating insulin release and suppressing glucagon secretion from the pancreas. However, GLP-1 is rapidly degraded and inactivated by dipeptidyl peptidase-4 (DPP-4) which is distributed throughout the body and has a short half-life of approximately 90 to 120 seconds. The product is a GLP-1 receptor agonist with structural similarities to exendin-4,[1] which is resistant to enzymatic cleavage by DPP-4, and stimulates insulin release by binding to the GLP-1 receptor and thus exerts a glucose-lowering effect.

[1] A peptide consisting of 39 amino acids, isolated from saliva of a kind of lizard (*Heloderma suspectum*)

The applicant has submitted a new drug application for Lyxumia as efficacy and safety have been confirmed for Lyxumia when used in combination with sulfonylureas (with or without biguanides) and with basal insulin (with or without sulfonylureas) for the treatment of type 2 diabetes mellitus. The applicant removed [redacted] as the proposed indication after the submission of the application.

As of March 2013, the product has been approved in Europe and is under review in the US.

As GLP-1 receptor agonists, liraglutide (genetical recombination) and exenatide have already been approved in Japan.

2. Data relating to quality

2.A Summary of the submitted data

2.A.(1) Drug substance

2.A.(1).1) Characterization

The drug substance is an amorphous, white powder. The physicochemical properties of the drug substance, including general properties (appearance, solubility, melting point, hygroscopicity [[redacted]], isoelectric point, biological activity, pH, and crystallographic property [X-ray powder diffraction]) have been determined. Its chemical structure has been elucidated by mass spectrometry (electrospray ionization [ESI]), peptide mapping (reversed-phase liquid chromatography [HPLC] and liquid chromatography/mass spectrometry [LC-MS]), amino acid compositional analysis, amino acid sequencing (Edman sequencing technique), infrared spectroscopy (IR), ultraviolet spectroscopy (UV), circular dichroism (CD) spectroscopy, nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, ^{15}N, NOESY), and fluorescence spectroscopy.

2.A.(1).2) Manufacturing process

Starting materials used for synthesis of drug substance are [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted] or [redacted], [redacted], [redacted], [redacted], [redacted], [redacted] and [redacted]. [redacted], [redacted], [redacted] and [redacted] have been defined as critical steps. As critical intermediates, [redacted], [redacted] and [redacted] are controlled.

2.A.(1).3) Control of drug substance

The specifications for drug substance include content, appearance, identification (amino acid sequencing, molecular mass), purity (acetate ion, related substances 1-3, isomers of constituent amino acids, high molecular weight proteins [all by HPLC], residual solvents ([redacted] [gas chromatography]), [redacted] (HPLC), water content, bacterial endotoxins, microbial limits, and assay (HPLC).

2.A.(1).4) Stability of drug substance

Stability studies on the drug substance are outlined in Table **1**. Photostability studies showed that the drug substance is photosensitive.

Table 1. Stability studies on drug substance

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term	3 pilot-scale batches	-20 ± 5°C	Ambient	[redacted] glass bottles [redacted]	36 months
Accelerated	3 pilot-scale batches	5 ± 3°C	Ambient		6 months
	3 pilot-scale batches	25 ± 2°C	60 ± 5%RH		1 month

Based on the above, a re-test period of 36 months was proposed for the drug substance when stored in a tight container, protected from light, at -20 ± 5°C.

2.A.(2) Drug product

2.A.(2).1) Description and composition of the drug product and formulation development

The drug product is a clear, colorless solution for subcutaneous injection containing 0.1 mg/mL of lixisenatide. The drug product contains L-methionine, sodium acetate hydrate, glycerol, hydrochloric acid and sodium hydroxide, *m*-cresol, and water for injection as excipients. The primary package for the drug product is a 3-mL cartridge closed with an aluminum flanged cap with a [redacted] rubber stopper which can be pierced by a needle and a [redacted] rubber plunger which contacts the injector. The drug product is a disposable kit product with a 3-mL cartridge assembled into a specific pen-injector, a lixisenatide pen. The lixisenatide pen has been certified in Japan (Certification No. 224AABZX00177000).

2.A.(2).2) Manufacturing process

The manufacturing process for the drug product consists of [redacted], sterile filtration, filling/sealing, assembly, labeling, packaging, and storage/testing. Critical steps are [redacted] step. There are two alternative manufacturing processes (Process A and Process B) for [redacted], which differ in [redacted] of [redacted].

Quality by Design (QbD) approaches were utilized, and [redacted] and [redacted] have been mainly identified as [redacted] were investigated as well.

2.A.(2).3) Control of drug product

The specifications for drug product include strength, appearance, identification (HPLC), pH, purity (related substances, high molecular weight proteins [both by HPLC]), bacterial endotoxins, extractable volume, foreign insoluble matter, insoluble particulate matter, sterility (membrane filtration method), and assay (lixisenatide, *m*-cresol, [redacted] [all by HPLC]).

2.A.(2).4) Stability of drug product

Stability studies on the drug product are outlined in Table 2. As a result of long-term, accelerated, stress, photostability and in-use stability studies,[2] and cycle tests[3], total related substances and high molecular weight proteins increased over time.

Table 2. Stability studies on drug product

Study	Primary batches	Manufacturing method	Temperature	Humidity	Storage package	Storage period
Long-term	3 production-scale batches	Process A	5 ± 3°C	Ambient	A colorless glass cartridge with an aluminum flanged cap with a [redacted] rubber stopper	36 months
Accelerated	3 production-scale batches	Process A	25 ± 2°C	60 ± 5%RH		6 months
Long-term	1 production-scale batch	Process B	5 ± 3°C	Ambient	A colorless glass cartridge with an aluminum flanged cap with a [redacted] rubber stopper assembled into a pen-injector	[redacted] months (Ongoing)
Accelerated	1 production-scale batch	Process B	25 ± 2°C	60 ± 5%RH		6 months

Based on the above, a shelf life of 36 months was proposed for the drug product when stored in a glass cartridge at 2°C to 8°C without freezing, protected from light. An in-use period of 30 days was proposed when stored at ≤25°C and not in a refrigerator, protected from light. The long-term stability study on the drug product manufactured by Process B will be continued up to [redacted] months.

2.B Outline of the review by PMDA

Based on the submitted data and the following reviews, PMDA concluded that the quality of drug substance and drug product is adequately controlled.

Biological activity

PMDA asked the applicant to justify not including biological activity in the specifications for drug substance and drug product.

The applicant responded as follows:

For drug substance, correlation between [redacted] by [redacted] and biological activity measured by [redacted] was investigated, using [redacted] samples and samples [redacted]. As a result, no significant decrease in biological activity was observed for the [redacted] samples with no correlation shown, while good correlation was shown in the evaluation using the samples [redacted]. For drug product, on the other hand, both investigations using [redacted] samples and [redacted] showed good correlation.

Taking into consideration that [redacted]

[2] Cartridges [redacted] assembled into pen-injectors were stored at [redacted]°C/[redacted]%RH for 30 days. During the 30-day in-use period, a needle was attached [redacted] times a day and [redacted] µg was expelled each time ([redacted]). Samples were [redacted] over a total of [redacted] days during the 30-day in-use period with [redacted].

[3] The samples of drug product after storage at [redacted]°C for [redacted], [redacted], and [redacted] months were stored at [redacted]°C/[redacted]%RH for [redacted] days and subsequently at [redacted]°C for [redacted] days. [redacted] was repeated [redacted].

[redacted], PMDA asked the applicant to explain how to ensure the biological activity of the product.

The applicant responded as follows:
The upper limits of acceptance criteria for [redacted] for the drug substance and drug product are [redacted]% and [redacted]%, respectively, and they only [redacted]; therefore it is considered that biological activity can be controlled by the [redacted] of [redacted]. Also, since lixisenatide [redacted], [redacted]. Therefore [redacted] (identification test, purity test) have been included. Taking the aforementioned into account, the applicant considers that biological activity can be ensured through control of [redacted] by assay. In addition, [redacted] is added to the specifications of [redacted] and [redacted].

PMDA considers as follows:
Sufficient correlation between [redacted] and biological activity was not shown, especially for the drug substance, due to biological activity of [redacted] or [redacted] of bioassay. However, taking it into consideration that [redacted] which may affect the biological activity are [redacted], biological activity can be ensured through the proposed control of the drug substance and drug product.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

Primary pharmacodynamic studies examined the effect of lixisenatide on GLP-1 receptor and its mode of action *in vitro* and the effects such as blood glucose-lowering in normal animals and animal models of type 2 diabetes mellitus *in vivo*. As secondary pharmacodynamic studies, pharmacological effects on the cardiovascular system and binding profile to various receptors were studied. Safety pharmacology studies examined effects on the central nervous system, cardiovascular system, and respiratory system.[4] No pharmacodynamic drug interaction studies were performed.

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1) *In vitro* studies

3.(i).A.(1).1).(a) Effects on human GLP-1 receptor (4.2.1.1-1, 4.2.1.1-2)

Using Chinese hamster ovary (CHO) cells expressing human GLP-1 receptor, the binding affinities of lixisenatide and native human GLP-1 to the GLP-1 receptor were determined. As a result, the IC_{50} values of lixisenatide and native human GLP-1 (mean ± standard error [SE]) were 1.43 ± 0.24 and 5.48 ± 1.28 nmol/L, respectively, and the Ki values were 1.33 ± 0.22 and 5.09 ± 1.19 nmol/L, respectively.

[4] Effects on hERG potassium current were not listed in the safety pharmacology core battery in "Safety Pharmacology Studies for Human Pharmaceuticals" (PMSB/ELD Notification No. 902 dated June 21, 2001, "Guideline for Safety Pharmacology") at the time of conducting a hERG assay. A study examining effects on arterial blood pressure and blood glucose levels in male rats was not conducted in compliance with GLP because the Guideline for Safety Pharmacology was not applicable at the time of study conduct.

3.(i).A.(1).1).(b) Effects on glucose-induced insulin secretion in perfused rat pancreatic preparation (4.2.1.1-3)

Pancreases isolated from male rats were perfused with perfusate[5] containing lixisenatide (10 nmol/L) or native human GLP-1 (10 nmol/L), and the effects on glucose-induced insulin secretion were examined by changing the glucose level in the perfusate.[6] As a result, neither lixisenatide nor native human GLP-1 induced insulin secretion at baseline. In contrast, at hyperglycemic glucose concentrations, lixisenatide and native human GLP-1 significantly increased insulin secretion as compared to control (perfusate only). The insulin $AUC_{10\text{-}60\ min}$ values (from 10 to 60 minutes after control, lixisenatide, and native human GLP-1 treatment) (median [interquartile range]) were 775.5 (629), 3784 (2146), and 2391.5 (1521) μg·min/L, respectively.

3.(i).A.(1).2) *In vivo* studies

3.(i).A.(1).2).(a) Effects in normal animals and animal models of diabetes mellitus (single-dose administration)

3.(i).A.(1).2).(a).i) Effects on blood glucose levels in db/db mice (4.2.1.1-4, 4.2.1.1-5)

A single intraperitoneal dose of lixisenatide (0.826, 8.26, 82.6, 826 μg/kg) or vehicle[7] was given to male db/db mice (8 weeks of age, 7-8 mice/group) under fasted conditions, and 15 minutes later, the mice were orally challenged with glucose solution (1 g/kg). Blood glucose was measured repeatedly for 240 minutes after the glucose challenge. As a result, glucose $AUC_{0\text{-}240\ min}$ for the difference in blood glucose measured at each timepoint and baseline (mean ± SE) was 294.05 ± 50.30 g·min/L in the control group, and 123.08 ± 33.37, 88.40 ± 30.49, 2.32 ± 11.74, and -21.32 ± 27.05 g·min/L in the lixisenatide 0.826, 8.26, 82.6, and 826 μg/kg groups, respectively. It was significantly lower in all lixisenatide groups as compared to the control group.

A single intraperitoneal dose of lixisenatide (486 μg/kg) or vehicle[8] was given to male db/db mice (11-18 weeks of age, 7-9 mice/group) under fasted conditions, and the mice were challenged with glucose (oral load of glucose solution [1 g/kg]) at different timepoints between 15 and 1440 minutes after administration.[9] Blood glucose was measured repeatedly for 240 minutes after each glucose load. As a result, the glucose $AUC_{0\text{-}240\ min}$ values in the mice receiving glucose load 15 to 720 minutes after lixisenatide administration were significantly lower as compared to the control group.

3.(i).A.(1).2).(a).ii) Effects on gastric emptying in normal mice (4.2.1.1-6)

A single intraperitoneal dose of lixisenatide (0.0024-4859 μg/kg), exendin-4 (0.4187-41866 μg/kg), or vehicle[8] was given to male mice (5-15 mice/group) under fasted conditions. Ten minutes after administration, 0.5 mL of pigment solution[10] was orally given, and 30 minutes later, the stomach was isolated to measure gastric residual pigment. As a result, lixisenatide and exendin-4 decreased the rate of gastric emptying. The ED_{50} and its 95% confidence interval (CI) were 31.05 [16.42, 58.79] and 54.00 [23.95, 121.83] μg/kg, respectively.

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[5] Krebs-Ringer-bicarbonate solution

[6] Pancreas was treated at the perfusate glucose concentration of 5.6 mmol/L (100.89 mg/dL) for 10 minutes and stimulated at 16.5 mmol/L (297.26 mg/dL) for 30 minutes and then treated at 5.6 mmol/L (100.89 mg/dL) for 20 minutes.

[7] Saline

[8] Phosphate buffered saline containing 0.1% bovine serum albumin (pH 7.4)

[9] The animals were orally challenged with glucose solution at 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720, 1080, 1260, and 1440 minutes after lixisenatide administration.

[10] Aqueous solution containing 1.5% methylcellulose and 0.05% methyl red

3.(i).A.(1).2).(a).iii) Effects on blood glucose levels in ZDF rats (4.2.1.1-7)

A single subcutaneous dose of lixisenatide (1, 5, 10 μg/kg) or vehicle[11] was given to male obese Zucker Diabetic Fatty (ZDF) rats (11 weeks of age, 8 rats/group) under fasted conditions and a single subcutaneous dose of vehicle was given to male lean ZDF rats (11 weeks of age, 8 rats). Thirty minutes later, the rats were orally challenged with glucose solution (2 g/kg). As a result, the difference in blood glucose at 0 and 30 minutes after the glucose challenge (blood glucose excursion) (mean ± SE) in the obese control group was 77.40 ± 5.73 mg/dL, which was significantly higher than that in the lean control group (23.74 ± 4.07 mg/dL). Blood glucose excursions in the obese lixisenatide groups (1, 5, 10 μg/kg) were 51.21 ± 11.67, 32.651 ± 7.85, and 22.79 ± 7.30 mg/dL, respectively, and it was significantly lower in the 5 and 10 μg/kg groups as compared to the obese control group.

3.(i).A.(1).2).(a).iv) Effects on blood glucose, plasma insulin, and plasma C-peptide levels in normal dogs (4.2.1.1-8)

A single subcutaneous dose of lixisenatide (0.146, 0.486, 1.46, 4.86 μg/kg), exendin-4 (0.126, 0.487, 1.256, 4.187 μg/kg), or vehicle[12] was given to male dogs (6-9 dogs/group) under fasted conditions, and 30 minutes later, the dogs were orally challenged with glucose solution (2 g/kg). As a result, blood glucose excursions were significantly reduced by both lixisenatide and exendin-4 compared to control, whereas no significant decrease was observed in glucose $AUC_{0\text{-}180\ min}$ (for 180 minutes after the glucose challenge) in all of the lixisenatide and exendin-4 groups as compared to control. The insulin $AUC_{0\text{-}180\ min}$ values (for 180 minutes after the glucose challenge) (mean ± SE) were 195.71 ± 29.17, 192.08 ± 41.63, 174.75 ± 36.29, and 148.09 ± 20.53 ng·min/mL in the 0.146, 0.486, 1.46, and 4.86 μg/kg lixisenatide groups, respectively, and 195.21 ± 30.34, 198.21 ± 20.92, 141.79 ± 23.74, and 145.11 ± 25.41 ng·min/mL in the 0.126, 0.487, 1.256, and 4.187 μg/kg exendin-4 groups, respectively. It was significantly lower in the 0.146, 1.46 and 4.86 μg/kg lixisenatide groups and all exendin-4 groups, as compared to that in the control group (403.16 ± 73.77 ng·min/mL). For plasma C-peptide $AUC_{0\text{-}180\ min}$ (for 180 minutes after the glucose challenge), there was no significant difference between any of the lixisenatide and exendin-4 groups and the control group.

3.(i).A.(1).2).(a).v) Comparison with liraglutide in normal dogs (4.2.1.1-9)

A single subcutaneous dose of lixisenatide (1 μg/kg), liraglutide (50 and 100 μg/kg), or vehicle[7] was given to male dogs (6 dogs/group) under fasted conditions, and 30 minutes later, the dogs were orally challenged with glucose solution (2 g/kg). As a result, the glucose $AUC_{0\text{-}270\ min}$ values (for 270 minutes after administration) (mean ± SE) were 240.69 ± 5.81 g·min/L in the lixisenatide group and 267.72 ± 6.39 and 258.53 ± 12.72 g·min/L in the liraglutide 50 and 100 μg/kg groups, respectively. As compared to the control group (277.09 ± 9.47 g·min/L), it was significantly lower in the lixisenatide group, but not in either of the liraglutide 50 or 100 μg/kg group. The glucagon $AUC_{0\text{-}270\ min}$ values (for 270 minutes after administration) were 317.3 ± 32.11 pg·h/mL in the lixisenatide group and 335.6 ± 24.34 and 343.7 ± 30.08 pg·h/mL in the liraglutide 50 and 100 μg/kg groups, respectively. It was significantly lower in all of the lixisenatide and liraglutide groups than in the control group (455.2 ± 35.57 pg·h/mL). There was no significant difference in insulin $AUC_{0\text{-}270\ min}$ or C-peptide $AUC_{0\text{-}270\ min}$ (for 270 minutes after administration) between the lixisenatide or liraglutide and control groups.

[11] Phosphate buffered saline (pH 4.5)
[12] HOE901 (insulin glargine) placebo solution (pH 4.5)

3.(i).A.(1).2).(b) Effects in animal models of diabetes mellitus (multiple-dose administration)

3.(i).A.(1).2).(b).i) Effects on blood glucose, fasting blood glucose, HbA1c, water intake, and pancreatic β-cells following 6 weeks of twice daily multiple-dose to db/db mice (4.2.1.1-10, 4.2.1.1-11)

Lixisenatide (4.86, 48.6, 486 μg/kg/dose) or vehicle[8] was intraperitoneally administered to male db/db mice (6-8 weeks of age, 15 mice/group) twice daily for 6 weeks. As a result, after all glucose challenges performed during the study period,[13] glucose $AUC_{0\text{-}240\ min}$ (for 240 minutes after the glucose challenge) was significantly decreased in all lixisenatide groups as compared to the control group. The glucose $AUC_{0\text{-}240\ min}$ values (mean ± SE) on Day 41 in the control and lixisenatide groups were 564.3 ± 42.7, 169.5 ± 33.8, 99.8 ± 27.7, and 184.1 ± 31.4 g·min/L, respectively. For fasting blood glucose,[14] there was no significant difference between any dose level of lixisenatide and vehicle on Day 14, while on Day 43 (the last observation day), it was significantly decreased by lixisenatide at all dose levels as compared to vehicle (260.5 ± 26.7, 149.1 ± 15.1, 146.0 ± 13.0, and 166.6 ± 20.7 mg/dL, respectively). HbA1c on the day of final dosing was decreased dose-dependently by lixisenatide and it was significantly lower in all lixisenatide groups than in the control group (8.4 ± 0.4%, 7.0 ± 0.3%, 6.5 ± 0.3%, and 6.2 ± 0.3%, respectively). Daily water intake was decreased dose-dependently by lixisenatide, and it was significantly lower in all lixisenatide groups than in the control group. In addition, on the day of final dosing, pancreas sections were prepared from 5 mice/group and immunostained using anti-insulin antibody to calculate the volume from the area of insulin-positive cells. As a result, there was no significant difference between the lixisenatide and control groups.

3.(i).A.(1).2).(b).ii) Effects on blood glucose, fasting blood glucose, HbA1c, water intake, body weight, and pancreatic β-cells following 90 days of once daily multiple-dose to db/db mice (4.2.1.1-12)

Lixisenatide (486 μg/kg) or vehicle[8] was intraperitoneally administered to male db/db mice (6-10 weeks of age, 9-11 mice/group) once daily for 90 days. Until Day 50 of administration, the animals were divided into 2 groups: lixisenatide and control groups, and from Days 51 to 90, each group was further divided into lixisenatide and control groups. Group 1 was treated with vehicle from Days 1 to 90 (G1), Group 2 was treated with vehicle from Days 1 to 50 and lixisenatide from Days 51 to 90 (G2), Group 3 was treated with lixisenatide from Days 1 to 50 and vehicle from Days 51 to 90 (G3), and Group 4 was treated with lixisenatide from Days 1 to 90 (G4). As a result, after glucose challenges[15] performed during the period from Days 51 to 90, glucose $AUC_{0\text{-}240\ min}$ (for 240 minutes after the glucose challenge) was significantly decreased in G2, G3, and G4 as compared to G1. Fasting blood glucose was significantly lower in the lixisenatide group than in the control group (Days 1 to 50). It was significantly decreased in G2, G3, and G4 compared to G1, while it was significantly increased in G3 compared to G4 (Days 51 to 90). HbA1c at the end of administration (mean ± SE) was decreased in G4 (6.65 ± 0.22%) compared to G1 (7.99 ± 0.51%), but there were no significant differences among the four groups. Daily water intake was significantly decreased in the lixisenatide group compared to the control group (Days 1 to 50), and it was decreased significantly in G2 and G4 compared to G1 (Days 51 to 90). Body weight was increased significantly in G4 compared to G1 (Days 51 to 90). The expression of insulin mRNA in pancreatic β-cells was increased in G4 (21.8 ± 4.1 pg/μg) as compared to G1 (10.7 ± 3.6 pg/μg), with no significant difference.

[13] Performed upon group assignment (3 days before study initiation) and on Study Days 1, 14, and 41. Glucose solution (1 g/kg) was orally challenged at 15 minutes after administration, and blood glucose was measured repeatedly for 240 minutes after the glucose challenge.
[14] Measured upon group assignment and on Study Days 1, 14, 41, and 43.
[15] Performed on Study Days 67, 78, and 90. Animals were fasted overnight after lixisenatide administration and orally challenged with glucose solution (1 g/kg) in the next morning. Blood glucose was measured repeatedly for 240 minutes after the glucose challenge.

3.(i).A.(1).2).(b).iii) Effects on blood glucose, HbA1c, food intake, body weight, and plasma insulin levels following 12 weeks of continuous subcutaneous infusion to ZDF rats (4.2.1.1-13)

Continuous subcutaneous infusion of lixisenatide (0.486, 4.86, 48.6 μg/kg/day), exendin-4 (4.187 μg/kg/day), or vehicle[16] was given to male obese ZDF rats (8 weeks of age, 8 rats/group) for 12 weeks and continuous subcutaneous infusion of lixisenatide (48.6 μg/kg/day) or vehicle was given to male lean ZDF rats (8 weeks of age, 8 rats/group) for 12 weeks. For obese rat groups, standard diet was switched to high-fat diet at 5.5 weeks after initiation of administration. As a result, regarding glucose $AUC_{0\text{-}180\ min}$ for 180 minutes after a glucose challenge performed 1 week after initiation of administration or 5.5 weeks after switching to high-fat diet (11 weeks after initiation of administration),[17] no significant decrease was observed in all obese lixisenatide and exendin-4 groups, as compared to the obese control group. HbA1c at the end of administration (mean ± SE) was 7.80 ± 0.12%, 7.70 ± 0.23%, 7.41 ± 0.30%, 5.16 ± 0.25%, and 7.25 ± 0.23% in the obese control, obese lixisenatide (0.486, 4.86, 48.6 μg/kg/day), and exendin-4 groups, respectively. HbA1c was significantly lower in the obese lixisenatide 48.6 μg/kg/day group than in the obese control group. In contrast, there was no significant difference between the lean control and lean lixisenatide groups. Mean food intake was 989.7 g/animal in the obese control group, and 1063.6, 998.9, 860.9, and 964.3 g/animal in the obese lixisenatide (0.486, 4.86, 48.6 μg/kg/day) and exendin-4 groups, respectively, and it was lower in the obese lixisenatide 48.6 μg/kg/day group compared to the control group. Similarly, mean food intake in the lean lixisenatide group (548.3 g/animal) was lower than that in the lean control group (651.8 g/animal). No significant decrease in body weight was observed in the obese lixisenatide and lean lixisenatide groups compared to the respective control groups throughout the study period. Plasma insulin levels at the end of the study were 20.682 ± 3.501, 27.927 ± 2.669, 27.279 ± 6.914, 58.828 ± 6.472, and 30.522 ± 5.593 ng/L in the obese control, obese lixisenatide (0.486, 4.86, 48.6 μg/kg/day), and exendin-4 groups, respectively. It was significantly increased in the obese lixisenatide 48.6 μg/kg/day group compared to the obese control group. In contrast, there was no significant difference between the lean control and lean lixisenatide groups.

3.(i).A.(1).2).(b).iv) Effects on pancreatic function in ZDF rats (4.2.1.1-14)

Continuous subcutaneous infusion of lixisenatide (50 μg/kg/day) or vehicle[11] was given to male obese ZDF rats (12 weeks of age, 6-7 rats/group) and male lean ZDF rats (12 weeks of age, 6 rats/group) for 6 weeks. At the end of infusion, pancreas were isolated from the animals and the effect on glucose-stimulated insulin secretion was examined by changing the glucose concentration in the perfusate.[6] As a result, the insulin $AUC_{10\text{-}60\ min}$ values (from 10 to 60 minutes after treatment) (mean ± SE) were 2026 ± 390 and 507 ± 137 μg·min/L in the obese lixisenatide and obese control groups, respectively and it was significantly increased in the obese lixisenatide group compared to the obese control group. In contrast, there was no significant difference between the lean lixisenatide and lean control groups.

[16] Water

[17] Animals were fasted overnight 1 week after initiation of administration and 5.5 weeks after switching to high-fat diet (11 weeks after initiation of administration) and orally challenged with glucose solution (2 g/kg). Blood glucose was measured repeatedly for 180 minutes after the glucose challenge.

3.(i).A.(2) Secondary pharmacodynamics

3.(i).A.(2).1) Effects on ischemia/reperfusion-induced injury in isolated rat heart (4.2.1.2-1)

Using isolated rat hearts, the left anterior descending artery was occluded. Lixisenatide (0.3 nmol/L), GLP-1 (0.3 nmol/L), liraglutide (0.3 nmol/L), or vehicle[18] was applied 35 minutes after the occlusion, and 10 minutes later, reperfusion was performed for 120 minutes. As a result, for the total and ischemic left ventricular areas, no significant differences between any of the treatment groups and the control group were observed. The left ventricular infarct area and the ratio of infarct/ischemic area were significantly decreased in all treatment groups compared to the control group, but for left ventricular pressure, contractility, coronary blood flow, and heart rate measured at 5 minutes after reperfusion, there were no significant differences between the treatment groups and the control group.

3.(i).A.(2).2) Effects on atherosclerotic plaque formation in apolipoprotein E knockout mice (4.2.1.2-2)

Continuous subcutaneous infusion of lixisenatide (3.6 μg/mouse/day for the first 4 weeks and 5.04 μg/mouse/day for subsequent 12 weeks) or vehicle[19] was given to male apolipoprotein E knockout (Apo E KO) mice (10 to 11 weeks of age, 17 to 18 mice/group) for 16 weeks. Wild type (WT) male mice (10 mice) served as untreated controls. As a result, total serum cholesterol levels prior to infusion and on Days 35 and 112 were significantly increased in the ApoE KO control group compared to the WT group. Total serum cholesterol levels on Days 35 and 112 were significantly decreased in the ApoE KO lixisenatide group compared to the ApoE KO control group. In addition, cholesterol distribution among lipoprotein fractions was determined using fast lipoprotein chromatography. As a result, treatment with lixisenatide resulted in a decrease in non-high-density lipoprotein (non-HDL) fractions (very-low-density lipoprotein and low-density lipoprotein [LDL]). For relative liver weight and hepatic cholesterol, triglyceride, and phospholipid concentrations on Day 112, there were no significant differences between the ApoE KO lixisenatide and ApoE KO control groups. Furthermore, atherosclerotic plaque formation was quantified by histology and MRI imaging on Day 112. As a result, the ratios of the plaque area to the total inner surface area of the aortic arch, the total inner surface area of the aorta, and the total area of the aortic root were significantly increased in the ApoE KO control group compared to the WT group, while those values were significantly decreased in the ApoE KO lixisenatide group compared to the ApoE KO control group.

3.(i).A.(2).3) Receptor binding profile (4.2.1.2-3 to 4.2.1.2-7)

Binding assays for 89 radioligands and 2 cellular functional assays were performed and the inhibition of specific radioligand binding to receptors, transporters, and ion channels by lixisenatide (100 nmol/L) was assessed. As a result, lixisenatide inhibited specific radioligand binding to the N-type Ca^{2+} channel by 71%, but did not cause >50% inhibition for others. In addition, the effect of lixisenatide (1 and 10 μmol/L) at native N-type Ca^{2+} channel in cultured neurons of rat dorsal root ganglia was examined using patch-clamp technique, and lixisenatide at 1 and 10 μmol/L showed 20% and 52% inhibition, respectively. The IC_{50} of lixisenatide for the N-type Ca^{2+} channel is approximately 10 μmol/L (48.6 μg/mL), which is 47,184-fold of the C_{max} following once-daily subcutaneous administration of 20 μg lixisenatide in humans.[20]

[18] 0.9% sodium chloride solution containing 0.1% bovine serum albumin

[19] Isotonic aqueous sodium acetate buffered glycerol solution (pH 4.5)

[20] C_{max} on the day of final dosing in Japanese type 2 diabetes patients (anti-lixisenatide antibody-positive patients) in Study PDY6797 (5.3.5.1-1) (1.03 ng/mL)

3.(i).A.(3) Safety pharmacology

3.(i).A.(3).1) Effects on the central nervous system

3.(i).A.(3).1).(a) Effects on the rat central nervous system (4.2.1.3-1)

A single intravenous dose of lixisenatide (0.1, 1, 10, 50, 150, 500 μg/kg) or vehicle[21] was administered to male rats (6 rats/group), and effects on general behavior and autonomic nervous system and motor function were examined by modified Irwin test. As a result, decreased muscle tone was observed 5 minutes post-dose in 1 rat at 1 μg/kg, and slight decreases in locomotor activity and muscle tone, slight apathy, abnormal dispersion within the home cage, and slight to moderate impairment of the righting reflex were observed at ≥10 μg/kg, but these signs resolved by Study Day 2. Slight and transient clonic convulsion was observed in 1 rat at 50 μg/kg. Estimated blood concentrations following single intravenous doses of 1 and 10 μg/kg of lixisenatide are approximately 20 and 200 ng/mL, respectively, which are 19- and 190-fold of the C_{max} following once daily subcutaneous administration of 20 μg lixisenatide in humans.[20]

3.(i).A.(3).1).(b) Effects on the mouse central nervous system (4.2.1.3-2)

A single subcutaneous dose of lixisenatide (20, 200, 2000 μg/kg) or vehicle[7] was given to male mice (8 mice/group) and effects on general behavior and autonomic nervous system and motor function were examined by modified Irwin test. As a result, there were no effects in all treatment groups. The exposure following once daily subcutaneous administration of 2000 μg/kg lixisenatide (AUC, 570 ng·h/mL) is 69.3-fold of that following once daily subcutaneous administration of 20 μg lixisenatide to humans.[22]

3.(i).A.(3).2) Effects on the cardiovascular system

3.(i).A.(3).2).(a) *In vitro* studies (4.2.1.3-3, 4.2.1.3-4)

Using CHO cells stably expressing hERG channels, effects of lixisenatide (10 and 30 μg/mL) on hERG potassium current were examined. As a result, lixisenatide 10 and 30 μg/mL inhibited hERG potassium currents by 12.5% and 37.3%, respectively.[23] Lixisenatide 10 μg/mL is 9709-fold of the C_{max} following once daily subcutaneous administration of 20 μg lixisenatide to humans.[20]

In addition, using rabbit Purkinje fibers, effects of lixisenatide (0.01, 0.1, 1 μg/mL) or vehicle[24] on resting membrane potential, amplitude, maximal rate of rise of action potential, and action potential duration were examined at stimulation rates of 3, 1, and 0.2 Hz. As a result, there were no marked changes in resting membrane potential or action potential parameters at any stimulation rate.

3.(i).A.(3).2).(b) *In vivo* studies

3.(i).A.(3).2).(b).i) Study in rats (4.2.1.3-5)

Cumulative doses of lixisenatide (50, 150, and 500 μg/kg, every 30 minutes) or vehicle[8] were intravenously administered to male Wistar rats (7 rats) to examine the effects on arterial blood pressure and blood glucose levels. As a result, lixisenatide 50 μg/kg caused a rapid and significant increase in mean arterial blood pressure (MAP) as compared to the control group, as well as a significant increase in blood glucose levels, but lixisenatide 150 and 500 μg/kg caused no further increases in MAP and blood glucose levels. In addition,

[21] Phosphate buffered saline (pH 7.4)

[22] Exposure on the day of final dosing in Japanese type 2 diabetes patients (anti-lixisenatide antibody-positive patients) in Study PDY6797 (5.3.5.1-1) (AUC, 8.22 ng·h/mL).

[23] Relative to the control value (obtained following perfusion of the same cells with the control solution [NaCl 130 mM; KCl 5 mM; sodium acetate 2.8 mM; $MgCl_2$ 1 mM; HEPES, 10 mM; glucose 10 mM; $CaCl_2$ 1 mM, pH7.4]).

[24] Krebs solution

lixisenatide (500 μg/kg) or vehicle[21] was intravenously administered to male Wistar rats and SD rats (4 rats each). As a result, no effects were observed on MAP and blood glucose levels in Wistar rats, whereas in SD rats, lixisenatide caused a significant increase in MAP and a persistent and significant increase in blood glucose levels, compared to the control group. The applicant explains that MAP increase observed following administration of lixisenatide to rats is known as a class effect of GLP-1 receptor agonists, but it is specific to rats because it has not been observed following administration of lixisenatide or other GLP-1 receptor agonists to other animal species.[25]

3.(i).A.(3).2).(b).ii) Studies in dogs (4.2.1.3-6, 4.2.1.3-7)

Lixisenatide or vehicle [21] was intravenously infused for 30 minutes to anesthetized dogs (2 dogs/sex/group) to examine the effects on the cardiovascular system. Each dog in the lixisenatide group received ascending doses of 0.1, 1.0, and 10 μg/kg of lixisenatide. As a result, no effects were observed on heart rate, arterial blood pressure (systolic blood pressure, diastolic blood pressure, mean blood pressure), electrocardiogram parameters (PR, QRS, QT, QTcB, and QTcF intervals), femoral artery blood flow, vascular resistance, and T wave morphology. The exposure following intravenous administration of 10 μg/kg lixisenatide (AUC, 118 ng·h/mL) is 14.4-fold of that following once-daily subcutaneous administration of 20 μg lixisenatide to humans.[22]

Anesthetized male dogs (8 dogs/group) were given intravenous infusion of lixisenatide 10 μg/kg or vehicle[26] for 30 minutes, and then an intravenous bolus dose of insulin glargine 0.1 IU/kg or vehicle.[27] Heart rate, electrocardiogram parameters (QRS, PQ, QTcF, and QTcW[28] intervals), and serum potassium concentrations were measured following coadministration of lixisenatide and insulin glargine. As a result, lixisenatide alone induced a significant increase in heart rate and a significant decrease of PQ interval duration compared to the control group during infusion and at the end of infusion, but no effects were observed on other parameters. The combination of lixisenatide with insulin glargine induced significant decreases in serum glucose and potassium concentrations, a significant increase in heart rate, reversible changes in electrocardiogram (ST segment elevation, changes in T wave morphology and amplitude) up to 60 minutes after administration, and a significant increase in QT interval duration (including QTcF and QTcW), compared to the control group.[29] These were similar to the effects observed with insulin glargine alone.

3.(i).A.(3).3) Effects on the respiratory system (4.2.1.3-6)

Lixisenatide or vehicle[21] was intravenously infused for 30 minutes to anesthetized dogs (2 dogs/sex/group) to examine the effects on the respiratory system. Each dog in the lixisenatide group received ascending doses of 0.1, 1.0, and 10 μg/kg of lixisenatide. As a result, lixisenatide had no significant effects on respiratory rate, tidal volume, minute volume, and peak inspiratory and expiratory flows, as compared to the control group. The exposure following intravenous administration of 10 μg/kg lixisenatide (AUC, 118 ng·h/mL) is 14.4-fold that following once-daily subcutaneous administration of 20 μg lixisenatide to humans.[22]

[25] Study in dogs (4.2.1.3-6), Liraglutide (Injection) for the treatment of Patients with Type 2 Diabetes. NDA 22-341 FDA briefing document, FDA: Center for Drug Evaluation and Research. NDA 21-773 - Pharmacology Review, Edwards CMB et al. *Exp Physio.* 1997;82:709-716.
[26] Lixisenatide placebo prepared by the applicant
[27] Insulin glargine placebo prepared by the applicant
[28] Calculated using Van de Water's correction formula.
[29] Group treated with lixisenatide placebo followed by insulin glargine placebo.

3.(i).B Outline of the review by PMDA

3.(i).B.(1) Duration of pharmacological action

PMDA asked the applicant to explain the relationship between the structure of lixisenatide and the duration of pharmacological action by comparing it with native human GLP-1 and exendin-4.

The applicant responded as follows:

Lixisenatide is a synthetic GLP-1 receptor agonist having a similar structure to exendin-4. It is comprised of 44 amino acids, and it is 84% homologous to exendin-4 with the 37 amino acids sequence from N terminal being identical, but it has different physico-chemical properties due to deletion of proline at the 38 position and insertion of 6 lysines to C-terminal, which has a higher stability in plasma than exendin-4. Homology of lixisenatide and GLP-1 (7-36) amide, the active form of native human GLP-1 in the circulating blood, is 47%, but based on the data in published literature,[30] the docking modeling conducted by the comparison of NMR structure suggested that the binding ability of lixisenatide to the GLP-1 receptor is similar to GLP-1. In addition, it is known that the substitution of the amino acid alanine in the second N-terminal position with glycine in GLP-1 confers resistance to DPP-4 degradation (Deacon CF et al. *Diabetologia*. 1998;41:271-278). In lixisenatide and exendin-4, the amino acid alanine in the second N-terminal position has been substituted with glycine, and improved stability of lixisenatide in plasma has been demonstrated in mice, rats, rabbits, dogs, pigs, and humans (4.2.2.4-2).

Based on the above, the substitution of the amino acid alanine in the second N-terminal position with glycine protects lixisenatide against DPP-4 degradation and in addition, lixisenatide has glutamate at the 16 position and a C-terminal extension, which are also considered to make lixisenatide more stable than native GLP-1 (Henchey LK et al. *Current Opinion in Chemical Biology*. 2008;12:692-697).

No study investigating the efficacy of lixisenatide administered subcutaneously once daily has been performed. While the exposure following single intraperitoneal administration of lixisenatide to db/db mice was higher than that of single subcutaneous administration, the elimination half-life was comparable.[31] Thus, the duration of exposure following intraperitoneal administration is considered to be longer than that of subcutaneous administration. Although direct comparison of the two routes of administration is unavailable, in a study in which oral glucose was loaded at different timepoints between 15 minutes and 24 hours after single intraperitoneal administration of lixisenatide to db/db mice, lixisenatide significantly reduced glucose AUC after a glucose challenge for 12 hours (4.2.1.1-5). Based on the results from studies of once daily intraperitoneal administration of lixisenatide or twice daily intraperitoneal administration of lixisenatide at the same dose in db/db mice (4.2.1.1-10, 4.2.1.1-12) and the results from a study of continuous subcutaneous infusion of lixisenatide in ZDF rats (4.2.1.1-13), it is considered that the efficacy of multiple-dose administration of lixisenatide has been demonstrated.

[30] Neidigh JW et al. *Biochemistry*. 2001;40:13188-13200, Runge S et al. *J Biol Chem*. 2008;283:11340-11347, Underwood CR et al. *J Biol Chem*. 2010;285:723-730

[31] C_{max}, AUC, and apparent elimination half-life following single intraperitoneal administration of lixisenatide 4860 μg/kg to male db/db mice were 2760.48 μg/L, 578.34 mg·min/L, and 103 min, respectively. C_{max}, AUC, and apparent elimination half-life following single subcutaneous administration of lixisenatide 4860 μg/kg were 1613.52 μg/L, 256.12 mg·min/L, and 69 min, respectively (4.2.2.2-1).

PMDA accepted the applicant's response on the relationship between the structure of lixisenatide and the duration of pharmacological action. However, the duration of pharmacological action up to 24 hours after lixisenatide administration and the efficacy of lixisenatide subcutaneously administered once daily have not been evaluated in non-clinical studies. Therefore, the efficacy of multiple-dose administration of lixisenatide will continue to be reviewed in the clinical section [see "4.iii).B.(3) Interpretation of results of multinational studies" for efficacy in humans].

3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

Pharmacokinetics was investigated after a single intravenous or subcutaneous administration of lixisenatide or ^{3}H- or ^{14}C-labeled lixisenatide to mice, rats, rabbits, dogs, and pigs. The pharmacokinetics of lixisenatide was also investigated after continuous subcutaneous infusion to rabbits and after repeated subcutaneous administration to mice, rats, and dogs. Plasma lixisenatide and total lixisenatide concentrations[32] were determined by LC/MS or double-sandwich enzyme-linked immunosorbent assay[33] (ELISA),[34] and anti-lixisenatide antibodies were determined by ELISA. The lower limit of quantification of LC/MS for plasma unchanged lixisenatide was 12.6, 16.5, and 19.9 ng/mL in rats, dogs, and pigs, respectively. The lower limit of quantification of ELISA for plasma unchanged lixisenatide was 50 pg/mL in mice and rats, and 40 pg/mL in rabbits and dogs. The lower limit of quantification of ELISA for plasma total lixisenatide was 100 pg/mL in mice and dogs, and 50 pg/mL in rats. Radioactivity in biological samples was determined by liquid scintillation counter and quantitative whole-body autoradiography. Metabolites were identified using high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results from the main studies are described below. Doses per administration in these studies are indicated.

3.(ii).A.(1) Absorption (4.2.2.2-1, 4.2.2.2-4, 4.2.2.2-8, 4.2.2.2-11, 4.2.2.2-13, 4.2.3.4.2-2, 4.2.3.2-6, 4.2.3.2-10)

The pharmacokinetic parameters of lixisenatide after a single intravenous or subcutaneous administration were as shown in Table 3.

Table 3. Pharmacokinetic parameters of lixisenatide after a single intravenous or subcutaneous administration to various animal species

Animal species	Sex	Administration route	Dose (μg/kg)	C_{max} (ng/mL)	AUC (min·μg/mL)	T_{max} (min)	$t_{1/2}$ (min)	CL (mL/min/kg)	V_{ss} (mL/kg)	F (%)
Rat	Male	s.c.	4860 (n = 6)	549 ± 199	41 ± 21	18.1 ± 4.1	30.5 ± 10.5	5.9 ± 1.4[a)]	284 ± 108[b)]	4.3 ± 1.9
	Male	i.v.	486 (n = 5)	8082 ± 2046	87 ± 19	0	48.6 ± 10.2	5.8 ± 1.4	98 ± 36	NC
Dog	Male	s.c.	199 (n = 4)	194 ± 53	131 ± 83	128 ± 26	342 ± 206	2.2 ± 1.6[a)]	790 ± 230[b)]	102 ± 75
	Male	i.v.	102[c)] (n = 4)	1050 ± 214	71 ± 11	30	60 ± 25	1.5 ± 0.2	104 ± 45	NC
Pig	Female	s.c.	530 (n = 3)	190 ± 15	75 ± 10	120 ± 23	159 ± 20	5.1 ± 0.2[a)]	1650 ± 360[b)]	71 ± 17
	Female	i.v.	88-97[c)] (n = 3)	413 ± 29	18-20	NC	53 ± 5	5.0 ± 0.6	192 ± 22	NC

Mean ± SD, NC: not calculated

s.c.: subcutaneous administration, i.v.: intravenous administration, C_{max}: maximum plasma concentration, AUC: area under the plasma concentration-time curve, T_{max}: time to maximum plasma concentration, $t_{1/2}$: elimination half-life, CL: apparent clearance, Vss: apparent volume of distribution, F: bioavailability

a) Corrected by CL × F.

[32] Total lixisenatide concentration includes both lixisenatide unbound and bound to anti-lixisenatide antibodies in plasma.

[33] As the ELISA method initially developed was revealed to be affected by anti-lixisenatide antibodies, the first-generation assay for the determination of total lixisenatide concentration was developed. The second-generation assay was subsequently developed, and similarity between the first- and second-generation assays was demonstrated. The third-generation assay was then developed, and the inter-laboratory reproducibility of this assay and the previous assays has been demonstrated.

[34] LC/MS was used in early nonclinical pharmacokinetic studies, and ELISA was used in toxicokinetic (TK) studies.

b) Corrected by V × F.
c) Intravenous infusion (30 minutes)

The pharmacokinetic parameters of lixisenatide after twice-daily subcutaneous administration to male and female mice, rats,[35] and dogs were as shown in Table 4.

Table 4. Pharmacokinetic parameters of lixisenatide after repeated subcutaneous administration to various animal species

Animal species	Dose (μg/kg BID)	Day of sampling	C_{max} (ng/mL)		$AUC_{0\text{-}24\,h}$ (ng·h/mL)	
			Male	Female	Male	Female
Mouse	200 (n = 3/time point)	1	140	108	202	151
		92	659	543	428	1410
	1000 (n = 3/time point)	1	666	544	895	753
		92	2250	2470	28400	6600
	2000 (n = 3/time point)	1	1080	1080	1670	1530
		92	4220	4260	25400	25700
Rat[a)]	200 (n = 3/time point)	1	41.5	41.1	113	108
		29	612	357	4570	4120
		92	1440	1460	19200	23500
	1000[b)] (n = 3/time point)	1	63.9	79.5	197	203
		29	1170	935	11100	16000
		92	5510	3930	35200	31600
	2000 (n = 3/time point)	1	140	121	287	518
		29	718	1960	9740	7220
		92	3170	2160	42800	14100
Dog	20 (n = 3)	1	22	26	215	208
		28	41	46	389	483
		91	175	110	3045	1249
	300/100[b)] (n = 3[c)])	1	150	165	1389	1313
		28	356	136	4906	1848
		91	798	211	15873	3600
	1000/400/250[d)] (n = 5[c)])	1	406	373	4514	3730
		28	138	198	1615	2619
		91	934	1432	19246	28103

Mean, BID: twice daily dosing
C_{max}: maximum plasma concentration (concentration after the first or second dose, whichever the higher), $AUC_{0\text{-}24\,h}$: area under the plasma concentration-time curve up to 24 hours after administration (including both the first and second doses)
a) Different batches of lixisenatide were used in Weeks ≤6 and Weeks ≥7, but a specific amount of the drug was administered to the animals. Due to the development of anti-lixisenatide antibodies, samples on Days 29 and 92 were reassayed after the establishment of a modified TK assay. As dosing solutions were prepared without correction for peptide content, the actual doses were 165.6, 828.2, and 1656.4 μg/kg.
b) The dose was reduced to 100 μg/kg BID from Day 29.
c) As the dose was reduced from Days 20 to 28 in one male, the numbers of males on Day 28 were 2 and 4 in the 300/100 and 1000/400/250 groups, respectively.
d) The dose was reduced to 400 μg/kg BID from Day 15 and from Day 29 to 250 μg/kg BID.

Pharmacokinetics was investigated after 76-hour continuous subcutaneous infusion of 71 μg/kg lixisenatide to rabbits (3 animals), and the plasma lixisenatide concentration at steady state was determined to be 2.36 ± 0.58 μg/L (normalized to an infusion rate of 1.0 μg/h/kg).

In mice, rats, and rabbits, exposure increased dose-proportionally in general after a single administration but nonlinearly after repeated administration. The results of TK assessment showed that most animals were positive for anti-lixisenatide antibodies, and exposure tended to increase with antibody formation. The

[35] A rat 4-week repeated intravenous dose toxicity study was conducted, but the data are not presented because TK samples were not analyzed within a sample stability period.

accumulation ratio of lixisenatide varied depending on the dose or exposure duration in all animal species. No consistent trend of gender-related differences in exposure was observed.

3.(ii).A.(2) Distribution (4.2.2.3-1 to 4.2.2.3-5)

Tissue distribution was investigated after a single intravenous or subcutaneous administration of ^{3}H- or ^{14}C-labeled lixisenatide at 1 mg/kg to male rats (1 animal/time point). At 15 minutes after subcutaneous administration of ^{3}H-labeled lixisenatide, the plasma radioactivity level was 0.2213 μgEq/g while the radioactivity levels in the pancreas, renal cortex, salivary gland, thyroid gland, and lung were as high as 2.98-, 2.40-, 2.19-, 1.83-, and 1.61-fold the plasma level, respectively; however, radioactivity was only widely distributed to other tissues in small amounts. Radioactivity levels reached a maximum at 336 and 168 hours post-dose in the brain/fat tissues and the spinal cord, respectively, and within 24 hours post-dose in other tissues. At 5 minutes after intravenous administration, systemic distribution was observed, and especially, the radioactivity levels in the renal cortex, kidney, renal medulla, adrenal gland, and thyroid gland were as high as 5.87-, 3.41-, 1.37-, 1.25-, and 1.30-fold of the plasma level, respectively. The radioactivity level at 15 minutes after subcutaneous administration of ^{14}C-labeled lixisenatide was 0.31 μgEq/g in plasma while the radioactivity levels in the renal cortex and kidney were as high as 10.68- and 5.10-fold (3.31 and 1.58 μgEq/g, respectively) of the plasma level, respectively. High levels of radioactivity were observed in the same organs after intravenous administration. The maximum radioactivity was observed within 48 hours after subcutaneous administration in tissues other than the lens; in the lens, however, the radioactivity level reached a maximum at 168 hours post-dose and was still high at 0.83 μgEq/g at 3 months post-dose (≤0.2 μgEq/g in all other tissues). Since approximately 2.4% of the lixisenatide concentration in plasma was found in the brain, the applicant has discussed that only a small amount of lixisenatide passes through the blood-brain barrier.

A single dose of 1 or 0.5 mg/kg of ^{14}C-labeled lixisenatide or lixisenatide was subcutaneously administered to pregnant rats (Day 17 of gestation, 2 animals/time point [^{14}C-labeled lixisenatide], 4 animals/time point [lixisenatide]) and pregnant rabbits (Day 18 of gestation, 1 animal/time point [^{14}C-labeled lixisenatide], 4 animals/time point [lixisenatide]) to investigate placental transfer. In rats, the administered radioactivity was widely distributed in the body of maternal animals, with a high radioactivity level in the kidney (5.34 μgEq/g). The plasma radioactivity ratio (fetal/maternal) and the fetus-to-placenta radioactivity ratio after administration of ^{14}C-labeled lixisenatide on Day 17 of gestation were 0.15 and 0.24, respectively, at 15 minutes, and 1.53 and 0.87, respectively, at 24 hours. The plasma lixisenatide concentration ratio (fetal/maternal) at 15 minutes after subcutaneous administration of lixisenatide was 0.0014, and lixisenatide was below the detection limit at 24 hours in the fetus and at both 15 minutes and 24 hours in the amniotic fluid. In rabbits, a high level of radioactivity was found in the fetal liver (3.41 μgEq/g) at 24 hours after subcutaneous administration of ^{14}C-labeled lixisenatide, but the plasma lixisenatide concentration ratio (fetal/maternal) was <0.0001 to 0.003 at 3 hours after administration of lixisenatide.

The binding of lixisenatide (0.05-10,000 ng/mL) to plasma protein (mean, ultracentrifugation) was 36% to 69% in dogs and 46% to 79% in rats [see “4.(ii).A.(1) Studies using human biomaterials” for human data].

3.(ii).A.(3) Metabolism (4.2.2.4-1, 4.2.2.4-2)

An investigation of the metabolism of lixisenatide using S9 kidney and liver fractions from mice, rats, rabbits, and dogs revealed that the unchanged lixisenatide accounted for ≥40% after 60 minutes of incubation in mouse and rat liver S9 fractions, indicating higher stability of lixisenatide in the mouse and rat compared to other animal species.

The stability of lixisenatide was investigated using the heparinized plasma from mice, rats, rabbits, dogs, and pigs, which revealed the plasma half-lives (mean ± standard deviation [SD]) of 212 ± 1,[36] 224 ± 19, 521 ± 66, 322 ± 3, and 564 ± 44 minutes, respectively [see "4.(ii).A.(1) Studies using human biomaterials" for human data].

3.(ii).A.(4) Excretion (4.2.2.5-1)

After a single subcutaneous administration of ^{14}C-labeled lixisenatide at 1 mg/kg to lactating rats (11 days after parturition, 3 animals), the milk/plasma radioactivity ratio was 3.2 at 24 hours, and approximately 9.4% of the administered radioactivity was observed in the milk by 24 hours. Radioactivity was detected in the gastric content of the pups at ≥2 hours post-dose, and approximately 0.01% of the radioactivity found in the gastric content at 24 hours post-dose was associated with the unchanged lixisenatide.

3.(ii).B Outline of the review by PMDA

Distribution of lixisenatide

Concerning the distribution of relatively high radioactivity to the tissues around the cartilages and lens at ≥24 hours after administration of radiolabeled lixisenatide, PMDA asked the applicant to explain the persistence and safety of lixisenatide in these tissues.

The applicant responded as follows:

Concerning the distribution of high radioactivity to the tissues around the cartilages and lens after subcutaneous administration of radiolabeled lixisenatide (after administration of ^{14}C-labeled lixisenatide) in rats and the radioactivity levels remaining above the lower limit of quantification even at 3 months post-dose, the stability of radiolabeled lixisenatide in the body must be considered. An early degradation of ^{14}C-labeled lixisenatide into volatile compounds (mostly estimated to be $^{14}CO_2$) after administration and the prolonged, wide distribution of radioactivity in the body suggest rapid metabolism of lixisenatide, and catabolism and protein synthesis by the incorporation of lixisenatide-derived ^{14}C-lysine or derivatives containing ^{14}C-lysine. Therefore, the distribution of low radioactivity is considered to be due to degradation products generated early after administration.

Lixisenatide and its metabolites containing ^{14}C have cationic properties because they have basic residues (lysine), and terminal metabolites are rapidly distributed to the whole body compared to the unchanged lixisenatide. In addition, radiolabeled lysine is considered to undergo catabolism and be incorporated into proteins. Meanwhile, the cartilages and melanin are considered to have affinity to cations because they overall have anionic properties. Therefore, it is considered that lixisenatide-derived basic metabolites are distributed as described above in the cartilages and tissues containing melanin because of their intrinsic affinity (including enrichment and long-term persistence) based on these properties. This has been reported

[36] Data in db/db mice. The plasma half-life in mice of mixed genetic strains was 476 ± 60 minutes.

for other weakly basic drugs as well (Leblanc B. et al. *Regulatory Toxicity and Pharmacology.* 1998;28:124-132). No abnormal findings were observed in the cartilages (histopathological examination) or eyes (ophthalmoscopy and histopathological examination) in the toxicity studies of lixisenatide, and it is considered that lixisenatide has no cartilaginous or ophthalmic toxicity.

The incidences of treatment-emergent adverse events (TEAEs) of "musculoskeletal and connective tissue disorders" in phase III studies[37] were 16.9% (25 of 148 patients) in the placebo group and 19.9% (36 of 181 patients) in the lixisenatide group in the Japanese population, 18.9% (173 of 913 patients) in the placebo group and 23.1% (449 of 1946 patients) in the lixisenatide group in the non-Japanese population, and 18.7% (198 of 1061 patients) in the placebo group and 22.8% (485 of 2127 patients) in the lixisenatide group in the overall population. These differences in the incidence of TEAEs were mainly due to back pain. They were slight differences and were not considered to be of any particular safety concern.

The incidences of TEAEs of "eye disorders" in phase III studies[37] were 12.2% (18 of 148 patients in the placebo group and 22 of 181 patients in the lixisenatide group) in both treatment groups in the Japanese population, 5.4% (49 of 913 patients) in the placebo group and 6.6% (129 of 1946 patients) in the lixisenatide group in the non-Japanese population, and 6.3% (67 of 1061 patients) in the placebo group and 7.1% (151 of 2127 patients) in the lixisenatide group in the overall population. The incidences of cataract were 0.0% (0 of 148 patients) in the placebo group and 1.7% (3 of 181 patients) in the lixisenatide group in the Japanese population, 0.9% (8 of 913 patients) in the placebo group and 1.2% (24 of 1946 patients) in the lixisenatide group in the non-Japanese population, and 0.8% (8 of 1061 patients) in the placebo group and 1.3% (27 of 2127 patients) in the lixisenatide group in the overall population.

Based on the above, the persistent distribution of low radioactivity after administration of radiolabeled lixisenatide was believed to be due to degradation products generated early after administration, and were not considered to be of any particular safety concern in the clinical setting either.

PMDA accepted the applicant's response.

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

Toxicity studies of lixisenatide conducted include single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, local tolerance, and other toxicity studies. Doses per administration in these studies are indicated.

3.(iii).A.(1) Single-dose toxicity

3.(iii).A.(1).1) Single-dose toxicity studies in mice (4.2.3.1-1, 4.2.3.1-2)

Studies were conducted in which a single dose of 500 μg/kg lixisenatide was subcutaneously or intravenously administered to male and female CD-1 mice (5 animals/sex). No mortality was observed, and transient lethargy was observed in one male after intravenous administration. Based on the above, the approximate lethal dose in mice was determined to be >500 μg/kg.

[37] Studies EFC6018 (5.3.5.1-3), EFC6015 (5.3.5.1-4), EFC10887 (5.3.5.1-5), EFC6014 (5.3.5.1-6), EFC6016 (5.3.5.1-7), and EFC10743 (5.3.5.1-8)

3.(iii).A.(1).2) Single-dose toxicity studies in rats (4.2.3.1-3 to 4.2.3.1-6)

Studies were conducted in which a single dose of 5000 μg/kg lixisenatide was subcutaneously or intravenously administered to male and female Wistar rats (5 animals/sex). No mortality was observed, and slight body weight loss or reduced body weight gain was observed. Although piloerection and decreased activity, etc. were observed after intravenous administration, all findings recovered.

A study was conducted in which a single dose of vehicle,[38] or 50, 125, or 500 μg/kg lixisenatide was subcutaneously administered to male and female Wistar rats (main study, 10 animals/sex/group; TK study, 10 animals/sex/group). No mortality was observed, and no effects of lixisenatide on clinical signs, etc. were observed.

A study was conducted in which a single dose of vehicle[38] or 1.0, 2.5, or 10 μg/kg lixisenatide was intravenously administered to male and female Wistar rats (main study, 10 animals/sex/group; TK study, 10 animals/sex/group). No mortality was observed, and no effects of lixisenatide on clinical signs, etc. were observed.

Based on the above, the approximate lethal dose by subcutaneous or intravenous administration in rats was determined to be >5000 μg/kg, and the no-observed-adverse-effect levels (NOAELs) for a single subcutaneous dose and a single intravenous dose were determined to be 500 and 10 μg/kg, respectively.

3.(iii).A.(1).3) Single-dose toxicity studies in dogs (4.2.3.1-7, 4.2.3.1-8)

A study was conducted in which a single dose of vehicle[38] or 10, 40, or 200 μg/kg lixisenatide were subcutaneously administered to male and female beagle dogs (4 animals/sex/group). No mortality was observed, and no effects of lixisenatide on clinical signs, etc. were observed.

A study was conducted in which a single dose of vehicle[38] or 5, 20, or 100 μg/kg lixisenatide was intravenously administered[39] to male and female beagle dogs (4 animals/sex/group). No mortality was observed, and, food consumption was reduced 1 to 2 days post-dose in females in the lixisenatide groups and males at ≥20 μg/kg.

Based on the above, the NOAELs for a single subcutaneous dose and a single intravenous dose in dogs were determined to be 200 and 100 μg/kg, respectively.

3.(iii).A.(2) Repeat-dose toxicity

Mouse (13 weeks), rat (5 days, 2 weeks, 13 weeks, 6 months), and dog (4 weeks, 13 weeks, 8 months, 12 months) repeated subcutaneous dose toxicity studies, and a rat (4 weeks) repeated intravenous dose toxicity study were conducted. Body weight loss or reduced body weight gain, and decreased food consumption due to the pharmacologic action of lixisenatide were observed in rats and dogs. Dilatation of seminiferous tubules and sperm stasis, etc. in the testis and epididymis were observed in dogs, and their relationships to lixisenatide were suggested. Anti-lixisenatide antibody formation was observed after ≥13 weeks of dosing in all animal species, but no findings suggestive of neutralization of lixisenatide activity or the deposition of antigen-antibody complexes (e.g., glomerulonephritis) were observed. The ratio of lixisenatide exposure

[38] Phosphate buffered saline
[39] A 30-minute intravenous infusion via the cephalic vein

($AUC_{0\text{-}24\ h}$) at the NOAEL of the mouse 13-week study, the rat 6-month study, or the dog 12-month study to the human exposure ($AUC_{0\text{-}24\ h}$) at the clinical dose[40] was as follows: 1015 and 647 for female and male mice, respectively, 2620 and 1770 for female and male rats, respectively, and 6.3 and 1690 for female and male dogs, respectively.

3.(iii).A.(2).1) Thirteen-week subcutaneous toxicity study in mice (4.2.3.4.2-2)

A study was conducted in which vehicle[41] or 20, 200, 1000, or 2000 μg/kg lixisenatide was subcutaneously administered BID for 13 weeks[42] to male and female CD-1 mice (main study, 10 animals/sex/group; TK study,[43] 6 animals/sex in the control group and 66 animals/sex/dose in lixisenatide groups). Markedly increased body weight gain during the early phase of administration and a trend towards higher incidences, and increased severity of, inflammation and fibrosis at the injection site were observed in the lixisenatide groups. Decreases in food and water consumption at Week 1 were observed in females treated with lixisenatide and males at ≥200 μg/kg. There was a trend towards an increase in glycogen vacuolation in the liver in females at ≥200 μg/kg and males at ≥1000 μg/kg, which was considered due to glycogen accumulation attributable to the pharmacologic action of lixisenatide. Based on the above, the NOAEL was determined to be 2000 μg/kg.

3.(iii).A.(2).2) Two-week subcutaneous toxicity study in rats with a two-week recovery period (4.2.3.2-4)

A study was conducted in which vehicle[41] or 2, 20, or 200 μg/kg lixisenatide was subcutaneously administered BID for 2 weeks to male and female SD rats (main study, 10 animals/sex/group; TK study, 12 animals/sex/dose in lixisenatide groups and 2 animals/sex in the control group; a 2-week recovery study, 5 animals/sex in each of the vehicle and 200 μg/kg groups). No mortality was observed. Reduced body weight gain and decreased food consumption in the lixisenatide groups; decreased splenic weight in males; hypoactivity in males at ≥2 μg/kg and females at ≥20 μg/kg; increased adrenal weight and decreased thymic weight in males at ≥20 μg/kg; and increased severity of mixed inflammatory cell infiltrate at the injection site at 200 μg/kg were observed. Reversibility was seen in recovery group animals. Based on the above, the NOAEL was determined to be 200 μg/kg.

3.(iii).A.(2).3) Thirteen-week subcutaneous toxicity study in rats with a four-week recovery period (4.2.3.2-14)

A study was conducted in which vehicle[41] or 5, 20, 200, 1000, or 2000 μg/kg lixisenatide was subcutaneously administered BID for 13 weeks[44] to male and female SD rats (main study, 10 animals/sex/group; TK study, 18 animals/sex/dose in lixisenatide groups and 3 animals/sex in the control group; a 4-week recovery study, 5 animals/sex in each of the vehicle, 200, and 2000 μg/kg groups). Lethargy, mouth rubbing, salivation, and paddling in the lixisenatide groups; and reduced body weight gain and decreased food consumption, a trend towards decreased white blood cell count, changes in red blood cell parameters, and increased adrenal weight, etc. in males were observed, which showed no definite dose-dependency and were not associated with

[40] Compared to the maximum exposure in Study ACT6011 (anti-lixisenatide antibody-positive patients, 20.1 ng·h/mL).
[41] 0.9% saline
[42] As dosing solutions were prepared without correction for peptide content, the actual doses were 16.6, 165.6, 828.2, and 1656.4 μg/kg.
[43] As the presence of anti-lixisenatide antibodies precluded analysis of the samples collected at Week 13, an additional study was conducted in which 0 (vehicle), 200, 1000, or 2000 μg/kg lixisenatide was administered BID for 13 weeks to male and female CD-1 mice (TK study, 66 animals/sex/dose in lixisenatide groups and 12 animals/sex in the control group; anti-lixisenatide antibody assay, 20 animals/sex/dose in lixisenatide groups and 20 animals/sex in the control group).
[44] As dosing solutions were prepared without correction for peptide content, the actual doses were 4.1, 16.6, 165.6, 828.2, and 1656.4 μg/kg.

histopathological changes. Thus, these findings were considered of low toxicological significance. Based on the above, the NOAEL was determined to be 2000 μg/kg.

3.(iii).A.(2).4) Six-month subcutaneous toxicity study in rats with a four-week recovery period (4.2.3.2-15)

A study was conducted in which vehicle[41] or 5, 100, or 2000 μg/kg lixisenatide was subcutaneously administered BID for 6 months to male and female SD rats (main study, 15 animals/sex/group; TK study, 18 animals/sex/dose in lixisenatide groups and 3 animals/sex in the control group; a 4-week recovery study, 5 animals/sex in each of the vehicle and 2000 μg/kg groups). Squeaky noises during subcutaneous administration, reduced body weight gain, decreased food consumption, increased adrenal weight, and decreased splenic weight in the lixisenatide groups; and increased incidence and severity of fibrosis at the injection site in males at ≥100 μg/kg were observed, but reversibility was suggested in recovery group animals. In addition, the incidences of atrophy/necrosis of seminiferous tubules and aspermia/oligospermia were increased in males at 2000 μg/kg (6 of 15 animals each), which were considered unrelated to lixisenatide because these findings were also observed in 4 of 15 control rats of the same strain in the same period at the testing facility and atrophy of seminiferous tubules is known as a background finding observed in aged rats (≥1 year-old).[45] Based on the above, the NOAEL was determined to be 2000 μg/kg.

3.(iii).A.(2).5) Four-week intravenous toxicity study in rats (4.2.3.2-10)

A study was conducted in which vehicle[46] or 3, 10, or 30 μg/kg lixisenatide was intravenously administered BID for 4 weeks to male and female SD rats (main study, 10 animals/sex/group; TK study, 9 animals/sex/dose in lixisenatide groups). No mortality was observed. Abnormal gait, piloerection, hypoactivity, irregular/fast respiration, hunched posture and eyelids partially closed, and decreased food consumption and increased water consumption during early phase of dosing in the lixisenatide groups; and reduced body weight gain in males in the lixisenatide groups were observed. Based on the above, the NOAEL was determined to be 30 μg/kg.

3.(iii).A.(2).6) Four-week subcutaneous toxicity study in dogs (4.2.3.2-17)

A study was conducted in which vehicle[41] or 10, 40, or 200 μg/kg lixisenatide was subcutaneously administered BID for 4 weeks to male and female beagle dogs (3 animals/sex/group). Markedly decreased food consumption during the early phase of administration and a trend towards higher incidences of inflammation and fibroblast proliferation at the injection site in the lixisenatide groups, and reduced body weight gain in females at ≥10 μg/kg and males at ≥40 μg/kg were observed. Based on the above, the NOAEL was determined to be 200 μg/kg.

3.(iii).A.(2).7) Thirteen-week subcutaneous toxicity study in dogs with a four-week recovery period (4.2.3.2-19)

A study was conducted in which vehicle[41] or 20, 300/100, or 1000/400/250[47] μg/kg lixisenatide was subcutaneously administered BID for 13 weeks to male and female beagle dogs (3 animals/sex/group; a 4-week recovery study, 2 animals/sex in each of the vehicle and 1000/400/250 μg/kg groups). Body weight

[45] Lee KP et al. *Toxicologic Pathology*. 1993;21:292-302, Greaves P & Facini JM., *Rat Histopathology*, Elsevier Science Publishers. 1984;163.
[46] Sodium citrate buffer
[47] High dose was reduced from 1000 μg/kg BID to 400 μg/kg BID from Day 15 and to 250 μg/kg from Day 29 and mid dose was reduced from 300 μg/kg BID to 100 μg/kg BID from Day 29, due to substantial body weight loss.

loss, markedly decreased food consumption during the early phase of dosing, and increased severity of inflammation and fibrosis at the injection site in the lixisenatide groups; emaciation in females in the lixisenatide groups and males in the mid and high dose groups; vomiting in females in the lixisenatide groups and males in the high dose group; and sperm stasis and dilatation of seminiferous tubules in the mid and high dose groups were observed. These findings were reversible after a recovery period. Based on the above, the NOAELs were determined to be 20 and 250 μg/kg in males and females, respectively.

3.(iii).A.(2).8) Twelve-month subcutaneous toxicity study in dogs (4.2.3.2-20)

A study was conducted in which vehicle[41] or 2, 200, or 1000 μg/kg lixisenatide was subcutaneously administered BID for 12 months[48] to male and female beagle dogs (4 animals/sex/group). Reduced body weight gain or reduced body weight, decreased food consumption, and a trend towards increases in vomiting and vocalization were observed in the lixisenatide groups. In addition, vacuolation and atrophy of seminiferous tubules; increased incidence and severity of hypospermatogenesis; and oligospermia, aspermia, and tubular dilation and epithelial degeneration in the epididymis were found in the mid and high dose groups. Increased incidence and severity of sperm stasis in the testis and increased incidence and severity of fibrosis in subcutaneous tissues at the injection site were observed in the high dose group. Based on the above, the NOAELs were determined to be 2 and 1000 μg/kg in males and females, respectively.

3.(iii).A.(2).9) Eight-month toxicity study in juvenile male dogs (4.2.3.5.4-2)

A study was conducted[49] in which vehicle[41] or 5, 20, or 200 μg/kg lixisenatide was subcutaneously administered BID or 200 μg/kg lixisenatide once daily (QD) for 8 months[50] to male beagle dogs (16-17 weeks of age; 4 animals/group) (a 8-week recovery study, 2 animals in each of the vehicle control, pair-fed vehicle control, 200 μg/kg QD, and 200 μg/kg BID groups). Emaciation, reduced skin elasticity, absent food intake, uncoordinated gait, trembling and twitching, and transient decrease in activity were observed in Weeks 1 and 2 in the 200 μg/kg BID group. In addition, reduced body weight gain in the lixisenatide groups and dilatation of seminiferous tubules, an increase in vacuolation, and sperm stasis in the testis, tubular dilatation and epithelial degeneration and oligospermia in the epididymis, and increased incidence and severity of mixed cellular infiltrate and fibrosis at the injection site at ≥20 μg/kg BID were observed. The changes noted during the dosing period were reversible. The mean body weight in the pair-fed vehicle control group was shown to be comparable to that in the 200 μg/kg BID group. Based on the above, the NOAEL was determined to be 5 μg/kg. The AUC was 49.4 ng·h/mL on Day 245 in the 5 μg/kg BID group, which was 2.5-fold of the human exposure at the clinical dose.[40]

3.(iii).A.(3) Genotoxicity (4.2.3.3.1-1 to 4.2.3.3.1-6, 4.2.3.3.2-1)

Bacterial reverse mutation tests, *in vitro* chromosomal aberration tests in human lymphocytes, and mouse bone marrow micronucleus test at single intravenous doses were conducted. All of these tests produced negative results and it was concluded that lixisenatide has no genotoxic potential.

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[48] Dose titration every 3 days in mid (200 μg/kg) and high dose (1000 μg/kg) groups; all dose groups started with 2 μg/kg BID, mid dose reached definitive dose after 31 days, high dose after 85 days.

[49] The pair-fed vehicle control group with the same amount of diet as the 200 μg/kg BID group was also included in the study to investigate the effect of restricted feeding.

[50] The dose was titrated upwards in 3 steps, 25%, 50%, and 100% of the final dose, at an interval of 4 days.

3.(iii).A.(4) Carcinogenicity

Mouse and rat 104-week subcutaneous carcinogenicity studies were conducted, in which an increase in thyroid C-cell adenoma was observed in male mice at 1000 μg/kg and male and female rats in the lixisenatide groups. Thyroid C-cell carcinoma occurred in male and female rats at ≥200 μg/kg. Since thyroid C-cell proliferation in rodents has also been reported with another GLP-1 receptor agonist[51] and thyroid C-cells show higher GLP-1 receptor expression in rodents compared to humans,[52] these findings were considered class effects. In addition, the results of mechanistic studies on the proliferative effects of lixisenatide on C-cells (4.2.3.4.3) supported that these effects were caused by a similar mechanism to that of other GLP-1 receptor agonists, and it was concluded that the proliferative changes in C-cells observed in the mouse and rat carcinogenicity studies are unlikely to cause a clinically relevant problem.

3.(iii).A.(4).1) One-hundred-four-week subcutaneous carcinogenicity study in mice (4.2.3.4.1-1)

A study was conducted in which vehicle[41] or 40, 200, or 1000 μg/kg lixisenatide was subcutaneously administered BID for 2 years to male and female CD-1 mice (main study, 60 animals/dose/sex/group; TK study, 51 animals/sex/dose in lixisenatide groups and 18 animals/sex in the control group; anti-lixisenatide antibody assay, 12 animals/sex/dose in lixisenatide groups and 6 animals/sex in the control group). Increased body weight gain and increased incidence of abdominal distension were observed in the lixisenatide groups. An increase in thyroid C-cell adenoma in males at 1000 μg/kg (4 of 60 animals), and an increase in focal C-cell hyperplasia in males at 200 μg/kg and females at 1000 μg/kg (8 of 59 animals and 11 of 59 animals, respectively) were observed. Although thyroid C-cell adenoma occurred in 1 male each in the 40 and 200 μg/kg groups, there were no significant differences in the total incidence of hyperplasia and adenoma between the control group and the 40 μg/kg group and the incidence was within the range of spontaneous incidence reported in the historical control database of the animal supplier (up to 2.0% in males and up to 3.3% in females) and that of the Registry of Industrial Toxicology Animal-data (RITA) (0%-1.7% in males and 0% in females); therefore, thyroid C-cell adenoma observed in 1 male in the 40 μg/kg group was considered to be incidental. In addition, endometrial adenocarcinoma occurred in the 200 and 1000 μg/kg groups (3 of 60 animals in the 200 μg/kg group, 2 of 60 animals in the 1000 μg/kg group), but these were considered spontaneous tumors because a significant difference was observed only for the 200 μg/kg group; the incidence (5%) at 200 μg/kg was within the range of the spontaneous incidence of endometrial adenocarcinoma in mice of the same strain in the historical control database of RITA (0%-8%); no increases were found in the incidence of endometrial gland hyperplasia; and no lixisenatide-related findings were observed in the ovary or vagina.[53] Based on the above, the NOAELs were determined to be 40 and 200 μg/kg in males and females, respectively. The $AUC_{0\text{-}24\ h}$ values on Day 176 were 67.3 and 706 ng·h/mL in males in the 40 μg/kg group and females in the 200 μg/kg group, respectively, which were 3.3- and 35-fold, respectively, of the human exposure at the clinical dose.[40]

3.(iii).A.(4).2) One-hundred-four-week subcutaneous carcinogenicity study in rats (4.2.3.4.1-2)

A study was conducted in which vehicle[41] or 40, 200, or 1000 μg/kg lixisenatide was subcutaneously administered BID for 2 years to male and female SD rats (main study, 60 animals/sex/group; TK study and

[51] FDA: Center for Drug Evaluation and Research. NDA 21-773 - Pharmacology Review. FDA: Liraglutide: Pharmacology/Toxicology review of thyroid c-cell tumors in rats and mice, 2009

[52] Korner M et al. *J Nucl Med.* 2007;48(5):736-743, Crespel A et al. *Endocrinology.* 1996;137:3674-3680.

[53] Squamous epithelial hyperplasia was observed in all groups, and the incidence was increased in the 1000 μg/kg group compared to the control group; however, these were spontaneous changes as observed also in the control group and considered of no toxicological significance.

anti-lixisenatide antibody assay, 18 animals/sex/dose in lixisenatide groups and 6 animals/sex in the control group).

Reduced body weight gain, abdominal distention, and salivation and increases in C-cell adenoma and focal hyperplasia in the lixisenatide groups, and thyroid C-cell carcinoma at ≥200 μg/kg (200 μg/kg group, 3 of 58 males and 1 of 55 females; 1000 μg/kg group, 1 of 60 males and 2 of 59 females) were observed. The NOAELs for C-cell adenoma and focal hyperplasia could not be determined, and the NOAEL for C-cell carcinoma was determined to be 40 μg/kg. The $AUC_{0\text{-}24\ h}$ values on Day 359 were 8290 and 6620 ng·h/mL in males and females, respectively, in the 40 μg/kg group, which were 412- and 329-fold, respectively, of the human exposure at the clinical dose.[40]

3.(iii).A.(5) Reproductive and developmental toxicity

A study of fertility and early embryonic development to implantation in rats, studies for effects on embryo-fetal development in rats, studies for effects on pre- and postnatal development, including maternal function, in rats, and studies for effects on embryo-fetal development in rabbits were conducted. Retarded ossification was observed in the studies for effects on embryo-fetal development in rats and rabbits, which was considered a secondary change associated with maternal toxicity for the following reasons: (1) lixisenatide is unlikely to directly affect the embryo and fetus because the placental transfer of lixisenatide is less likely (4.2.2.3-3 to 4.2.2.3-4) and (2) retardation of fetal growth and ossification associated with a decrease in maternal food consumption has been reported with other GLP-1 receptor agonists as well.[54]

3.(iii).A.(5).1) Study of fertility and early embryonic development to implantation in rats (4.2.3.5.1-1)

A study was conducted in which vehicle[41] or 2, 29, or 414 μg/kg lixisenatide was subcutaneously administered BID[55] to male and female SD rats (24 animals/sex/group). Subdued behavior and salivation in the lixisenatide groups; reduced body weight gain and decreased food consumption from 1 to 29 days after the initiation of administration (pre-mating period) in males in the lixisenatide groups; decreased food consumption during the pre-mating period and gestation in females in the lixisenatide groups; and changes in body weight gain during gestation (reduction from Days 3 to 6 of gestation, and increase from Days 6 to 10 of gestation) in the 414 μg/kg group were observed. Based on the above, the NOAEL was determined to be 414 μg/kg.

3.(iii).A.(5).2) Study for effects on embryo-fetal development in pregnant rats (a dose-finding study) (4.2.3.5.2-1)

A study was conducted in which vehicle[41] or 70, 1000, or 2000 μg/kg lixisenatide was subcutaneously administered BID from Days 6 to 17 of gestation to female SD rats that were confirmed to have mated (6 animals/group). In maternal animals, hypoactivity, bristling coat, decreased food consumption, reduced body weight gain, and decreased placental weight were observed in the lixisenatide groups. In the embryo and fetus, fetal growth retardation (decreased fetal body weight and decreased crown-rump length) in the lixisenatide groups, and early resorption and increased mortality at ≥1000 μg/kg were observed.

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[54] Brent RL et al. *Birth Defects:* Original Article Series. 1985;21(5):1-8. Cappon GD et al. *Birth Defects Research (Part B).* 2005;74:424-430. Chahoud I.et al. *Brazilian Journal Medical and Biological Research.* 2005; 38:565-75. FDA: Center for Drug Evaluation and Research. NDA 21-773 - Pharmacology Review. Available.

[55] From 4 weeks prior to mating, throughout mating, and until the day before post-mating autopsy for males and from 2 weeks prior to mating, throughout mating, and until Day 6 of gestation for females.

3.(iii).A.(5).3) Study for effects on embryo-fetal development in rats (4.2.3.5.2-2)

A study was conducted in which vehicle[41] or 2.5, 35, or 500 μg/kg lixisenatide was subcutaneously administered BID from Days 6 to 17 of gestation (until Day 12 of gestation in the TK group) to female SD rats that were confirmed to have mated (main study, 32 animals in the control group and 30 animals/dose in lixisenatide groups; TK study, 3 animals in the control group and 15 animals/dose in lixisenatide groups). In maternal animals, decreased motor activity, piloerection, and reduced body weight gain throughout the dosing period, and markedly decreased food consumption during early phase of dosing were observed in the lixisenatide groups. In the embryo and fetus, a trend towards retarded ossification (including multiple skeletal malformations observed in 1 animal in each group) in the lixisenatide groups, and body weight loss and decreased fetal crown-rump length in the 500 μg/kg group were observed. Skeletal malformations were considered to be hypoplasias rather than malformations for the reason that malformations mainly consisted of long bone bent at the site of insufficient ossification in the fetuses and incomplete ossification was generally noted. Based on the above, the NOAELs for maternal and embryo-fetal developmental toxicity were determined to be <2.5 μg/kg.

3.(iii).A.(5).4) Study for effects on embryo-fetal development in pregnant rabbits (a dose-finding study) (4.2.3.5.2-5)

A study was conducted in which vehicle[41] or 40, 200, or 1000 μg/kg lixisenatide was subcutaneously administered BID from Days 6 to 18 of gestation to female Himalayan rabbits that were confirmed to have mated (6 animals/group). In maternal animals, hypoactivity, bristling coat, body weight loss, and decreased food consumption were observed in the lixisenatide groups, and premature delivery was noted in the 1000 μg/kg group. In the embryo and fetus, body weight loss and decreased crown-rump length were noted in the 1000 μg/kg group.

3.(iii).A.(5).5) Study for effects on embryo-fetal development in rabbits (4.2.3.5.2-6)

A study was conducted in which vehicle[41] or 2.5, 25, or 250 μg/kg lixisenatide was subcutaneously administered BID from Days 6 to 18 of gestation (from Days 6 to 12 of gestation in the TK group) to female Himalayan rabbits that were confirmed to have mated (main study, 20 animals/group; TK study, 3 animals in the control group and 9 animals/dose in lixisenatide groups). In maternal animals, decreased motor activity, piloerection, body weight loss, and decreased food consumption were observed in the lixisenatide groups during the dosing period. In the embryo and fetus, multiple malformations[56] (2 animals each in the 2.5 and 25 μg/kg groups) and retarded ossification in the lixisenatide groups; small gallbladder and absent gallbladder in the 25 and 250 μg/kg groups (1 animal each in the 25 μg/kg group, 2 animals each in the 250 μg/kg group); and a trend towards decreased survival rate and increased incidence of supernumerary 13th rib in the 250 μg/kg group were observed. Four cases of multiple malformations in the lixisenatide groups were not dose-related and multiple malformations were seen only in the control group in a second study[57]; therefore, these were considered attributable to genetic and environmental factors. The small gallbladder was considered incidental because it spontaneously occurs in rabbits of the same strain,[58] and the supernumerary rib was considered related to maternal toxicity.[59] Based on the above, the NOAEL could not be determined, and a second study was conducted.

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[56] Thoracoabdominal closure defects, absence of the trunk, and neural tube closure defect, etc.
[57] Multiple malformations, including thoracogastroschisis, absent trachea, and absent gallbladder, etc.
[58] Viertel B et al. *Laboratory Animal.* 2003;37(1):19-36
[59] Beyer PE et al. *Teratogenesis Carcinogenesis and Mutagenesis.* 1986;6:419-429

3.(iii).A.(5).6) Study for effects on embryo-fetal development in rabbits (a second study) (4.2.3.5.2-9)

A study was conducted in which vehicle[41] or 0.15, 1, or 2.5 μg/kg lixisenatide was subcutaneously administered BID from Days 6 to 18 of gestation (from Days 6 to 12 of gestation in the TK group) to female Himalayan rabbits that were confirmed to have mated (main study, 20 animals/group; TK study, 3 animals in the control group and 9 animals/dose in lixisenatide groups). In maternal animals, decreased motor activity, piloerection, body weight loss, and decreased food consumption were observed at ≥1μg/kg. Based on the above, the NOAELs for maternal and embryo-fetal toxicity were determined to be 0.15 and 2.5 μg/kg, respectively. The $AUC_{0\text{-}24\,h}$ values at the NOAELs on Day 12 of gestation were 0.929 and 41.5 ng·h/mL in the 0.15 and 2.5 μg/kg groups, respectively, which were 1.1- and 49-fold, respectively, of exposure[60] at the clinical dose.

3.(iii).A.(5).7) Study for effects on pre- and postnatal development, including maternal function, in rats (a dose-finding study) (4.2.3.5.3-1)

A study was conducted in which a vehicle[42], or 2.5, 35, or 500 μg/kg lixisenatide was subcutaneously administered BID from Day 6 of gestation to Day 3 of lactation to pregnant SD rats (6 animals/group). In maternal animals, decreased motor activity, piloerection, body weight loss, decreased food consumption, and reduced rearing and suckling behavior were observed in the lixisenatide groups. In the F_1 litters, a trend towards growth retardation was noted in the lixisenatide groups, and a decrease in postnatal survival to day 4 was observed in the 500 μg/kg group.

3.(iii).A.(5).8) Study for effects on pre- and postnatal development, including maternal function, in rats (4.2.3.5.3-2)

A study was conducted in which vehicle[41] or 2, 20, or 200 μg/kg lixisenatide was subcutaneously administered BID from Day 6 of gestation to Day 20 of lactation to female SD rats that were confirmed to have mated (24 animals/group). In maternal animals, decreased motor activity, piloerection, body weight loss, and decreased food consumption were observed in the lixisenatide groups. In the F_1 litters, a trend towards reduced body weight gain until postnatal day 14 in males in the lixisenatide groups; delayed coat growth at ≥20 μg/kg; and increased pup mortality in the 200 μg/kg group were observed. No effects of lixisenatide were observed in F1 animals after weaning. Based on the above, the NOAEL for maternal toxicity could not be determined, and the NOAEL for pre-and postnatal developmental toxicity was determined to be 2 μg/kg.

3.(iii).A.(6) Local tolerance (4.2.3.6-1 to 4.2.3.6-3)

A study was conducted in which 0.5 mL of a control solution (saline) and a formulation containing 100 μg/mL lixisenatide was given by subcutaneous, intramuscular, intravenous, or intraarterial administration, or 0.1 mL by paravenous administration to female New Zealand White rabbits (4 animals/group) (including an evaluation of the reversibility of local lesions by histology at 120 hours post-dose [2 animals/group]). Lixisenatide showed moderate local tolerability (minimal to mild necrosis and fibrosis with mixed inflammatory cells) after subcutaneous administration, good or limited local tolerability (partial changes in connective tissues with mixed inflammatory cells) after intravenous or intraarterial administration, respectively, and poor local tolerability (vascular necrosis with mixed inflammatory cells, etc.) after paravenous and intramuscular administration. When the local tolerability of a placebo formulation not

[60] Compared with the exposure in anti-lixisenatide antibody-negative patients (AUC, 0.848 ng·h/mL) as the duration of exposure in embryo-fetal toxicity studies was considered insufficient to induce adequate anti-lixisenatide antibody formation.

containing lixisenatide was tested under the same conditions, moderate local tolerability of placebo was observed after subcutaneous administration, good local tolerability of placebo after intravenous administration, poor local tolerability of placebo after intramuscular and paravenous administration, and limited local tolerability of placebo after intraarterial administration. When a modified formulation in which EDTA and histidine were removed without changing the concentration of lixisenatide was administered, the modified formulation showed good local tolerability after intramuscular and subcutaneous administration and poor local tolerability after paravenous administration. Based on the above, the local irritation observed with the unmodified formulation (including the placebo formulation) was considered attributable to specific excipients (EDTA and histidine), and thus they were not used in the formulation for clinical studies or the proposed commercial formulation.

3.(iii).A.(7) Other toxicity studies

3.(iii).A.(7).1) Mechanistic studies on the proliferative effects of lixisenatide on thyroid C-cells

The following studies were conducted to investigate the mechanism of the proliferative effects of lixisenatide on thyroid C-cells observed in the carcinogenicity studies of lixisenatide.

3.(iii).A.(7).1).(a) GLP-1 receptor expression in thyroid C-cells and follicular cells of rats and humans (4.2.3.4.3-3 to 4.2.3.4.3-6, Reference data)

A study was conducted to quantify calcitonin and GLP-1 receptor expression using RNA samples isolated from C-cells and follicular cells in thyroid tissues from untreated male rats (4 and 12 months of age).[61] In rats, GLP-1 receptor expression was observed in thyroid C cells while little GLP-1 receptor expression was detected in follicular cells. In addition, a study was conducted to quantify calcitonin, thyroglobulin, and GLP-1 receptor expression using RNA samples isolated from C-cells and follicular cells in thyroid tissues from humans with no pathology (formalin-fixed or frozen tissues).[61] As a result, little or no GLP-1 receptor expression was detected in either C-cells or follicular epithelial cells in human thyroid tissues.

3.(iii).A.(7).1).(b) Functional activity of lixisenatide in rat versus human C-cells (4.2.3.4.3-7, Reference data)

Lixisenatide, GLP-1 (7-36) amid, exendin-4, and liraglutide (which are GLP-1 receptor agonists) and GIP (a GIP receptor agonist) were tested in a functional cAMP assay for effects on a rat thyroid C-cell carcinoma cell line [redacted] (a rat cell line) and a human medullary thyroid carcinoma cell line [redacted] (a human cell line). There was a strong response to all the four GLP-1 receptor agonists in the rat cell line compared to a weak response in the human cell line. In contrast, GIP as a control induced strong responses in both cell lines. In conclusion, there are species differences in GLP-1 receptor responsiveness between rats and humans.

3.(iii).A.(7).1).(c) Effects on calcitonin release and proliferation markers in mice (4.2.3.4.3-8)

A study was conducted in which vehicle[41] QD, 2000 μg/kg lixisenatide QD, or 2000 μg/kg lixisenatide BID was subcutaneously administered for 1 day to male and female CD-1 mice (main study, 10 animals/sex/group; measurement of calcitonin, 12 animals/sex in the control group and 24 animals/sex in each of the lixisenatide QD and BID groups), and reversibility was observed after a 5-day recovery period (5 animals/sex/group). Lixisenatide injection resulted in increased calcitonin values during the test period of 24 hours when compared to pre-test or control values (approximately 2-fold).

[61] Calcitonin expression levels were used as an index of purity of each fraction.

3.(iii).A.(7).1).(d) Effects on calcitonin release and proliferation markers in rats (4.2.3.4.3-9)

A study was conducted in which vehicle[41] QD, 2000 μg/kg lixisenatide QD, or 2000 μg/kg lixisenatide BID was subcutaneously administered for 1 day to male and female SD rats (main study, 10 animals/sex/group; calcitonin study, 6 and 12 animals/each of the lixisenatide QD and BID groups), and reversibility was observed after a 5-day recovery period (5 animals/sex/group). No increased calcitonin levels were observed after administration of lixisenatide compared with pre-test or control values.

3.(iii).A.(7).1).(e) Calcitonin release in CD-1 mice and GLP-1R(-/-)KO mice (4.2.3.4.3-10, Reference data)

A study was conducted in which vehicle[41] or 1000 μg/kg lixisenatide was subcutaneously administered BID for 2 weeks to male and female CD-1 mice and GLP-1 receptor knockout mice (KO mice) (8 animals/group). In CD-1 mice, body weight loss and decreased food consumption were observed in males in the lixisenatide group, and increased calcitonin concentration at the end of the dosing period was noted in males and females in the lixisenatide group. In KO mice, body weight was reduced and calcitonin concentration remained low in males and females in the lixisenatide group. Calcitonine concentrations observed in CD-1 and KO mice from the control groups were similar. The above findings suggested that the effects of lixisenatide on thyroid C-cells are mediated by the GLP-1 receptor.

3.(iii).A.(7).2) Mechanistic study on testicular and epididymal findings in dogs (4.2.3.7.3-1, Reference data)

GLP-1 receptor expression in the testis and epididymis (rats, dogs, humans) was measured using RNA samples to assess the human relevance of testicular and epididymal findings observed in dog repeat-dose toxicity studies of ≥13 weeks duration. By segment (caput, corpus, cauda) of the epididymis, there were large species differences in GLP-1 receptor expression in the caput epididymidis and the GLP-1 receptor was expressed ≥4000-fold higher in dogs compared to rats and 3.8-fold higher compared to humans. In whole epididymis, the GLP-1 receptor was expressed 184-fold higher in dogs compared to rats and 10-fold higher compared to humans. In testicular samples, the GLP-1 receptor was expressed 100-fold higher in dogs compared to rats and 10-fold higher compared to humans. Based on the above, the expression levels of the GLP-1 receptor in the epididymis and testis were higher in dogs compared to rats and humans, suggesting that dogs may be more susceptible for testicular and epididymal GLP-1 receptor activation than rats and humans.

3.(iii).A.(7).3) Qualification of impurities, etc.

The following studies were conducted to qualify impurities and batches for use in clinical studies (clinical batches).

3.(iii).A.(7).3).(a) Fourteen-day repeated subcutaneous dose toxicity study in mice with batch stored under stress conditions (4.2.3.7.6-1)

A study was conducted in which vehicle[41] or 2, 20, or 200 μg/kg lixisenatide[62] was subcutaneously administered BID for 14 days to male and female CD-1 mice (10 animals/sex/group). At ≥20 μg/kg, increased body weight gain was observed as in a mouse 13-week repeated subcutaneous dose toxicity study

[62] Batch stored under stress conditions at 40°C for 1 month (the impurity content was 10.4%).

(4.2.3.4.2-2), but no effects of impurities were found. Based on the above, the NOAEL was determined to be 200 μg/kg.

3.(iii).A.(7).3).(b) Fourteen-day repeated subcutaneous dose toxicity study in rats with clinical batch (4.2.3.7.6-2)

A study was conducted in which vehicle[41] or 2, 20, or 200 μg/kg lixisenatide[63] was subcutaneously administered BID for 14 days to male and female SD rats (10 animals/sex/group). In the lixisenatide groups, reduced body weight gain, decreased food consumption, and increased water consumption were observed, but no effects of impurities were found. Based on the above, the NOAEL was determined to be 200 μg/kg.

3.(iii).A.(7).3).(c) Thirteen-week repeated subcutaneous dose toxicity study in rats for qualification of impurities (4.2.3.7.6-3)

A study was conducted in which vehicle[41] or 200 μg/kg lixisenatide[64] was subcutaneously administered BID for 13 weeks to male and female SD rats (10 animals/sex/group). Salivation, reduced body weight gain, decreased food consumption, and injection site fibrosis were observed in the lixisenatide group, but no differences of toxicological significance were found between the drug products stored under non-stress and stress conditions.

3.(iii).B Outline of the review by PMDA

3.(iii).B.(1) Testicular findings

Regarding the testicular and epididymal findings observed in dog repeat-dose toxicity studies of ≥13 weeks duration, PMDA asked the applicant to explain whether there are any concerns regarding safety in the testis of humans, taking account of the binding affinity of lixisenatide to the target site in dogs and humans, species differences in pharmacologic activity, differences in GLP-1 receptor expression in epididymal tissues (caput, corpus, cauda), clinical study results of lixisenatide, and clinical experience with other GLP-1 receptor agonists, etc.

The applicant responded as follows:

Although the binding affinity of lixisenatide to the GLP-1 receptor in other animal species (non-human) has not been determined, it has been reported that the binding affinity of other GLP-1 receptor agonists to the GLP-1 receptor was comparable across mammalian species (pigs, rabbits, mice) (Kunuddsen L. et al. *Regulatory Peptides.* 2012;175:21-29). Thus, it is inferred that any GLP-1 receptor agonist with human GLP-1 receptor activity has comparable activity at the GLP-1 receptor across mammalian species. An analysis of GLP-1 receptor expression in human, dog, and rat epididymal tissues (caput, corpus, cauda) revealed that the highest expression level of the receptor was observed in the epididymal caput of dogs, which was 3.8-fold higher compared to the epididymal caput of humans. This finding suggests that dogs are more susceptible for epididymal GLP-1 receptor activation. Furthermore, we requested an expert in male reproductive toxicity to review in detail the results of dog repeat-dose toxicity studies of lixisenatide, etc. As a result, the findings were considered to represent secondary changes (tubular dilation and epithelial degeneration, etc. in the epididymis) associated with increased back pressure resulting from increased fluid in the epididymis due to the inhibition of fluid resorption in the efferent ducts or the epididymal caput.

[63] Clinical batch (the impurity content was 0.6%).

[64] Batch stored under stress conditions at 40°C for 2 weeks (impurity concentration, 7.1%; impurity Des-(1-3) concentration, 0.54%) or batch of the same formulation as proposed for marketing, except for the lixisenatide concentration.

Although it is not clear if GLP-1 is involved in the regulation of fluids, secretin, which belongs to the glucagon family as does GLP-1, is known to control fluid and electrolyte transport in the caput epididymidis (Chow BK. et al. *Biological Reprod.* 2004;70:1594-1599) and considered to inhibit fluid resorption by stimulating chloride secretion into the lumen. Based on these findings, the testicular and epididymal effects observed in dogs are considered to be secondary changes due to GLP-1 receptor-mediated inhibitory effects of lixisenatide on fluid resorption. A study in healthy subjects to assess the effects of long-term treatment with lixisenatide on spermatogenesis (Study TDR11215,[65] 5.3.4.1-4) indicated that lixisenatide has no relevant effects on human spermatogenesis. Moreover, as no effects on sperm parameters (sperm concentration,[66] total sperm count, sperm motility, normal sperm morphology rate) or reproductive hormone concentrations[67] (total serum testosterone, serum free testosterone, follicle-stimulating hormone [FSH], luteinizing hormone [LH]) were observed, it was considered that lixisenatide has no adverse effects on human male reproductive function. Based on the above, the findings observed in dogs, including hypospermatogenesis, are species-specific and not considered to cause a clinically relevant problem.

PMDA considers as follows:
Since the non-inferiority of lixisenatide compared to placebo with respect to spermatogenesis was demonstrated and no effects were observed on sperm parameters or reproductive hormone concentrations etc. in Study TDR11215 in healthy subjects, the findings in dogs are unlikely to cause a clinically relevant problem. However, as the relationship between the inhibition of fluid resorption in the caput epididymidis and GLP-1 receptor activation by lixisenatide is undefined, the mechanism of development has not been adequately explained and in view of the ratio of lixisenatide exposure ($AUC_{0\text{-}24\ h}$) at the NOAEL of a dog repeated subcutaneous dose toxicity study to the human exposure ($AUC_{0\text{-}24\ h}$) in a clinical study,[68] safety in humans has not been adequately explained from a toxicological point of view.

Given that lixisenatide is intended to be administered for long periods and that the severity of testicular and epididymal findings tended to increase with prolonged treatment in dogs, PMDA asked the applicant to explain if a caution statement, etc. in the package insert are needed.

The applicant responded as follows:
Although a comparison by treatment duration indicated increasing seriousness of the testicular and epididymal findings, not all dogs with tubular dilation had degeneration of the initial segment of the epididymis in each study. Tubular dilation is considered to be caused by fluid pressure disturbance due to the dysfunction of the efferent ducts and initial segment, and epithelial degeneration is considered to be a delayed and progressive consequence of this functional disturbance. As it was considered that Study TDR11215 should cover multiple spermatogenic cycles in order to accurately assess potential effects on human spermatogenesis, a treatment duration of 26 weeks was selected for this clinical study so that subjects

[65] Lixisenatide 20 μg or placebo was subcutaneously administered QD for 26 weeks and the effects of lixisenatide on spermatogenesis were assessed based on the proportion (%) of subjects with ≥50% reduction in sperm concentration from baseline at the end of a 26-week treatment period. Non-inferiority was declared if the upper limit of the two-sided 95% CI for the difference for lixisenatide versus placebo was below 20%.
[66] Sperm analysis was performed twice at an interval of ≥48 hours and <120 hours at both baseline and Week 26 and means were calculated.
[67] Samples for measurement of sex hormones were collected pre-dose on Day 1 and on Days 28, 56, 84, 112, 140 and 181 and the final observation day (Day 196).
[68] Compared to the maximum exposure in Study ACT6011 (AUC in anti-lixisenatide antibody-positive patients, 20.1 ng·h/mL), the exposure at the NOAEL of a 12-month repeated subcutaneous dose toxicity study was approximately 6-fold and the exposure at the NOAEL of a 8-month repeated subcutaneous dose toxicity study in juvenile dogs was 2.5-fold.

were exposed to lixisenatide for 2 spermatogenic cycles[69] and no findings suggestive of the effects of lixisenatide on spermatogenesis and other sperm-related parameters, including reproductive hormones, were observed. Thus, it is unlikely that the findings in dogs cause a clinically relevant problem, but a caution statement will be included in the package insert from the standpoint of providing safety information.

PMDA accepted the applicant's response.

3.(iii).B.(2) Thyroid C-cell tumor

PMDA understands the opinion of the applicant that thyroid C-cell tumors observed in rat and mouse carcinogenicity studies are unlikely to cause a clinically relevant problem, based on the results of mechanistic studies on the proliferative effects of lixisenatide on C-cells (4.2.3.4.3) and the occurrence of thyroid C-cell tumors with other GLP-1 receptor agonists[70]; however, PMDA asked the applicant to explain if a caution statement in the package insert is needed, in view of the ratio of lixisenatide exposure ($AUC_{0\text{-}24\ h}$) at the NOAEL of a carcinogenicity study to the human exposure ($AUC_{0\text{-}24\ h}$) at the clinical dose.[71]

The applicant responded as follows:

In the mouse carcinogenicity study, C-cell carcinoma was not found, and the NOAELs for focal C-cell hyperplasia and C-cell adenoma were 40 and 200 μg/kg BID in males and females, respectively, which were 9.3- and 97-fold, respectively, of the human exposure ($AUC_{0\text{-}24\ h}$) at the clinical dose.[72] In the rat carcinogenicity study, the NOAEL for C-cell carcinoma was 40 μg/kg BID and the exposures at the NOAEL in males and females were 1140- and 913-fold the human exposure at the clinical dose ($AUC_{0\text{-}24\ h}$) and the NOAELs for focal C-cell hyperplasia and C-cell adenoma could not be determined. The proliferative changes in thyroid C-cells are considered to be caused by a nongenotoxic mechanism to which rodents are particularly sensitive, suggesting that there is no concern for humans at the intended clinical dose. However, a caution statement will be included in the package insert from the standpoint of providing safety information.

PMDA accepted the applicant's response. Tumorigenic risk in humans should continue to be reviewed in the clinical section [see "4.(iii). B.(5).9) Relationship with tumor development"]

4. Clinical Data

4.(i) Summary of biopharmaceutic studies and associated analytical methods

4.(i).A Summary of the submitted data

For the clinical development of lixisenatide, [redacted] A or B and a solution for injection were used (Table 5), and a solution for injection was used in Japanese clinical studies (evaluation data).

[69] Differentiation from spermatogonia to mature sperms requires approximately 3 months.
[70] FDA: Center for Drug Evaluation and Research. NDA 21-773 - Pharmacology Review. FDA: Liraglutide: Pharmacology/Toxicology review of thyroid c-cell tumors in rats and mice, 2009
[71] Compared to the maximum exposure in Study ACT6011 (anti-lixisenatide antibody-positive patients, 20.1 ng·h/mL), the exposures in males and females at the NOAELs of a mouse carcinogenicity study were 3.3- and 35-fold, respectively.
[72] Comparison with the mean exposure in Study ACT6011 (AUC in anti-lixisenatide antibody-positive patients, 7.25 ng·h/mL)

Table 5. Formulations used in studies

Type of study	Formulation	Concentration	Study No.
Phase I	[redacted] A	25 μg/mL[a)]	01-016
	Solution for injection	100 μg/mL	BDR6864, INT10408, INT10409, INT10782, INT10783, INT6052, INT6863, PDY10433, PDY11431, PDY11941, PDY11824, POP11320, POP11814, POP6053, TES6865, TES11807, TDR11215
Phase II	[redacted] B	25 μg/mL[a)]	ACT6011
	Solution for injection	100 μg/mL	PDY10931, PDY6797[b)], DRI6012
Phase III	Solution for injection	100 μg/mL	EFC6015[b)], EFC6018[b)], EFC6019, EFC10887[b)], EFC6014, EFC6016, EFC10743, EFC10780, LTS10888[b)]
Bioequivalence	Solution for injection	50 μg/mL	BEQ11094
		100 μg/mL	

Composition is different between [redacted] A and B.
a) Concentration when [redacted] with [redacted] mL
b) Japanese clinical studies (including multinational studies)

ELISA method (1st to 3rd generation) was used for quantitative determination of lixisenatide in human biomaterials, and the lower limit of quantification for total lixisenatide concentration in plasma was 20 pg/mL for the 1st generation and 11.87 pg/mL for the 2nd and 3rd generations.

As the reference data on biopharmaceutics, the results from a bioequivalence study[73] (BEQ11094) and a relative bioavailability study of lixisenatide administered at different injection sites in obese subjects (BDR6864) were submitted. The results from Study BDR6864 are described below.

Relative bioavailability study of lixisenatide administered at different injection sites in obese subjects (5.3.1.2-2, BDR6864 [[redacted] 20[redacted] to [redacted] 20[redacted]] Reference data)

A randomized, open-label, three-way, cross-over study was conducted in non-Japanese obese subjects[74] (target sample size of 42 subjects) to assess the relative bioavailability of a single subcutaneous dose of lixisenatide administered at different injection sites (the abdomen, upper arm, thigh).

A single dose of 10 μg of lixisenatide was administered to subjects in the supine position by subcutaneous injection in the periumbilical abdomen, upper arm, or thigh in each period, and a 48-hour washout period was included between periods.

All of 43 treated subjects were included in the pharmacokinetic[75] and safety analysis populations.

Pharmacokinetic analysis showed that the point estimates of the ratios of C_{max} of unchanged lixisenatide in plasma (upper arm/abdomen, thigh/abdomen) and their two-sided 90% CI were 1.00 [0.92, 1.09] after subcutaneous injection of lixisenatide in the upper arm versus the abdomen and 0.86 [0.79, 0.94] after subcutaneous injection of lixisenatide in the thigh versus the abdomen and the point estimates of the AUC_{∞} ratios and their two-sided 90% CIs were 0.99 [0.89, 1.11] and 1.02 [0.90, 1.14], respectively.

[73] A bioequivalence study comparing the 50 μg/mL formulation and 100 μg/mL formulation of lixisenatide in non-Japanese healthy subjects
[74] Healthy male and female subjects with BMI of ≥27.0 and ≤45.0 kg/m^2
[75] One subject who was discontinued from the study after receiving lixisenatide injection in the thigh in Period 1 was excluded from the analysis for other injection sites.

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

The results from Studies PDY6797, DRI6012, TES6865, and TES11807 as the evaluation data and the results from 17 foreign clinical studies as the reference data were submitted. The results from the main studies are described below. Doses per administration in these studies are indicated.

4.(ii).A.(1) Studies using human biomaterials (4.2.2.4-1, 4.2.2.4-2, 5.3.2.1-1, 5.3.2.2-1 to 5.3.2.2-3, 5.3.2.3-2)

The binding of lixisenatide (500-50,000 pg/mL) to human plasma protein (mean, ultracentrifugation) was 52.5% to 56.2%. The stability of lixisenatide (4850 ng/mL) was investigated using human heparinized plasma, which revealed the plasma half-life (mean ± SD) of 2094 ± 598 minutes. The metabolism of lixisenatide was studied using S9 liver and kidney fractions from humans. In human S9 fractions, 28 metabolites were detected, of which 10 metabolites were detected in both liver and kidney fractions. Amino acids associated with the main cleavage sites in the protein were phenylalanine and alanine.

The potential for lixisenatide (180-180,000 pg/mL) to induce various metabolizing enzymes (CYP1A, 2B6, 2C9, 3A) was evaluated using human hepatocytes, which indicated that lixisenatide has no potential to induce these enzymes. The potential for lixisenatide (0-9.7 × 10^7 pg/mL) to inhibit CYP isoenzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A) was evaluated using human liver microsomes. As a result, lixisenatide showed little inhibition of any of the CYP isoenzymes (IC_{50} values >97 μg/mL [CYP1A2, 2B6, 2C9, 2C19, 2D6, 3A]; IC_{50} value ≥9.7 × 10^8 pg/mL [CYP2C8]).

Using cells expressing human organic cation transporter (hOCT) 2 and human organic anion transporting polypeptide (hOATP) 1B1 (CHO-hOCT2 and HEK-hOATP1B1), the potential for lixisenatide (180-180,000 pg/mL) to inhibit these uptake transporters was evaluated. As a result, lixisenatide did not inhibit hOCT2 or hOATP1B1.

4.(ii).A.(2) Healthy adult subject studies

Phase I multiple-dose study (5.3.3.3-3, Study POP11320 [to 20] Reference data)

An open-label, uncontrolled study was conducted in non-Japanese (Chinese) healthy adult subjects (target sample size of 22 subjects) to assess the pharmacokinetics of lixisenatide after multiple subcutaneous administration.

Lixisenatide 10 μg was subcutaneously administered once daily 30 minutes prior to breakfast on Days 1 to 7 and lixisenatide 20 μg was subcutaneously administered once daily 30 minutes prior to breakfast on Days 8 to 14.[76]

All of 22 treated subjects were included in the safety analysis population, and 21subjects were included in the pharmacokinetic analysis population. Excluded was 1 subject who was withdrawn from the study for a personal reason.

[76] Unless otherwise specified, subjects were subcutaneously injected in the abdomen.

The pharmacokinetic parameters of unchanged lixisenatide in plasma after administration of multiple subcutaneous doses of 10 and 20 μg by antibody status were as shown in Table 6.

Table 6. Pharmacokinetic parameters of unchanged lixisenatide in plasma after administration of multiple subcutaneous doses of 10 and 20 μg by antibody status

	Day of sampling	C_{max} (pg/mL)	AUC_{τ} (pg·h/mL)	T_{max} (h)	CL_{SS}/F (L/h)	$t_{1/2z}$ (h)
Antibody-negative (n = 19)	Day 7	86.2 ± 27.6	332 ± 289[a)]	1.00 (0.50, 2.50)	42.5 ± 21.3[a)]	1.58 ± 0.527[a)]
	Day 14	160 ± 50.1	607 ± 361[b)]	1.50 (1.00, 3.00)	39.5 ± 14.3[b)]	1.80 ± 0.491[b)]
Antibody-positive (n = 2)[c)]	Day 7	54.8 ± 12.7	188 ± 28.3	1.00 (1.00, 1.00)	53.9 ± 8.12	1.79 ± 0.206
	Day 14	122 ± 67.8	411 ± 83.6	1.00 (1.00, 1.00)	49.7 ± 10.1	1.95 ± 0.885

Mean ± SD, T_{max}: median (Min, Max)
C_{max}: maximum plasma concentration, AUCτ: area under the plasma concentration-time curve in the dosing interval, T_{max}: time to reach maximum plasma concentration, CL_{ss}/F: total body clearance, $t_{1/2z}$: apparent terminal elimination half-life
a) n = 17, b) n = 18, c) Subjects who were antibody-positive at the time of pharmacokinetic assessment (both subjects were antibody-positive at the initiation of the study).

4.(ii).A.(3) Patient studies

4.(ii).A.(3).1) Study investigating insulin secretory response (5.3.4.2-1, Study PDY10433 [to 20] Reference data)

A placebo-controlled, randomized, double-blind, two-way cross-over study was conducted in non-Japanese patients with type 2 diabetes mellitus[77] (target sample size of 20 subjects) to investigate the effect of lixisenatide on insulin secretion.

A single subcutaneous dose of placebo or lixisenatide 20 μg was administered under fasting conditions. Continuous intravenous insulin infusion was started 1.5 hours before administration of placebo or lixisenatide to adjust the glucose level (99.1 mg/dL), and continued for about 3 hours. Two hours after administration of placebo or lixisenatide, 0.3 g/kg of a 50% glucose solution for injection was intravenously injected over ≥30 seconds. A wash-out period of 1 to 7 days was included between periods.

All of 22 treated patients were included in the pharmacokinetic and safety analysis populations, and 20 patients were included in the pharmacodynamic analysis population. Excluded were 2 subjects who were withdrawn from the study due to adverse events.[78]

Pharmacokinetic analysis showed that the C_{max} (mean ± SD) was 83.9 ± 21.3 pg/ml, the $t_{1/2z}$ was 2.56 ± 0.68 hours, the AUC_{last} was 449 ± 149 pg·h/mL, the AUC_{∞} was 529 ± 165 pg·h/mL, and the T_{max} (median [Min, Max]) was 1.98 (1.00, 4.00) hours.

Pharmacodynamic analysis showed that the estimated geometric mean ratio of insulin $AUC_{0\text{-}10\ min}$ for lixisenatide versus placebo (lixisenatide/placebo) and its two-sided 90% CI were 6.60 [5.00, 8.72], showing a marked increase in first-phase insulin release after administration of lixisenatide. Likewise, the insulin $AUC_{10\text{-}120\ min}$ ratio was 2.96 [2.65, 3.29], the C-peptide $AUC_{0\text{-}10\ min}$ ratio was 6.09 [4.20, 8.83], the C-peptide

[77] Patients with type 2 diabetes mellitus on dietary and exercise therapy alone or metformin hydrochloride who were aged 18 to 65 years and had BMI of 25.0 to 35.0 kg/m^2 and HbA1c of 6.0% to 8.5%.
[78] One patient treated with placebo only and the other patient treated with lixisenatide only.[79] Patients with type 2 diabetes mellitus on dietary and exercise therapy and (or) one oral glucose-lowering agent other than insulin who were aged 18 to 65 years and had BMI of ≤40 kg/m^2 and fasting plasma glucose at screening of 125 to 250 mg/dL (110-250 mg/dL for patients on medications).

$AUC_{10\text{-}120\ min}$ ratio was 2.08 [1.88, 2.31], and the ratio of glucose elimination rate constant was 1.75 [1.62, 1.89]. On the other hand, lixisenatide did not affect glucagon release, as compared to placebo.

4.(ii).A.(3).2) Phase I single subcutaneous dose study (5.3.3.2-1, Study 01-016 [20 to 20] Reference data)

A placebo-controlled, randomized, double-blind study was conducted in non-Japanese patients with type 2 diabetes mellitus[79] (target sample size of 36 subjects) to assess the safety, tolerability, and maximum tolerated dose of lixisenatide after a single subcutaneous administration.

A single subcutaneous dose of placebo or lixisenatide (1, 3, 10, 20, 40 μg) was administered (in the thigh)[80] and a meal[81] was given 1 hour after injection of placebo or lixisenatide.

All of 28 treated patients were included in the pharmacodynamic and safety analysis populations.

Regarding pharmacodynamics, there was no increase in plasma glucose after meal loading in the lixisenatide ≥10 μg groups. Plasma glucose levels 1 hour post-dose tended to decrease with increasing dose (placebo group [mean ± SD], 181 ± 45.2 mg/dL; 1 μg group, 176 ± 37.4 mg/dL; 3 μg group, 180 ± 39.2 mg/dL; 10 μg group, 170 ± 96.5 mg/dL; 20 μg group, 123 ± 24.6 mg/dL; 40 μg group, 123 ± 19.8 mg/dL). Plasma insulin increased 2 to 3 hours post-dose (about 40-50 μIU/mL) and then decreased in the low-dose groups (1 and 3 μg groups), whereas plasma insulin leveled off or gradually increased in the high-dose groups (10-40 μg groups), suggesting suppression of insulin release. Like insulin, C-peptide increased 2 to 4 hours post-dose in the low-dose groups (1 and 3 μg groups), but decreased or leveled off in the high-dose groups (10-40 μg groups). Glucagon concentrations tended to increase (about 90 pg/mL) after meal loading but returned to the pre-meal levels by 3 hours post-dose in the low-dose groups (1 and 3 μg groups), and there were no major changes in the high-dose groups (10-40 μg groups).

4.(ii).A.(3).3) Multinational phase II study (5.3.5.1-1, Study PDY6797 [20 to 20])

A placebo-controlled, randomized, double-blind, parallel-group, comparative study was conducted in Japanese and non-Japanese patients with type 2 diabetes mellitus (target sample size of 120 subjects [60 Japanese and 60 non-Japanese]) to assess the efficacy, safety, and pharmacokinetics of lixisenatide [see "4.(iii).A.(2).1) Multinational phase II study" for the study design and efficacy and safety].

The pharmacokinetic parameters on the last day of administration by antibody status were as shown in Table 7.

[79] Patients with type 2 diabetes mellitus on dietary and exercise therapy and (or) one oral glucose-lowering agent other than insulin who were aged 18 to 65 years and had BMI of ≤40 kg/m^2 and fasting plasma glucose at screening of 125 to 250 mg/dL (110-250 mg/dL for patients on medications).
[80] Although the 60 μg dose was planned to be administered, due to the occurrence of adverse events that met the criteria for stopping enrollment and treatment (nausea after administration of lixisenatide) in the 40 μg group, enrollment and treatment were stopped for the remaining subjects in the 40 μg group and for the 60 μg group.
[81] A blended meal containing carbohydrate 82 g, protein 20 g, and fat 8 g.

Table 7. Pharmacokinetic parameters on the last day of administration by antibody status

	Population	Dosage regimen	N	C_{max} (pg/mL)	AUC_{τ} (pg·h/mL)	$AUC_{0\text{-}10h}$ (pg·h/mL)	T_{max} (h)	$CL_{(\tau)}/F$ (L/h)	$t_{1/2}$ (h)
Antibody-negative	Japanese	10 μg QD	20	80.4 (31.3)	367 (38.0)	362 (37.1)	1.50 (0.48, 2.50)	32.3 (50.6)	2.19 (27.4)
		20 μg QD	16	172 (35.5)	869 (46.6)	807 (42.6)	1.75 (0.48, 2.50)	28.1 (45.0)	2.50 (21.5)
		30 μg QD	9	194 (27.6)	1100 (30.5)	989 (28.9)	1.47 (0.92, 2.50)	30.1 (36.3)	3.11 (21.6)
		10 μg BID	18	73.7 (45.4)	330 (51.1)	—	1.50 (0.47, 2.50)	44.2 (99.8)	2.73 (33.6)
		20 μg BID	16	145 (56.8)	658 (45.7)	—	1.50 (0.00, 4.50)	39.0 (59.3)	2.64 (29.1)
		30 μg BID	10	184 (34.5)	899 (22.9)	—	1.50 (0.48, 2.50)	35.0 (23.2)	2.68 (18.9)
	Non-Japanese	10 μg QD	16	61.6 (51.1)	325 (49.3)	295 (54.4)	2.50 (1.00, 3.50)	40.1 (64.8)	3.82 (67.7)
		20 μg QD	14	133 (89.0)	941 (113)	813 (102)	2.49 (1.52, 3.50)	35.6 (61.7)	3.05 (18.3)
		30 μg QD	8	95.8 (44.1)	574 (61.3)	523 (56.7)	3.00 (1.50, 3.50)	96.9 (104)	2.84 (30.3)
		10 μg BID	17	48.2 (55.9)	255 (72.7)	—	2.00 (0.00, 3.50)	95.3 (116)	5.30 (71.5)[c)]
		20 μg BID	16	101 (36.9)	571 (38.3)[a)]	—	1.98 (0.48, 2.50)	42.2 (55.1)[a)]	3.13 (26.3)[a)]
		30 μg BID	13	151 (34.0)	897 (37.5)	—	1.50 (0.98, 3.50)	38.3 (40.8)	3.12 (26.1)
Antibody-positive	Japanese	10 μg QD	0	—	—	—	—	—	—
		20 μg QD	1	1030	8220	6520	2.58	2.43	4.17
		30 μg QD	2	1700 (103)	26400 (123)	13500 (109)	2.50 (2.50, 2.50)	4.66 (123)	9.23 (106)
		10 μg BID	1	81.0	467	—	1.50	21.4	2.82
		20 μg BID	3	260 (36.6)	1450 (45.0)	—	1.00 (1.00, 2.00)	16.0 (47.4)	3.51 (12.9)
		30 μg BID	5	542 (116)	4010 (132)[b)]	—	2.50 (0.48, 6.50)	19.4 (70.9)[b)]	4.82 (44.4)[b)]
	Non-Japanese	10 μg QD	0	—	—	—	—	—	—
		20 μg QD	1	138	—[d)]	118	0.480	—[d)]	—[d)]
		30 μg QD	6	972 (82.6)	9530 (95.5)	6850 (90.3)	3.54 (2.50, 4.50)	6.78 (103)	5.32 (51.3)
		10 μg BID	0	—	—	—	—	—	—
		20 μg BID	2	676 (126)	5170 (134)	—	1.26 (0.00, 2.52)	38.8 (134)	7.24 (72.1)
		30 μg BID	4	2670 (110)	22200 (109)	—	4.00 (0.48, 4.50)	15.3 (148)	10.2 (75.3)

Mean (coefficient of variation %), - : not calculated or not applicable, T_{max}: median (Min, Max), QD: τ = 24 h, BID: τ = 10 h
C_{max}: maximum plasma concentration, AUCτ: area under the plasma concentration-time curve in the dosing interval, $AUC_{0\text{-}10\,h}$: area under the plasma concentration-time curve from 0 to 10 hours, T_{max}: time to reach maximum plasma concentration, $CL_{(\tau)}/F$: total body clearance, $t_{1/2}$: apparent terminal elimination half-life
a) n = 15, b) n = 4, c) n = 16, d) n = 0

4.(ii).A.(3).4) Phase II study (5.3.4.2-2, Study ACT6011 [[redacted] to [redacted] 20[redacted]] Reference data)

A placebo-controlled, randomized, double-blind, parallel-group, comparative study was conducted in non-Japanese patients with type 2 diabetes mellitus[82] (target sample size of 60 subjects [20 subjects per group]) to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of dose titration of lixisenatide.

The study consisted of a run-in period (2 days), a study treatment period (28 days), and a follow-up period (up to 14 days).

In the lixisenatide groups, lixisenatide was started at 5 μg followed by titration to 20 μg or the highest tolerable dose in increments of 2.5 μg at intervals of 4 days and subjects were subcutaneously administered lixisenatide twice daily (BID) 15 minutes prior to breakfast and evening meal or lixisenatide once daily (QD) 15 minutes prior to breakfast and placebo 15 minutes prior to evening meal. In the placebo group, placebo was subcutaneously administered BID 15 minutes prior to breakfast and evening meal.

[82] Patients with type 2 diabetes mellitus on ≤2 oral glucose-lowering agents (SU agent or metformin hydrochloride) who were aged 18 to 70 years and had BMI of ≤35 kg/m^2 and HbA1c of 7.0% to 10%.

All of 64 treated patients were included in the pharmacodynamic and safety analysis populations. And 37 patients were included in the pharmacokinetic analysis[83] population.

The pharmacokinetic parameters on Days, 4, 12, and 28 of treatment by antibody status were as shown in Table 8.

Table 8. Pharmacokinetic parameters on Days, 4, 12, and 28 of treatment by antibody status

	Day of sampling	Dosage regimen	$C_{max0:14\text{-}23:55\ h}$ (pg/mL)	$C_{max0:14\text{-}9:55\ h}$ (pg/mL)	$C_{max9:55\text{-}23:55\ h}$ (pg/mL)	$AUC_{0:14\text{-}23:55\ h}$ (pg·h/mL)	$AUC_{0:14\text{-}9:55\ h}$ (pg·h/mL)	$AUC_{9:55\text{-}23:55\ h}$ (pg·h/mL)	$T_{max0:14\text{-}23:55\ h}$ (h)	$T_{max0:14\text{-}9:55\ h}$ (h)	$T_{max9:55\text{-}23:55\ h}$ (h)
Antibody-negative	Day 4	5 μg QD (n = 9)	37.3 ± 11.8	37.3 ± 11.8	—	267.6 ± 129.6[b)]	198.4 ± 60.2	—	1.25 (0.75, 1.25)	1.25 (0.75, 1.25)	—
		5 μg BID (n = 11)	50.9 ± 17.2	47.3 ± 16.9	41.9 ± 14.3	512.6 ± 152.3	228.6 ± 88.0	269.2 ± 97.9	2.25 (0.75, 14.25)	1.75 (0.75, 2.25)	11.25 (10.75, 14.25)
	Day 12	10 μg QD (n = 9)	82.7 ± 36.9	82.7 ± 36.9	—	420.5 ± 178.2	357.1 ± 133.9	—	1.25 (0.75, 1.75)	1.25 (0.75, 1.75)	—
		10 μg BID (n = 11)	108.6 ± 23.8	102.8 ± 23.3	90.9 ± 26.3	916.0 ± 249.2	468.6 ± 132.2	438.2 ± 150.7	2.25 (1.25, 11.25)	1.75 (0.75, 2.25)	11.25 (10.75, 13.25)
	Day 28	20 μg QD (n = 9)	187.2 ± 69.8	187.2 ± 69.8	—	847.8 ± 337.3	749.0 ± 280.4	—	1.25 (0.75, 2.25)	1.25 (0.75, 2.25)	—
		20 μg BID (n = 11)	234.4 ± 90.3	216.1 ± 100.6	187.4 ± 54.0	1788.6 ± 709.2	841.4 ± 368.5	936.3 ± 425.1	1.25 (0.75, 13.25)	1.25 (0.75, 2.38)	11.25 (10.75, 13.25)
Antibody-positive	Day 4	5 μg QD (n = 8)	40.6 ± 11.7	40.6 ± 11.7	—	229.2 ± 104.1	178.6 ± 48.3	—	1.50 (0.75, 3.25)	1.50 (0.75, 3.25)	—
		5 μg BID (n = 9)	51.2 ± 20.3	48.1 ± 15.3	43.6 ± 23.4	506.7 ± 160.9	223.4 ± 73.6	260.2 ± 108.1	1.75 (0.75, 11.75)	1.75 (0.75, 3.25)	11.25 (10.75, 13.25)
	Day 12	10 μg QD (n = 8)	93.7 ± 29.0	93.7 ± 29.0	—	411.9 ± 130.3	353.3 ± 106.2	—	1.25 (0.75, 1.75)	1.25 (0.75, 1.75)	—
		10 μg BID (n = 9)	104.3 ± 40.4	94.8 ± 33.8	100.3 ± 41.9	945.9 ± 338.3	427.3 ± 160.9	501.6 ± 198.2	10.75 (1.25, 11.75)	1.75 (0.75, 2.25)	11.25 (10.75, 14.25)
	Day 28	20 μg QD (n = 10)	703.6 ± 525.4	696.3 ± 521.9	—	7250.7 ± 7027.5	4395.8 ± 3606.1	—	2.75 (0.75, 20.00)	2.25 (0.75, 6.25)	—
		20 μg BID (n = 8)	2504.1 ± 2938.7	2066.2 ± 2494.2	2485.2 ± 2913.8	40904.7 ± 52422.1	16179.1 ± 21144.8	24725.6 ± 31479.7	1.75 (1.25, 4.92)	2.25 (1.25, 4.92)	1.75 (1.25, 4.92)

C_{max}, AUC: mean ± SD, T_{max}: median (Min, Max), - : not calculated, C_{max}: maximum plasma concentration, T_{max}: time to reach maximum plasma concentration, AUC: area under the plasma concentration-time curve
a) n = 9, b) n = 8

Regarding pharmacodynamics, the changes from baseline to Day 4 in the postprandial blood glucose $AUC_{0:14\text{-}4:55\ h}$ at breakfast (mean ± SE) were 3.0 ± 44.62 mg·h/dL (n = 22) in the placebo group, -378.3 ± 44.58 mg·h/dL (n = 21) in the QD group, and -360.0 ± 45.97 mg·h/dL (n = 21) in the BID group. The influence of lixisenatide on gastric emptying was estimated by means of a ^{13}C-octanoic acid breath test.[84] As a result, the changes from baseline in the lag-time and half-life of gastric emptying were -14 to 11 minutes and -24 to 5 minutes, respectively, in the placebo group, but >100 minutes and >160 minutes, respectively, in the QD and BID groups, and lixisenatide was shown to delay gastric emptying.

4.(ii).A.(3).5) Foreign phase II study (5.3.5.1-2, Study DRI6012 [[redacted]20[redacted] to [redacted] 20[redacted]])

A placebo-controlled, randomized, double-blind, parallel-group, comparative study was conducted in non-Japanese patients with type 2 diabetes mellitus (target sample size of 500 subjects) to assess the efficacy,

[83] There were no antibody-positive patients on Day 14. Patients tested positive on Day 29 were excluded from the analysis for Day 28.
[84] After ingestion of a standard breakfast test meal containing 99%^{13}C-octanoic acid 100 mg, 99%^{13}C-octanoic acid is absorbed in the duodenum and oxidized in the liver. Thus, breath samples were collected from subjects and the gastric emptying rate was calculated by measuring the $^{13}CO_2/^{12}CO_2$ ratio using an infrared isotope analyzer.

safety, and dose-response relationship of lixisenatide [see "4.(iii).A.(2).2) Foreign phase II study" for the details of the study design, the dosage regimen, and efficacy and safety].

The pharmacokinetic parameters by antibody status were as shown in Table 9.

Table 9. Pharmacokinetic parameters by antibody status

	Dosage regimen	N	C_{max} (pg/mL)	$AUC_{0\text{-}4.5\,h}$ (pg·h/mL)	T_{max} (h)
Antibody-negative	5 μg QD	5	28.36 (10.21)	89.78 (3.56)[a)]	2.58 (1.3, 4.5)
	10 μg QD	13	52.16 (48.55)	174.75 (51.73)[b)]	2.00 (0.4, 3.6)
	20 μg QD	11	108.39 (39.11)	298.37 (35.50)[c)]	2.45 (0.6, 3.9)
	30 μg QD	7	116.24 (37.32)	406.13 (37.48)	1.02 (0.9, 2.5)
	5 μg BID	5	35.27 (20.50)	107.69 (24.68)[a)]	3.47 (1.5, 4.5)
	10 μg BID	6	58.06 (42.36)	210.91 (47.48)[d)]	1.94 (0.9, 3.5)
	20 μg BID	4	155.00 (73.95)	516.98 (67.55)	1.00 (0.4, 2.5)
	30 μg BID	2	178.62 (9.74)	583.86 (24.10)	1.31 (1.0, 1.6)
Antibody-positive	5 μg QD	7	210.85 (149.16)	1097.48 (133.72)[e)]	4.48 (2.5, 4.6)
	10 μg QD	5	434.72 (114.21)	2401.94 (102.65)[e)]	3.50 (1.1, 4.8)
	20 μg QD	6	538.35 (117.20)	948.78 (103.17)[e)]	2.38 (1.0, 4.5)
	30 μg QD	9	1195.95 (81.48)	4031.60 (89.98)[f)]	2.53 (0.4, 4.5)
	5 μg BID	14	231.86 (102.92)	987.69 (102.31)[g)]	2.53 (0.5, 4.5)
	10 μg BID	12	498.44 (119.45)	955.77 (61.53)[d)]	3.42 (1.0, 4.6)
	20 μg BID	9	678.35 (95.73)	3848.80 (94.00)[c)]	2.47 (0.4, 4.5)
	30 μg BID	11	1534.08 (80.23)	6271.90 (83.18)[c)]	2.58 (0.5, 4.4)

Geometric mean (coefficient of variation %), T_{max}: median (Min, Max), $C_{max:}$ maximum plasma concentration, $AUC_{0\text{-}4.5\,h}$: area under the plasma concentration-time curve from 0 to 4.5 hours, T_{max}: time to reach maximum plasma concentration
a) n = 3, b) n = 10, c) n = 7, d) n = 5, e) n = 4, f) n = 8, g) n = 10

4.(ii).A.(3).6) Phase II active-controlled study (5.3.4.2-3, Study PDY10931 [to 20] Reference data)

A randomized, open-label, parallel-group, comparative study was conducted in non-Japanese patients with type 2 diabetes mellitus[85] (target sample size of 120 subjects [60 in the lixisenatide group, 60 in the liraglutide group]) to investigate the effect of lixisenatide on postprandial plasma glucose.

The study consisted of a starting dose period (2 weeks) and a maintenance dose period (2 weeks).

During the starting dose period, lixisenatide 10 μg was subcutaneously administered once daily under fasting conditions for 2 weeks or liraglutide 0.6 mg was subcutaneously administered once daily under fasting conditions for 1 week followed by liraglutide 1.2 mg once daily under fasting conditions for 1 week. During the maintenance dose period, lixisenatide 20 μg or liraglutide 1.8 mg was subcutaneously administered once daily under fasting conditions.

All of 148 treated patients were included in the safety analysis population and 143 patients[86] were included in the pharmacodynamic analysis population.

[85] Patients with type 2 diabetes mellitus not adequately controlled on ≥1.5 g/day of metformin hydrochloride who were aged 18 to 74 years and had HbA1c of 6.5% to 9.0%. Patients with BMI of ≤20 kg/m^2 or ≥37 kg/m^2 were excluded.
[86] Four patients withdrawn from the study due to adverse events (2 in the lixisenatide group, 2 in the liraglutide group) and 1 patient withdrawn due to a protocol deviation (liraglutide group) were excluded.

Regarding pharmacodynamics, the changes in postprandial plasma glucose (PPG) ($AUC_{0:30\text{-}4:30\ h}$ corrected for pre-meal value) from baseline to Day 28 (mean ± SE) were -227.25 ± 9.93 mg·h/dL (n = 75) in the lixisenatide group and -72.83 ± 10.30 mg·h/dL (n = 68) in the liraglutide group, showing a statistically significant reduction in the lixisenatide group as compared to the liraglutide group (*P* <0.0001, analysis of covariance [ANCOVA] model[87]). The $AUC_{0:30\text{-}4:30\ h}$ of free insulin was 5.34 μIU·h/mL in the liraglutide group compared to -64.22 μIU-h·mL in the lixisenatide group. There were no major changes from baseline in the proinsulin $AUC_{0:30\text{-}4:30\ h}$ in the lixisenatide group or the liraglutide group. While lixisenatide resulted in a reduction in the C-peptide $AUC_{0:30\text{-}4:30\ h}$ from baseline (-5.03 ng·h/mL), the C-peptide $AUC_{0:30\text{-}4:30\ h}$ increased in the liraglutide group (1.04 ng·h/mL). The glucagon $AUC_{0:30\text{-}4:30\ h}$ decreased in both treatment groups, but the decrease was greater in the lixisenatide group (lixisenatide group, -46.71 pg·h/mL; liraglutide group, -25.28 pg·h/mL) and the difference between the two treatments was statistically significant.

4.(ii).A.(4) Intrinsic factor pharmacokinetic studies

4.(ii).A.(4).1) Phase I single-dose study in elderly and young subjects (5.3.3.3-1, Study POP11814 [redacted] Reference data)

An open-label study was conducted in non-Japanese healthy elderly and young subjects[88] (target sample size of 36 subjects) to assess the pharmacokinetics of lixisenatide after a single subcutaneous administration.

A single subcutaneous dose of lixisenatide 20 μg was administered 30 minutes prior to breakfast.

All of 36 treated subjects were included in the pharmacokinetic and safety analysis populations.

The pharmacokinetic parameters of unchanged lixisenatide in plasma after administration of a single subcutaneous dose of 20 μg were as shown in Table 10.

Table 10. Pharmacokinetic parameters of unchanged lixisenatide in plasma after administration of a single subcutaneous dose of 20 μg

	N	C_{max} (pg/mL)	AUC_{∞} (pg·h/mL)	AUC_{last} (pg·h/mL)	CL/F (L/h)	V_z/F (L)	$t_{1/2z}$ (h)	T_{max} (h)
Young	18	179 ± 50.0	776 ± 297	733 ± 291	27.9 (51.3)	69.7 (44.6)	1.77 ± 0.379	1.51 (0.50, 3.0)
Elderly	18	173 ± 46.1	1060 ± 440	970 ± 394	20.6 (53.3)	82.2 (36.1)	2.83 ± 0.607	1.75 (1.00, 3.02)

Mean ± SD, CL/F, Vz/F: geometric mean (coefficient of variation %), T_{max}: median (Min, Max), C_{max}: maximum plasma concentration, AUC_{∞}: area under the plasma concentration-time curve, AUC_{last}: area under the plasma concentration-time curve from time zero to the final quantifiable (above the lower limit of quantification) time point, CL/F: apparent total body clearance, V_z/F: apparent volume of distribution, $t_{1/2z}$: apparent terminal elimination half-life, T_{max}: time to reach maximum plasma concentration

Pharmacokinetic analysis showed that the point estimates of the ratios (elderly/young) of C_{max}, AUC_{last}, AUC_{∞}, and $t_{1/2}$ of unchanged lixisenatide in plasma and their two-sided 90% CIs were 0.94 [0.81, 1.09], 1.26 [1.03, 1.55], 1.29 [1.06, 1.57], and 1.57 [1.41, 1.75], respectively.

4.(ii).A.(4).2) Single subcutaneous dose study in subjects with renal impairment (5.3.3.3-2, Study POP6053 [redacted] Reference data)

An open-label study was conducted to assess the pharmacokinetics of lixisenatide in non-Japanese subjects with renal impairment[89] (target sample size of 32 subjects; 8 per group).

[87] ANCOVA model with treatment and trial site as fixed effects and baseline $AUC_{0:30\text{-}4:30\ h}$ as a covariate.

[88] Elderly: ≥65 years (including at least 30% subjects aged ≥75 years), Young: 18 to 45 years.

A single subcutaneous dose of lixisenatide 5 μg was administered 30 minutes prior to breakfast.

All of 32 treated subjects were included in the pharmacokinetic and safety analysis populations.

The pharmacokinetic parameters of lixisenatide by degree of renal impairment were as shown in Table 11.

Table 11. Pharmacokinetic parameters by degree of renal impairment

Parameter	Subjects with normal renal function (n = 8)	Subjects with mild renal impairment (n = 8)	Subjects with moderate renal impairment (n = 8)	Subjects with severe renal impairment (n = 8)
C_{max} (pg/mL)	54.4 ± 28.2	50.4 ± 18.7	54.1 ± 27.0	64.4 ± 15.3
AUC_{last} (pg·h/mL)	210 ± 89.7	211 ± 104	274 ± 116	346 ± 116
AUC_{∞} (pg·h/mL)	270 ± 92.6	285 ± 94.2	336 ± 104	397 ± 129
$AUC_{0\text{-}24\ h}$ (pg·h/mL)	219 ± 91.2	221 ± 107	285 ± 116	358 ± 120
T_{max} (h)	2.25 (0.50, 3.00)	2.25 (0.50, 3.00)	2.29 (1.50, 3.50)	1.77 (1.50, 3.52)
$t_{1/2}$ (h)	2.62 ± 0.996	2.41 ± 1.21	2.62 ± 0.844	2.87 ± 1.15
CL/F (L/h)	20.3 ± 6.20	19.3 ± 6.54	16.3 ± 5.66	14.1 ± 5.39
V_z/F (L)	67.3 ± 23.6	55.4 ± 21.0	55.8 ± 20.7	53.3 ± 13.7
MRT (h)	4.42 ± 1.42	4.40 ± 0.989	5.02 ± 1.03	5.29 ± 1.55

Mean ± SD, T_{max}: median (Min, Max), C_{max}: maximum plasma concentration, AUC_{last}: area under the plasma concentration-time curve from time zero to the time corresponding to the last concentration above the lower limit of quantification, AUC_{∞}: area under the plasma concentration-time curve, $AUC_{0\text{-}24\ h}$: area under the plasma concentration-time curve from 0 to 24 hours, T_{max}: time to reach maximum plasma concentration, $t_{1/2}$: elimination half-life, CL/F: total body clearance, V_z/F: apparent volume of distribution during terminal phase, MRT: mean residence time

The point estimates of the geometric mean ratios of C_{max}, $AUC_{0\text{-}24\ h}$, AUC_{last}, and AUC_{∞} for subjects with different degrees of renal impairment versus subjects with normal renal function and their 90% CIs were as shown in Table 12.

Table 12. Ratios of pharmacokinetic parameters for subjects with different degrees of renal impairment versus subjects with normal renal function

Geometric mean ratio	Subjects with mild renal impairment /Subjects with normal renal function	Subjects with moderate renal impairment /Subjects with normal renal function	Subjects with severe renal impairment /Subjects with normal renal function
C_{max} ratio	0.98 [0.68, 1.41]	0.99 [0.69, 1.43]	1.29 [0.90, 1.86]
$AUC_{0\text{-}24\ h}$ ratio	0.95 [0.64, 1.41]	1.28 [0.86, 1.91]	1.66 [1.11, 2.47]
AUC_{last} ratio	0.94 [0.62, 1.41]	1.28 [0.85, 1.93]	1.67 [1.12, 2.51]
AUC_{∞} ratio	1.05 [0.76, 1.45]	1.24 [0.91, 1.70]	1.46 [1.08, 1.97]
CL/F ratio	0.95 [0.69, 1.31]	0.80 [0.59, 1.10]	0.68 [0.51, 0.92]

4.(ii).A.(5) Drug-drug interaction studies

Drug-drug interaction studies (5.3.3.4-1 to 5.3.3.4-6, Study INT10408 [to 20], Study INT10409 [20 to 20], Study INT10782 [to 20], Study INT10783 [to 20], Study INT6052 [to 20], Study INT6863 [20 to 20] Reference data)

Drug-drug interaction studies were conducted in non-Japanese healthy adult subjects (non-Japanese healthy adult male subjects were included in Study INT10408 and Study INT10409 and non-Japanese postmenopausal healthy adult female subjects were included in Study INT6052). The results of these studies were as shown in Table 13.

[89] Subjects who were aged 18 to 75 years and had BMI of 18.5 to 35 kg/m^2 and body weight >50 kg. Patients with type 2 diabetes mellitus were eligible if they had HbA1c of ≤10.0% and fasting plasma glucose of ≤250 mg/dL and were on ≤2 oral glucose-lowering agents. Normal renal function = CLcr >80 mL/min, mild impairment = CLcr 50 to 80 mL/min, moderate impairment = CLcr ≥30 and <50 mL/min, severe impairment = CLcr <30 mL/min not requiring hemodialysis.

Table 13. Results of drug-drug interaction studies (non-Japanese)

Study No.	Dosage regimen[a)]	Drug studied for interactions and its dose	Analyte	N	C_{max} ratio[b)] [90% CI]	AUC ratio[b)] [90% CI]	T_{max} (h) after single-agent administration (or in combination with placebo) (median [Min, Max])	T_{max} (h) after co-administration (median [Min, Max])
INT10408	20 µg QD[c)]	Warfarin (25 mg)	S-warfarin[d)]	16	0.81 [0.68, 0.96][e)]	1.03 [0.87, 1.22]	1.00 (1.00, 8.00)	8.00 (1.00, 12.05)
INT10409	20 µg QD[c)]	Atorvastatin[f)] (40 mg)	Atorvastatin (morning dosing)	18	0.69 [0.55, 0.86]	1.08 [0.99, 1.18][g)]	1.50 (0.50, 4.00)	4.03 (0.50, 10.03)
			Atorvastatin (evening dosing)	18	1.66 [1.36, 2.03]	1.27 [1.18, 1.36][g)]	2.00 (1.0, 4.00)	1.76 (1.00, 4.02)
			p-hydroxy atorvastatin (morning dosing)	18	1.44 [1.27, 1.62]	1.47 [1.32, 1.63][g)]	6.00 (0.50, 9.98)	10.00 (4.00, 24.00)
			p-hydroxy atorvastatin (evening dosing)	18	1.52 [1.32, 1.75]	1.48 [1.37, 1.60][g)]	4.00 (1.00, 24.00)	3.02 (1.00, 23.98)
			o-hydroxy atorvastatin (morning dosing)	18	0.92 [0.79, 1.07]	1.23 [1.14, 1.33][g)]	3.00 (0.50, 9.98)	8.00 (4.00, 10.03)
			o-hydroxy atorvastatin (evening dosing)	18	1.37 [1.16, 1.61]	1.31 [1.21, 1.43][g)]	4.00 (1.02, 6.00)	3.00 (1.00, 6.00)
INT10782	20 µg QD[c)]	Ramipril[h)] (5 mg)	Ramipril	26	0.37 [0.29, 0.46]	1.21 [1.06, 1.39][g)]	0.50 (0.25, 0.75)	2.78 (0.52, 6.02)
			Ramiprilat[i)]	26	1.02 [0.92, 1.14]	1.11 [1.06, 1.16][g)]	2.50 (1.50, 5.00)	5.02 (2.03, 8.02)
INT10783	20 µg QD[c)]	Digoxin[j)] (0.25 mg)	Digoxin	24	0.74 [0.64, 0.86]	0.94 [0.87, 1.01][g)]	0.52 (0.50, 6.00)	2.00 (0.50, 4.02)
INT6052	10 µg QD	Oral contraceptive (ethinyl estradiol [EE] 0.03 mg, levonorgestrel [LV] 0.15 mg)	EE (11 hours after lixisenatide dosing)	25	0.99 [0.90, 1.09]	1.03 [0.97, 1.09]	2.00 (0.97, 2.00)	2.00 (0.97, 3.00)
			EE (1 hour before lixisenatide dosing)	25	0.93 [0.84, 1.02]	1.00 [0.94, 1.06]		2.00 (0.50, 2.00)
			EE (1 hour after lixisenatide dosing)	25	0.48 [0.43, 0.53]	1.06 [1.00, 1.12]		4.00 (0.93, 12.00)
			EE (4 hours after lixisenatide dosing)	25	0.61 [0.56, 0.68]	0.96 [0.91, 1.02]		3.00 (2.00, 8.00)
INT6052	10 µg QD	Oral contraceptive (ethinyl estradiol [EE] 0.03 mg, levonorgestrel [LV] 0.15 mg)	LV (11 hours after lixisenatide dosing)	25	1.00 [0.88, 1.15]	1.02 [0.94, 1.11]	0.97 (0.97, 2.00)	1.00 (0.92, 6.00)
			LV (1 hour before lixisenatide dosing)	25	1.01 [0.89, 1.16]	0.99 [0.92, 1.08]		0.97 (0.50, 2.00)
			LV (1 hour after lixisenatide dosing)	25	0.54 [0.48, 0.62]	1.20 [1.10, 1.31]		4.00 (0.50, 8.00)
			LV (4 hours after lixisenatide dosing)	25	0.80 [0.70, 0.92]	1.14 [1.05, 1.24]		2.00 (2.00, 8.00)
INT6863	10 µg QD	Acetaminophen (1000 mg)	Acetaminophen (1 hour before lixisenatide dosing)	15	0.97 [0.78, 1.19]	0.97 [0.92, 1.02]	0.25[k)] (0.25, 2.00)	0.50 (0.25, 2.00)
			Acetaminophen (1 hour after lixisenatide dosing)	15	0.71 [0.57, 0.87]	0.95 [0.91, 1.00]		4.50 (0.50, 6.00)
			Acetaminophen (4 hours after lixisenatide dosing)	15	0.69 [0.56, 0.85]	0.95 [0.91, 1.00]		2.00 (0.50, 4.00)

a) Dose of co-administered lixisenatide
b) Point estimate of geometric mean ratio. AUC refers to AUC_{∞}.
c) Lixisenatide 10 µg QD followed by 20 µg QD.
d) More potent enantiomer of racemic warfarin.
e) Including 1 subject who had plasma exposure of R- and S- warfarin that was only about 20% of the mean level without an increase in INR (the subject was administered warfarin alone followed by co-administration). The results were unaffected when this subject was excluded.
f) In the first period, the morning dosing group received oral atorvastatin 40 mg once daily in the morning for 6 days; and the evening dosing group received oral atorvastatin 40 mg once daily in the evening for 6 days. In the 2nd period, the morning dosing group received subcutaneous lixisenatide 10 µg once daily in the morning for 7 days, followed by subcutaneous lixisenatide 20 µg once daily in the morning for 7 days with

atorvastatin 40 mg once daily in the morning on Days 2 to 7; and the evening dosing group received subcutaneous lixisenatide 10 μg once daily in the morning for 7 days, followed by subcutaneous lixisenatide 20 μg once daily for 8 days with atorvastatin 40 mg once daily in the evening on Days 2 to 7. A 5-day wash-out period was included between periods.

g) Point estimate of geometric mean ratio of AUC_{τ}
h) Regarding pharmacodynamics, the point estimate of ratio of change from baseline in $AUC_{0\text{-}24\,h}$ of AcSDKP (N-Acetyl-Seryl-Aspartyl-Lysyl-Proline) for co-administration versus single-agent administration of ramipril (co-administration/single-agent administration of ramipril) and its two-sided 90% CI were 1.19 [1.03, 1.37], and the C_{max} was slightly increased after co-administration and the T_{max} was prolonged to 4 hours.
i) Active metabolite of ramipril
j) Digoxin was dosed with 240 mL of non-carbonated water at breakfast or evening meal (a moderately fatty meal). The data included 1 subject who had a low plasma concentration of digoxin after both single-agent administration of digoxin and co-administration with lixisenatide, but the results were unaffected when this subject was excluded.
k) Data when acetaminophen was dosed 1 hour before placebo dosing.

4.(ii).A.(6) Pharmacodynamic studies

4.(ii).A.(6).1) Hypoglycemic clamp study (5.3.4.1-2, Study PDY11941 [to 20] Reference data)

A placebo-controlled, randomized, double-blind, two-period, cross-over study was conducted in non-Japanese healthy adult subjects (target sample size of 18 subjects) to assess the pharmacodynamic effects of a single subcutaneous dose of lixisenatide, using a hypoglycemic clamp procedure.

As hypoglycemic clamp steps, a single subcutaneous dose of placebo or lixisenatide 20 μg was administered after the baseline step (60 minutes) and then, a 60-minute clamp was performed in each of Step 1 to Step 3 (glucose concentration was 3.9 mmol/L in Step 1, 3.1 mmol/L in Step 2, and 2.5 mmol/L in Step 3) and after Step 3, a recovery step (120 minutes) was included. A wash-out period of 4 to 5 weeks was included between periods.

All of 18 treated subjects were included in the pharmacokinetic and safety analysis populations, and 17 subjects were included in the pharmacodynamic analysis population. Excluded was 1 subject who was withdrawn due to an adverse event.

Pharmacokinetic analysis showed that the C_{max} (mean ± SD) after administration of lixisenatide was 140 ± 46.2 pg/mL, the $t_{1/2}$ was 2.30 ± 0.64 hours, the AUC_{last} was 612 ± 201 pg·h/mL, the AUC_{∞} was 697 ± 178 pg·h/mL, and the T_{max} (median [Min, Max]) was 1.50 (1.00, 3.00) hours.

Regarding pharmacodynamics, the point estimate of the geometric mean ratio (lixisenatide/placebo) of the mean glucagon concentration during the 30 minutes of the 2.5 mmol/L clamp period and its two-sided 90% CI were 1.04 [0.97, 1.12]. The point estimates of the geometric mean ratios for cortisol, epinephrine, and norepinephrine (lixisenatide/placebo) ranged from 0.88 to 1.43 throughout all steps. On the other hand, the ratios for growth hormone were 1.45 in the baseline step, >2 in Steps 1 and 2, and 0.93 in Step 3, and the ratio was decreased to 0.66 in the recovery step. The ratios for C-peptide and free insulin decreased with decreasing concentration of glucose, and the ratios during the 2.5 mmol/L clamp period were 1.49 and 1.00, respectively.

2) QT/QTc evaluation study (5.3.4.1-6: Study TES11807 [20 to 20])

A randomized, double-blind, parallel-group, comparative study versus placebo and moxifloxacin (positive control) was conducted in non-Japanese healthy adult subjects (target number of subjects: 260) to investigate the effect of repeated subcutaneous administration of lixisenatide on QT/QTc interval.[90]

In the 20 μg QD group, lixisenatide 10 μg was subcutaneously administered once daily for 7 days followed by lixisenatide 15 μg subcutaneously administered once daily for 7 days and then lixisenatide 20 μg subcutaneously administered once daily for 14 days (15 minutes prior to breakfast for all sessions of dosing). As for placebo administration, lixisenatide placebo was subcutaneously administered once daily on Days 1 to 27 (15 minutes prior to evening meal) and moxifloxacin placebo was orally administered once daily for 28 days (1 hour prior to breakfast). In the lixisenatide 30 μg BID group, lixisenatide 10 μg was subcutaneously administered twice daily for 7 days followed by lixisenatide 20 μg subcutaneously administered twice daily for 7 days and then lixisenatide 30 μg subcutaneously administered twice daily for 14 days (15 minutes prior to breakfast and evening meal for all sessions of dosing [only in the morning on Day 28]). As for placebo administration, moxifloxacin placebo was orally administered once daily (1 hour prior to breakfast) on Days 1 to 28. In the placebo group, lixisenatide placebo was subcutaneously administered twice daily for 28 days (15 minutes prior to breakfast and evening meal [only in the morning on Day 28]) and moxifloxacin placebo was orally administered once daily for 28 days (1 hour prior to breakfast). In the moxifloxacin group, moxifloxacin placebo was orally administered once daily for 27 days and a single oral dose of moxifloxacin 400 mg was administered (1 hour prior to breakfast) on Day 28. As for placebo administration, lixisenatide placebo was subcutaneously administered twice daily for 27 days (15 minutes prior to breakfast and evening meal [only in the morning on Day 28]).

All of 264 treated subjects (159 males and 105 females) were included in the safety analysis population, and 244 subjects in the pharmacodynamic analysis population[91] and 123 subjects in the pharmacokinetic[92] analysis population.

The pharmacokinetic parameters after administration of lixisenatide by antibody status were as shown in Table14.

Table 14. Pharmacokinetic parameters after administration of lixisenatide by antibody status

	Dose	N	C_{max} (pg/mL)	AUC_{last} (h·pg/mL)	AUC_{τ} (h·pg/mL)	T_{max} (h)	$t_{1/2}$ (h)
Antibody-negative	20 μg QD	32	144 ± 56.7	705 ± 331	765 ± 357	1.52 (1.00, 5.02)	2.29 ± 0.515
	30 μg BID	28	209 ± 87.7	979 ± 346	960 ± 320	1.50 (0.50, 3.00)	2.57 ± 0.452
Antibody-positive[a)]	20 μg QD	27	662 ± 655	8700 ± 11700	8710 ± 11700	2.02 (1.00, 6.00)	7.91 ± 6.44
	30 μg BID	33	945 ± 1080	12500 ± 19100	7090 ± 9390	2.00 (0.50, 5.02)	8.51 ± 7.01

Mean ± SD, T_{max}: median (Min, Max)

C_{max}: maximum plasma concentration, AUC_{last}: area under the plasma concentration-time curve from time zero to the time corresponding to the last concentration above the lower limit of quantification, AUC_{τ}: area under the plasma concentration-time curve in the dosing interval, T_{max}: time to reach maximum plasma concentration, $t_{1/2}$: apparent terminal elimination half-life

a) Antibody-positive subjects on Day 28.

[90] Before the conduct of Study TES11807, Study TES6865 was conducted as a parallel-group, comparative study in 22 subjects per group (target sample size of 88 subjects) with the same design as Study TES11807 (The actual total number of subjects treated was 91). However, due to the limited number of subjects and variability in measurement results, Study TES11807 was conducted as a second QT/QTc evaluation study .

[91] 17 subjects who withdrew consent, 2 subjects with substantial missing ECG data on Day 28, and 1 subject withdrawn due to vomiting within 1 hour after dosing on Day 28 were excluded from the pharmacodynamic analysis.

[92] 17 subjects who withdrew consent by Day 28 were excluded from the pharmacokinetic analysis.

Regarding ECG, the largest time-matched baseline-adjusted least-squares mean differences in QTcF interval[93] on Day 28 between lixisenatide 20 μg QD and placebo and between lixisenatide 30 μg BID and placebo (ΔΔQTcF) and their two-sided 90% CIs[94] were 4.60 [2.34-6.87] ms and 5.48 [3.22-7.75] ms, respectively, at 3 hours post-dose. For both lixisenatide groups, the upper bound of the CI was below 10 ms. The least-squares mean of ΔΔQTcF at 3 to 5 hours post-dose for the moxifloxacin group and its two-sided 90% CI were 10.61 [8.66-12.55] ms and the lower bound of the 90% CI was greater than 5 ms.

Regarding safety, 159 adverse events occurred in 38 of 66 subjects in the placebo group, 583 in 54 of 68 subjects in the 20 μg QD group, 662 in 55 of 65 subjects in the 30 μg BID group; and 102 in 34 of 65 subjects and 22 in 15 of 63 subjects in the moxifloxacin group (before/after administration), respectively. Of these, adverse drug reactions were reported as follows: 137 in 35 of 66 subjects in the placebo group, 564 in 52 of 68 subjects in the 20 μg QD group, 642 in 52 of 65 subjects in the 30 μg BID group; and 91 in 31 of 65 subjects and 16 in 9 of 63 subjects in the moxifloxacin group (before/after administration), respectively. A serious adverse event (hospitalization due to peritonsillar abscess) was reported in 1 subject in the 20 μg QD group, but the causal relationship was denied. There were no deaths or other serious adverse events. Adverse events leading to treatment discontinuation occurred in 3 subjects (1 subject each with urethral abscess, hypersensitivity, and abdominal pain) in the placebo group, 5 subjects (3 subjects with neutropenias, and 1 subject each with peritonsillar abscess, and dermatitis allergic/pruritus/feeling hot) in the 20 μg QD group, 3 subjects (1 subject each with neutropenias, hypersensitivity, and abdominal pain) in the 30 μg BID group, and 2 subjects (1 subject each with rash pruritic and abdominal pain upper) in the moxifloxacin group.

Regarding anti-lixisenatide antibody, 27 subjects in the 20 μg QD group and 33 subjects in the 30 μg BID group were antibody-positive on Day 28.

4.(ii).A.(7) Other studies

4.(ii).A.(7).1) Spermatogenesis study (5.3.4.1-4, Study TDR11215 [20 to 20] Reference data)

A placebo-controlled, randomized, double-blind, parallel-group, comparative study was conducted in non-Japanese overweight or obese adult male subjects[95] (target sample size of 254 subjects) to assess the effects of multiple doses of lixisenatide on sperm concentration.

Placebo or lixisenatide 10 μg was subcutaneously administered once daily within 1 hour prior to breakfast for 3 days, followed by placebo or lixisenatide 15 μg subcutaneously administered once daily within 1 hour prior to breakfast for 4 days and then placebo or lixisenatide 20 μg subcutaneously administered once daily within 1 hour prior to breakfast for 175 days.

All of 275 treated subjects were included in the safety analysis population, and 217 subjects were included in the pharmacodynamic analysis population.

Regarding pharmacodynamics, the point estimate of the geometric mean ratio of sperm concentration[96] at Week 26 (lixisenatide group/placebo group) and its two-sided 90% CI were 1.02 [0.91, 1.13]. The primary

[93] QT interval corrected according to Fridericia's formula.
[94] A linear fixed effects model with treatment and gender as fixed effects and time-matched baseline QTcF as a covariate.
[95] Adult male subjects with BMI of 27.0 to 37.0 kg/m^2.

endpoint of the proportion (%) of subjects with ≥50% reduction in sperm concentration from baseline to Week 26 was 8.93% (10 of 112 subjects) in the lixisenatide group and 3.81% (4 of 105 subjects) in the placebo group; and the non-inferiority of lixisenatide compared to placebo was demonstrated, as the upper limit of the two-sided 95% CI for the difference in the proportion for lixisenatide versus placebo was 12.44%, which was below the pre-specified non-inferiority margin of 20%. In addition, lixisenatide had no effects on total sperm count, sperm motility, normal sperm morphology rate, reproductive hormones (total and free testosterone, FSH, LH), or neutral α-glucosidase and inhibin B in seminal plasma.

4.(ii).A.(7).2) Population pharmacokinetic analysis (5.3.3.5-1 to 5.3.3.5-4)

Using the plasma unchanged lixisenatide concentration data (4313 concentrations from 503 subjects) from phase I studies (BDR6864, POP6053) and phase II studies (PDY6797, DRI6012), a population pharmacokinetic (PPK) analysis was performed using non-linear mixed effect modeling (software, NONMEM [version 7.1]). The base model was a 1-compartment model. Predicted normal weight (PNWT), renal clearance (CL_R), creatinine clearance (CL_{cr}), and non-renal clearance (CL_N) as covariates on apparent clearance (CL/F), PNWT as a covariate on apparent volume of distribution (V/F), and PNWT, injection site (thigh, f_{thigh}), and race (non-Japanese [Asian], f_{Asian}) as covariates on mean absorption time (MAT) were evaluated using the stepwise method. As a result, PNWT and CL_{cr} as covariates on CL/F, PNWT as a covariate on V/F, and injection site and race as covariates on MAT were included in the final model.

Simulations using the final model predicted that when PNWT is decreased from 70 kg to 35 kg, in a healthy adult with CL_{cr} of 6 L/h, an individual with severe renal impairment with CL_{cr} of 2 L/h, and an individual with end-stage renal disease with CL_{cr} of 0 L/h, AUC would increase 1.2-, 1.4-, and 1.7-fold, respectively; and when PNWT is increased from 70 kg to 140 kg, AUC would decrease 0.78-, 0.68-, and 0.59-fold, respectively; there would be changes in AUC of up to 42%, 72%, and 111%, respectively. Based on the PK model obtained from the above PPK analysis, using phase III study data[97] (EFC6018, EFC6015, EFC10887), a mixed-effect model analysis was performed using NONMEM in order to search for new covariates, but no new covariates were identified.

4.(ii).B Outline of the review by PMDA

4.(ii).B.(1) Differences in pharmacokinetics and pharmacodynamics between Japanese and non-Japanese populations

Since there was a tendency toward higher exposure in the Japanese population as compared to the non-Japanese population, PMDA asked the applicant to explain differences in the pharmacokinetics and pharmacodynamics of lixisenatide between the Japanese and non-Japanese populations.

The applicant responded as follows:

Regarding pharmacokinetics, the AUC (geometric mean) in the Japanese population in Study PDY6797 tended to be slightly higher although not being obvious (the AUC in antibody-negative patients in the 20 μg QD group, 785 pg·h/mL [Japanese population], 691 pg·h/mL [non-Japanese population]). However, there should be no clear differences in the AUC across dose levels between the Japanese and non-Japanese populations with QD and BID dosing, considering the following points: the inter-individual variability of

[96] Sperm analysis was performed twice at an interval of ≥48 hours and <120 hours at both baseline and Week 26 and means were calculated.

[97] 393 plasma concentrations from 184 subjects in Study EFC6018, 734 plasma concentrations from 401 subjects in Study EFC6015, and 330 plasma concentrations from 135 subjects in Study EFC10887 were used.

pharmacokinetic parameters was relatively high in both populations; the range of exposure largely overlapped between the Japanese and non-Japanese populations; and the difference in the AUC (geometric mean) between the Japanese and non-Japanese populations was not dose-related.

Regarding pharmacodynamics, the change from baseline in the AUC of postprandial plasma glucose was greater in the Japanese population than in the non-Japanese population in Study PDY6797 (the mean change in plasma glucose [2 hours after breakfast] in the 20 μg QD group, -166.9 mg/dL [Japanese population]; -110.7 mg/dL [non-Japanese population]). And there was a reduction in the insulin AUC in the Japanese population, but not in the non-Japanese population (Japanese population, -53.8 to -12.4 μIU·h/mL; non-Japanese population, -21.9 to 28.8 μIU·h/mL). The AUC of postprandial glucagon was significantly decreased in the Japanese and non-Japanese populations (Japanese population, -86.3 to -31.1 pg·h/mL; non-Japanese population, -86.6 to -26.8 pg·h/mL). The reduction in postprandial plasma glucose after administration of 20 μg QD or BID was comparable to the reduction after administration of 30 μg in each population.

A PPK analysis of the data obtained from phase III studies (EFC6018, EFC6015, EFC10887) was performed based on the PPK model constructed with the use of four studies - phase I and phase II - including Study PDY6797. As a result, race (Japanese/non-Japanese), other than renal function and body weight, was found not to be an independent confounder among the covariates tested for their effect on the exposure to lixisenatide. Fasting plasma glucose (FPG) during up-titration of lixisenatide was evaluated by PK/PD modeling with the use of Study PDY6797 data and when race, gender, and body weight were investigated as potential covariates, the effect of gender and race disappeared by incorporating body weight as a covariate. In addition, according to the Study PDY6797 data, a difference was seen in the change from baseline in the $AUC_{0:29\text{-}4:30\ h}$ of postprandial plasma glucose (PPG) between the Japanese and non-Japanese populations, suggesting an effect of body weight, but when this was corrected for FPG, the effect of race or body weight on PPG disappeared.

Based on the above, the greater change in postprandial plasma glucose in the Japanese population was considered due to differences in fasting plasma glucose in addition to a lower mean body weight and a higher blood concentration of lixisenatide in the Japanese population.

Regarding safety, in Study PDY6797,[98] there was no special concern about the Japanese population, nor were there ethnicity-related differences, when analysing TEAEs (Japanese population [placebo group, QD group (lixisenatide 10-15 μg), BID group (lixisenatide 10-15 μg)], 61.9%, 85.0%, 77.3%, respectively; non-Japanese population, 84.2%, 94.7%, 73.7%, respectively), and nausea (Japanese population, 0%, 50.0%, 18.2%, respectively; non-Japanese population, 5.3%, 31.6%, 21.1%, respectively), vomiting (Japanese population, 0%, 10.0%, 18.2%, respectively; non-Japanese population, 0%, 15.8%, 10.5%, respectively), diarrhoea (Japanese population, 4.8%, 5.0%, 22.7%, respectively; non-Japanese population, 21.1%, 10.5%, 21.1%, respectively), and hypoglycaemia (Japanese population, 14.3%, 20.0%, 31.8%, respectively; non-Japanese population, 0%, 21.1%, 10.5%, respectively), not only by ethnicity but also by dose level.

[98] Japanese population (placebo group, n = 21; lixisenatide QD group, n = 20; lixisenatide BID group, n = 22), non-Japanese population (placebo group, n = 19; lixisenatide QD group, n = 19; lixisenatide BID group, n = 19)

PMDA considers as follows:
The applicant's explanation that body weight is a factor contributing to pharmacodynamic differences between the Japanese and non-Japanese populations is acceptable. However, according to the results from Study PDY6797, the pharmacokinetic data showed that individual exposure levels (AUC and C_{max}) tended to be higher in Japanese patients than in non-Japanese patients and the observed pharmacodynamic differences were large. Thus, it is difficult to conclude that there are no pharmacokinetic differences between the Japanese and non-Japanese populations. Based on the above, differences in the pharmacokinetics and pharmacodynamics of lixisenatide between the Japanese and non-Japanese populations will continue to be reviewed from an efficacy and safety point of view in the clinical section [see "4.(iii).B.(3) Interpretation of results of multinational studies].

4.(ii).B.(2) Influence of antibody formation on pharmacokinetics
The applicant explained about the influence of anti-lixisenatide antibodies on the pharmacokinetics of lixisenatide as follows:
In healthy adult subjects (20 μg QD), the AUC_{τ} increased about 7-fold and the C_{max} increased about 3-fold in antibody-positive subjects (about 30%) as compared to antibody-negative subjects in Study TES6865 (duration of treatment, 4 weeks). Similarly, there were about 5- and 3-fold increases, respectively, in Study TES11807 (duration of treatment, 4 weeks; antibody-positive subjects, about 40%-50%) and about 5- and 4-fold increases, respectively, in Study TDR11215 (duration of treatment, 26 weeks; antibody-positive subjects, about 75%-80%). In studies with a short treatment duration (up to 17 days), there were antibody-positive subjects but the presence of antibodies had no relevant effect on the mean plasma concentration of lixisenatide.

In patients with type 2 diabetes mellitus, an obvious elevation was seen in the exposure to lixisenatide only when the duration of treatment was longest at the highest dose in Study ACT6011. In antibody-positive patients as compared to antibody-negative patients, the AUC and the C_{max} increased about 6- and 3-fold, respectively, in the 20 μg QD group and about 8- and 5-fold, respectively, in the 20 μg BID group, with a reduction in the CL/F and a prolongation of the $t_{1/2}$. In Study PDY6797, the number of antibody-positive patients was small (11 of 42 in the Japanese population, 11 of 38 in the non-Japanese population), but there were substantial increases in the AUC and the C_{max} in the 30 μg QD and BID groups (the highest dose and the longest duration of treatment). As compared to antibody-negative patients, the AUC_{τ} and the C_{max} in antibody-positive patients were about 12- and 6-fold, respectively, in the 30 μg QD group and about 3- and 2-fold, respectively, in the 30 μg BID group in the Japanese population and about 15- and 8-fold, respectively, in the 30 μg QD group and about 9- and 7-fold, respectively, in the 30 μg BID group in the non-Japanese population. In addition, a reduction in the CL/F and a prolongation of the $t_{1/2}$ were also seen. In Study DRI6012, no consistent relationship between the $AUC_{0\text{-}4.5\ h}$ or the C_{max} and dose levels was observed, and there were about 3- to 14-fold increases in antibody-positive patients as compared to antibody-negative patients.

Furthermore, in Study EFC10887, as plasma concentrations of lixisenatide were less than the lower limit of quantification before administration in all antibody-negative patients, the applicant considered that there was no accumulation in these subjects. On the other hand, the accumulation ratios in antibody-positive patients were 5.1 at 1 to 2 hours after administration and 8.8 at 4 to 6 hours after administration.

PMDA asked the applicant to explain about factors contributing to an increase in the exposure to lixisenatide in antibody-positive subjects and the impact of an increase in exposure on efficacy and safety.

The applicant responded as follows:
Lixisenatide bound to anti-lixisenatide antibodies in human plasma was characterized by surface plasmon resonance with the use of samples in Study TES11807, which showed that after 28-day administration, anti-lixisenatide antibodies were bound reversibly to lixisenatide. When plasma concentrations of total lixisenatide are measured, lixisenatide that has formed a complex with anti-lixisenatide antibodies is also measured as total lixisenatide in antibody-positive subjects, resulting in a higher apparent exposure. A prolongation of the half-life of lixisenatide was seen in antibody-positive subjects, but the prolongation being less than 2-fold is considered to suggest release of lixisenatide from the antibody-drug complex. According to the combined data of Study EFC6015 and Study EFC10887, the least-squares mean change in HbA1c at Week 24 and its 95% CI[99] were -0.74 [-0.962,-0.516]% (81 antibody-positive patients) and -0.86 [-1.178, -0.545]% (33 antibody-negative patients) in the Japanese population, and -0.80 [-0.944, -0.659]% (312 antibody-positive patients) and -0.76 [-0.930, -0.588]% (169 antibody-negative patients) in the overall population. In Study EFC6015, the changes in HbA1c at Week 76 were -0.64 [-0.988, -0.289]% (26 antibody-positive patients) and -1.97 [-5.170, 1.237]% (3 antibody negative-patients) in the Japanese population, and -0.77 [-0.908, -0.633]% (180 antibody-positive patients) and -0.97 [-1.254, -0.690]% (60 antibody-negative patients) in the overall population. The changes in HbA1c at Week 24 and Week 76 were comparable regardless of anti-lixisenatide antibody status in both the Japanese population and the overall population, showing no clear relationship between antibody concentration and the change in HbA1c. Therefore, antibody status is not considered a predictor of the efficacy of lixisenatide for individual subjects.

Regarding safety, the incidences of TEAEs by anti-lixisenatide antibody status in phase III clinical studies were 90.8% (167 of 184 patients) in antibody-positive patients and 90.2% (55 of 61 patients) in antibody-negative patients in the Japanese population, and 82.0% (1217 of 1484 patients) in antibody-positive patients and 73.6% (420 of 571 patients) in antibody-negative patients in the non-Japanese population, showing similar incidences regardless of antibody status. The incidences of injection site reactions were 8.2% (15 of 184 patients) in antibody-positive patients and 3.3% (2 of 61 patients) in antibody-negative patients in the Japanese population, and 5.7% (84 of 1484 patients) in antibody-positive patients and 2.5% (14 of 571 patients) in antibody-negative patients in the non-Japanese population, showing a slightly higher incidence in antibody-positive patients in both populations, but most of the events were mild in severity regardless of antibody status.

Based on the above, the apparent exposure is considered to be increased in antibody-positive patients, but with no impact on the efficacy and safety of lixisenatide.

PMDA considers as follows:
In clinical studies, even when the pharmacokinetics of lixisenatide was influenced by antibody formation, there was no major impact on efficacy and safety. However, since lixisenatide is intended to be administered for a long period of time and the duration of treatment and the number of subjects in clinical studies were

[99] A meta-analysis based on an inverse variance weighted parametric model.

limited, it is necessary to continue to collect information on the influence of antibody formation after the market launch. The influence of antibody formation will continue to be reviewed in the clinical section [see "4.(iii).B.(4).3) Influence of anti-lixisenatide antibody titer on efficacy evaluation, and 4.(iii).B.(5).5) Anti-lixisenatide antibody development and immune reactions"].

4.(ii).B.(3) Drug-drug interactions

PMDA asked the applicant to explain about drug-drug interactions associated with delayed gastric emptying by lixisenatide, taking into account the results of drug-drug interactions studies.

The applicant responded as follows:

With respect to drug interactions with oral contraceptive (OC) or acetaminophen, administration of lixisenatide within 4 hours before administration of acetaminophen or OC resulted in prolonged T_{max} (coadministration with acetaminophen, 1.75-4.25 hours; coadministration with OC, 1-3 hours) and decreased C_{max} (coadministration with acetaminophen, about 30% decrease; coadministration with OC, about 20%-50% decreases); but there were no effects when acetaminophen or OC was administered 1 hour before lixisenatide administration or when OC was administered 11 hours after lixisenatide administration. Based on these study results, the exposure (AUC) was unaffected by the timing of lixisenatide administration, but delayed gastric emptying by lixisenatide provides a potential mechanism for drug-drug interactions distinct from effects on pathway of metabolism or transport. In Study ACT6011, there were effects of delayed gastric emptying on plasma glucose excursions after a standardized test breakfast following once-daily subcutaneous administration of lixisenatide in the morning. Since these effects faded at lunch and dinner, lixisenatide does not cause a sustained delay in the absorption of concomitant drugs that are absorbed from the gastrointestinal tract over ≥4 hours for efficacy. However, a precaution statement regarding drugs whose effect may be reduced by delayed absorption needs to be included in the section of drug interactions in the package insert.

PMDA asked the applicant to also explain about the influence of co-administration with an enteric-coated or sustained-release formulation of a drug, taking into account that lixisenatide delays gastric emptying.

The applicant responded as follows:

Using the data from Study EFC6015 where Japanese patients participated, HbA1c changes in patients treated with a sustained-release SU as background therapy (60 of 286 patients in the placebo group and 149 of 570 patients [including 3 Japanese patients] in the lixisenatide group were taking sustained-release SU at screening) were analyzed. As a result, the least-squares mean change in HbA1c[100] in the lixisenatide group ranged from -0.87% to -0.73% in all populations (Japanese population, non-Japanese population, overall population), regardless of the dosage form of SU (immediate-release or sustained-release formulation), and consistently similar values were obtained. In the placebo group, there were differences in the least-squares mean among subgroups; and in the non-Japanese population and the overall population, the least-squares mean change in HbA1c in patients treated with a sustained-released SU (non-Japanese population and overall population, -0.26%) was greater than that in patients treated with an immediate-release SU (non-Japanese population, -0.11%; overall population, -0.04%). In the Japanese population, HbA1c in patients treated with an immediate-release SU in the placebo group increased from baseline (least-squares

[100] ANCOVA model with treatment, HbA1c at screening (<8.0%, ≥8.0%), use of metformin hydrochloride at screening, country, category of SU (immediate-release or sustained-release formulation), and interaction between treatment group and category of SU as fixed-effects, and baseline HbA1c as a covariate.

mean [SE] was 0.23% [0.137]; mean ± SD was 0.19 ± 0.78). Based on the above results, there was a variability in the least-square mean change in HbA1c in the placebo group, but efficacy was consistently seen regardless of the dosage form of concomitant SU in the lixisenatide group.

Regarding safety, the incidence of TEAEs during the main treatment period of Study EFC6015 tended to be lower in the overall population of patients treated with a sustained-release SU than in patients treated with an immediate-release SU (patients treated with a sustained-release SU, 59.7% in the placebo group [37 of 62 patients] and 55.9% in the lixisenatide group [85 of 152 patients]; patients treated with an immediate-release SU, 61.4% in the placebo group [137 of 223 patients] and 72.7% in the lixisenatide group [307 of 422 patients]). This trend was also seen during the entire treatment period in the lixisenatide group (patients treated with a sustained-release SU, 71.1% [108 of 152 patients]; patients treated with an immediate-release SU, 85.3% [360 of 422 patients]). A subgroup analysis within the Japanese population alone in this study was not meaningful because of a small number of patients treated with a sustained-release formulation of SU.

With respect to co-administration with an enteric-coated or sustained-release formulation of a non-diabetic drug, 7 Japanese subjects[101] in Studies EFC6015, EFC10887, and LTS10888 received concomitant sustained-release formulations of drugs (5 subjects treated with a sustained-release formulation of nifedipine [NF], 1 subject treated with a sustained-release formulation of bezafibrate [BF], 1 subject treated with a sustained-release formulation of NF/a sustained-release formulation of BF). Six subjects treated with a sustained-release formulation of NF had no dose change and stable blood pressures during the study, but 1 subject[102] (a subject treated with a sustained-release formulation of NF) had dose increase on Day 6. The subject treated with a sustained-release formulation of NF/a sustained-release formulation of BF had stable serum lipids. In these 7 subjects, no adverse events of safety concern were reported.

In the clinical studies in which Japanese subjects participated, there were no subjects treated with an enteric-coated formulation of a drug. As for enteric-coated formulations of drugs or drugs that are absorbed only in the upper small intestine, there is a report that even when an enteric-coated formulation of a drug stayed in the stomach longer, the C_{max} after repeated dosing was similar to the C_{max} at steady state after administration under fasting conditions (Willis JV et al. *Eur. J. Clin.Pharmacol.* 1981;19:33-7). Thus, it is inferred that unless the absorption of active ingredient is site-specific, delayed gastric emptying is unlikely to affect the plasma concentration at steady state.

Based on the above, although lixisenatide is unlikely to produce adverse effects when coadministered with a sustained-release or enteric-coated formulation of a drug, given that the data are limited, information will be collected through post-marketing pharmacovigilance activities.

PMDA accepted the applicant's response, considering that as lixisenatide delays gastric emptying, it is important to collect information on concomitant drugs via post-marketing surveillance and it is necessary to include a precaution statement regarding lixisenatide interactions with therapeutic drugs whose effect may be reduced by delayed absorption in the package insert etc.

[101] One subject treated with a sustained-release formulation of NF was withdrawn from the study due to a deviation and another subject did not consent to an extension of treatment period and was withdrawn from the study.
[102] The subject's blood pressure remained around 150 mmHg for 4 weeks from the run-in phase, and remained around 130 mmHg thereafter.

4.(iii) Summary of clinical efficacy and safety

4.(iii).A Summary of the submitted data

As the evaluation data, the results from foreign clinical pharmacology studies (TES6865, TES11807), a multinational Phase II study (PDY6797), a foreign Phase II study (DRI6012), three multinational Phase III studies (EFC6015, EFC10887, EFC6018), and a Japanese Phase III open-label study (LTS10888) were submitted. As the reference data, the results from 24 foreign clinical studies were submitted. In the following sections, HbA1c results are reported in NGSP units.

4.(iii).A.(1) Clinical pharmacology studies

See "4.(ii).A.(6).2) QT/QTc evaluation study" for TES11807.[103]

4.(iii).A.(2) Phase II studies

4.(iii).A.(2).1) Multinational Phase II study (5.3.5.1-1, PDY6797 [20 to 20])

A randomized, double-blind,[104] placebo-controlled, parallel-group study was conducted to evaluate the efficacy, safety, and pharmacokinetics of lixisenatide in Japanese and non-Japanese[105] patients with type 2 diabetes mellitus[106] (target sample size of 120 subjects; 60 Japanese subjects and 60 non-Japanese subjects).

The study consisted of a single-dose period and a multiple-dose period.

In the single-dose period, a single subcutaneous dose of lixisenatide 5 or 10 μg was administered. In the multiple-dose period, lixisenatide was subcutaneously administered 30 minutes prior to breakfast and evening meal in the BID group, lixisenatide was subcutaneously administered 30 minutes prior to breakfast and placebo was subcutaneously administered 30 minutes prior to evening meal in the QD group, and placebo was subcutaneously administered 30 minutes prior to breakfast and evening meal in the placebo group. In both the lixisenatide QD and BID groups, the starting dose was 5 or 10 μg, and the dose was increased by 5 μg/week up to 30 μg. The duration of treatment was 6 weeks for subjects who received a starting dose of 5 μg and 5 weeks for subjects who received a starting dose of 10 μg.

The disposition of 120 treated patients (40 patients in the placebo group and 80 patients in the lixisenatide group [39 patients in the lixisenatide QD group and 41 patients in the lixisenatide BID group]) was as follows: 21 Japanese patients in the placebo group, 20 Japanese patients in the lixisenatide QD group, and 22 Japanese patients in the lixisenatide BID group; and 19 non-Japanese patients in the placebo group, 19 non-Japanese patients in the lixisenatide QD group, and 19 non-Japanese patients in the lixisenatide BID group. All the treated patients were included in the safety population. The primary efficacy population was the per-protocol (PP) population. Excluded were 10 patients with protocol deviations (5 Japanese patients [3 patients in the lixisenatide QD group and 2 patients in the lixisenatide BID group]; and 5 non-Japanese patients [1 patient in the placebo group, 2 patients in the lixisenatide QD group, and 2 patients in the lixisenatide BID group]).

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[103] Due to the limited number of subjects and variability in measurement results, Study TES11807 was conducted as a second QT/QTc evaluation study after the conduct of Study TES6865 [See footnote 90]. In this review report, only the results of Study TES11807 are presented.

[104] Blinding between doses could not be maintained, but double-blinding was maintained with regard to assignment of placebo or lixisenatide and dosing frequency.

[105] Germany, Holland, Australia, and South Africa

[106] Patients aged 20 to 75 years with type 2 DM being treated with SU alone or SU in combination with metformin hydrochloride (at a stable dose for at least 3 months prior to screening, with no other antidiabetic drugs), with BMI ≤35 kg/m^2 and HbA1c of ≥7.0% and ≤10.0%.

The primary efficacy endpoint of the change in the AUC of postprandial plasma glucose after a standardized breakfast (PPG-$AUC_{0:29\text{-}4:30\ h}$) from baseline to endpoint at the highest well tolerated dose in the PP population was as shown in Table 15.

Table 15. Change in PPG-$AUC_{0:29\text{-}4:30\ h}$ from baseline to endpoint at the highest well tolerated dose (PDY6797, Overall population, PP population)

	Placebo (n = 39)	Lixisenatide QD (n = 34)	Lixisenatide BID (n = 37)
Baseline[a) c)]	887.0 ± 166.9	830.0 ± 156.1	870.8 ± 175.6
Endpoint[a) c)]	772.4 ± 140.5	420.4 ± 105.7	473.5 ± 126.9
Change from baseline[a) c)]	-114.5 ± 126.6	-409.6 ± 170.6	-397.3 ± 202.8
Difference vs. placebo [95% CI][b) c)]	—	-333.4 [-386.78, -280.00]	-288.8 [-340.63, -237.00]

a) Mean ± SD (mg･h/dL)
b) ANCOVA model with treatment, cohort, Japanese/non-Japanese, and interaction between treatment and Japanese/non-Japanese as fixed effects and baseline value as a covariate
c) One patient in the placebo group was excluded from the analysis due to missing baseline data.

The results in the Japanese and non-Japanese populations were as shown in Table 16.

Table 16. Change in PPG-$AUC_{0:29\text{-}4:30\ h}$ from baseline to endpoint at the highest well tolerated dose (PDY6797, Japanese and non-Japanese populations, PP population)

	Japanese population			Non-Japanese population		
	Placebo (n = 21)	Lixisenatide QD (n = 17)	Lixisenatide BID (n = 20)	Placebo (n = 18)	Lixisenatide QD (n = 17)	Lixisenatide BID (n = 17)
Baseline[a) c)]	871.6 ± 156.3	877.0 ± 157.8	861.3 ± 197.1	905.9 ± 182.1	782.9 ± 143.6	881.9 ± 151.7
Endpoint[a) c)]	783.8 ± 136.6	377.2 ± 109.2	434.9 ± 130.5	758.4 ± 148.2	463.6 ± 84.6	518.9 ± 109.5
Change from baseline[a) c)]	-87.8 ± 127.6	-499.8 ± 155.3	-426.4 ± 240.9	-147.6 ± 121.0	-319.3 ± 136.4	-363.0 ± 146.1
Difference vs. placebo [95% CI][b) c)]	—	-406.7 [-479.53, -333.77]	-346.3 [-416.04, -276.56]	—	-260.1 [-338.52, -181.73]	-231.3 [-307.96, -154.71]

a) Mean ± SD (mg·h/dL)
b) ANCOVA model with treatment, cohort, Japanese/non-Japanese, and interaction between treatment and Japanese/non-Japanese as fixed effects and baseline value as a covariate
c) One non-Japanese patient in the placebo group was excluded from the analysis due to missing baseline data.

The results of key secondary endpoints were as shown in Table 17.

Table 17 Results of key secondary endpoints (PDY6797, Japanese and non-Japanese populations, PP population)

		Japanese population			Non-Japanese population		
		Placebo	Lixisenatide QD	Lixisenatide BID	Placebo	Lixisenatide QD	Lixisenatide BID
PPG-$AUC_{0:29\text{-}4.30h}$[a)]	10 μg dose	-81.1 ± 68.7 (n = 20)	-417.7 ± 135.0 (n = 17)	-325.8 ± 244.3 (n = 19)	-79.6 ± 81.9 (n = 17)	-225.6 ± 143.4 (n = 17)	-218.9 ± 107.5 (n = 17)
	20 μg dose	-43.8 ± 154.1 (n = 20)	-509.0 ± 132.1 (n = 17)	-398.5 ± 274.1 (n = 19)	-111.5 ± 138.3 (n = 17)	-283.5 ± 166.6 (n = 17)	-308.2 ± 132.4 (n = 17)
	30 μg dose	-85.0 ± 130.3 (n = 20)	-462.1 ± 124.6 (n = 11)	-426.9 ± 251.8 (n = 16)	-147.6 ± 121.0 (n = 17)	-328.6 ± 151.0 (n = 13)	-363.0 ± 146.1 (n = 17)
FPG[b)]	Highest well tolerated dose	-7.8 ± 25.6 (n = 21)	-34.6 ± 22.6 (n = 17)	-51.2 ± 25.2 (n = 20)	-25.3 ± 44.4 (n = 18)	-31.0 ± 28.7 (n = 17)	-38.9 ± 46.2 (n = 17)

a) Change in PPG-$AUC_{0:29\text{-}4.30h}$ after a standardized breakfast from baseline to endpoint at each dose level. Mean ± SD (mg·h/dL)
b) Change in fasting plasma glucose (FPG) from baseline to endpoint at the highest well tolerated dose. Mean ± SD (mg/dL)

With regard to safety, the incidences of adverse events were 61.9% (13 of 21 subjects) in the placebo group, 85.0% (17 of 20 subjects) in the lixisenatide QD group, and 77.3% (17 of 22 subjects) in the lixisenatide BID group in the Japanese population, and 84.2% (16 of 19 subjects) in the placebo group, 94.7% (18 of 19

subjects) in the lixisenatide QD group, and 73.7% (14 of 19 subjects) in the lixisenatide BID group in the non-Japanese population. The incidences of adverse drug reactions were 38.1% (8 of 21 subjects) in the placebo group, 70.0% (14 of 20 subjects) in the lixisenatide QD group, and 72.7% (16 of 22 subjects) in the lixisenatide BID group in the Japanese population, and 21.1% (4 of 19 subjects) in the placebo group, 68.4% (13 of 19 subjects) in the lixisenatide QD group, and 47.4% (9 of 19 subjects) in the lixisenatide BID group in the non-Japanese population. The main adverse events were gastrointestinal disorders and the event with the highest incidence was nausea. Adverse events and adverse drug reactions reported by ≥2 patients in any of the treatment groups in the Japanese population or non-Japanese population are summarized in Tables 18 and 19, respectively.

Table 18. Adverse events reported by ≥2 patients in any treatment group in Japanese population or non-Japanese population (PDY6797, Safetypopulation)

Event term	Japanese population			Non-Japanese population		
	Placebo (n = 21)	Lixisenatide QD (n = 20)	Lixisenatide BID (n = 22)	Placebo (n = 19)	Lixisenatide QD (n = 19)	Lixisenatide BID (n = 19)
Any event	61.9 (13)	85.0 (17)	77.3 (17)	84.2 (16)	94.7 (18)	73.7 (14)
Nasopharyngitis	14.3 (3)	20.0 (4)	4.5 (1)	10.5 (2)	0.0 (0)	5.3 (1)
Hypoglycaemia	9.5 (2)	20.0 (4)	31.8 (7)	0.0 (0)	26.3 (5)	10.5 (2)
Decreased appetite	0.0 (0)	15.0 (3)	4.5 (1)	5.3 (1)	10.5 (2)	21.1 (4)
Headache	4.8 (1)	15.0 (3)	0.0 (0)	36.8 (7)	15.8 (3)	21.1 (4)
Dizziness	4.8 (1)	15.0 (3)	0.0 (0)	0.0 (0)	5.3 (1)	5.3 (1)
Tremor	0.0 (0)	10.0 (2)	0.0 (0)	5.3 (1)	5.3 (1)	5.3 (1)
Cough	0.0 (0)	0.0 (0)	0.0 (0)	10.5 (2)	0.0 (0)	10.5 (2)
Oropharyngeal pain	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	10.5 (2)
Nausea	0.0 (0)	50.0 (10)	18.2 (4)	5.3 (1)	31.6 (6)	21.1 (4)
Diarrhoea	4.8 (1)	5.0 (1)	22.7 (5)	21.1 (4)	10.5 (2)	21.1 (4)
Vomiting	0.0 (0)	10.0 (2)	18.2 (4)	0.0 (0)	15.8 (3)	10.5 (2)
Constipation	4.8 (1)	10.0 (2)	13.6 (3)	0.0 (0)	0.0 (0)	5.3 (1)
Abdominal distension	0.0 (0)	10.0 (2)	0.0 (0)	5.3 (1)	10.5 (2)	5.3 (1)
Dyspepsia	0.0 (0)	0.0 (0)	9.1 (2)	0.0 (0)	10.5 (2)	5.3 (1)
Eructation	0.0 (0)	10.0 (2)	0.0 (0)	0.0 (0)	0.0 (0)	10.5 (2)
Abdominal discomfort	4.8 (1)	5.0 (1)	9.1 (2)	0.0 (0)	0.0 (0)	5.3 (1)
Flatulence	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	15.8 (3)
Abdominal pain	4.8 (1)	10.0 (2)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Hyperhidrosis	0.0 (0)	15.0 (3)	18.2 (4)	0.0 (0)	10.5 (2)	15.8 (3)
Cold sweat	4.8 (1)	0.0 (0)	9.1 (2)	0.0 (0)	0.0 (0)	0.0 (0)
Fatigue	0.0 (0)	0.0 (0)	9.1 (2)	5.3 (1)	5.3 (1)	15.8 (3)
Chills	0.0 (0)	15.0 (3)	4.5 (1)	0.0 (0)	0.0 (0)	0.0 (0)
Feeling abnormal	0.0 (0)	0.0 (0)	9.1 (2)	0.0 (0)	0.0 (0)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

Table 19. Adverse drug reactions reported by ≥2 patients in any treatment group in Japanese population or non-Japanese population (PDY6797, Safetypopulation)

Event term	Japanese population			Non-Japanese population		
	Placebo (n = 21)	Lixisenatide QD (n = 20)	Lixisenatide BID (n = 22)	Placebo (n = 19)	Lixisenatide QD (n = 19)	Lixisenatide BID (n = 19)
Any event	38.1 (8)	70.0 (14)	72.7 (16)	21.1 (4)	68.4 (13)	47.4 (9)
Hypoglycaemia	9.5 (2)	15.0 (3)	27.3 (6)	0.0 (0)	21.1 (4)	10.5 (2)
Decreased appetite	0.0 (0)	15.0 (3)	4.5 (1)	5.3 (1)	10.5 (2)	21.1 (4)
Headache	0.0 (0)	10.0 (2)	0.0 (0)	5.3 (1)	5.3 (1)	0.0 (0)
Dizziness	4.8 (1)	15.0 (3)	0.0 (0)	0.0 (0)	5.3 (1)	0.0 (0)
Tremor	0.0 (0)	10.0 (2)	0.0 (0)	5.3 (1)	5.3 (1)	5.3 (1)
Nausea	0.0 (0)	50.0 (10)	18.2 (4)	5.3 (1)	31.6 (6)	15.8 (3)
Diarrhoea	0.0 (0)	0.0 (0)	13.6 (3)	5.3 (1)	5.3 (1)	0.0 (0)
Vomiting	0.0 (0)	10.0 (2)	18.2 (4)	0.0 (0)	10.5 (2)	5.3 (1)
Constipation	0.0 (0)	10.0 (2)	13.6 (3)	0.0 (0)	0.0 (0)	0.0 (0)
Abdominal distension	0.0 (0)	10.0 (2)	0.0 (0)	0.0 (0)	10.5 (2)	0.0 (0)
Dyspepsia	0.0 (0)	0.0 (0)	9.1 (2)	0.0 (0)	5.3 (1)	5.3 (1)
Eructation	0.0 (0)	10.0 (2)	0.0 (0)	0.0 (0)	0.0 (0)	10.5 (2)
Abdominal discomfort	4.8 (1)	5.0 (1)	9.1 (2)	0.0 (0)	0.0 (0)	5.3 (1)
Flatulence	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	10.5 (2)
Abdominal pain	0.0 (0)	10.0 (2)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Hyperhidrosis	0.0 (0)	15.0 (3)	13.6 (3)	0.0 (0)	10.5 (2)	5.3 (1)
Cold sweat	4.8 (1)	0.0 (0)	9.1 (2)	0.0 (0)	0.0 (0)	0.0 (0)
Chills	0.0 (0)	15.0 (3)	4.5 (1)	0.0 (0)	0.0 (0)	0.0 (0)
Feeling abnormal	0.0 (0)	0.0 (0)	9.1 (2)	0.0 (0)	0.0 (0)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

No death occurred. No serious adverse events were reported in the Japanese population while serious adverse events occurred in 1 patient (coronary artery disease) in the placebo group and 1 patient (atrioventricular block second degree) in the lixisenatide BID group in the non-Japanese population. Both events led to treatment discontinuation, but their causal relationship to study drug was denied. Adverse events leading to treatment discontinuation occurred in 1 patient (gastroenteritis viral) in the lixisenatide BID group in the Japanese population, and 1 patient (coronary artery disease) in the placebo group and 1 patient (hypertension/atrioventricular block second degree) in the lixisenatide BID group in the non-Japanese population.

With regard to hypoglycaemia, the incidences of symptomatic hypoglycaemia[107] were 14.3% (3 of 21 subjects) in the placebo group, 20.0% (4 of 20 subjects) in the lixisenatide QD group, and 31.8% (7 of 22 subjects) in the lixisenatide BID group in the Japanese population, and 0.0% (0 of 19 subjects) in the placebo group, 21.1% (4 of 19 subjects) in the lixisenatide QD group, and 10.5% (2 of 19 subjects) in the lixisenatide BID group in the non-Japanese population. All of the subjects who developed symptomatic hypoglycaemia were taking concomitant SU. No severe symptomatic hypoglycaemia[108] occurred.

There were no clinically meaningful changes in vital signs or laboratory values. There were no clinically meaningful changes in ECG except for a serious adverse event of coronary artery disease. A QTcF interval of

[107] Symptomatic hypoglycaemia was defined as symptoms consistent with hypoglycaemia, with an accompanying plasma glucose <60 mg/dL (3.3 mmol/L) or prompt recovery with oral carbohydrate, intravenous glucose, or glucagon administration if no plasma glucose value was available.
[108] Severe symptomatic hypoglycaemia was defined as an episode with symptoms consistent with acute neuroglycopenia in which the subject was unable to self-treat, requiring the assistance of another person, associated with either of the following:
• Plasma glucose <36 mg/dL (2.0 mmol/L).
• Prompt recovery with oral carbohydrate, intravenous glucose, or glucagon administration if no plasma glucose value was available.

≥500 ms was noted in 1 subject in the placebo group. An increase in QTcF interval of >60 ms from baseline was seen in 1 subject in the placebo group.

Injection site reactions occurred in 1 patient (application site erythema) in the placebo group in the Japanese population, and 1 patient (injection site pain and injection site reaction) in the lixisenatide QD group in the non-Japanese population.

With regard to anti-lixisenatide antibody, 26.2% (11 of 42 patients) of the Japanese population and 28.9% (11 of 38 patients) of the non-Japanese population were antibody-positive at the end of treatment. One patient in the Japanese population was antibody-positive at baseline and the antibody titer remained low throughout the study period.

4.(iii).A.(2).2) Foreign phase II study (5.3.5.1-2, Study DRI6012 [20 to 20])

A randomized, double-blind,[104] placebo-controlled, parallel-group study was conducted to evaluate the efficacy, safety, and dose-response relationship of lixisenatide in non-Japanese[109] patients with type 2 diabetes mellitus[110] (target sample size of 500 subjects).

Following a 2-week placebo run-in period, placebo (placebo administered prior to breakfast and evening meal), lixisenatide 5, 10, 20, or 30 μg QD (lixisenatide administered prior to breakfast and placebo administered prior to evening meal), or lixisenatide 5, 10, 20, or 30 μg BID (prior to breakfast and evening meal) was subcutaneously administered. For subjects randomized to 20 and 30 μg dose levels of the study drug (both QD and BID groups), the dose was initiated at 10 μg and increased by 5 μg/week up to the randomized dose. The duration of treatment was 13 weeks.

All of 542 treated patients (placebo group, 109 patients; lixisenatide 5 μg QD group, 55 patients; lixisenatide 10 μg QD group, 52 patients; lixisenatide 20 μg QD group, 55 patients; lixisenatide 30 μg QD group, 54 patients; lixisenatide 5 μg BID group, 53 patients; lixisenatide 10 μg BID group, 56 patients; lixisenatide 20 μg BID group, 54 patients; lixisenatide 30 μg BID group, 54 patients) were included in the safety population. Of these, 529 patients were included in the Intent-to-treat (ITT) population. Excluded were 13 patients with no post-baseline values for efficacy variables (placebo group, 1 patient; lixisenatide 10 μg QD group, 1 patient; lixisenatide 20 μg QD group, 2 patients; lixisenatide 30 μg QD group, 2 patients; lixisenatide 5 μg BID group, 2 patients; lixisenatide 10 μg BID group, 2 patients; lixisenatide 20 μg BID group, 2 patients; lixisenatide 30 μg BID group, 1 patient). The primary efficacy population was the ITT population.

The primary efficacy endpoint of the change in HbA1c from baseline to endpoint in the ITT population was as shown in Table 20. When a trend test using contrast coefficients of [-2, -1, 0, 1, 2] for the BID groups (placebo group, lixisenatide 5 μg BID group, lixisenatide 10 μg BID group, lixisenatide 20 μg BID group, lixisenatide 30 μg BID group) was statistically significant, a trend test using contrast coefficients of [-2, -1, 0, 1, 2] for the QD groups (placebo group, lixisenatide 5 μg QD group, lixisenatide 10 μg QD group,

[109] The US, Brazil, Canada, Poland, Rumania, Russia, and Ukraine

[110] Patients aged 30 to 75 years with type 2 diabetes mellitus being treated with a stable dose of metformin hydrochloride (≥1 g/day) for at least 3 months prior to screening, with BMI of 25 to 40 kg/m^2 and HbA1c of ≥7.0% and <9.0%.

lixisenatide 20 μg QD group, lixisenatide 30 μg QD group) was to be performed. As a result, both trend tests were statistically significant (*P* <0.0001 each, two-sided significance level of 5%).

Table 20. Change in HbA1c from baseline to endpoint (DRI6012, Overall population: ITT population)

Treatment group	Baseline	Endpoint	Change from baseline	Difference vs. placebo [95% CI][a)]	*P*-value[a), b)]	*P*-value[a), b)]
Placebo (n = 108)	7.53 ± 0.63	7.32 ± 0.73	-0.21 ± 0.71	—	—	—
Lixisenatide 5 μg QD (n = 55)	7.58 ± 0.72	7.08 ± 0.75	-0.50 ± 0.58	-0.28 [-0.472,-0.091]	<0.0001	—
Lixisenatide 10 μg QD (n = 51)	7.53 ± 0.64	7.01 ± 0.67	-0.52 ± 0.55	-0.31 [-0.508, -0115]		—
Lixisenatide 20 μg QD (n = 53)	7.58 ± 0.66	6.85 ± 1.03	-0.73 ± 0.73	-0.50 [-0.695, -0.310]		—
Lixisenatide 30 μg QD (n = 52)	7.53 ± 0.67	6.75 ± 0.78	-0.78 ± 0.63	-0.57 [-0.768, -0.377]		—
Lixisenatide 5 μg BID (n = 51)	7.58 ± 0.56	6.90 ± 0.76	-0.68 ± 0.64	-0.47 [-0.661, -0.271]	—	<0.0001
Lixisenatide 10 μg BID (n = 54)	7.53 ± 0.61	6.73 ± 0.63	-0.80 ± 0.44	-0.59 [-0.784, -0.401]	—	
Lixisenatide 20 μg BID (n = 52)	7.62 ± 0.67	6.82 ± 0.61	-0.80 ± 0.62	-0.57 [-0.760, -0.372]	—	
Lixisenatide 30 μg BID (n = 53)	7.47 ± 0.52	6.59 ± 0.55	-0.88 ± 0.55	-0.69 [-0.878, -0.492]	—	

Mean ± SD (%)

a) ANCOVA model with treatment and country as fixed effects and baseline HbA1c as a covariate

b) Two-sided significance level of 5%. When a trend test using contrast coefficients of [-2, -1, 0, 1, 2] for BID groups (placebo group, lixisenatide 5 μg BID group, lixisenatide 10 μg BID group, lixisenatide 20 μg BID group, lixisenatide 30 μg BID group) was statistically significant, a trend test using contrast coefficients of [-2, -1, 0, 1, 2] for QD groups (placebo group, lixisenatide 5 μg QD group, lixisenatide 10 μg QD group, lixisenatide 20 μg QD group, lixisenatide 30 μg QD group) was to be performed.

With regard to key secondary endpoints, the changes (mean ± SD) in fasting plasma glucose (FPG) from baseline to endpoint were -3.97 ± 35.80 mg/dL in the placebo group, -8.66 ± 31.60 mg/dL in the lixisenatide 5 μg QD group, -9.22 ± 25.93 mg/dL in the lixisenatide 10 μg QD group, -11.88 ± 30.23 mg/dL in the lixisenatide 20 μg QD group, -18.37 ± 25.93 mg/dL in the lixisenatide 30 μg QD group, -3.46 ± 30.59 mg/dL in the lixisenatide 5 μg BID group, -19.68 ± 23.30 mg/dL in the lixisenatide 10 μg BID group, -22.29 ± 27.46 mg/dL in the lixisenatide 20 μg BID group, and -26.53 ± 40.43 mg/dL in the lixisenatide 30 μg BID group. Similarly, the changes in 2-hour post-prandial plasma glucose were -2.28 ± 61.05 mg/dL in the placebo group, -43.84 ± 53.13 mg/dL in the lixisenatide 5 μg QD group, -73.86 ± 56.33 mg/dL in the lixisenatide 10 μg QD group, -57.97 ± 63.23 mg/dL in the lixisenatide 20 μg QD group, -76.23 ± 39.46 mg/dL in the lixisenatide 30 μg QD group, -37.09 ± 57.86 mg/dL in the lixisenatide 5 μg BID group, -56.57 ± 46.47 mg/dL in the lixisenatide 10 μg BID group, -73.33 ± 55.72 mg/dL in the lixisenatide 20 μg BID group, and -87.07 ± 72.47 mg/dL in the lixisenatide 30 μg BID group.

With regard to safety, adverse events and adverse drug reactions reported by ≥3 patients in any of the treatment groups are summarized in Tables 21 and 22, respectively.

Table 21. Adverse events reported by ≥3 patients in any treatment group (DRI6012, Safety population)

Event term	Placebo (n = 109)	Lixisenatide QD				Lixisenatide BID			
		5 μg (n = 55)	10 μg (n = 52)	20 μg (n = 55)	30 μg (n = 54)	5 μg (n = 53)	10 μg (n = 56)	20 μg (n = 54)	30 μg (n = 54)
Any event	59.6 (65)	56.4 (31)	50.0 (26)	67.3 (37)	77.8 (42)	56.6 (30)	57.1 (32)	70.4 (38)	74.1 (40)
Influenza	4.6 (5)	0.0 (0)	1.9 (1)	1.8 (1)	3.7 (2)	1.9 (1)	1.8 (1)	5.6 (3)	1.9 (1)
Nasopharyngitis	4.6 (5)	0.0 (0)	3.8 (2)	1.8 (1)	1.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)	3.7 (2)
Decreased appetite	1.8 (2)	1.8 (1)	3.8 (2)	3.6 (2)	5.6 (3)	3.8 (2)	5.4 (3)	9.3 (5)	7.4 (4)
Hypoglycaemia	0.9 (1)	1.8 (1)	3.8 (2)	3.6 (2)	3.7 (2)	5.7 (3)	1.8 (1)	5.6 (3)	1.9 (1)
Headache	10.1 (11)	12.7 (7)	5.8 (3)	12.7 (7)	13.0 (7)	13.2 (7)	8.9 (5)	11.1 (6)	7.4 (4)
Dizziness	6.4 (7)	1.8 (1)	7.7 (4)	7.3 (4)	11.1 (6)	5.7 (3)	8.9 (5)	3.7 (2)	9.3 (5)
Tremor	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	1.9 (1)	3.8 (2)	7.1 (4)	3.7 (2)	0.0 (0)
Dysgeusia	0.9 (1)	0.0 (0)	0.0 (0)	5.5 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	1.9 (1)
Bundle branch block left	2.8 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Nausea	4.6 (5)	7.3 (4)	11.5 (6)	25.5 (14)	35.2 (19)	7.5 (4)	14.3 (8)	22.2 (12)	33.3 (18)
Diarrhoea	7.3 (8)	5.5 (3)	7.7 (4)	9.1 (5)	7.4 (4)	5.7 (3)	7.1 (4)	11.1 (6)	25.9 (14)
Vomiting	0.9 (1)	3.6 (2)	5.8 (3)	5.5 (3)	18.5 (10)	5.7 (3)	7.1 (4).	7.4 (4)	3.7 (2)
Dyspepsia	1.8 (2)	1.8 (1)	3.8 (2)	3.6 (2)	3.7 (2)	1.9 (1)	3.6 (2)	7.4 (4)	3.7 (2)
Abdominal pain	1.8 (2)	1.8 (1)	1.9 (1)	1.8 (1)	7.4 (4)	0.0 (0)	7.1 (4)	1.9 (1)	5.6 (3)
Abdominal pain upper	0.0 (0)	3.6 (2)	0.0 (0)	5.5 (3)	3.7 (2)	1.9 (1)	0.0 (0)	3.7 (2)	5.6 (3)
Constipation	1.8 (2)	1.8 (1)	1.9 (1)	0.0 (0)	5.6 (3)	0.0 (0)	0.0 (0)	5.6 (3)	3.7 (2)
Hyperhidrosis	0.9 (1)	3.6 (2)	0.0 (0)	1.8 (1)	5.6 (3)	1.9 (1)	3.6 (2)	5.6 (3)	7.4 (4)
Back pain	0.9 (1)	0.0 (0)	3.8 (2)	3.6 (2)	7.4 (4)	1.9 (1)	1.8 (1)	3.7 (2)	1.9 (1)
Pain in extremity	2.8 (3)	1.8 (1)	1.9 (1)	3.6 (2)	3.7 (2)	1.9 (1)	0.0 (0)	3.7 (2)	0.0 (0)
Arthralgia	3.7 (4)	1.8 (1)	0.0 (0)	1.8 (1)	0.0 (0)	1.9 (1)	3.6 (2)	3.7 (2)	1.9 (1)
Asthenia	0.0 (0)	0.0 (0)	3.8 (2)	9.1 (5)	5.6 (3)	0.0 (0)	3.6 (2)	3.7 (2)	9.3 (5)
Injection site haematoma	0.9 (1)	3.6 (2)	1.9 (1)	0.0 (0)	3.7 (2)	3.8 (2)	3.6 (2)	5.6 (3)	3.7 (2)
Injection site pain	0.9 (1)	0.0 (0)	0.0 (0)	1.8 (1)	7.4 (4)	0.0 (0)	1.8 (1)	0.0 (0)	5.6 (3)
Fatigue	2.8 (3)	0.0 (0)	1.9 (1)	1.8 (1)	0.0 (0)	1.9 (1)	0.0 (0)	1.9 (1)	0.0 (0)
Overdose	4.6 (5)	0.0 (0)	0.0 (0)	9.1 (5)	7.4 (4)	0.0 (0)	1.8 (1)	9.3 (5)	9.3 (5)

Incidence % (n), MedDRA/J (ver.13.1)

Table 22. Adverse drug reactions reported by ≥3 patients in any treatment group (DRI6012, Safety population)

Event term	Placebo (n = 109)	Lixisenatide QD				Lixisenatide BID			
		5 μg (n = 55)	10 μg (n = 52)	20 μg (n = 55)	30 μg (n = 54)	5 μg (n = 53)	10 μg (n = 56)	20 μg (n = 54)	30 μg (n = 54)
Any event	22.9 (25)	18.2 (10)	36.5 (19)	38.2 (21)	53.7 (29)	18.9 (10)	32.1 (18)	48.1 (26)	51.9 (28)
Decreased appetite	1.8 (2)	1.8 (1)	3.8 (2)	1.8 (1)	5.6 (3)	3.8 (2)	3.6 (2)	9.3 (5)	7.4 (4)
Hypoglycaemia	0.9 (1)	1.8 (1)	1.9 (1)	3.6 (2)	1.9 (1)	1.9 (1)	1.8 (1)	5.6 (3)	1.9 (1)
Headache	4.6 (5)	1.8 (1)	1.9 (1)	1.8 (1)	5.6 (3)	5.7 (3)	0.0 (0)	7.4 (4)	5.6 (3)
Dizziness	3.7 (4)	0.0 (0)	5.8 (3)	7.3 (4)	7.4 (4)	3.8 (2)	5.4 (3)	3.7 (2)	3.7 (2)
Tremor	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	1.9 (1)	1.9 (1)	5.4 (3)	1.9 (1)	0.0 (0)
Nausea	2.8 (3)	5.5 (3)	9.6 (5)	18.2 (10)	33.3 (18)	3.8 (2)	10.7 (6)	20.4 (11)	24.1 (13)
Diarrhoea	0.9 (1)	3.6 (2)	7.7 (4)	5.5 (3)	1.9 (1)	0.0 (0)	1.8 (1)	5.6 (3)	9.3 (5)
Vomiting	0.9 (1)	0.0 (0)	1.9 (1)	3.6 (2)	16.7 (9)	3.8 (2)	3.6 (2)	5.6 (3)	3.7 (2)
Dyspepsia	1.8 (2)	0.0 (0)	3.8 (2)	3.6 (2)	1.9 (1)	0.0 (0)	1.8 (1)	7.4 (4)	1.9 (1)
Abdominal pain	0.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)	1.9 (1)	0.0 (0)	1.8 (1)	1.9 (1)	5.6 (3)
Constipation	0.9 (1)	1.8 (1)	1.9 (1)	0.0 (0)	1.9 (1)	0.0 (0)	0.0 (0)	5.6 (3)	1.9 (1)
Hyperhidrosis	0.9 (1)	0.0 (0)	0.0 (0)	0.0 (0)	5.6 (3)	0.0 (0)	3.6 (2)	5.6 (3)	5.6 (3)
Asthenia	0.0 (0)	0.0 (0)	1.9 (1)	1.8 (1)	1.9 (1)	0.0 (0)	1.8 (1)	1.9 (1)	5.6 (3)
Injection site pain	0.9 (1)	0.0 (0)	0.0 (0)	1.8 (1)	7.4 (4)	0.0 (0)	1.8 (1)	0.0 (0)	5.6 (3)
Fatigue	2.8 (3)	0.0 (0)	0.0 (0)	1.8 (1)	0.0 (0)	0.0 (0)	0.0 (0)	1.9 (1)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

No death occurred. Serious adverse events occurred in 3 patients (appendicitis perforated, squamous cell carcinoma, and cerebrovascular accident in 1 patient each) in the placebo group, 1 patient (anaphylactic reaction) in the lixisenatide 10 μg QD group, 1 patient (acute myeloid leukaemia) in the lixisenatide 20 μg QD group, 3 patients (basal cell carcinoma, loss of consciousness, and syncope in 1 patient each) in the lixisenatide 30 μg QD group, 1 patient (non-cardiac chest pain) in the lixisenatide 10 μg BID group, and 2 patients (gallbladder perforation and chest pain in 1 patient each) in the lixisenatide 20 μg BID group. Of these, the events reported by 1 patient (anaphylactic reaction) in the lixisenatide 10 μg QD group and 1 patient (loss of consciousness) in the lixisenatide 30 μg QD group were classified as adverse drug reactions. Adverse events leading to treatment discontinuation occurred in 2 patients (appendicitis perforated and urticaria in 1 patient each) in the placebo group, 1 patient (hypersensitivity) in the lixisenatide 5 μg QD group, 2 patients (anaphylactic reaction and dizziness/tachycardia in 1 patient each) in the lixisenatide 10 μg QD group, 2 patients (nausea and acute myeloid leukaemia in 1 patient each) in the lixisenatide 20 μg QD group, 6 patients (nausea/vomiting in 2 patients, vomiting in 2 patients, loss of consciousness and transaminases increased in 1 patient each) in the lixisenatide 30 μg QD group, 2 patients (nausea and urticaria in 1 patient each) in the lixisenatide 10 μg BID group, 8 patients (nausea in 2 patients, nausea/vomiting projectile/decreased appetite, diarrhoea/flatulence/headache/somnolence/hyperhidrosis, diarrhoea, gallbladder perforation, fatigue, and injection site hypersensitivity in 1 patient each) in the lixisenatide 20 μg BID group, and 4 patients (injection site reaction in 2 patients, nausea and nausea/dizziness/vomiting in 1 patient each) in the lixisenatide 30 μg BID group. Of these, the events reported by 1 patient (urticaria) in the placebo group, 1 patient (hypersensitivity) in the lixisenatide 5 μg QD group, 2 patients (anaphylactic reaction, dizziness/tachycardia) in the lixisenatide 10 μg QD group, 6 patients (nausea/vomiting in 2 patients, vomiting in 2 patients, loss of consciousness, transaminases increased) in the lixisenatide 30 μg QD group, 2 patients (nausea and urticaria in 1 patient each) in the lixisenatide 10 μg BID group, 7 patients (nausea in 2 patients, nausea/vomiting projectile/decreased appetite, flatulence/headache/somnolence/hyperhidrosis, diarrhoea, fatigue, injection site hypersensitivity) in the lixisenatide 20 μg BID group, and 4 patients (injection site reaction in 2 patients, nausea and nausea/dizziness/vomiting in 1 patient each) in the lixisenatide 30 μg BID group were classified as adverse drug reactions. In the lixisenatide 5 μg BID group, there was no adverse event leading to treatment discontinuation.

With regard to hypoglycaemia, the incidences of symptomatic hypoglycaemia were 0.9% (1 of 109 subjects) in the placebo group, 1.8% (1 of 55 subjects) in the lixisenatide 5 μg QD group, 3.8% (2 of 52 subjects) in the lixisenatide 10 μg QD group, 1.8% (1 of 55 subjects) in the lixisenatide 20 μg QD group, 1.9% (1 of 54 subjects) in the lixisenatide 30 μg QD group, 5.7% (3 of 53 subjects) in the lixisenatide 5 μg BID group, 1.8% (1 of 56 subjects) in the lixisenatide 10 μg BID group, 5.6% (3 of 54 subjects) in the lixisenatide 20 μg BID group, and 1.9% (1 of 54 subjects) in the lixisenatide 30 μg BID group. No severe symptomatic hypoglycaemia occurred.

With regard to vital signs, the incidences of potentially clinically significant abnormalities for increases in systolic blood pressure (≥160 mmHg and an increase of ≥20 mmHg at any time point during the study period) were 9.2% (10 of 109 subjects) in the placebo group and 5.5% (24 of 433 subjects) in the lixisenatide group. Potentially clinically significant abnormalities for decreases in systolic blood pressure (≤95 mmHg and a decrease of ≥20 mmHg from baseline) were noted in 11 subjects only in the lixisenatide group.

Potentially clinically significant abnormalities for increases in heart rate (≥120 beats/min and an increase of ≥20 beats/min from baseline) were seen in 1 subject each in the lixisenatide 10 and 20 μg BID groups. With regard to ECG findings, A potentially clinically significant abnormality for decreases in heart rate (≤50 beats/min and a decrease of ≥20 beats/min from baseline) was noted in 1 subject in the lixisenatide 10 μg QD group.

The QTcF interval was ≥500 ms in 3 subjects in the placebo group and 1 subject in the lixisenatide 20 μg QD group. The QTcF interval increased by >60 ms from baseline in 4 subjects in the placebo group, 2 subjects in the lixisenatide 5 μg QD group, 1 subject in the lixisenatide 20 μg QD group, 2 subjects in the lixisenatide 5 μg BID group, and 3 subjects in the lixisenatide 30 μg BID group.

Injection site reactions occurred in 1 patient in the placebo group and 4 patients in the lixisenatide 30 μg BID group.

With regard to anti-lixisenatide antibody, the percentages of antibody-positive subjects at the end of treatment were 58.2% (32 of 55 subjects) in the lixisenatide 5 μg QD group, 43.1% (22 of 51 subjects) in the lixisenatide 10 μg QD group, 49.1% (26 of 53 subjects) in the lixisenatide 20 μg QD group, 57.7% (30 of 52 subjects) in the lixisenatide 30 μg QD group, 62.7% (32 of 51 subjects) in the lixisenatide 5 μg BID group, 66.7% (36 of 54 subjects) in the lixisenatide 10 μg BID group, 71.2% (37 of 52 subjects) in the lixisenatide 20 μg BID group, and 62.3% (33 of 53 subjects) in the lixisenatide 30 μg BID group.

4.(iii).A.(3) Phase III studies

4.(iii).A.(3).1) Multinational phase III study (in combination with SU with or without metformin hydrochloride[111]) (5.3.5.1-4, Study EFC6015 [20 to 20])

A randomized, double-blind, placebo-controlled, parallel-group study was conducted to evaluate the efficacy and safety of lixisenatide in Japanese and non-Japanese[112] patients with type 2 diabetes mellitus[113] (target sample size of 855 subjects [285 subjects in the placebo group and 570 subjects in the lixisenatide group]).

Following a one-week placebo run-in period, placebo or lixisenatide was subcutaneously administered (self-injection) QD within 1 hour prior to breakfast in the upper arm, abdomen, or thigh. The starting dose was 10 μg, and the dose was increased by 5 μg/week up to 20 μg. The duration of treatment was ≥76 weeks. The dose of SU was reduced by 25% to 50% in patients with screening HbA1c <8.0% and it was allowed to be increased to the dose at screening between Week 4 and 12 if no hypoglycaemia occurred and the majority of fasting plasma glucose self-monitoring readings were ≥126 mg/dL. If severe symptomatic hypoglycaemia or ≥2 episodes of suspected symptomatic hypoglycaemia occurred, the SU dose was to be reduced, as appropriate.

All of 859 treated patients (286 patients in the placebo group and 573 patients in the lixisenatide group) were included in the safety population (285 patients in the placebo group and 574 patients in the lixisenatide

[111] Hereinafter metformin hydrochloride is referred to as metformin.
[112] The US, Germany, Bulgaria, Czech Republic, Holland, Rumania, Russia, South Korea, Taiwan, Thailand, India, Israel, Turkey, Egypt, and Tunisia
[113] Patients with type 2 diabetes mellitus diagnosed ≥1 year before screening, being treated with SU at a stable dose (at least the maximum recommended dose in each region) for at least 3 months prior to screening or SU in combination with metformin at a stable dose of ≥1.5 g/day (except ≥0.75 g/day in Japan and ≥1.0 g/day in South Korea) for at least 3 months prior to screening, with HbA1c of ≥7.0% and ≤10.0% at screening.

group[115]). Of these, 856 patients were included in the modified intent-to-treat (mITT) population. Excluded were 3 patients with no post-baseline values for efficacy variables in the lixisenatide group. The primary efficacy population was the mITT population.

The primary efficacy endpoint of the change in HbA1c from baseline to Week 24 in the mITT population was as shown in Table 23. In the overall population, the difference of lixisenatide versus placebo was statistically significant (*P* <0.0001, two-sided significance level of 5%, ANCOVA model[114]).

Table 23. Change in HbA1c from baseline to Week 24 (EFC6015, Overall population and Japanese population, mITT population)

	Treatment group	Baseline	Week 24 (LOCF)	Change from baseline (LOCF)	Difference vs. placebo [95% CI][b)]	*P*-value[b)]
Overall population[a)]	Placebo (n = 274)	8.22 ± 0.83	8.10 ± 1.11	-0.12 ± 0.82	-0.74 [-0.867, -0.621]	<0.0001
	Lixisenatide (n = 544)	8.28 ± 0.86	7.40 ± 1.00	-0.88 ± 0.93		
Japanese population	Placebo (n = 51)	8.62 ± 0.79	8.81 ± 1.15	0.19 ± 0.78	-1.10 [-1.407, -0.803]	—
	Lixisenatide (n = 76)	8.38 ± 0.92	7.53 ± 1.03	-0.86 ± 0.84		

Mean ± SD (%)

a) Twelve patients in the placebo group and 26 patients in the lixisenatide group were excluded from the analysis because only measurements after the introduction of rescue medication or after treatment cessation plus 3 days were available.

b) Two-sided significance level of 5%, ANCOVA model

A plot of change in HbA1c from baseline by visit was as illustrated in Figure 1.

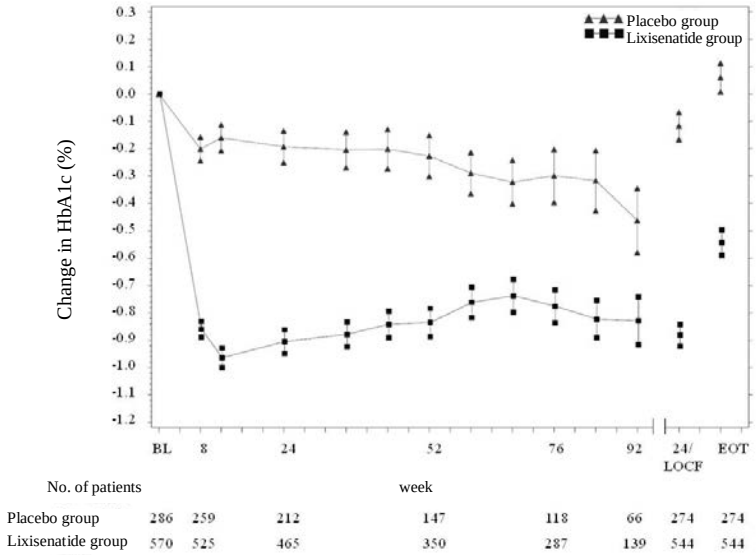

Figure 1. Plot of change in HbA1c from baseline by visit (mean ± SE) (EFC6015)

The results of key secondary endpoints were as shown in Table 24.

[114] ANCOVA model with treatment (lixisenatide or placebo), HbA1c (<8.0% or ≥8.0%) at screening, metformin use at screening, and country (not included in the analysis of the Japanese population) as fixed effects and baseline HbA1c as a covariate.

Table 24. Results of key secondary endpoints (EFC6015, Overall population, mITT population)

Endpoint		Placebo (n = 286)	Lixisenatide (n = 570)
FPG (mg/dL)	Baseline	167.44 ± 42.70 (n = 283)	174.22 ± 40.35 (n = 564)
	Change at Week 24 (LOCF)	-1.86 ± 36.05 (n = 283)	-16.75 ± 42.68 (n = 564)
	Change at Week 76	-7.64 ± 44.3 (n = 119)	-16.3 ± 43.9 (n = 287)
2-hour PPG (mg/dL)	Baseline	298.20 ± 67.46 (n = 120)	299.31 ± 73.61 (n = 249)
	Change at Week 24 (LOCF)	2.10 ± 66.79 (n = 120)	-108.10 ± 97.14 (n = 249)
	Change at Week 76	-21.3 ± 72.44 (n = 46)	-73.5 ± 90.59 (n = 107)
Body weight (kg)	Baseline	84.52 ± 22.81 (n = 278)	82.58 ± 21.88 (n = 554)
	Change at Week 24 (LOCF)	-0.89 ± 2.48 (n = 278)	-1.67 ± 3.08 (n = 554)
	Change at Week 76	-1.89 ± 3.86 (n = 117)	-2.14 ± 3.83 (n = 287)
Percentage of subjects achieving HbA1c <7.0% at Week 24 (%) (LOCF)[a)]		13.5% (37/274)	36.4% (198/544)
Percentage of subjects achieving HbA1c ≤6.5% at Week 24 (%) (LOCF)[a)]		4.7% (13/274)	19.3% (105/544)
Percentage of subjects requiring rescue therapy during 24-week treatment period		12.6% (36/286)	4.0% (23/570)

Mean ± SD

a) Subjects requiring rescue therapy and subjects who stopped study treatment for ≥3 days were excluded.

With regard to safety, the incidences of adverse events were 75.8% (216 of 285 subjects[115]) in the placebo group and 81.5% (468 of 574 subjects) in the lixisenatide group and the incidences of adverse drug reactions were 31.9% (91 of 285 subjects) in the placebo group and 48.4% (278 of 574 subjects) in the lixisenatide group. Adverse events and/or adverse drug reactions reported by ≥3% of patients in either treatment group in the overall population, and adverse events and/or adverse drug reactions reported by ≥5% of patients in either treatment group in the Japanese population are summarized in Tables 25 and 26, respectively.

[115] One subject was assigned to the placebo group but mistakenly received lixisenatide kit thus used lixisenatide during the majority of the study period (543 of 561 days). Therefore, this subject was included in the placebo group for the mITT population for efficacy analysis and in the lixisenatide group for the safety population.

Table 25. Adverse events and/or adverse drug reactions reported by ≥3% of patients in either treatment group (EFC6015, Overall population, Safety population)

Event term	Placebo (n = 285)		Lixisenatide (n = 574)	
	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions
Any event	75.8 (216)	31.9 (91)	81.5 (468)	48.4 (278)
Nasopharyngitis	20.4 (58)	0.0 (0)	15.9 (91)	0.0 (0)
Upper respiratory tract infection	7.4 (21)	0.0 (0)	7.5 (43)	0.0 (0)
Influenza	3.9 (11)	0.0 (0)	5.2 (30)	0.0 (0)
Bronchitis	2.5 (7)	0.0 (0)	4.4 (25)	0.0 (0)
Hypoglycaemia	19.3 (55)	12.6 (36)	24.6 (141)	19.3 (111)
Decreased appetite	2.8 (8)	1.8 (5)	4.5 (26)	4.0 (23)
Dizziness	6.3 (18)	1.8 (5)	10.5 (60)	5.6 (32)
Headache	7.0 (20)	2.5 (7)	7.7 (44)	2.1 (12)
Tremor	1.1 (3)	1.1 (3)	3.1 (18)	1.7 (10)
Hypertension	3.9 (11)	0.7 (2)	4.4 (25)	0.2 (1)
Cough	4.2 (12)	0.0 (0)	2.4 (14)	0.0 (0)
Nausea	8.8 (25)	4.2 (12)	28.0 (161)	23.3 (134)
Diarrhoea	9.5 (27)	3.9 (11)	12.4 (71)	3.7 (21)
Vomiting	5.3 (15)	1.1 (3)	10.6 (61)	7.1 (41)
Dyspepsia	1.4 (4)	0.4 (1)	5.9 (34)	3.8 (22)
Constipation	3.9 (11)	2.1 (6)	5.2 (30)	2.6 (15)
Abdominal pain	2.8 (8)	1.1 (3)	3.8 (22)	0.9 (5)
Abdominal distension	1.1 (3)	0.4 (1)	3.7 (21)	1.9 (11)
Abdominal pain upper	2.5 (7)	0.7 (2)	3.0 (17)	0.7 (4)
Back pain	4.2 (12)	0.4 (1)	6.3 (36)	0.0 (0)
Arthralgia	3.9 (11)	0.0 (0)	3.5 (20)	0.0 (0)
Pain in extremity	2.1 (6)	0.0 (0)	3.0 (17)	0.0 (0)
Fatigue	2.1 (6)	0.4 (1)	4.4 (25)	1.4 (8)
Asthenia	2.5 (7)	1.1 (3)	4.2 (24)	2.3 (13)
Blood glucose decreased	3.9 (11)	2.1 (6)	5.2 (30)	3.3 (19)
Fall	4.2 (12)	0.0 (0)	1.7 (10)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

Table 26. Adverse events and/or adverse drug reactions reported by ≥5% of patients in either treatment group (EFC6015, Japanese population, Safety population)

Event term	Placebo (n = 51)		Lixisenatide (n = 76)	
	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions
Any event	94.1 (48)	45.1 (23)	97.4 (74)	65.8 (50)
Nasopharyngitis	56.9 (29)	0.0 (0)	46.1 (35)	0.0 (0)
Influenza	0.0 (0)	0.0 (0)	5.3 (4)	0.0 (0)
Bronchitis	2.0 (1)	0.0 (0)	6.6 (5)	0.0 (0)
Gastroenteritis	3.9 (2)	2.0 (1)	5.3 (4)	1.3 (1)
Cystitis	0.0 (0)	0.0 (0)	5.3 (4)	0.0 (0)
Cellulitis	5.9 (3)	0.0 (0)	0.0 (0)	0.0 (0)
Hypoglycaemia	17.6 (9)	5.9 (3)	28.9 (22)	25.0 (19)
Decreased appetite	0.0 (0)	0.0 (0)	6.6 (5)	6.6 (5)
Dizziness	2.0 (1)	2.0 (1)	7.9 (6)	6.6 (5)
Headache	9.8 (5)	3.9 (2)	5.3 (4)	0.0 (0)
Diabetic retinopathy	9.8 (5)	5.9 (3)	9.2 (7)	2.6 (2)
Hypertension	5.9 (3)	3.9 (2)	2.6 (2)	0.0 (0)
Nausea	2.0 (1)	0.0 (0)	26.3 (20)	25.0 (19)
Diarrhoea	15.7 (8)	7.8 (4)	18.4 (14)	6.6 (5)
Vomiting	7.8 (4)	2.0 (1)	5.3 (4)	3.9 (3)
Dyspepsia	0.0 (0)	0.0 (0)	14.5 (11)	13.2 (10)
Constipation	15.7 (8)	9.8 (5)	15.8 (12)	13.2 (10)
Dental caries	3.9 (2)	0.0 (0)	6.6 (5)	0.0 (0)
Back pain	7.8 (4)	0.0 (0)	11.8 (9)	0.0 (0)
Arthralgia	5.9 (3)	0.0 (0)	3.9 (3)	0.0 (0)
Pain in extremity	2.0 (1)	0.0 (0)	5.3 (4)	0.0 (0)
Trigger finger	7.8 (4)	2.0 (1)	0.0 (0)	0.0 (0)
Fatigue	0.0 (0)	0.0 (0)	5.3 (4)	1.3 (1)
Hunger	3.9 (2)	0.0 (0)	5.3 (4)	2.6 (2)
Malaise	2.0 (1)	2.0 (1)	5.3 (4)	2.6 (2)
Fall	7.8 (4)	0.0 (0)	2.6 (2)	0.0 (0)
Contusion	5.9 (3)	0.0 (0)	2.6 (2)	0.0 (0)
Road traffic accident	2.0 (1)	0.0 (0)	5.3 (4)	0.0 (0)
Percutaneous coronary intervention	5.9 (3)	2.0 (1)	1.3 (1)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

Death occurred in 2 patients (myocardial infarction, sudden death) in the lixisenatide group, but their causal relationship to study drug was denied.[116] The incidences of serious adverse events were 12.3% (35 of 285 subjects) in the placebo group and 10.1% (58 of 574 subjects) in the lixisenatide group. The incidences of adverse events classified as “Cardiac disorders,” the System Organ Class (SOC) with the highest incidence of serious adverse events, were 1.8% (5 of 285 subjects) in the placebo group and 2.1% (12 of 574 subjects) in the lixisenatide group. The incidences of adverse events leading to treatment discontinuation were 7.7% (22 of 285 subjects) in the placebo group and 12.4% (71 of 574 subjects) in the lixisenatide group. The incidences of nausea, which was the most frequently reported event leading to treatment discontinuation in the lixisenatide group, were 0.4% (1 of 285 subjects) in the placebo group and 4.2% (24 of 574 subjects) in the lixisenatide group.

With regard to hypoglycaemia, the incidences of symptomatic hypoglycaemia were 17.9% (51 of 285 subjects) in the placebo group and 22.1% (127 of 574 subjects) in the lixisenatide group. The incidences of severe symptomatic hypoglycaemia were 0.4% (1 of 285 subjects) in the placebo group and 0.3% (2 of 574 subjects) in the lixisenatide group.

[116] In addition to these 2 deaths, 1 patient (respiratory failure) in the placebo group and 1 patient (multi-organ failure) in the lixisenatide group died after the discontinuation of study treatment, but their causal relationship to study drug was denied.

With regard to vital signs, the mean changes in blood pressures (in the order of systolic and diastolic) from baseline to the end of treatment were -2.2 and -1.4 mmHg, respectively, in the placebo group, and -1.8 and -1.0 mmHg, respectively, in the lixisenatide group; there were slight decreases in both groups. The changes (mean) in heart rate from baseline to the end of treatment were 0.1 beats/min in the placebo group and -0.1 beats/min in the lixisenatide group; the changes were minimal in both groups. With regard to changes in ECG, 5 patients in the placebo group and 2 patients in the lixisenatide group had no potentially clinically significant abnormalities at baseline and had potentially clinically significant abnormalities during the study treatment period.[117]

The incidences of injection site reactions were 2.8% (8 of 285 subjects) in the placebo group and 4.9% (28 of 574 subjects) in the lixisenatide group and the events led to treatment discontinuation in 3 patients in the lixisenatide group, but none was reported as serious.

With regard to anti-lixisenatide antibody, the percentages of antibody-positive patients were 2.0% (5 of 252 subjects) in the placebo group and 6.7% (34 of 504 subjects) in the lixisenatide group at baseline, 9.2% (22 of 238 subjects) in the placebo group and 62.3% (215 of 345 subjects) in the lixisenatide group at Week 24, and 8.6% (15 of 175 subjects) in the placebo group and 77.2% (261 of 338 subjects) in the lixisenatide group at Week 76.

4.(iii).A.(3).2) Multinational phase III study (in combination with insulin with or without SU) (5.3.5.1-5, Study EFC10887 [[redacted] 20[redacted] to [redacted] 20[redacted]])

A randomized, double-blind, placebo-controlled, parallel-group study was conducted to evaluate the efficacy and safety of lixisenatide in Japanese and non-Japanese[118] patients with type 2 diabetes mellitus[119] (target sample size of 300 subjects, 150 subjects per group).

Following a one-week placebo run-in period, placebo or lixisenatide was subcutaneously administered (self-injection) QD within 1 hour prior to breakfast in the upper arm, abdomen, or thigh. The starting dose was 10 μg, and the dose was increased by 5 μg/week up to 20 μg. The duration of treatment was 24 weeks. The dose of basal insulin was reduced by 20% in patients with screening HbA1c ≤7.5% and if no hypoglycaemia occurred, it was allowed to be increased to the dose at screening between Week 4 and 12 and then the insulin dose was to be kept stable within ± 20% of the dose at screening. The dose of SU was reduced by at least 25% (or stopped in case of minimum dose) in patients with screening HbA1c <8.0% and if no hypoglycaemia occurred and the majority of fasting plasma glucose self-monitoring readings were ≥126 mg/dL, it was allowed to be increased to the dose at screening between Week 4 and 12. If ≥2 episodes of symptomatic hypoglycaemia or ≥1 episode of severe symptomatic hypoglycaemia occurred, the dose of SU was to be decreased by ≥25% (or stopped in case of minimum dose) first, and then if further ≥2 episodes of symptomatic hypoglycaemia or ≥1 episode of severe symptomatic hypoglycaemia occurred, the dose of basal insulin was to be, as necessary, decreased.

[117] Heart rate: ≤50 bpm and a decrease of ≥20 bpm from baseline, or ≥120 bpm and an increase of ≥20 bpm from baseline, PR interval: ≥220 ms and an increase of ≥20 ms from baseline, QRS interval: ≥120 ms, QTc interval: >450 ms (male), >470 ms (female), >500 ms (male and female) or an increase of >60 ms from baseline.

[118] South Korea, Philippines, and Taiwan

[119] Patients with type 2 diabetes mellitus diagnosed ≥1 year before screening, being treated with basal insulin for at least 3 months prior to screening and at a dose of ≥10 U/day for at least 2 months prior to screening, with HbA1c of ≥7.0% and ≤10.0% (at screening).

All of 311 treated patients (157 patients in the placebo group and 154 patients in the lixisenatide group) were included in the safety population and mITT population. The primary efficacy population was the mITT population.

The primary efficacy endpoint of the change in HbA1c from baseline to Week 24 in the mITT population was as shown in Table 27. In the overall population, the difference of lixisenatide versus placebo was statistically significant (P <0.0001, two-sided significance level of 5%, ANCOVA model[120]).

Table 27. Change in HbA1c from baseline to Week 24 (EFC10887, Overall population and Japanese population, mITT population)

	Treatment group	Baseline	Week 24 (LOCF)	Change from baseline (LOCF)	Difference vs. placebo [95% CI][b)]	*P*-value[b)]
Overall population[a)]	Placebo (n = 154)	8.53 ± 0.78	8.55 ± 1.09	0.02 ± 0.85	-0.88 [-1.116, -0.650]	<0.0001
	Lixisenatide (n = 146)	8.53 ± 0.73	7.64 ± 1.24	-0.90 ± 1.22		
Japanese population	Placebo (n = 87)	8.53 ± 0.80	8.79 ± 1.03	0.26 ± 0.75	-1.12 [-1.429, -0.809]	—
	Lixisenatide (n = 72)	8.55 ± 0.71	7.70 ± 1.26	-0.85 ± 1.25		

Mean ± SD (%)

a) Three patients in the placebo group and 8 patients in the lixisenatide group were excluded from the analysis because only measurements after the introduction of rescue medication or after treatment cessation plus 3 days were available.

b) Two-sided significance level of 5%, ANCOVA model

A plot of change in HbA1c from baseline by visit was as illustrated in Figure 2.

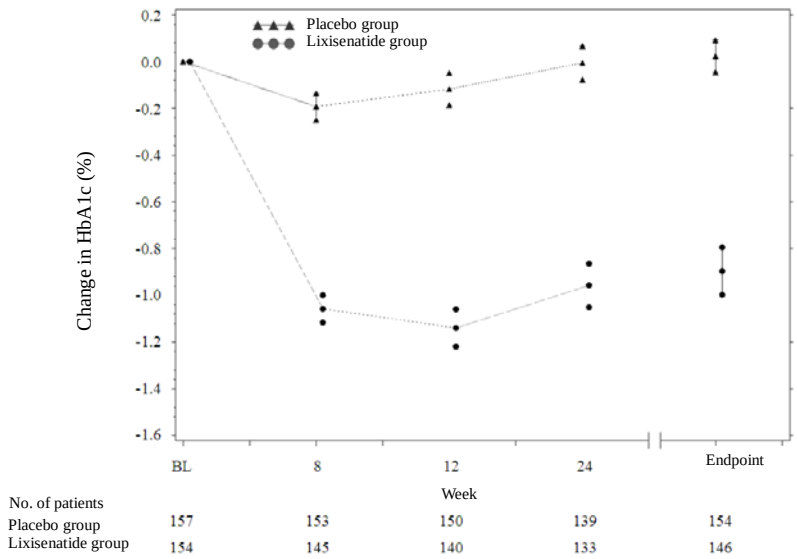

Figure 2. Plot of change in HbA1c from baseline by visit (mean ± SE) (EFC10887)

The results of key secondary endpoints were as shown in Table 28.

[120] Calculated using an ANCOVA model with treatment (lixisenatide or placebo), HbA1c (<8.0% or ≥8.0%) at screening, SU use at screening, and country (not included in the analysis of the Japanese population) as fixed effects and baseline HbA1c as a covariate.

Table 28. Results of key secondary endpoints (EFC10887, Overall population, mITT population)

Endpoint		Placebo (n = 157)	Lixisenatide (n = 154)
FPG (mg/dL)	Baseline	139.69 ± 40.48 (n = 157)	137.70 ± 41.57 (n = 148)
	Change at Week 24 (LOCF)	8.76 ± 49.63 (n = 157)	-4.87 ± 48.35 (n = 148)
2-hour PPG (mg/dL)	Baseline	324.05 ± 65.86 (n = 142)	322.19 ± 58.87 (n = 131)
	Change at Week 24 (LOCF)	-6.64 ± 71.52 (n = 142)	-148.24 ± 102.40 (n = 131)
Body weight (kg)	Baseline	65.60 ± 12.47 (n = 157)	65.99 ± 12.94 (n = 150)
	Change (LOCF)	-0.02 ± 1.54 (n = 157)	-0.39 ± 2.68 (n = 150)
Basal insulin dose (U/day)	Baseline	24.11 ± 14.18 (n = 157)	24.87 ± 14.02 (n = 151)
	Change (LOCF)	-0.01 ± 2.69 (n = 157)	-1.42 ± 4.42 (n = 151)
Percentage of subjects achieving HbA1c <7.0% at Week 24 (%) (LOCF)[a)]		5.2 (8/154)	35.6 (52/146)
Percentage of subjects achieving HbA1c ≤6.5% at Week 24 (%) (LOCF)[a)]		1.3 (2/154)	17.8 (26/146)
Percentage of subjects requiring rescue therapy		3.2% (5/157)	1.3% (2/154)

Mean ± SD

a) Subjects requiring rescue therapy and subjects who stopped study treatment for ≥3 days were excluded.

With regard to safety, the incidences of adverse events during the entire treatment period were 70.1% (110 of 157 subjects) in the placebo group and 89.0% (137 of 154 subjects) in the lixisenatide group and the incidences of adverse drug reactions were 24.8% (39 of 157 subjects) in the placebo group and 66.2% (102 of 154 subjects) in the lixisenatide group. Adverse events and/or adverse drug reactions reported by ≥3% of patients in either treatment group in the overall population, and adverse events and/or adverse drug reactions reported by ≥5% of patients in either treatment group in the Japanese population are summarized in Tables 29 and 30, respectively.

Table 29. Adverse events and/or adverse drug reactions reported by ≥3% of patients in either treatment group (EFC10887, Overall population, Safety population)

Event term	Placebo (n = 157)		Lixisenatide (n = 154)	
	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions
Any event	70.1 (110)	24.8 (39)	89.0 (137)	66.2 (102)
Nasopharyngitis	12.7 (20)	0.0 (0)	13.6 (21)	0.0 (0)
Upper respiratory tract infection	0.6 (1)	0.0 (0)	4.5 (7)	0.0 (0)
Hypoglycaemia	23.6 (37)	13.4 (21)	43.5 (67)	29.2 (45)
Decreased appetite	0.0 (0)	0.0 (0)	6.5 (10)	5.2 (8)
Headache	1.9 (3)	0.6 (1)	10.4 (16)	4.5 (7)
Dizziness	5.1 (8)	1.3 (2)	8.4 (13)	3.9 (6)
Tremor	3.2 (5)	1.9 (3)	4.5 (7)	2.6 (4)
Vision blurred	0.0 (0)	0.0 (0)	3.2 (5)	1.3 (2)
Palpitations	0.6 (1)	0.0 (0)	3.9 (6)	1.3 (2)
Hypertension	4.5 (7)	0.0 (0)	1.3 (2)	0.0 (0)
Nausea	4.5 (7)	3.8 (6)	39.6 (61)	35.7 (55)
Vomiting	1.9 (3)	0.6 (1)	18.2 (28)	16.9 (26)
Abdominal discomfort	0.6 (1)	0.6 (1)	7.1 (11)	5.8 (9)
Dyspepsia	0.0 (0)	0.0 (0)	7.1 (11)	5.8 (9)
Diarrhoea	2.5 (4)	1.3 (2)	6.5 (10)	2.6 (4)
Constipation	2.5 (4)	1.3 (2)	5.2 (8)	4.5 (7)
Abdominal pain upper	0.6 (1)	0.0 (0)	3.2 (5)	0.0 (0)
Hyperhidrosis	1.3 (2)	0.6 (1)	3.2 (5)	1.3 (2)
Asthenia	7.6 (12)	3.8 (6)	6.5 (10)	3.9 (6)
Fall	1.3 (2)	0.0 (0)	3.2 (5)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

Table 30. Adverse events and/or adverse drug reactions reported by ≥5% of patients in either treatment group (EFC10887, Japanese population, Safety population)

Event term	Placebo (n = 87)		Lixisenatide (n = 72)	
	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions
Any event	66.7 (58)	21.8 (19)	87.5 (63)	72.2 (52)
Nasopharyngitis	19.5 (17)	0.0 (0)	19.4 (14)	0.0 (0)
Hypoglycaemia	17.2 (15)	10.3 (9)	36.1 (26)	23.6 (17)
Decreased appetite	0.0 (0)	0.0 (0)	9.7 (7)	8.3 (6)
Tremor	3.4 (3)	2.3 (2)	5.6 (4)	4.2 (3)
Nausea	3.4 (3)	2.3 (2)	36.1 (26)	34.7 (25)
Vomiting	3.4 (3)	1.1 (1)	18.1 (13)	18.1 (13)
Abdominal discomfort	0.0 (0)	0.0 (0)	12.5 (9)	11.1 (8)
Diarrhoea	3.4 (3)	2.3 (2)	5.6 (4)	4.2 (3)
Constipation	3.4 (3)	2.3 (2)	8.3 (6)	8.3 (6)
Back pain	2.3 (2)	0.0 (0)	5.6 (4)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

Death occurred in 1 patient (completed suicide) in the placebo group, but its causal relationship to study drug was denied. Serious adverse events (including deaths) occurred in 9 patients (pneumonia, breast cancer, colonic polyp/rectal cancer, completed suicide, skin laceration/hyphaema/retinal haemorrhage, asthma, deviation of the nasal septum, liver disorder, and lower limb fracture in 1 patient each) in the placebo group and 10 patients (cerebral infarction, cerebral infarction/respiratory failure/salivary hypersecretion [respiratory failure/salivary hypersecretion occurred after treatment discontinuation], upper respiratory tract infection/hand fracture, herpes zoster oticus, vomiting/hypertensive crisis, colonic polyp, nausea, uterine prolapse, cartilage injury, and joint injury in 1 patient each) in the lixisenatide group. Of these, the events reported by 1 patient (liver disorder) in the placebo group and 3 patients (colonic polyp, nausea, vomiting) in the lixisenatide group were classified as adverse drug reactions. The incidences of adverse events leading to treatment discontinuation were 3.2% (5 of 157 subjects) in the placebo group and 9.1% (14 of 154 subjects) in the lixisenatide group. Nausea and vomiting were the most frequently reported events leading to treatment discontinuation, but nausea or vomiting was not noted in the placebo group and the incidences of nausea and vomiting in the lixisenatide group were 3.9% (6 of 154 subjects) and 2.6% (4 of 154 subjects), respectively.

With regard to hypoglycaemia, the incidences of symptomatic hypoglycaemia were 23.6% (37 of 157 subjects) in the placebo group and 42.9% (66 of 154 subjects) in the lixisenatide group. No severe symptomatic hypoglycaemia occurred.

Vital sign and ECG data were reviewed for potentially clinically significant abnormalities[117]. As a result, there were no clinically relevant changes in vital signs or ECG in the lixisenatide group.

The incidences of injection site reactions were 1.3% (2 of 157 subjects) in the placebo group and 1.3% (2 of 154 subjects) in the lixisenatide group. None of the events was serious or severe or led to treatment discontinuation.

With regard to anti-lixisenatide antibody, the percentages of antibody-positive patients in the lixisenatide group were 3.3% (5 of 153 subjects) at baseline and 78.0% (110 of 141 subjects) at Week 24. In the placebo group, 7 patients were antibody-positive at baseline, and 5 patients remained antibody-positive throughout the treatment period.

4.(iii).A.(3).3) Multinational phase III study (monotherapy) (5.3.5.1-3, Study EFC6018 [20 to 20])

A randomized, double-blind,[121] placebo-controlled, parallel-group study was conducted to evaluate the efficacy and safety of lixisenatide monotherapy in Japanese and non-Japanese[122] patients with type 2 diabetes mellitus[123] (target sample size of 360 subjects, 120 subjects per treatment group).

Following a one-week placebo run-in period, placebo or lixisenatide was subcutaneously administered (self-injection) QD within 1 hour prior to breakfast in the upper arm, abdomen, or thigh. One-step titration consisted of injections of lixisenatide 10 μg for 2 weeks followed by 20 μg and two-step titration was 10 μg for 1 week, then 15 μg for 1 week and then 20 μg. When uptitration was not tolerated, the dose was allowed to be maintained at 10 μg in the one-step titration group and 10 or 15 μg in the two-step titration group. When uptitration to 15 μg was not tolerated, the dose was allowed to be decreased to 10 μg. When uptitration to 20 μg was not tolerated, the dose was allowed to be reduced to 10 μg in the one-step titration group, and to 15 μg, then if necessary, to 10 μg in the two-step titration group. The duration of treatment was 12 weeks.

All of 361 treated patients (combined placebo group, 122 patients [61 patients in the placebo one-step titration group and 61 patients in the placebo two-step titration group]; lixisenatide one-step titration group, 119 patients; lixisenatide two-step titration group, 120 patients) were included in the safety population. Of these, 359 patients were included in the mITT population. Excluded were 2 patients with no post-baseline values for efficacy variables (1 patient each in the placebo one-step titration group and lixisenatide one-step titration group). The primary efficacy population was the mITT population.

The primary efficacy endpoint of the change in HbA1c from baseline to Week 12 was as shown in Table 31. In the overall population, the lixisenatide two-step titration group was first compared with the combined placebo group; if the test was statistically significant, then the lixisenatide one-step titration group was compared with the combined placebo group. As a result, there was a statistically significant difference between each of the lixisenatide groups and the combined placebo group (P <0.0001 each, two-sided significance level of 5%, ANCOVA model[124]).

[121] The titration regimens were not blinded, but double-blinding was maintained with regard to assignment of placebo or lixisenatide.
[122] The US, Poland, Belgium, Rumania, Ukraine, Russia, South Korea, India, Israel, Mexico, and Tunisia
[123] Patients with type 2 diabetes mellitus diagnosed ≥2 months before screening, not treated with antidiabetic drugs for 3 months at screening, with BMI >20 kg/m^2 and HbA1c ≥7.0% and ≤10.0%.
[124] Calculated using an ANCOVA model with treatment (two-step titration lixisenatide and placebo, one-step titration lixisenatide and placebo), HbA1c (<8.0% or ≥8.0%) at screening, BMI (<30 kg/m^2 or ≥30 kg/m^2), and country (not included in the analysis of the Japanese population) as fixed effects, and baseline HbA1c as a covariate.

Table 31. Change in HbA1c from baseline to Week 12 (EFC6018, Overall population and Japanese population, mITT population)

	Treatment group	Baseline	Endpoint (LOCF)	Change from baseline (LOCF)	Difference vs. placebo [95% CI][b)]	*P*-value[b)]
Overall population[a)]	Placebo combined (n = 112)	8.07 ± 0.92	7.80 ± 1.35	-0.27 ± 1.09	—	—
	Lixisenatide one-step titration (n = 114)	8.06 ± 0.85	7.11 ± 0.89	-0.94 ± 0.72	-0.66 [-0.903, -0.423]	<0.0001
	Lixisenatide two-step titration (n = 113)	7.97 ± 0.91	7.20 ± 1.19	-0.77 ± 0.94	-0.54 [-0.785, -0.300]	<0.0001
Japanese population	Placebo combined (n = 10)	8.17 ± 0.81	7.98 ± 0.85	-0.19 ± 0.38	—	—
	Lixisenatide one-step titration (n = 16)	8.28 ± 0.53	7.01 ± 0.63	-1.27 ± 0.59	-1.12 [-1.862, -0.381]	—
	Lixisenatide two-step titration (n = 17)	8.45 ± 0.86	7.75 ± 1.25	-0.70 ± 1.09	-0.41 [-1.123, 0.306]	—

Mean ± SD (%)

a) Nine patients in the placebo group, 4 patients in the lixisenatide one-step titration group, and 7 patients in the lixisenatide two-step titration group were excluded from the analysis because their data were only available from measurements after the introduction of rescue medication or after treatment cessation plus 3 days.

b) Two-sided significance level of 5%, ANCOVA model

A plot of change in HbA1c from baseline by visit was as illustrated in Figure 3.

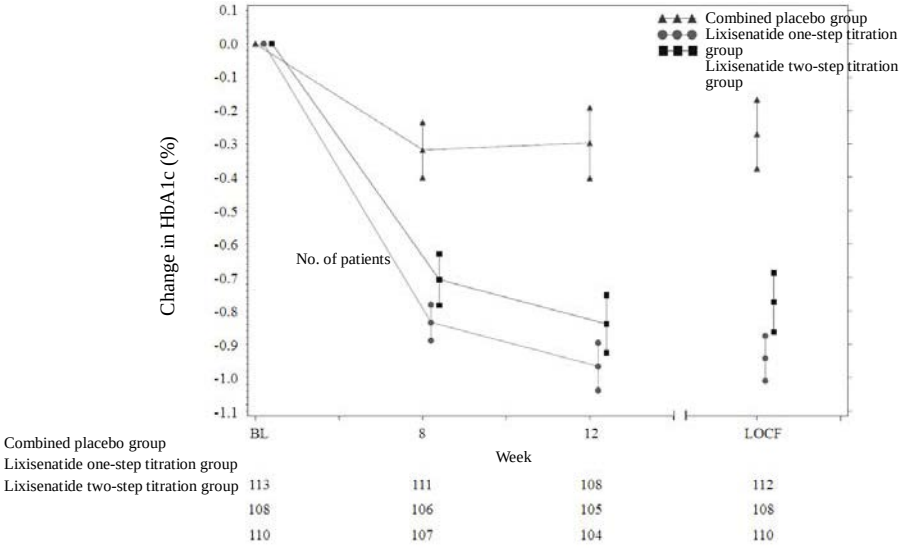

Figure 3. Plot of change in HbA1c from baseline by visit (mean ± SE) (EFC6018)

With regard to key secondary endpoints, the changes in FPG from baseline to endpoint (mean ± SD) were 4.43 ± 45.41 mg/dL in the combined placebo group, -15.63 ± 29.11 mg/dL in the lixisenatide one-step titration group, and -11.81 ± 35.12 mg/dL in the lixisenatide two-step titration group. Similarly, the changes in 2-hour PPG (mean ± SD) were -10.28 ± 79.97 mg/dL in the combined placebo group, -104.02 ± 70.20 mg/dL in the lixisenatide one-step titration group, and -85.93 ± 81.53 mg/dL in the lixisenatide two-step titration group. The changes in body weight (mean ± SD) were -1.98 ± 2.77 kg in the combined placebo group, -1.92 ± 2.78 kg in the lixisenatide one-step titration group, and -2.01 ± 2.68 kg in the lixisenatide two-step titration group. The percentages of subjects achieving HbA1c <7.0% at endpoint were 26.8% (30 of 112 subjects) in the combined placebo group, 46.5% (53 of 114 subjects) in the lixisenatide one-step titration group, and 52.2% (59 of 113 subjects) in the lixisenatide two-step titration group. The percentages of subjects achieving HbA1c ≤6.5% were 12.5% (14 of 112 subjects) in the combined placebo group, 25.4% (29 of 114 subjects) in the lixisenatide one-step titration group, and 31.9% (36 of 113 subjects) in the lixisenatide two-step titration group. The percentages of subjects requiring rescue therapy were 2.5% (3 of

121 subjects) in the combined placebo group, 0.8% (1 of 118 subjects) in the lixisenatide one-step titration group, and 1.7% (2 of 120 subjects) in the lixisenatide two-step titration group.

With regard to safety, the incidences of adverse events were 45.1% (55 of 122 subjects) in the combined placebo group, 54.6% (65 of 119 subjects) in the lixisenatide one-step titration group, and 52.5% (63 of 120 subjects) in the lixisenatide two-step titration group and the incidences of adverse drug reactions were 13.1% (16 of 122 subjects) in the combined placebo group, 33.6% (40 of 119 subjects) in the lixisenatide one-step titration group, and 27.5% (33 of 120 subjects) in the lixisenatide two-step titration group. Adverse events and/or adverse drug reactions reported by ≥3 patients in any of the treatment groups in the overall population, and adverse events and/or adverse drug reactions reported by ≥2 patients in any of the treatment groups in the Japanese population are summarized in Tables 32 and 33, respectively.

Table 32. Adverse events and/or adverse drug reactions reported by ≥3 patients in any treatment group

(EFC6018, Overall population, Safety population)

Event term	Placebo		Lixisenatide			
	Combined (n = 122)		One-step titration (n = 119)		Two-step titration (n = 120)	
	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions
Any event	45.1 (55)	13.1 (16)	54.6 (65)	33.6 (40)	52.5 (63)	27.5 (33)
Nasopharyngitis	3.3 (4)	0.0 (0)	4.2 (5)	0.0 (0)	5.0 (6)	0.0 (0)
Upper respiratory tract infection	0.0 (0)	0.0 (0)	3.4 (4)	0.0 (0)	1.7 (2)	0.8 (1)
Pharyngitis	2.5 (3)	0.0 (0)	0.0 (0)	0.0 (0)	1.7 (2)	0.0 (0)
Gastroenteritis	2.5 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.8 (1)	0.0 (0)
Decreased appetite	0.8 (1)	0.0 (0)	4.2 (5)	4.2 (5)	2.5 (3)	2.5 (3)
Hypoglycaemia	1.6 (2)	0.0 (0)	0.8 (1)	0.8 (1)	3.3 (4)	2.5 (3)
Headache	11.5 (14)	3.3 (4)	7.6 (9)	3.4 (4)	8.3 (10)	4.2 (5)
Dizziness	2.5 (3)	1.6 (2)	3.4 (4)	2.5 (3)	7.5 (9)	4.2 (5)
Palpitations	0.0 (0)	0.0 (0)	2.5 (3)	1.7 (2)	0.8 (1)	0.0 (0)
Oropharyngeal pain	2.5 (3)	0.0 (0)	2.5 (3)	0.0 (0)	0.8 (1)	0.0 (0)
Nausea	4.1 (5)	2.5 (3)	20.2 (24)	18.5 (22)	24.2 (29)	21.7 (26)
Vomiting	0.0 (0)	0.0 (0)	6.7 (8)	6.7 (8)	7.5 (9)	7.5 (9)
Diarrhoea	2.5 (3)	1.6 (2)	3.4 (4)	0.8 (1)	2.5 (3)	0.0 (0)
Abdominal pain upper	0.0 (0)	0.0 (0)	1.7 (2)	1.7 (2)	3.3 (4)	1.7 (2)
Constipation	0.8 (1)	0.8 (1)	1.7 (2)	0.8 (1)	3.3 (4)	0.8 (1)
Abdominal distension	0.0 (0)	0.0 (0)	2.5 (3)	2.5 (3)	0.8 (1)	0.8 (1)
Back pain	1.6 (2)	0.0 (0)	1.7 (2)	0.0 (0)	3.3 (4)	0.0 (0)
Injection site pruritus	0.0 (0)	0.0 (0)	3.4 (4)	3.4 (4)	1.7 (2)	1.7 (2)
Fatigue	0.8 (1)	0.0 (0)	0.8 (1)	0.0 (0)	3.3 (4)	0.8 (1)
Asthenia	0.8 (1)	0.0 (0)	2.5 (3)	0.8 (1)	0.8 (1)	0.0 (0)
Fall	2.5 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

Table 33. Adverse events and/or adverse drug reactions reported by ≥2 patients in any treatment group
(EFC6018, Japanese population, Safety population)

Event term	Placebo		Lixisenatide			
	Combined (n = 10)		One-step titration (n = 16)		Two-step titration (n = 17)	
	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions
Any event	50.0 (5)	0.0 (0)	81.3 (13)	75.0 (12)	82.4 (14)	47.1 (8)
Nasopharyngitis	30.0 (3)	0.0 (0)	6.3 (1)	0.0 (0)	23.5 (4)	0.0 (0)
Decreased appetite	0.0 (0)	0.0 (0)	18.8 (3)	18.8 (3)	11.8 (2)	11.8 (2)
Headache	0.0 (0)	0.0 (0)	12.5 (2)	12.5 (2)	11.8 (2)	11.8 (2)
Nausea	0.0 (0)	0.0 (0)	56.3 (9)	56.3 (9)	35.3 (6)	35.3 (6)
Vomiting	0.0 (0)	0.0 (0)	25.0 (4)	25.0 (4)	17.6 (3)	17.6 (3)
Dyspepsia	0.0 (0)	0.0 (0)	12.5 (2)	12.5 (2)	5.9 (1)	5.9 (1)
Abdominal distension	0.0 (0)	0.0 (0)	18.8 (3)	18.8 (3)	5.9 (1)	5.9 (1)
Eructation	0.0 (0)	0.0 (0)	12.5 (2)	12.5 (2)	0.0 (0)	0.0 (0)
Rash	0.0 (0)	0.0 (0)	12.5 (2)	12.5 (2)	0.0 (0)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

No death occurred. Serious adverse events occurred in 5 patients (colon cancer stage III, acute myocardial infarction, ileus, blood glucose increased, and ulna fracture in 1 patient each) in the placebo group and 1 patient (goitre) in the lixisenatide two-step titration group and the event reported by 1 patient (acute myocardial infarction) in the placebo group was classified as an adverse drug reaction. Adverse events leading to treatment discontinuation occurred in 1 patient (colon cancer stage III) in the placebo group, 3 patients (nausea in 2 patients, hypertension/vomiting/abdominal pain upper/nausea in 1 patient) in the lixisenatide one-step titration group, and 5 patients (nausea in 2 patients, decreased appetite/nausea/vomiting, colitis, and haematochezia in 1 patient each) in the lixisenatide two-step titration group and all events except for those reported by 1 patient (colon cancer stage III) in the placebo group and 2 patients (haematochezia and colitis in 1 patient each) in the lixisenatide two-step titration group, were classified as adverse drug reactions.

With regard to hypoglycaemia, the incidences of symptomatic hypoglycaemia were 1.6% (2 of 122 subjects) in the placebo group, 0.8% (1 of 119 subjects) in the lixisenatide one-step titration group, and 2.5% (3 of 120 subjects) in the lixisenatide two-step titration group. No severe symptomatic hypoglycaemia was noted.

Vital sign and ECG data were reviewed for potentially clinically significant abnormalities.[117] As a result, there were no clinically relevant changes in vital signs or ECG.

No injection site reaction occurred in the placebo group, and the incidences of injection site reactions were 5.9% (7 of 119 subjects) in the lixisenatide one-step titration group and 3.3% (4 of 120 subjects) in the lixisenatide two-step titration group. None of the events was serious or led to treatment discontinuation.

With regard to anti-lixisenatide antibody, the percentage of antibody-positive patients at Week 12 were 59.8% (52 of 87 subjects) in the lixisenatide one-step titration group and 55.7% (49 of 88 subjects) in the lixisenatide two-step titration group.

4.(iii).A.(3).4) Japanese phase III, open-label study (5.3.5.2-1, Study LTS10888 [20 to 20])

A randomized, open-label, parallel-group study was conducted to evaluate the safety of once daily lixisenatide monotherapy using a one-step or two-step titration regimen in Japanese patients with type 2 diabetes mellitus[125] (target sample size of 66 subjects [33 subjects each in the one-step and two-step titration groups]).

Following a one-week placebo run-in period, lixisenatide was subcutaneously administered (self-injection) QD within 1 hour prior to breakfast in the upper arm, abdomen, or thigh. One-step titration consisted of injections of lixisenatide 10 μg for 2 weeks followed by 20 μg and two-step titration was 10 μg for 1 week, then 15 μg for 1 week and then 20 μg. The total duration of treatment was 76 weeks.

All of 69 treated patients (one-step titration group, 36 patients; two-step titration group, 33 patients) were included in the safety population and mITT population. The efficacy population was the mITT population.

With regard to efficacy, the changes in HbA1c from baseline to last value on treatment (mean ± SD [95% CI]) were -0.44 ± 1.11 [-0.82, -0.06]% in the one-step titration group (n = 35) and -0.18 ± 1.35 [-0.67, 0.31]% in the two-step titration group (n = 32). A plot of change in HbA1c from baseline by visit in the pooled titration group in the mITT population was as illustrated in Figure 4.

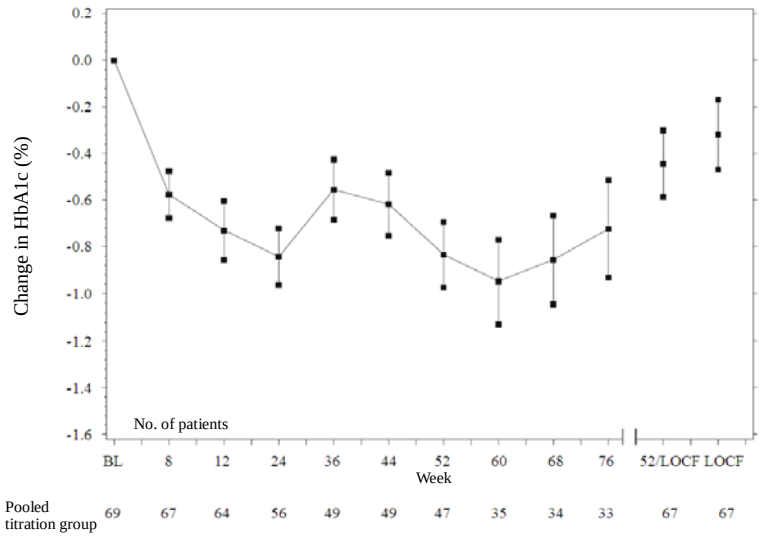

Figure 4. Change in HbA1c from baseline by visit to Week 76 (LTS10888, mITT population) (mean ± SE)

The primary safety endpoint of the incidence of adverse events during the 24-week treatment period was 88.9% (32 of 36 subjects) in the one-step titration group and 81.8% (27 of 33 subjects) in the two-step titration group. A key secondary endpoint of the incidence of adverse events during the 76-week treatment

[125] Patients with type 2 diabetes mellitus diagnosed ≥2 months before screening, not treated with antidiabetic drugs excluding a stable dose of an SU or an α-glucosidase inhibitor for 3 months before screening (SUs and α-glucosidase inhibitors were discontinued before the placebo run-in period), with HbA1c of ≥7.0% and ≤10.0%.

period was 91.3% (63 of 69 subjects) in the pooled titration group (94.4% [34 of 36 subjects] in the one-step titration group, 87.9% [29 of 33 subjects] in the two-step titration group).

Adverse events and/or adverse drug reactions reported by ≥5% of patients in either titration group at Week 24, and adverse events and/or adverse drug reactions reported by ≥5% of patients in the pooled titration group at Week 76 are summarized in Tables 34 and 35, respectively.

Table 34. Adverse events and/or adverse drug reactions reported by ≥5% of patients in either titration group (LTS10888, 24-week treatment period)

Event term	One-step titration (n = 36)		Two-step titration (n = 33)	
	Adverse events	Adverse drug reactions	Adverse events	Adverse drug reactions
Any event	88.9 (32)	80.6 (29)	81.8 (27)	66.7 (22)
Nasopharyngitis	8.3 (3)	0.0 (0)	9.1 (3)	0.0 (0)
Decreased appetite	19.4 (7)	19.4 (7)	18.2 (6)	18.2 (6)
Hypoglycaemia	2.8 (1)	2.8 (1)	9.1 (3)	6.1 (2)
Headache	5.6 (2)	2.8 (1)	0.0 (0)	0.0 (0)
Yawning	0.0 (0)	0.0 (0)	6.1 (2)	6.1 (2)
Nausea	50.0 (18)	47.2 (17)	36.4 (12)	36.4 (12)
Abdominal discomfort	16.7 (6)	16.7 (6)	12.1 (4)	12.1 (4)
Dyspepsia	2.8 (1)	2.8 (1)	12.1 (4)	12.1 (4)
Vomiting	2.8 (1)	2.8 (1)	12.1 (4)	9.1 (3)
Abdominal distension	0.0 (0)	0.0 (0)	9.1 (3)	9.1 (3)
Constipation	5.6 (2)	5.6 (2)	6.1 (2)	6.1 (2)
Toothache	0.0 (0)	0.0 (0)	6.1 (2)	0.0 (0)
Abdominal pain upper	5.6 (2)	5.6 (2)	3.0 (1)	0.0 (0)
Diarrhoea	8.3 (3)	2.8 (1)	3.0 (1)	0.0 (0)
Back pain	0.0 (0)	0.0 (0)	6.1 (2)	0.0 (0)
Fatigue	5.6 (2)	5.6 (2)	6.1 (2)	3.0 (1)
Early satiety	11.1 (4)	11.1 (4)	3.0 (1)	3.0 (1)
Contusion	5.6 (2)	0.0 (0)	3.0 (1)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

Table 35. Adverse events and/or adverse drug reactions reported by ≥5% of patients in the pooled titration group (LTS10888, 76-week treatment period)

Event term	Pooled titration group (n = 69)	
	Adverse events	Adverse drug reactions
Any event	91.3 (63)	76.8 (53)
Nasopharyngitis	31.9 (22)	0.0 (0)
Decreased appetite	18.8 (13)	18.8 (13)
Hypoglycaemia	8.7 (6)	7.2 (5)
Nausea	43.5 (30)	42.0 (29)
Abdominal discomfort	15.9 (11)	15.9 (11)
Constipation	7.2 (5)	7.2 (5)
Dyspepsia	7.2 (5)	7.2 (5)
Vomiting	7.2 (5)	5.8 (4)
Abdominal pain upper	5.8 (4)	2.9 (2)
Diarrhoea	5.8 (4)	1.4 (1)
Early satiety	7.2 (5)	7.2 (5)
Fatigue	5.8 (4)	4.3 (3)
Contusion	7.2 (5)	0.0 (0)

Incidence % (n), MedDRA/J (ver.13.1)

No death occurred. Serious adverse events occurred in 2 patients (gastroenteritis and skin laceration in 1 patient each) in the two-step titration group during the 24-week treatment period, and 3 patients (gastroenteritis, skin laceration, and cataract/intervertebral disc protrusion in 1 patient each) in the pooled titration group during the entire 76-week treatment period, but a causal relationship to study drug was denied for all events. Adverse events leading to treatment discontinuation occurred in 4 patients (nausea, abdominal discomfort, urticaria, and neutrophil count decreased in 1 patient each) in the one-step titration group and 3 patients (diabetic retinopathy, nausea, and nausea/constipation/vomiting in 1 patient each) in the two-step titration group during the 24-week treatment period, and 10 patients (neutrophil count decreased in 3 patients, nausea in 2 patients, diabetic retinopathy, nausea/constipation/vomiting, abdominal discomfort, urticaria, and non-cardiac chest pain in 1 patient each) in the pooled titration group during the entire 76-week treatment period.

With regard to hypoglycaemia, the incidences of symptomatic hypoglycaemia were 2.8% (1 of 36 subjects) in the one-step titration group and 6.1% (2 of 33 subjects) in the two-step titration group during the 24-week treatment period, and 7.2% (5 of 69 subjects) in the pooled titration group during the entire 76-week treatment period. No severe symptomatic hypoglycaemia occurred.

Vital sign and ECG data were reviewed for potentially clinically significant abnormalities.[117] As a result, there were no clinically relevant changes in vital signs or ECG.

The incidences of injection site reactions were 11.1% (4 of 36 subjects) in the one-step titration group and 9.1% (3 of 33 subjects) in the two-step titration group during the 24-week treatment period, and 10.1% (7 of 69 subjects) in the pooled titration group during the entire 76-week treatment period. All of the events were mild in severity, and none of them was serious or led to treatment discontinuation.

With regard to anti-lixisenatide antibody, there was no antibody-positive patient at baseline. Subsequently, the percentages of antibody-positive patients were 57.1% (20 of 35 subjects) in the one-step titration group and 71.0% (22 of 31 subjects) in the two-step titration group at Week 24, 80.6% (25 of 31 subjects) in the one-step titration group and 84.6% (22 of 26 subjects) in the two-step titration group at Week 52, and 63.0% (17 of 27 subjects) in the one-step titration group and 70.0% (14 of 20 subjects) in the two-step titration group at Week 76.

4.(iii).B Outline of the review by PMDA

4.(iii).B.(1) Adequacy of clinical data package for registration of lixisenatide

PMDA requested the applicant to provide the basis for determining that of lixisenatide can be evaluated based on the submitted data package despite the fact that the number of patients treated with lixisenatide at the proposed dosage regimen was small, i.e. in Study .

The applicant responded as follows:

For the clinical development of lixisenatide, administration of lixisenatide to Japanese patients was planned in accordance with the . As a result, Japanese data were obtained from 63 patients in Study EFC6015 (lixisenatide in combination with SU with

or without metformin) and 57 patients (one-step titration group, 31 patients; two-step titration group, 26 patients) in LTS10888 (lixisenatide [redacted]). However, in consideration of PMDA's comment, additional clinical study data are necessary for adequately evaluating the [redacted] of lixisenatide [redacted] in Japanese patients, and therefore the indication proposed in this application will be changed so that lixisenatide will be used in combination with SUs (with or without biguanides) and with basal insulin (with or without SUs). Additional clinical study data will be obtained in the future development to gain approval of lixisenatide [redacted] for the treatment of "type 2 diabetes mellitus" in accordance with the "Guideline for Clinical Evaluation of Oral Hypoglycemic Agents" (PFSB/ELD Notification No. 0709-1 dated July 9, 2010).

PMDA considers as follows:
While lixisenatide was developed through multinational clinical studies, a two-step titration regimen, instead of a one-step titration regimen employed overseas, has been selected in Japan based on the incidences of "gastrointestinal disorders" and "nausea," etc. [see "4.(iii).B.(7) Dosage and administration"]. Taking also into consideration that lixisenatide [redacted] is unapproved overseas,[126] the clinical data package for registration of lixisenatide [redacted] is inadequate and thus a further investigation of the [redacted] safety and efficacy of lixisenatide [redacted] in Japanese patients is necessary. In this regard, the applicant's response (the indication proposed in the application will be changed and additional clinical study data will be obtained.) is appropriate.

Based on the above, PMDA has decided to review the application for lixisenatide in combination with SUs (with or without biguanides) and with basal insulin (with or without SUs).

4.(iii).B.(2) Clinical positioning
PMDA considers that lixisenatide, a GLP-1 receptor agonist, could be a new therapeutic option for patients with type 2 diabetes mellitus because the efficacy of lixisenatide has been demonstrated in the phase III multinational studies [see "4.(iii).B.(3) Interpretation of results of multinational studies" and "4.(iii).B.(4) Efficacy"] and its safety is acceptable [see "4.(iii).B.(3) Interpretation of results of multinational studies" and "4.(iii).B.(5) Safety"].

4.(iii).B.(3) Interpretation of results of multinational studies
For the interpretation of the results of multinational phase III studies (EFC6015 and EFC10887), PMDA conducted the following reviews based on "Basic Principles on Global Clinical Trials" (PFSB/ELD Notification No. 0928010 dated September 28, 2007) and the ICH-E5 guideline.

4.(iii).B.(3).1) Intrinsic and extrinsic ethnic factors
PMDA asked the applicant to explain the influence of intrinsic and extrinsic ethnic differences among the countries participating in multinational studies on the evaluation of the efficacy and safety of lixisenatide.

The applicant responded as follows:
There should be no differences in the definition of type 2 diabetes mellitus, diagnostic criteria, drug

[126] The applicant explained that lixisenatide [redacted] has not been filed with the EMA [redacted].

evaluation, treatment goals, or treatments between Japan and other participating countries. With respect to intrinsic and extrinsic ethnic differences, there were some differences in the baseline demographic characteristics of subjects (intrinsic ethnic factors: age, gender, body weight; extrinsic ethnic factors: doses and types of concomitant medications) between the Japanese and overall populations, which were not considered to significantly influence the efficacy and safety evaluation of lixisenatide [to be described later in "Efficacy in Japanese population and overall population" and "Safety in Japanese population and overall population"].

Given that dietary habit is different between other countries participating in multinational studies and Japan and that it is unknown whether or not there are ethnic differences in native GLP-1, which is relevant to the mechanism of action of lixisenatide, PMDA considers that it cannot be concluded at present that intrinsic and extrinsic ethnic factors are similar. However, the definition, diagnostic criteria, pathogenesis, treatments, etc. of type 2 diabetes mellitus should be similar among the participating countries including Japan.In addition, although it is difficult to conclude that there are no differences in the pharmacokinetics and pharmacodynamics of lixisenatide between the Japanese and non-Japanese populations [see "4.(ii).B.(1) Differences in pharmacokinetics and pharmacodynamics between Japanese and non-Japanese populations"], no major differences have been observed for clinical efficacy and safety. In light of these points, PMDA accepted the applicant's response. In the sections below, the efficacy and safety of lixisenatide were evaluated, taking also account of the influence of differences in the demographic characteristics of subjects enrolled into each multinational study.

4.(iii).B.(3).2) Add-on to SU (with or without metformin) study (EFC6015)

4.(iii).B.(3).2).(a) Efficacy in Japanese population and overall population

The applicant explained as follows:

For the primary endpoint of HbA1c change from baseline to Week 24, as shown in Table 36, lixisenatide in combination with SU with or without metformin showed consistent efficacy between the Japanese and overall populations and similar efficacy also in the non-Japanese population. As for other endpoints, the treatment difference in the change in fasting plasma glucose from baseline to Week 24 (lixisenatide minus placebo) and its 95% CI were -11.71 [-23.179, -0.246] mg/dL in the Japanese population and -11.40 [-16.563, -6.241] mg/dL in the overall population. The percentage of subjects achieving HbA1c ≤6.5% at Week 24 in the lixisenatide group was 16.4% (12 of 73 subjects) in the Japanese population and 19.3% (105 of 544 subjects) in the overall population, and the percentage of subjects achieving HbA1c <7.0% in the lixisenatide group was 31.5% (23 of 73 subjects) in the Japanese population and 36.4% (198 of 544 subjects) in the overall population. There were no major differences between the populations. Furthermore, the HbA1c reduction was maintained over the 76-week treatment period in both the Japanese and overall populations (HbA1c change from baseline to Week 76 [mean ± SE], -0.41 ± 0.97% in the placebo group [n = 19] and -0.49 ± 1.17% in the lixisenatide group [n = 46] in the Japanese population; -0.30 ± 1.05% in the placebo group [n = 118] and -0.78 ± 1.02% in the lixisenatide group [n = 287] in the overall population).

Table 36. HbA1c change from baseline to Week 24
(EFC6015, Japanese, non-Japanese, and overall populations, mITT population)

	Japanese population		Non-Japanese population[a)]		Overall population	
	Placebo (n = 51)	Lixisenatide (n = 76)	Placebo (n = 235)	Lixisenatide (n = 494)	Placebo (n = 286)	Lixisenatide (n = 570)
HbA1c change	0.19 ± 0.78	-0.86 ± 0.84	-0.19 ± 0.81	-0.89 ± 0.95	-0.12 ± 0.82	-0.88 ± 0.93
Difference vs. placebo [95% CI][b)]	-1.10 [-1.407, -0.803]		-0.66 [-0.791, -0.522]		-0.74 [-0.867, -0.621]	
P-value[b)]	<0.0001		<0.0001		<0.0001	

Mean ± SD (%), LOCF
a) As the Basic Principles on Global Clinical Trials state that a global trial should be designed so that consistency can be obtained between results from the Japanese population and the entire population, this review report basically presents the results from the Japanese population and the overall population accordingly, but the results from the non-Japanese population are also included for the primary endpoint of HbA1c change, for reference.
b) Calculated using an ANCOVA model with treatment (lixisenatide or placebo), HbA1c at screening (<8.0% or ≥8.0%), metformin use at screening, and country (only for the analysis of the overall population) as fixed effects and baseline HbA1c as a covariate.

The baseline demographic characteristics of subjects were as shown in Table 37 and there was a trend towards a lower body weight, a higher proportion of men, and a higher proportion of subjects aged ≥65 years in the Japanese population compared to the overall population. The proportion of subjects on "a combination of SU and metformin" was lower in the Japanese population than in the overall population. In 20[redacted] to 20[redacted] when patients were enrolled into the study, >750 mg/day of metformin was unapproved in Japan. Consequently, the number of Japanese subjects on >750 mg/day of metformin was as small as 4 in the placebo group and 2 in the lixisenatide group, whereas there were no subjects on ≤750 mg/day of metformin in the non-Japanese population. The dose of SU was higher in the overall population because the approved doses were different between Japan and overseas. However, since patients on less than the maximum recommended dose of SU at enrollment were excluded, there were no patients on low-dose SU.

Table 37. Baseline demographic characteristics of subjects (EFC6015, Japanese population and overall population, mITT population)

		Japanese population (n = 127)		Overall population (n = 856)	
		Placebo (n = 51)	Lixisenatide (n = 76)	Placebo (n = 286)	Lixisenatide (n = 570)
HbA1c (%)		8.59 ± 0.81	8.41 ± 0.93	8.21 ± 0.84	8.27 ± 0.86
Body weight (kg)		69.92 ± 16.61	65.30 ± 11.48	84.34 ± 22.83	82.42 ± 21.78
BMI (kg/m^2)	<25	45.1 (23)	56.6 (43)	23.4 (67)	23.3 (133)
	≥25 and <30	43.1 (22)	36.8 (28)	30.1 (86)	33.2 (189)
	≥30	11.8 (6)	6.6 (5)	46.5 (133)	43.5 (248)
Gender	Male	68.6 (35)	63.2 (48)	52.4 (150)	49.8 (284)
	Female	31.4 (16)	36.8 (28)	47.6 (136)	50.2 (286)
Age	<65 years	60.8 (31)	64.5 (49)	74.1 (212)	77.0 (439)
	≥65 years	39.2 (20)	35.5 (27)	25.9 (74)	23.0 (131)
Background antidiabetic medications	SU	29.4 (15)	38.2 (29)	16.1 (46)	15.4 (88)
	SU and metformin	70.6 (36)	61.8 (47)	83.9 (240)	84.6 (482)
Metformin dose (mg/day)	≤750	88.9 (32)	95.7 (45)	13.3 (32)	9.3 (45)
	>750 and ≤1500	11.1 (4)	4.3 (2)	22.1 (53)	21.0 (101)
	>1500	0.0 (0)	0.0 (0)	64.6 (155)	69.7 (336)

Mean ± SD, Proportion % (n)

Subgroup analyses of the primary efficacy endpoint, HbA1c changes, were performed using demographic characteristics (gender, body weight, age, and metformin use), which were different between the Japanese and overall populations. As a result, the efficacy of lixisenatide was demonstrated across all subgroups in both populations (Table 38).

Table 38. Comparison of HbA1c changes by gender, body weight, age, and concomitant medication (EFC6015, Japanese population and overall population, mITT population)

Subgroup		Japanese population		Overall population	
		Placebo (n = 50)	Lixisenatide (n = 73)	Placebo (n = 274)	Lixisenatide (n = 544)
Gender	Male	0.13 ± 0.80 (n = 35)	-0.76 ± 0.93 (n = 47)	-0.12 ± 0.83 (n = 142)	-0.96 ± 0.90 (n = 274)
	Female	0.34 ± 0.75 (n = 15)	-1.04 ± 0.64 (n = 26)	-0.12 ± 0.81 (n = 132)	-0.80 ± 0.97 (n = 270)
Body weight (kg)	<60	0.52 ± 0.82 (n = 13)	-1.09 ± 0.93 (n = 29)	0.05 ± 0.85 (n = 36)	-1.05 ± 0.81 (n = 76)
	≥60 and ≤80	-0.00 ± 0.75 (n = 27)	-0.64 ± 0.78 (n = 38)	-0.18 ± 0.77 (n = 97)	-0.85 ± 0.88 (n = 214)
	>80	0.31 ± 0.74 (n = 10)	-1.08 ± 0.34 (n = 6)	-0.12 ± 0.84 (n = 141)	-0.85 ± 1.01 (n = 254)
Age	<65 years	0.24 ± 0.84 (n = 31)	-0.74 ± 0.87 (n = 47)	-0.08 ± 0.85 (n = 204)	-0.86 ± 0.97 (n = 419)
	≥65 years	0.13 ± 0.69 (n = 19)	-1.07 ± 0.76 (n = 26)	-0.23 ± 0.73 (n = 70)	-0.96 ± 0.82 (n = 125)

Metformin use	Yes	0.02 ± 0.76 (n = 35)	-0.87 ± 0.71 (n = 44)	-0.16 ± 0.80 (n = 232)	-0.91 ± 0.91 (n = 458)
	No	0.59 ± 0.70 (n = 15)	-0.84 ± 1.03 (n = 29)	0.13 ± 0.89 (n = 42)	-0.74 ± 1.03 (n = 86)

Mean ± SD

PMDA considers as follows:

There was no clear discrepancy in efficacy between the Japanese and overall populations, thus allowing the interpretation that the trend was consistent between the Japanese and overall populations, since (1) the superiority of lixisenatide over placebo on the primary endpoint of HbA1c change in the overall population was demonstrated; (2) no major differences between the Japanese and overall populations were observed; and (3) it was confirmed that differences in the demographic characteristics of subjects have no clinically significant impact on HbA1c change. Hence, it may be concluded that the efficacy of lixisenatide in combination with SU (with or without metformin) has been demonstrated.

4.(iii).B.(3).2).(b) Safety in Japanese population and overall population

The applicant explained as follows:

In Study EFC6015 (the entire treatment period), the incidences of adverse events were 94.1% (48 of 51 subjects) in the placebo group and 97.4% (74 of 76 subjects) in the lixisenatide group in the Japanese population and 75.8% (216 of 285 subjects) in the placebo group and 81.5% (468 of 574 subjects) in the lixisenatide group in the overall population, and the incidence was higher in the Japanese population than in the overall population for both treatment groups. The incidences of serious adverse events were 13.7% (7 of 51 subjects) in the placebo group and 13.2% (10 of 76 subjects) in the lixisenatide group in the Japanese population and 12.3% (35 of 285 subjects) in the placebo group and 10.1% (58 of 574 subjects) in the lixisenatide group in the overall population, and the incidence was similar between the placebo and lixisenatide groups in both populations. The incidences of adverse events leading to treatment discontinuation were 5.9% (3 of 51 subjects) in the placebo group and 17.1% (13 of 76 subjects) in the lixisenatide group in the Japanese population, and 7.7% (22 of 285 subjects) in the placebo group and 12.4% (71 of 574 subjects) in the lixisenatide group in the overall population. The incidences of the following events in the SOCs in the lixisenatide group were higher in the Japanese population than in the overall population: “infections and infestations,” “eye disorders,” “gastrointestinal disorders,” “musculoskeletal and connective tissue disorders,” “general disorders and administration site conditions,” and “injury, poisoning and procedural complications”. Of these, adverse events in the SOCs of “gastrointestinal disorders” and “general disorders and administration site conditions” (whose incidences were different between the lixisenatide and placebo groups) that were reported at a ≥5% higher incidence in the Japanese population than in the overall population were diarrhoea, dyspepsia, constipation, and dental caries. When the incidences were examined by treatment period, many of these events occurred within 12 weeks of treatment and there was no trend towards a marked increase in incidence in the lixisenatide group compared to the placebo group during and after the main treatment period (24 weeks) (Table 39).

Table 39. Incidence of main gastrointestinal disorders by treatment period in Japanese population and overall population
(EFC6015, Japanese population and overall population, Safety population)

Event term	Treatment period	Japanese population		Overall population	
		Placebo (n = 51)	Lixisenatide (n = 76)	Placebo (n = 285)	Lixisenatide (n = 574)
Nausea	12 weeks of treatment	0.0 (0)	23.7 (18)	5.3 (15)	23.3 (134)
	Main treatment period[a)]	2.0 (1)	25.0 (19)	7.0 (20)	25.3 (145)
	Entire treatment period[b)]	2.0 (1)	26.3 (20)	8.8 (25)	28.0 (161)
Diarrhoea	12 weeks of treatment	5.9 (3)	10.5 (8)	4.9 (14)	6.1 (35)
	Main treatment period[a)]	7.8 (4)	11.8 (9)	6.7 (19)	8.9 (51)
	Entire treatment period[b)]	15.7 (8)	18.4 (14)	9.5 (27)	12.4 (71)
Vomiting	12 weeks of treatment	2.0 (1)	5.3 (4)	2.1 (6)	7.5 (43)
	Main treatment period[a)]	3.9 (2)	5.3 (4)	3.5 (10)	8.7 (50)
	Entire treatment period[b)]	7.8 (4)	5.3 (4)	5.3 (15)	10.6 (61)
Dyspepsia	12 weeks of treatment	0.0 (0)	11.8 (9)	0.4 (1)	4.2 (24)
	Main treatment period[a)]	0.0 (0)	11.8 (9)	0.4 (1)	5.4 (31)
	Entire treatment period[b)]	0.0 (0)	14.5 (11)	1.4 (4)	5.9 (34)
Constipation	12 weeks of treatment	7.8 (4)	6.6 (5)	2.1 (6)	3.0 (17)
	Main treatment period[a)]	11.8 (6)	7.9 (6)	2.8 (8)	3.5 (20)
	Entire treatment period[b)]	15.7 (8)	15.8 (12)	3.9 (11)	5.2 (30)
Dental caries	12 weeks of treatment	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	Main treatment period[a)]	0.0 (0)	1.3 (1)	0.0 (0)	0.2 (1)
	Entire treatment period[b)]	3.9 (2)	6.6 (5)	0.7 (2)	1.2 (7)

Incidence % (n)
a) 24 weeks
b) ≥76 weeks

Subgroup analyses of the incidences of adverse events were performed by gender, age, body weight, and metformin use, which were different between the Japanese and overall populations. As a result, there were no major differences between the populations within each subgroup (Table 40). Therefore, these differences in the demographic characteristics of subjects were not considered to significantly influence the safety evaluation of lixisenatide.

Table 40. Incidence of adverse events by gender, age, body weight, and metformin use
(EFC6015, Japanese population and overall population, Safety population)

		Japanese population		Overall population	
		Placebo (n = 51)	Lixisenatide (n = 76)	Placebo (n = 285)	Lixisenatide (n = 574)
Gender	Male	94.3 (33/35)	97.9 (47/48)	78.0 (117/150)	84.5 (240/284)
	Female	93.8 (15/16)	96.4 (27/28)	73.3 (99/135)	78.6 (228/290)
Age	<65 years	96.8 (30/31)	95.9 (47/49)	77.3 (163/211)	81.3 (360/443)
	≥65 years	90.0 (18/20)	100.0 (27/27)	71.6 (53/74)	82.4 (108/131)
Body weight (kg)	<60	100.0 (13/13)	96.9 (31/32)	86.8 (33/38)	89.3 (75/84)
	≥60 and ≤80	96.4 (27/28)	100.0 (38/38)	71.3 (72/101)	82.4 (187/227)
	>80	80.0 (8/10)	83.3 (5/6)	76.0 (111/146)	78.3 (206/263)
Metformin use	Yes	91.7 (33/36)	97.9 (46/47)	77.0 (184/239)	81.9 (398/486)
	No	100.0 (15/15)	96.6 (28/29)	69.6 (32/46)	79.5 (70/88)

Incidence % (Number of patients with events/Number of patients in each category)

The main gastrointestinal disorders with a ≥5% difference in incidence in the lixisenatide group during the

entire treatment period between the Japanese and overall populations were diarrhoea, dyspepsia, constipation, and dental caries. The differences in incidence were largely due to events occurring within 12 weeks of treatment (Table 39) and the differences observed during long-term treatment thereafter were unlikely to be clinically meaningful and were not considered to significantly influence the evaluation of the long-term safety of lixisenatide. The incidences of gastrointestinal disorders by metformin use were 36.1% (13 of 36 subjects) in the placebo group and 68.1% (32 of 47 subjects) in the lixisenatide group when using metformin and 60.0% (9 of 15 subjects) in the placebo group and 62.1% (18 of 29 subjects) in the lixisenatide group when not using metformin in the Japanese population, and 29.3% (70 of 239 subjects) in the placebo group and 49.2% (239 of 486 subjects) in the lixisenatide group when using metformin and 30.4% (14 of 46 subjects) in the placebo group and 47.7% (42 of 88 subjects) in the lixisenatide group when not using metformin in the overall population. In both populations, there was no trend towards a major difference in incidence in the lixisenatide group according to metformin use.

For the incidence of symptomatic hypoglycaemia in the lixisenatide group, there were no clear differences between the Japanese and overall populations in either the add-on to SU setting or the add-on to SU and metformin setting and many events tended to occur during the main treatment period (Table 41). In the add-on to SU setting, the number of symptomatic hypoglycemic events per 100 patient-years in the lixisenatide group was higher in the Japanese population than in the overall population. Severe symptomatic hypoglycaemia occurred in 1 subject in the placebo group (a Japanese subject) and 2 subjects in the lixisenatide group (non-Japanese subjects) in the add-on to SU and metformin setting. Hypoglycaemia leading to treatment discontinuation occurred in 1 subject treated with lixisenatide as add-on to SU (a non-Japanese subject) only and was uncommon in both populations.

Table 41. Incidence of symptomatic hypoglycaemia by treatment period in Japanese population and overall population (EFC6015, Japanese population and overall population, Safety population)

	Treatment period	Japanese population		Overall population	
		Placebo (n = 51)	Lixisenatide (n = 76)	Placebo (n = 285)	Lixisenatide (n = 574)
Add on to SU	12 weeks of treatment	20.0 (3/15)	20.7 (6/29)	8.7 (4/46)	17.0 (15/88)
	Main treatment period[a)]	20.0 (3/15)	20.7 (6/29)	8.7 (4/46)	18.2 (16/88)
	Entire treatment period[b)]	20.0 (3/15)	24.1 (7/29)	15.2 (7/46)	22.7 (20/88)
		4 [14.8]	46 [99.7]	22 [32.4]	77 [61.5]
Add on to SU and metformin	12 weeks of treatment	5.6 (2/36)	14.9 (7/47)	9.6 (23/239)	11.1 (54/486)
	Main treatment period[a)]	5.6 (2/36)	14.9 (7/47)	13.0 (31/239)	14.8 (72/486)
	Entire treatment period[b)]	13.9 (5/36)	23.4 (11/47)	18.4 (44/239)	22.0 (107/486)
		12 [21.1]	28 [37.1]	208 [60.7]	312 [44.3]

Upper row: incidence % (Number of patients with events/Number of patients in each category),
Lower row for the entire treatment period: total number of events [Number of events/100 patient-years]
a) 24 weeks
b) ≥76 weeks

The incidences of injection site reactions during the main treatment period were 3.9% (2 of 51 subjects) in the placebo group and 6.6% (5 of 76 subjects) in the lixisenatide group in the Japanese population, and 1.8% (5 of 285 subjects) in the placebo group and 4.5% (26 of 574 subjects) in the lixisenatide group in the overall population. The incidences during the entire treatment period were 5.9% (3 of 51 subjects) in the placebo group and 9.2% (7 of 76 subjects) in the lixisenatide group in the Japanese population, and 2.8% (8 of 285 subjects) in the placebo group and 4.9% (28 of 574 subjects) in the lixisenatide group in the overall population. Although the incidences during the main treatment period and during the entire treatment period were higher in the Japanese population than in the overall population for both treatment groups, no major

differences between the lixisenatide and placebo groups were seen for both populations. In both populations, most of the events were mild in severity and none of the events were severe or serious. A small number of subjects in the lixisenatide group had events leading to treatment discontinuation, i.e. 1 subject in the Japanese population and 2 subjects in the overall population. Furthermore, most of the events in the lixisenatide group occurred during the main treatment period in both populations and there was no trend towards a marked increase in incidence during the entire treatment period compared with the main treatment period. Therefore, the observed differences in incidence between the populations were not considered to significantly influence the evaluation of the long-term safety of lixisenatide.

Based on the above, the generalization of the results from the overall population to Japanese patients for the safety evaluation of lixisenatide is possible.

PMDA considers as follows:
There is no particular problem with the applicant's explanation that observed differences in the incidences of some adverse events between the Japanese and overall populations are not considered clinically significant differences in safety. It may be interpreted that there are no safety concerns for Japanese patients. Since the limited number of Japanese patients were treated with lixisenatide in combination with SU and high-dose (>750 mg/day) metformin in the study, it is necessary to continue to collect information on the safety of lixisenatide in combination with SU and high-dose (>750 mg/day) metformin via post-marketing surveillance.

4.(iii).B.(3).3) Add-on to basal insulin (with or without SU) study (EFC10887)

4.(iii).B.(3).3).(a) Efficacy in Japanese population and overall population

PMDA asked the applicant to explain the efficacy of lixisenatide in combination with basal insulin with or without SU in the Japanese and overall populations.

The applicant responded as follows:
The primary endpoint for Study EFC10887, HbA1c change from baseline to Week 24 was as shown in Table 42 and lixisenatide in combination with basal insulin with or without SU showed consistent efficacy between the Japanese and overall populations and similar efficacy also in the non-Japanese population. The percentage of subjects achieving HbA1c ≤6.5% at Week 24 in the lixisenatide group was 17.8% (26 of 154 subjects) in the overall population and 12.9% (9 of 72 subjects) in the Japanese population, and the percentage of subjects achieving HbA1c <7.0% at Week 24 in the lixisenatide group was 35.6% (52 of 154 subjects) in the overall population and 31.4% (22 of 72 subjects) in the Japanese population. The treatment difference in the change in fasting plasma glucose from baseline to Week 24 (lixisenatide minus placebo) and its 95% CI were -17.27 [-30.781, -3.763] mg/dL in the Japanese population and -12.04 [-22.062, -2.015] mg/dL in the overall population. Similarly, the treatment difference for the change in the daily basal insulin dose and its 95% CI were -0.70 [-1.297, -0.112] U/day in the Japanese population and -1.29 [-2.097, -0.477] U/day in the overall population. No major differences between the populations were observed for all endpoints.

Table 42. HbA1c change from baseline to Week 24
(EFC10887, Japanese, non-Japanese, and overall populations, mITT population)

	Japanese population (n = 159)		Non-Japanese population (n = 152)[a)]		Overall population (n = 311)	
	Placebo (n = 87)	Lixisenatide (n = 72)	Placebo (n = 70)	Lixisenatide (n = 82)	Placebo (n = 157)	Lixisenatide (n = 154)
HbA1c change	0.26 ± 0.75	-0.85 ± 1.25	-0.28 ± 0.87	-0.94 ± 1.21	0.02 ± 0.85	-0.90 ± 1.22
Difference vs. placebo [95% CI][b)]	-1.12 [-1.429, -0.809]		-0.62 [-0.973, -0.260]		-0.88 [-1.116, -0.650]	
P-value[b)]	<0.0001		0.0008		<0.0001	

Mean ± SD (%), LOCF

a) As the Basic Principles on Global Clinical Trials state that a global trial should be designed so that consistency can be obtained between results from the entire population and the Japanese population, this review report basically presents the results from the Japanese population and the overall population accordingly, but the results from the non-Japanese population are also included for the primary endpoint of HbA1c change, for reference.

b) Calculated using an ANCOVA model with treatment (lixisenatide or placebo), HbA1c at screening (<8.0% or ≥8.0%), SU use at screening, and country (only for the analysis of the overall population) as fixed effects and baseline HbA1c as a covariate.

The baseline demographic characteristics of subjects were as shown in Table 43 and the proportion of male subjects tended to be higher in the Japanese population compared to the overall population. The proportion of subjects on basal insulin alone was higher in the Japanese population than in the overall population, while the dose level of basal insulin and the proportion of subjects on the combination of basal insulin and high-dose SU tended to be higher in the overall population.

Table 43. Baseline demographic characteristics of subjects (EFC10887, Japanese population and overall population, mITT population)

		Japanese population (n = 159)		Overall population (n = 311)	
		Placebo (n = 87)	Lixisenatide (n = 72)	Placebo (n = 157)	Lixisenatide (n = 154)
HbA1c (%)		8.52 ± 0.80	8.53 ± 0.71	8.52 ± 0.78	8.54 ± 0.73
Body weight (kg)		66.73 ± 14.21	67.35 ± 13.90	65.60 ± 12.47	65.93 ± 13.00
Age (years)		57.1 ± 10.7	59.1 ± 10.5	58.0 ± 10.1	58.7 ± 10.2
Gender	Male	65.5 (57)	66.7 (48)	51.0 (80)	44.8 (69)
	Female	34.5 (30)	33.3 (24)	49.0 (77)	55.2 (85)
Background antidiabetic medications	Basal insulin alone	40.2 (35)	43.1 (31)	29.3 (46)	29.9 (46)
	Basal insulin and SU	59.8 (52)	56.9 (41)	70.7 (111)	70.1 (108)
Basal insulin dose (U/day)		20.61 ± 13.82	18.57 ± 8.89	24.11 ± 14.18	24.85 ± 13.96
High-dose SU[a)] use		46.2 (24/52)	48.8 (20/41)	59.5 (66/111)	61.1 (66/108)

Mean ± SD, Proportion % (n)

a) High-dose SU: glimepiride ≥3 mg/day, glibenclamide ≥5 mg/day, gliclazide ≥80 mg/day

The influences of gender, SU use, and the dose of SU on efficacy evaluation were assessed (Table 44). As a result, although HbA1c change tended to be greater in female subjects in the Japanese population, the efficacy of lixisenatide in other subgroups was also demonstrated consistently in both populations.

Table 44. HbA1c change by gender, SU use, and the dose of SU (EFC10887, Japanese population and overall population, mITT population)

		Japanese population		Overall population	
		Placebo (n = 86)	Lixisenatide (n = 70)	Placebo (n = 154)	Lixisenatide (n = 146)
Gender	Male	0.26 ± 0.76 (n = 57)	-0.71 ± 1.37 (n = 46)	0.06 ± 0.88 (n = 80)	-0.84 ± 1.29 (n = 65)
	Female	0.26 ± 0.74 (n = 29)	-1.12 ± 0.96 (n = 24)	-0.02 ± 0.82 (n = 74)	-0.94 ± 1.17 (n = 81)
SU use	Yes	0.32 ± 0.81 (n = 52)	-0.84 ± 1.49 (n = 40)	0.04 ± 0.88 (n = 109)	-0.91 ± 1.36 (n = 103)
	No	0.16 ± 0.65 (n = 34)	-0.87 ± 0.85 (n = 30)	-0.01 ± 0.76 (n = 45)	-0.87 ± 0.83 (n = 43)
High-dose SU use[a)]	Yes	0.43 ± 0.83 (n = 24)	-1.21 ± 0.87 (n = 19)	0.11 ± 0.90 (n = 65)	-1.21 ± 1.06 (n = 61)
	No	0.19 ± 0.79 (n = 25)	-0.50 ± 1.85 (n = 21)	-0.01 ± 0.82 (n = 37)	-0.54 ± 1.60 (n = 38)

Mean ± SD

a) High-dose SU: glimepiride ≥3 mg/day, glibenclamide ≥5 mg/day, gliclazide ≥80 mg/day

PMDA considers as follows:

There was no clear discrepancy in efficacy between the Japanese and overall populations, thus allowing the

interpretation that the trend was consistent between the Japanese and overall populations, since (1) the superiority of lixisenatide over placebo on the primary endpoint of HbA1c change in the overall population was demonstrated; (2) no major differences between the Japanese and overall populations or between the Japanese and non-Japanese populations were observed; and (3) it was confirmed that differences in the demographic characteristics of subjects have no clinically significant impact on HbA1c change. Hence, it may be concluded that the efficacy of lixisenatide in combination with basal insulin (with or without SU) has been demonstrated.

4.(iii).B.(3).3).(b) Safety in Japanese population and overall population

In Study EFC10887 (the entire treatment period [24 weeks]), the incidences of adverse events were 66.7% (58 of 87 subjects) in the placebo group and 87.5% (63 of 72 subjects) in the lixisenatide group in the Japanese population, and 70.1% (110 of 157 subjects) in the placebo group and 89.0% (137 of 154 subjects) in the lixisenatide group in the overall population. The incidences of serious adverse events were 6.9% (6 of 87 subjects) in the placebo group and 2.8% (2 of 72 subjects) in the lixisenatide group in the Japanese population and 5.7% (9 of 157 subjects) in the placebo group and 6.5% (10 of 154 subjects) in the lixisenatide group in the overall population, and the incidence was similar between the placebo and lixisenatide groups. The incidences of adverse events leading to treatment discontinuation were 4.6% (4 of 87 subjects) in the placebo group and 6.9% (5 of 72 subjects) in the lixisenatide group in the Japanese population and 3.2% (5 of 157 subjects) in the placebo group and 9.1% (14 of 154 subjects) in the lixisenatide group in the overall population.

The main adverse events reported in the lixisenatide group were hypoglycaemia and events in the SOC of gastrointestinal disorders in both populations. The main adverse events reported at a higher incidence in the Japanese population than in the overall population (the entire treatment period) were nasopharyngitis (19.5% [17 of 87 subjects] in the placebo group and 19.4% [14 of 72 subjects] in the lixisenatide group in the Japanese population; 12.7% [20 of 157 subjects] in the placebo group and 13.6% [21 of 154 subjects] in the lixisenatide group in the overall population) and abdominal discomfort (0.0% [0 of 87 subjects] in the placebo group and 12.5% (9 of 72 subjects) in the lixisenatide group in the Japanese population; 0.6% [1 of 157 subjects] in the placebo group and 7.1% [11 of 154 subjects] in the lixisenatide group in the overall population), but the incidence of nasopharyngitis was similar between the placebo and lixisenatide groups. The incidences of hypoglycaemia (17.2% [15 of 87 subjects] in the placebo group and 36.1% [26 of 72 subjects] in the lixisenatide group in the Japanese population; 23.6% [37 of 157 subjects] in the placebo group and 43.5% [67 of 154 subjects] in the lixisenatide group in the overall population) and headache (2.3% [2 of 87 subjects] in the placebo group and 2.8% [2 of 72 subjects] in the lixisenatide group in the Japanese population; 1.9% [3 of 157 subjects] in the placebo group and 10.4% [16 of 154 subjects] in the lixisenatide group in the overall population) were higher in the overall population than in the Japanese population.

Concerning the main demographic characteristics of subjects, the gender distribution was different between the Japanese and overall populations. In the Japanese population, the incidences of adverse events were 66.7% (38 of 57 subjects) in the placebo group and 85.4% (41 of 48 subjects) in the lixisenatide group among male subjects, and 66.7% (20 of 30 subjects) in the placebo group and 91.7% (22 of 24 subjects) in the lixisenatide group among female subjects. In the overall population, the incidences of adverse events were 67.5% (54 of 80 subjects) in the placebo group and 85.5% (59 of 69 subjects) in the lixisenatide group

among male subjects, and 72.7% (56 of 77 subjects) in the placebo group and 91.8% (78 of 85 subjects) in the lixisenatide group among female subjects. There were no major gender differences in the incidence of adverse events for both populations and the observed differences in the gender distribution between the populations were not considered to significantly influence the safety evaluation of lixisenatide.

The gastrointestinal disorders mainly observed were similar between the populations and no major differences between the populations were observed for the incidences in the placebo and lixisenatide groups. Most of the events reported in the lixisenatide group occurred within 12 weeks of treatment (Table 45). The incidence of abdominal discomfort in the lixisenatide group was ≥5% higher in the Japanese population than in the overall population, but the difference in incidence was due to events occurring within 12 weeks of treatment. Gastrointestinal disorders leading to treatment discontinuation also occurred within 12 weeks of treatment in all 3 subjects in the Japanese population, and within 12 weeks of treatment in 8 subjects and during the 24-week treatment period in 9 subjects in the overall population, and most of the events occurred within 12 weeks of treatment in both populations.

Table 45. Incidence of main gastrointestinal disorders[a)] by treatment period in Japanese population and overall population (EFC10887, Japanese population and overall population, Safety population)

Event term	Treatment period	Japanese population		Overall population	
		Placebo (n = 87)	Lixisenatide (n = 72)	Placebo (n = 157)	Lixisenatide (n = 154)
Nausea	12 weeks of treatment	2.3 (2)	36.1 (26)	3.8 (6)	38.3 (59)
	Entire treatment period[b)]	3.4 (3)	36.1 (26)	4.5 (7)	39.6 (61)
Vomiting	12 weeks of treatment	1.1 (1)	16.7 (12)	0.6 (1)	16.9 (26)
	Entire treatment period[b)]	3.4 (3)	18.1 (13)	1.9 (3)	18.2 (28)
Abdominal discomfort	12 weeks of treatment	0.0 (0)	12.5 (9)	0.6 (1)	7.1 (11)
	Entire treatment period[b)]	0.0 (0)	12.5 (9)	0.6 (1)	7.1 (11)
Dyspepsia	12 weeks of treatment	0.0 (0)	2.8 (2)	0.0 (0)	6.5 (10)
	Entire treatment period[b)]	0.0 (0)	4.2 (3)	0.0 (0)	7.1 (11)
Diarrhoea	12 weeks of treatment	3.4 (3)	5.6 (4)	1.9 (3)	6.5 (10)
	Entire treatment period[b)]	3.4 (3)	5.6 (4)	2.5 (4)	6.5 (10)
Constipation	12 weeks of treatment	3.4 (3)	8.3 (6)	2.5 (4)	5.2 (8)
	Entire treatment period[b)]	3.4 (3)	8.3 (6)	2.5 (4)	5.2 (8)

Incidence % (n)
a) Gastrointestinal disorders reported by ≥5% of subjects in either population
b) 24 weeks

In the add-on to basal insulin alone setting, no major differences between the populations were observed for the incidence of symptomatic hypoglycaemia and the number of symptomatic hypoglycemic events per 100 patient-years in the lixisenatide group. In the add-on to basal insulin and SU setting, the incidence of symptomatic hypoglycaemia was higher in the overall population than in the Japanese population for both the placebo and lixisenatide groups, and the incidence of symptomatic hypoglycaemia in the lixisenatide group was higher compared to the add-on to basal insulin alone setting. Especially when using high-dose SU compared to when not using it, the incidences of symptomatic hypoglycaemia in the lixisenatide and placebo groups both tended to be higher (Table 46). As to the influence of the basal insulin dose, in both the Japanese and overall populations, regardless of the use of SU, the incidence of symptomatic hypoglycaemia and the

number of events per 100 patient-years tended to be higher when using high-dose basal insulin compared to when not using it, for both the lixisenatide and placebo groups.

Table 46. Incidence of symptomatic hypoglycaemia in Japanese population and overall population
(EFC10887, Japanese population and overall population, Safety population)

		Japanese population		Overall population	
		Placebo (n = 87)	Lixisenatide (n = 72)	Placebo (n = 157)	Lixisenatide (n = 154)
Add on to basal insulin alone		22.9 (8/35)	32.3 (10/31)	28.3 (13/46)	32.6 (15/46)
		16 [103.5]	19 [137.4]	28 [135.7]	29 [147.9]
Add on to basal insulin and SU		13.5 (7/52)	39.0 (16/41)	21.6 (24/111)	47.2 (51/108)
		12 [51.0]	54 [301.8]	74 [147.8]	165 [353.5]
High-dose SU[a)] use	Yes	16.7 (4/24)	40.0 (8/20)	24.2 (16/66)	53.0 (35/66)
		9 [79.4]	30 [349.9]	55 [182.4]	118 [426.0]
	No	8.0 (2/25)	38.1 (8/21)	13.2 (5/38)	39.5 (15/38)
		2 [18.5]	24 [257.6]	14 [84.3]	45 [261.7]
High-dose basal insulin[b)] use	Yes	22.6 (7/31)	45.5 (10/22)	30.8 (24/78)	55.0 (44/80)
		15 [104.8]	25 [286.9]	71 [198.8]	125 [377.9]
	No	14.3 (8/56)	32.0 (16/50)	16.5 (13/79)	29.7 (22/74)
		13 [52.7]	48 [208.6]	31 [88.6]	69 [207.8]

Upper row: incidence % (Number of patients with events/Number of patients in each category)
Lower row: total number of events [Number of events/100 patient-years]
a) High-dose SU: glimepiride ≥3 mg/day, glibenclamide ≥5 mg/day, gliclazide ≥80 mg/day
b) High-dose basal insulin: >20 U/day

No severe hypoglycaemia was reported and hypoglycaemia leading to treatment discontinuation occurred in 3 subjects treated with lixisenatide in combination with basal insulin and SU (including 2 Japanese subjects). The incidences of hypoglycaemia were examined by treatment period. As a result, in both populations, the incidence was high in the early phase of treatment in the lixisenatide group, and low in the later phase in both the lixisenatide and placebo groups.

As described above, the occurrence of hypoglycaemia was considered similar between the Japanese and overall populations.

The incidences of injection site reactions were 1.1% (1 of 87 subjects) in the placebo group and 2.8% (2 of 72 subjects) in the lixisenatide group in the Japanese population, and 1.3% (2 of 157 subjects) in the placebo group and 1.3% (2 of 154 subjects) in the lixisenatide group in the overall population. All events occurred within 12 weeks of treatment and were mild in severity. None led to treatment discontinuation.

PMDA considers as follows:
There is no particular problem with the applicant's explanation that observed differences in the incidences of some adverse events between the Japanese and overall populations are not considered clinically significant differences in safety. It may be interpreted that there are no safety concerns for Japanese patients.

Based on the review described in the above 4.(iii).B.(3).1) to 4.(iii).B.(3).3), there is no major problem with the generalization of the results from the overall population in Studies EFC6015 and EFC10887 to Japanese patients with type 2 diabetes mellitus.

4.(iii).B.(4) Efficacy

4.(iii).B.(4).1) Lixisenatide in combination with SU (with or without metformin)

PMDA considers that the efficacy of lixisenatide in combination with SU (with or without metformin) has been demonstrated in Study EFC6015 that showed the superiority of lixisenatide over placebo [see

"4.(iii).B.(3) Interpretation of results of multinational studies"].

4.(iii).B.(4).2) Lixisenatide in combination with basal insulin (with or without SU)

PMDA considers that the efficacy of lixisenatide in combination with basal insulin (with or without SU) has been demonstrated in Study EFC10887 that showed the superiority of lixisenatide over placebo [see "4.(iii).B.(3) Interpretation of results of multinational studies"].

4.(iii).B.(4).3) Influence of anti-lixisenatide antibody titers on efficacy evaluation

The applicant explained as follows:

HbA1c changes from baseline to Week 24 (LOCF) in Studies EFC10887 and EFC6015 were assessed by antibody status (positive or negative). According to the pooled data from Studies EFC6015 and EFC10887, the least-squares mean changes in HbA1c [95% CI] among lixisenatide-treated subjects with antibody assay results and HbA1c values at Week 24 (481 subjects in the overall population, 114 subjects in the Japanese population) were -0.80 [-0.944, -0.659]% in antibody-positive subjects (n = 312) and -0.76 [-0.930, -0.588]% in antibody-negative subjects (n = 169) in the overall population, and -0.74 [-0.962, -0.516]% in antibody-positive subjects (n = 81) and -0.86 [-1.178, -0.545]% in antibody-negative subjects (n = 33) in the Japanese population. There were no major differences according to antibody status in both populations. In Study EFC6015, the mean changes in HbA1c [95% CI] among lixisenatide-treated subjects with antibody assay results and HbA1c values at Week 76 (240 subjects in the overall population, 29 subjects in the Japanese population) were -0.77 [-0.908, -0.633]% in antibody-positive subjects (n = 180) and -0.97 [-1.254, -0.690]% in antibody-negative subjects (n = 60) in the overall population, and -0.64 [-0.988, -0.289]% in antibody-positive subjects (n = 26) and -1.97 [-5.170, 1.237]% in antibody-negative subjects (n = 3) in the Japanese population, which showed a similar trend as the results up to Week 24 (the pooled data from Studies EFC6015 and EFC10887).

PMDA considers as follows:

There was no trend towards marked rises in antibody titers in clinical studies and no clear relationship between the level of antibody formation and efficacy was observed. As the information on antibody formation during long-term treatment with lixisenatide in Japanese patients is limited, it is necessary to continue to collect information on the relationship between antibody formation and efficacy via post-marketing surveillance [see "4.(iii).B.(5).5) Anti-lixisenatide antibody development and immune reactions" for the relationship between antibody formation and safety].

4.(iii).B.(5) Safety

Based on the occurrence of adverse events and adverse drug reactions in multinational phase III studies (EFC6015 and EFC10887) (Tables 25, 26, 29, and 30), PMDA considers that the safety of lixisenatide is acceptable, provided that appropriate cautions and information will be provided, but individual events were further analyzed.

4.(iii).B.(5).1) Hypoglycaemia

The applicant explained as follows:

Lixisenatide in combination with SU (with or without metformin) was studied in Study EFC6015. As a result,

the incidence of symptomatic hypoglycaemia was higher in the lixisenatide group than in the placebo group while severe symptomatic hypoglycaemia and hypoglycaemia leading to treatment discontinuation were uncommon. Lixisenatide in combination with basal insulin (with or without SU) was studied in Study EFC10887. As a result, the incidence of symptomatic hypoglycaemia was higher in the lixisenatide group than in the placebo group and the incidence tended to be higher especially when lixisenatide was used in combination with basal insulin and SU. Meanwhile, severe symptomatic hypoglycaemia and hypoglycaemia leading to treatment discontinuation were uncommon [see "4.(iii).B.(3).2).(b) Safety in Japanese population and overall population" and "4.(iii).B.(3).3).(b) Safety in Japanese population and overall population"].

As described above, when lixisenatide was used in combination with SU or basal insulin, the incidence of symptomatic hypoglycaemia was higher in the lixisenatide group than in the placebo group and when lixisenatide was used in combination with both basal insulin and SU, the incidence was particularly high. The incidence of symptomatic hypoglycaemia was slightly higher during the early phase of treatment. In the add-on to basal insulin alone setting, the incidence was higher when using high-dose basal insulin compared to when not using it. Taking account of the above, when lixisenatide is added to existing therapy of an SU or a basal insulin, a reduction in the dose of the SU or the basal insulin should be considered, as necessary, in order to lessen the risk of hypoglycaemia.

PMDA considers as follows:
Although there has been no major concern about the occurrence of hypoglycaemia, it is necessary to provide appropriate caution and information regarding the risk of hypoglycaemia and continue to collect information on hypoglycaemia via post-marketing surveillance, since hypoglycaemia is an important adverse drug reaction associated with the long-term prognosis for patients; the use of lixisenatide in combination with SU or basal insulin requires special attention; the number of patients included in clinical studies is limited, etc.

4.(iii).B.(5).2) Gastrointestinal disorders
The applicant explained as follows:
According to the pooled data from phase III placebo-controlled studies,[127] the incidences of nausea during the main treatment period were 6.2% (66 of 1061 subjects) in the placebo group and 26.1% (556 of 2127 subjects) in the lixisenatide group, and the incidences of nausea during the entire treatment period were 7.7% (82 of 1061 subjects) in the placebo group and 28.5% (607 of 2127 subjects) in the lixisenatide group. The incidences of vomiting during the main treatment period were 1.8% (19 of 1061 subjects) in the placebo group and 10.5% (224 of 2127 subjects) in the lixisenatide group and the incidences of vomiting during the entire treatment period were 2.8% (30 of 1061 subjects) in the placebo group and 12.4% (263 of 2127 subjects) in the lixisenatide group. Both nausea and vomiting occurred mostly during the main treatment period. Serious nausea and serious vomiting occurred in 2 lixisenatide-treated subjects each (all non-Japanese subjects) during the main treatment period, but no new events occurred thereafter. Nausea leading to treatment discontinuation occurred in 65 subjects in the lixisenatide group (13 Japanese subjects, 52 non-Japanese subjects) during the main treatment period and in 72 subjects (13 Japanese subjects, 59 non-Japanese subjects) during the entire treatment period. Vomiting leading to treatment discontinuation occurred in 26 subjects (2 Japanese subjects, 24 non-Japanese subjects) during the main treatment period and

[127] Multinational studies including non-Japanese and Japanese patients (EFC6015, EFC10887, EFC6018) and foreign clinical studies (EFC6014, EFC6016, EFC10743)

in 28 subjects (2 Japanese subjects, 26 non-Japanese subjects) during the entire treatment period.

As described above, the main adverse events of gastrointestinal disorders observed during treatment with lixisenatide, i.e. nausea and vomiting occurred mostly during the early phase of treatment, but serious events and events leading to treatment discontinuation were uncommon.

According to the results from multinational studies in which Japanese patients participated (EFC6015 and EFC10887) [see "4.(iii).B.(3).2).(b) Safety in Japanese population and overall population" and "4.(iii).B.(3).3).(b) Safety in Japanese population and overall population"], although the incidences of gastrointestinal disorders were higher in the Japanese population compared with the overall population, as the differences in incidence were due to events occurring within 12 weeks of treatment and serious events and events leading to treatment discontinuation were uncommon in both populations, these differences were not considered a particular problem.

PMDA considers as follows:
Many of the gastrointestinal disorders reported in multinational studies in which Japanese patients participated were mild or moderate in severity and clinical studies showed no clinically relevant concern. However, as there was a consistent trend towards a higher incidence in the Japanese population compared with the overall population and the number of Japanese patients included in clinical studies is limited, it is necessary to include an appropriate precaution statement in the package insert and continue to collect information on gastrointestinal disorders via post-marketing surveillance.

4.(iii).B.(5).3) Pancreatitis (including elevations of amylase and lipase)
The applicant explained as follows:
With regard to adverse events potentially related to pancreatitis,[128] according to the pooled data from phase III placebo-controlled studies (the entire treatment period), the incidences of adverse events specific to pancreatitis (Class 2) such as pancreatitis, acute pancreatitis, and chronic pancreatitis were 0.2% (2 of 1061 subjects; pancreatitis and acute pancreatitis in 1 subject each) in the placebo group and 0.4% (9 of 2127 subjects; pancreatitis in 5 subjects, acute pancreatitis in 2 subjects, chronic pancreatitis in 2 subjects) in the lixisenatide group and no adverse events specific to pancreatitis (Class 2) occurred in the Japanese population. Among the 5 cases of pancreatitis in the lixisenatide group, 2 cases were confirmed and the remaining 3 cases were reported as suspected pancreatitis. In the 2 subjects with acute pancreatitis in the lixisenatide group, time to the onset of symptoms was >1 year after starting treatment with lixisenatide. The rates of adverse events potentially related to pancreatitis were 0.65 cases per 100 patient-years in the placebo group and 1.20 cases per 100 patient-years in the lixisenatide group and the relative risk for lixisenatide compared to placebo and its 95% CI were 1.92 [0.91, 4.48]. The differences between the lixisenatide and placebo groups were considered due mainly to Class 3 adverse events less specific to diagnosis. Also,

[128] Adverse events potentially related to pancreatitis were identified according to the following criteria.
Class 1: subjects with preferred terms related to abdominal pain due to acute pancreatitis in the SMQ "acute pancreatitis" (Category C) (abdominal distension, abdominal pain, abdominal pain upper, abdominal rigidity, abdominal tenderness, acute abdomen, gastrointestinal pain, abdominal rebound tenderness, abdominal compartment syndrome, abdominal discomfort, abdominal pain lower, or abdominal symptom) and lipase or amylase ≥3 times the upper limit of normal.
Class 2: subjects with preferred terms in the narrow SMQ "acute pancreatitis" (Category A) (pancreatitis, acute pancreatitis, chronic pancreatitis, pancreatitis relapsing, etc.).
Class 3: subjects with terms in the broad SMQ "acute pancreatitis" (Category B and Category C), regardless of the time of onset.

according to the pooled data from phase II and phase III studies[129] (the entire treatment period), no adverse events potentially related to pancreatitis occurred in the Japanese population and the incidence was 1.2% (38 of 3304 subjects) in the lixisenatide group in the overall population.

With respect to laboratory tests (pancreatic enzymes), according to the pooled data from phase III placebo-controlled studies (the entire treatment period), the percentages of subjects with elevated amylase ≥3 times the upper limit of normal were 0.2% (2 of 1051 subjects) in the placebo group and 0.4% (9 of 2100 subjects) in the lixisenatide group. The percentages of subjects with elevated lipase ≥3 times the upper limit of normal were 2.0% (21 of 1051 subjects) in the placebo group and 2.3% (48 of 2100 subjects) in the lixisenatide group. One subject in the lixisenatide group who had elevations in both amylase and lipase ≥3 times the upper limit of normal was diagnosed with acute pancreatitis, leading to lixisenatide discontinuation. The elevations in lipase ≥3 times the upper limit of normal observed in 4 subjects in the lixisenatide group were determined to be adverse events of lipase increased and led to lixisenatide discontinuation. No elevations in amylase or lipase ≥3 times the upper limit of normal occurred in the Japanese population.

According to active-controlled phase III studies (EFC6019 and EFC10780), pancreatic enzymes increased (lipase or amylase) occurred in 1.6% of the lixisenatide group (5 of 318 subjects) and 2.8% of the exenatide group (9 of 316 subjects) in Study EFC6019, but only 1 subject in the exenatide group (mild chemical pancreatitis) had a confirmed diagnosis of pancreatitis. As an adverse event potentially related to pancreatitis, severe lipase increased occurred in 1 subject in the lixisenatide group. No serious adverse events were reported. In Study EFC10780, pancreatic enzymes increased specific to pancreatitis or related to suspected pancreatitis were observed in 3.8% of the lixisenatide group (6 of 158 subjects) and 1.2% of the sitagliptin phosphate hydrate group (2 of 161 subjects). No events of pancreatitis were confirmed and there were no serious adverse events potentially related to pancreatitis, severe adverse events, or adverse events leading to treatment discontinuation.

The above results did not indicate an increased risk of pancreatitis.

PMDA considers as follows:
There were only a few confirmed cases of pancreatitis reported during lixisenatide treatment and also there were no confirmed diagnoses of pancreatitis in the Japanese population. However, given that the pooled data from phase III placebo-controlled studies showed a trend towards a higher risk of adverse events potentially related to pancreatitis in the lixisenatide group compared with the placebo group, it is necessary to include a precaution statement regarding pancreatitis in the package insert and continue to collect information on pancreatitis via post-marketing surveillance.

4.(iii).B.(5).4) Injection site reactions
The applicant explained as follows:
According to the pooled data from phase III placebo-controlled studies, the incidences of injection site reactions during the main treatment period were 1.4% (15 of 1061 subjects) in the placebo group and 3.9% (83 of 2127 subjects) in the lixisenatide group and the incidences of injection site reactions during the entire

[129] Multinational studies including non-Japanese and Japanese patients (PDY6797, EFC6015, EFC10887, EFC6018), a Japanese clinical study (LTS10888), and foreign clinical studies (ACT6011, DRI6012, PDY10931, EFC6014, EFC6016, EFC10743, EFC6019, EFC10780)

treatment period were 1.9% (20 of 1061 subjects) in the placebo group and 4.7% (101 of 2127 subjects) in the lixisenatide group. Although the incidence was higher in the lixisenatide group than in the placebo group during both treatment periods, there was no trend towards a marked increase in incidence during the entire treatment period compared with the main treatment period. Most of the events were mild in severity and events leading to treatment discontinuation occurred in 9 subjects (1 Japanese subject, 8 non-Japanese subjects) during the entire treatment period, but no serious events were reported. Furthermore, most of the subjects (80 of 101 subjects) having an injection site reaction in the lixisenatide group experienced only once during the entire treatment period. The pooled data from phase II and phase III studies showed a similar trend, i.e. the incidence in the lixisenatide group (the entire treatment period) was 5.4% (160 of 2950 subjects).

PMDA considers as follows:
In Japanese and foreign clinical studies, the incidence of injection site reactions during treatment with lixisenatide was low and there was no trend towards a marked increase in incidence with continued treatment. No serious events were reported. Thus, there is no major concern. However, as the incidence tended to be higher in the lixisenatide group than in the placebo group, it is necessary to include a precaution statement in the package insert and continue to collect information on injection site reactions via post-marketing surveillance.

4.(iii).B.(5).5) Anti-lixisenatide antibody development and immune reactions
The applicant explained as follows:
According to the pooled data from phase III studies[130] (the entire treatment period), the incidences of adverse events by antibody status were 83.0% (1384 of 1668 subjects) in antibody-positive subjects and 75.2% (475 of 632 subjects) in antibody-negative subjects in the overall population. In the Japanese population, the incidences of adverse events were 90.8% (167 of 184 subjects) in antibody-positive subjects and 90.2% (55 of 61 subjects) in antibody-negative subjects. There were no differences according to antibody status in both populations. The incidences of injection site reactions were 5.9% (99 of 1668 subjects) in antibody-positive subjects and 2.5% (16 of 632 subjects) in antibody-negative subjects in the overall population. In the Japanese population, the incidences of injection site reactions were 8.2% (15 of 184 subjects) in antibody-positive subjects and 3.3% (2 of 61 subjects) in antibody-negative subjects. Although antibody-positive subjects had a higher incidence in both populations, most of the events were mild in severity regardless of antibody status. The incidences of adverse events adjudicated by the allergic reaction assessment committee (ARAC) as allergic reactions by antibody status were 2.0% (33 of 1668 subjects) in antibody-positive subjects and 1.9% (12 of 632 subjects) in antibody-negative subjects in the overall population. In the Japanese population, the incidences were 3.3% (6 of 184 subjects) in antibody-positive subjects and 3.3% (2 of 61 subjects) in antibody-negative subjects. There were no differences according to antibody status. Based on the analysis of the data from phase III studies in which Japanese patients participated (Table 47), there were no major differences in the incidence of symptomatic hypoglycaemia according to antibody status.

[130] Multinational studies including non-Japanese and Japanese patients (EFC6015, EFC10887, EFC6018), a Japanese clinical study (LTS10888), and foreign clinical studies (EFC6014, EFC6016, EFC10743, EFC6019, EFC10780)

Table 47. Incidences of symptomatic hypoglycaemia in lixisenatide group by antibody status in phase III studies in which Japanese patients participated (Entire treatment period, Safety population)

Study ID		Japanese population		Overall population	
		Antibody-positive	Antibody-negative	Antibody-positive	Antibody-negative
EFC6015	Add on to SU alone	26.3 (5/19)	22.2 (2/9)	20.3 (12/59)	29.6 (8/27)
	Add on to SU and metformin	26.3 (10/38)	12.5 (1/8)	22.9 (84/367)	19.4 (21/108)
EFC10887	Add on to basal insulin alone	28.0 (7/25)	50.0 (3/6)	28.6 (10/35)	50.0 (5/10)
	Add on to basal insulin and SU	26.7 (8/30)	72.7 (8/11)	40.8 (31/76)	62.5 (20/32)
EFC6018[a)]		0.0 (0/15)	0.0 (0/15)	0.8 (1/122)	2.9 (3/105)
LTS10888[a)]		7.0 (4/57)	8.3 (1/12)	–	–

Incidence % (Number of subjects with symptomatic hypoglycaemia/Number of subjects in each category)
a) Results of the pooled titration group are indicated.

PMDA considers as follows:

Although there was a trend towards rises in antibody titers during treatment with lixisenatide, antibody formation had no clear influence on safety. However, as the information on antibody formation during long-term treatment with lixisenatide in Japanese patients is limited, it is necessary to continue to collect information on antibody development and immune reactions via post-marketing surveillance.

4.(iii).B.(5).6) Effects on renal function

The applicant explained as follows:

According to the pooled data from phase III placebo-controlled studies (the entire treatment period), the incidences of renal adverse events were 1.3% (14 of 1061 subjects) in the placebo group and 0.9% (20 of 2127 subjects) in the lixisenatide group in the overall population. The events that were commonly reported in the lixisenatide group and occurred at a higher incidence in the lixisenatide group than in the placebo group were blood creatinine increased (1 placebo-treated subject, 7 lixisenatide-treated subjects) and acute renal failure (1 placebo-treated subject, 3 lixisenatide-treated subjects). In the Japanese population, only 1 subject in the lixisenatide group had diabetic nephropathy. The incidence of renal adverse events in the lixisenatide group was low and most of the events were mild in severity. The incidences of potentially clinically significant abnormalities (PCSAs) (≥30% changes from baseline) for serum creatinine (the entire treatment period) were 11.8% (121 of 1023 subjects) in the placebo group and 14.6% (291 of 1997 subjects) in the lixisenatide group in the overall population. In the Japanese population, the incidences were 3.4% (5 of 146 subjects) in the placebo group and 6.3% (11 of 174 subjects) in the lixisenatide group. No clear differences between the treatment groups were observed for the both populations. With regard to the incidences in the lixisenatide group, the pooled data from phase II and phase III studies also showed a similar trend as the pooled data from phase III placebo-controlled studies.

PMDA considers as follows:

There is no particular problem with the applicant's explanation that the incidences of renal adverse events and PCSAs for serum creatinine were low in both the lixisenatide and placebo groups and no clear differences between the treatment groups were observed. However, as the incidences of adverse events of serum creatinine increased and acute renal failure tended to be higher in the lixisenatide group, it is necessary to continue to collect information on effects on the renal function via post-marketing surveillance.

4.(iii).B.(5).7) Hypersensitivity reactions

The applicant explained as follows:

According to the pooled data from phase III placebo-controlled studies (the entire treatment period), the incidences of adverse events adjudicated by the ARAC as allergic reactions were 1.2% (13 of 1061 subjects)

in the placebo group and 1.8% (39 of 2127 subjects) in the lixisenatide group and a causal relationship to study drug could not be denied for the events reported by 1 subject in the placebo group and 10 subjects in the lixisenatide group. In the Japanese population, adverse events adjudicated by the ARAC as allergic reactions occurred in 3 subjects in the lixisenatide group only (contact dermatitis, anaphylactic shock, angioedema) and a causal relationship to study drug could not be denied for 1 case (angioedema). Most events were classified as severity grade 1[131] or 2,[132] and 9 events reported by 8 subjects (including 1 Japanese subject) were classified as serious adverse events. Two events of anaphylactic shock occurred in the lixisenatide group (1 event was reported by a Japanese subject), but a causal relationship to study drug was denied for both cases. According to the pooled data from phase II and phase III studies (the entire treatment period), the incidence in the lixisenatide group was 1.8% (59 of 3262 subjects), which was comparable to that derived from the pooled data of phase III placebo-controlled studies.

PMDA considers as follows:
Although the applicant's explanation is largely acceptable, given that angioedema for which a causal relationship to lixisenatide could not be denied and serious allergic reactions have been reported, it is necessary to include a precaution statement regarding hypersensitivity reactions in the package insert and continue to collect information on hypersensitivity reactions via post-marketing surveillance.

4.(iii).B.(5).8) Cardiovascular risk
The applicant explained as follows:
According to the pooled data from phase III placebo-controlled studies (the entire treatment period), the incidences of cardiovascular (including cerebrovascular disorders) adverse events[133] were 5.1% (54 of 1061 subjects, including 6 Japanese subjects) in the placebo group and 6.8% (145 of 2127 subjects, including 12 Japanese subjects) in the lixisenatide group and there were no major differences between the treatment groups.

Concerning changes in lipid parameters, according to the pooled data from phase III placebo-controlled studies (the entire treatment period), the incidences of PCSAs for total cholesterol (≥7.74 mmol/L) were 4.4% (41 of 939 subjects) in the placebo group and 3.6% (65 of 1811 subjects) in the lixisenatide group. The incidences of PCSAs for triglycerides (≥4.6 mmol/L) were 10.0% (94 of 940 subjects) in the placebo group and 10.8% (196 of 1817 subjects) in the lixisenatide group and there were no clear differences between the treatment groups. As to changes in lipid parameters in the lixisenatide group, the pooled data from phase II and phase III studies also showed similar results as the pooled data from phase III placebo-controlled studies. No clinically meaningful mean changes were observed for total cholesterol, HDL-C, LDL-C, and triglycerides over time compared to baseline values in both treatment groups during the entire treatment period.

Concerning vital signs, according to the pooled data from phase III placebo-controlled studies (the entire treatment period), the percentages of subjects with systolic blood pressure ≤95 mmHg and a ≥20 mmHg decrease from baseline were 2.4% (25 of 1057 subjects) in the placebo group and 3.0% (64 of 2110 subjects) in the lixisenatide group. The percentages of subjects with systolic blood pressure ≥160 mmHg and a ≥20

[131] When no systemic drugs were administered or antihistamines only were administered.
[132] When catecholamines or corticosteroids were given systemically.
[133] Adverse events in the SOC of "Cardiac disorders" or the HLT of "Central nervous system haemorrhages and cerebrovascular accidents"

mmHg increase from baseline were 10.3% (109 of 1057 subjects) in the placebo group and 10.4% (219 of 2110 subjects) in the lixisenatide group. There were no clear differences between the treatment groups. Only a few subjects had PCSAs for diastolic blood pressure and heart rate (diastolic blood pressure, ≤45 mmHg and a ≥10 mmHg decrease from baseline, ≥110 mmHg and a ≥10 mmHg increase from baseline; heart rate, ≤50 bpm and a ≥20 bpm decrease from baseline, ≥120 bpm and a ≥20 bpm increase from baseline) in both treatment groups. As to vital signs in the lixisenatide group, the pooled data from phase II and phase III studies also showed similar results as the pooled data from phase III placebo-controlled studies. No clinically meaningful mean changes were observed for systolic blood pressure, diastolic blood pressure, and heart rate over time compared to baseline values in both treatment groups during the entire treatment period.

Concerning ECGs, in Study EFC6018 in which Japanese patients participated, there were no subjects with a QTcF interval[93] increase from baseline of >60 ms in the overall population and the percentages of subjects with a QTcF interval increase from baseline of 30 to 60 ms were 0.9% (1 of 113 subjects) in the placebo group and 0.9% (2 of 221 subjects) in the lixisenatide group, but a QTcF interval increase from baseline of 30 to 60 ms was not reported in the Japanese population. The percentages of subjects with PR interval ≥220 ms and a ≥20 ms increase from baseline were 1.8% (2 of 113 subjects [1 Japanese subject]) in the placebo group and 0.5% (1 of 218 subjects [Japanese subject]) in the lixisenatide group. According to the pooled data from phase III placebo-controlled studies excluding Study EFC6018 (the entire treatment period), the percentages of subjects with a shift in the interpretation of their ECG results from normal or missing at baseline to abnormal after starting the study drug were 14.7% (93 of 634 subjects) in the placebo group and 15.9% (208 of 1306 subjects) in the lixisenatide group and there were no clear differences between the treatment groups.

In phase III placebo-controlled studies in which Japanese patients participated, the least-squares mean body weight changes from baseline to Week 24 (Week 12 for Study EFC6018) were 0.06 kg to -1.98 kg in the placebo group and -0.38 kg to -1.96 kg in the lixisenatide group and there was no trend towards increased body weight following treatment with lixisenatide.

As described above, the occurrence of adverse events and the results of laboratory tests, vital signs, and ECGs posed no safety concern regarding the cardiovascular effects of lixisenatide.

PMDA considers as follows:
As no relevant changes in blood pressure or heart rate or no increases in lipid parameters etc. were observed following treatment with lixisenatide, the applicant’s explanation is largely acceptable. However, since the number of patients included in clinical studies is limited, it is necessary to continue to collect information on cardiovascular risk via post-marketing surveillance, in addition to an ongoing cardiovascular event study (EFC11319, a planned enrollment of 6000 patients including 120 Japanese patients).

4.(iii).B.(5).9) Relationship with tumor development
The applicant explained as follows:
According to the pooled data from phase III placebo-controlled studies, the incidences of events in the SOC of “Neoplasms benign, malignant and unspecified (incl cysts and polyps)” during the entire treatment period were 2.7% (29 of 1061 subjects) in the placebo group and 2.5% (54 of 2127 subjects) in the lixisenatide

group and the rates were 2.9 cases per 100 patient-years in the placebo group and 2.6 cases per 100 patient-years in the lixisenatide group. There were no major differences between the treatment groups. In the Japanese population, events in the SOC of “Neoplasms benign, malignant and unspecified (incl cysts and polyps)” occurred in 3 subjects in the placebo group (rectal cancer, renal cell carcinoma, colon adenoma) and 4 subjects in the lixisenatide group (gastric cancer, glottis carcinoma, uterine leiomyoma, lipoma). The most commonly reported events were adverse events of thyroid neoplasm (thyroid neoplasm and benign neoplasm of thyroid gland) and thyroid cancer was reported by 1 subject in the placebo group, but not in the lixisenatide group. Pancreatic carcinoma was reported by 3 subjects in the lixisenatide group. A causal relationship to study drug was not denied for 1 of the 3 events. The pooled data from phase II and phase III studies showed a similar incidence of events in the SOC of “Neoplasms benign, malignant and unspecified (incl cysts and polyps)” in the lixisenatide group (2.0%; 67 of 3304 subjects, including 4 Japanese subjects).

According to active-controlled phase III studies (EFC6019 and EFC10780), the incidences of events in the SOC of “Neoplasms benign, malignant and unspecified (incl cysts and polyps)” were 3.1% (10 of 318 subjects) in the lixisenatide group and 1.6% (5 of 316 subjects) in the exenatide group in Study EFC6019. Malignant events were reported by 1 of the 10 subjects in the lixisenatide group (metastatic neoplasm) and 4 of the 5 subjects in the exenatide group (prostate cancer, basal cell carcinoma, thyroid cancer, pancreatic carcinoma). In Study EFC10780, no events in the SOC of “Neoplasms benign, malignant and unspecified (incl cysts and polyps)” were reported.

According to the pooled data from phase III placebo-controlled studies, the incidences of adverse events potentially related to thyroid C-cell proliferation during the entire treatment period and the post-treatment follow-up period were 0.8% in the placebo group (9 of 1061 subjects; goitre in 5 subjects, thyroid neoplasm in 2 subjects, benign neoplasm of thyroid gland in 1 subject, thyroid cancer in 1 subject) and 0.9% in the lixisenatide group (19 of 2127 subjects; thyroid neoplasm in 10 subjects, goitre in 6 subjects, benign neoplasm of thyroid gland in 2 subjects, thyroid C-cell hyperplasia in 1 subject) and there were no differences between the treatment groups. The pooled data from phase II and phase III studies showed a similar incidence of adverse events potentially related to thyroid C-cell proliferation in the lixisenatide group (0.7%; 23 of 3304 subjects) (thyroid neoplasm in 11 subjects, goitre in 7 subjects, benign neoplasm of thyroid gland in 3 subjects, thyroid C-cell hyperplasia in 1 subject, thyroid cyst in 1 subject) and no adverse events potentially related to thyroid C-cell proliferation were reported in the Japanese population.

Concerning laboratory values (calcitonin levels), according to the pooled data from phase III placebo-controlled studies (the entire treatment period), calcitonin values ≥20 ng/L and <50 ng/L were observed in 1.8% of the placebo group (16 of 867 subjects), which was similar to 1.5% of the lixisenatide group (26 of 1682 subjects). In 7 subjects with calcitonin values ≥20 ng/L (1 subject in the placebo group and 6 subjects in the lixisenatide group, all non-Japanese subjects), blood calcitonin increased (non-serious) led to treatment discontinuation. One subject in the lixisenatide group already had a high calcitonin level of 24 ng/L on Treatment Day 2 and was diagnosed with thyroid C-cell hyperplasia (serious) and discontinued from study treatment on Treatment Day 64. Calcitonin values ≥50 ng/L were observed in 0.1% of the placebo group (1 of 867 subjects), which was similar to 0.5% of the lixisenatide group (8 of 1682 subjects), but the baseline values were unknown for most subjects. The subject with a calcitonin value of ≥50 ng/L in the placebo group was diagnosed with thyroid cancer (medullary thyroid cancer, serious), leading to treatment

discontinuation. In the Japanese population, there were no subjects with elevated calcitonin ≥20 ng/L. According to the pooled data from phase II and phase III studies (the entire treatment period), calcitonin values ≥20 ng/L and <50 ng/L were observed in 1.5% of the lixisenatide group (32 of 2166 subjects) and calcitonin values ≥50 ng/L were observed in 0.4% of the lixisenatide group (8 of 2166 subjects). In the Japanese population, calcitonin values ≥20 ng/L were not observed.

According to active-controlled phase III studies (EFC6019 and EFC10780), blood calcitonin increased (calcitonin values ≥20 ng/L and <50 ng/L) were observed in 4 subjects each in the lixisenatide and exenatide groups in Study EFC6019. One subject in the exenatide group was diagnosed with thyroid cancer on Treatment Day 500 with no increase in calcitonin. In Study EFC10780, calcitonin increased was not observed in the lixisenatide group and was observed in 1 subject in the sitagliptin group, but the event was non-serious and did not lead to treatment discontinuation.

As described above, the occurrence of adverse events of neoplasms, events potentially related to thyroid C-cell proliferation, and laboratory abnormalities suggested no increased risk of tumors including thyroid tumors associated with lixisenatide.

PMDA considers as follows:
Although there has been no apparent increased risk of tumor development associated with lixisenatide at present, it is necessary to include a precaution statement regarding thyroid effects in the package insert and continue to collect information on the relationship with tumor development (especially, thyroid malignant tumors and pancreatic malignant tumors, etc.) via post-marketing surveillance.

4.(iii).B.(5).10) Long-term safety of lixisenatide in combination with basal insulin
PMDA asked the applicant to explain the long-term safety of lixisenatide in combination with basal insulin in Japanese patients.

The applicant responded as follows:
Because the long-term safety of lixisenatide in combination with basal insulin had been evaluated in Foreign Study EFC6016,[134] the safety profiles over 24 weeks were compared between the Japanese population in Study EFC10887 (24-week treatment) and the overall population (non-Japanese subjects) in Foreign Study EFC6016 to determine whether the results from Study EFC6016 can be generalized to the Japanese population. Regarding the demographic characteristics of subjects, although there were differences in the gender distribution and body weight between the two populations as in the Japanese population and the overall population in Study EFC6015, there were no major differences in the incidence of adverse events between the subgroups in Study EFC6015 [see "4.(iii).B.(3).2).(b) Safety in Japanese population and overall population"]. Therefore, differences between the Japanese population in Study EFC10887 and the overall population in Study EFC6016 were also not considered to significantly influence the safety evaluation of lixisenatide. The basal insulin dose was higher in the overall population in Study EFC6016. The incidences of symptomatic hypoglycaemia in subjects treated with lixisenatide as add-on to basal insulin alone (the treatment common to both studies) were examined by basal insulin dose. As a result, in the Japanese

[134] A double-blind, comparative, 24-week study of lixisenatide or placebo as add-on to basal insulin (with or without metformin) in non-Japanese patients with type 2 diabetes mellitus not adequately controlled with basal insulin (with or without metformin), followed by an extension (≥76 weeks).

population in Study EFC10887, the incidences were 16.7% (3 of 18 subjects) in the placebo group and 27.3% (3 of 11 subjects) in the lixisenatide group when using lower-dose basal insulin (≤15 U/day) and 29.4% (5 of 17 subjects) in the placebo group and 35.0% (7 of 20 subjects) in the lixisenatide group when using higher-dose basal insulin (>15 U/day). In the overall population in Study EFC6016, the incidences were 21.1% (4 of 19 subjects) in the placebo group and 26.5% (9 of 34 subjects) in the lixisenatide group when using lower-dose basal insulin (≤40 U/day) and 23.5% (4 of 17 subjects) in the placebo group and 36.4% (12 of 33 subjects) in the lixisenatide group when using higher-dose basal insulin (>40 U/day). In both populations, the incidence of symptomatic hypoglycaemia tended to be higher in subjects treated with lixisenatide in combination with higher-dose basal insulin.

Concerning the occurrence of adverse events, the main adverse events reported in the lixisenatide group were hypoglycaemia and events in the SOC of "Gastrointestinal disorders" in both populations. The incidence of events in the SOC of "Gastrointestinal disorders" was 61.1% (44 of 72 subjects) in the Japanese population in Study EFC10887, which was higher than 40.2% (132 of 328 subjects) in the overall population in Study EFC6016, but the difference in incidence was due mainly to events occurring within 12 weeks of treatment, and severe events and events leading to treatment discontinuation were uncommon in both populations. The incidences of symptomatic hypoglycaemia among subjects treated with lixisenatide as add-on to basal insulin alone were 22.9% (8 of 35 subjects) in the placebo group and 32.3% (10 of 31 subjects) in the lixisenatide group in the Japanese population in Study EFC10887 and 22.2% (8 of 36 subjects) in the placebo group and 31.3% (21 of 67 subjects) in the lixisenatide group in the overall population in Study EFC6016. There were no clear differences between the populations.

The incidences of adverse events by treatment period in Study EFC6016 were 68.3% (114 of 167 subjects) in the placebo group and 73.5% (241 of 328 subjects) in the lixisenatide group during the main treatment period (24 weeks) and 85.6% (143 of 167 subjects) in the placebo group and 87.5% (287 of 328 subjects) in the lixisenatide group during the entire treatment period (≥76 weeks). The incidences of symptomatic hypoglycaemia among subjects treated with lixisenatide as add-on to basal insulin alone were 22.2% (8 of 36 subjects) in the placebo group and 31.3% (21 of 67 subjects) in the lixisenatide group during the main treatment period and 33.3% (12 of 36 subjects) in the placebo group and 43.3% (29 of 67 subjects) in the lixisenatide group during the entire treatment period. No major differences between the main treatment period and the entire treatment period were observed for the treatment differences. There were no adverse events with a clearly higher incidence with long-term treatment with lixisenatide compared to placebo.

As described above, since the safety profiles over 24 weeks were similar between the Japanese population in Study EFC10887 and the overall population in Study EFC6016, the generalization of the results from Foreign Study EFC6016 to Japanese patients for the evaluation of the long-term safety of lixisenatide in combination with basal insulin is possible. As there were no adverse events with a clearly higher incidence with long-term treatment with lixisenatide compared to placebo in Study EFC6016, the applicant considered that its long-term safety has been demonstrated also in Japanese patients.

PMDA considers as follows:
There is no major safety concern as long as appropriate caution regarding the long-term use of lixisenatide in combination with basal insulin is provided and lixisenatide in combination with basal insulin is administered

carefully. However, as the long-term safety of lixisenatide in combination with basal insulin in Japanese patients has not been studied, it is necessary to collect information via post-marketing surveillance.

4.(iii).B.(6) Indication

PMDA considers as follows:

Since the efficacy of lixisenatide in combination with SU (with and without metformin) and lixisenatide in combination with basal insulin (with and without SU) has been demonstrated [see "4.(iii).B.(4) Efficacy"] and the safety is acceptable [see "4.(iii).B.(5) Safety"], there is no major problem with the indication of "Type 2 diabetes mellitus: Lyxumia should be used only when either of the following therapies does not provide adequate glycemic control:

(a) diet and exercise plus sulfonylureas (with or without biguanides) or

(b) diet and exercise plus soluble prolonged-acting or intermediate-acting insulin (with or without sulfonylureas)."

As the efficacy and safety of lixisenatide in combination with basal insulin in insulin-dependent patients with type 2 diabetes mellitus have not been studied, it is necessary to advise physicians to check whether their patient is insulin-dependent and to carefully determine whether or not lixisenatide should be used in combination with basal insulin. A final conclusion will be made, taking account of comments from the Expert Discussion.

4.(iii).B.(7) Dosage and administration

PMDA asked the applicant to provide the rationale for selecting a two-step titration regimen in Japan, instead of a one-step titration regimen employed overseas.

The applicant responded as follows:

Phase III studies, EFC6018 and LTS10888, evaluated the efficacy and safety of lixisenatide with 2 titration regimens. In the overall population in Study EFC6018, the changes in HbA1c from baseline to Week 12 (mean ± SD) were -0.27 ± 1.09% in the combined placebo group (n = 112), -0.94 ± 0.72% in the lixisenatide one-step titration group (n = 114), and -0.77 ± 0.94% in the lixisenatide two-step titration group (n = 113). In Study LTS10888, the changes in HbA1c from baseline to Week 24 (mean ± SD) were -0.74 ± 0.79% in the lixisenatide one-step titration group (n = 33) and -0.99 ± 1.07% in the lixisenatide two-step titration group (n = 23). No major differences were observed in both studies. Regarding safety, the incidences of commonly reported SOC of gastrointestinal disorders and nausea were examined for both studies. In Study EFC6018, the incidences of gastrointestinal disorders were 75.0% (12 of 16 subjects) in the one-step titration group and 41.2% (7 of 17 subjects) in the two-step titration group and the incidences of nausea were 56.3% (9 of 16 subjects) in the one-step titration group and 35.3% (6 of 17 subjects) in the two-step titration group in the Japanese population. In the non-Japanese population, the incidences of gastrointestinal disorders were 25.2% (26 of 103 subjects) in the one-step titration group and 31.1% (32 of 103 subjects) in the two-step titration group and the incidences of nausea were 14.6% (15 of 103 subjects) in the one-step titration group and 22.3% (23 of 103 subjects) in the two-step titration group. The incidences of both adverse events were higher in the Japanese population than in the non-Japanese population and the incidences were higher in the one-step titration group than in the two-step titration group in the Japanese population. In Study LTS10888, the incidences of gastrointestinal disorders were 63.6% (21 of 33 subjects) in the two-step titration group and 77.8% (28 of 36 subjects) in the one-step titration group and the incidences of nausea were 36.4% (12 of 33

subjects) in the two-step titration group and 50.0% (18 of 36 subjects) in the one-step titration group. The incidences were higher in the one-step titration group than in the two-step titration group. The percentages of subjects who had achieved the target maintenance dose of 20 μg at the end of treatment in Study EFC6018 were 100% (17 of 17 subjects) in the two-step titration group and 75.0% (12 of 16 subjects) in the one-step titration group in the Japanese population and 89.3% (92 of 103 subjects) in the two-step titration group and 90.3% (93 of 103 subjects) in the one-step titration group in the non-Japanese population. In Study LTS10888, the percentages of subjects who had achieved the target maintenance dose of 20 μg at the end of treatment were 81.8% (27 of 33 subjects) in the two-step titration group and 72.2% (26 of 36 subjects) in the one-step titration group. In both the Japanese population in Study EFC6018 and Study LTS10888 (Japanese subjects only), the percentage was higher in the two-step titration group than in the one-step titration group.

As described above, gastrointestinal disorders such as nausea are more likely to occur in Japanese patients than in non-Japanese patients. However, a two-step titration regimen is expected to reduce gastrointestinal disorders in Japanese patients and allows for optimum therapeutic effects of lixisenatide by increasing the likelihood that the recommended dose of 20 μg will be reached and maintained. Therefore, a two-step titration regimen has been selected for Japanese patients.

PMDA asked the applicant to provide the basis for determining that the same starting and maintenance doses as overseas can be selected also in Japan.

The applicant responded as follows:
A dose response study (Foreign Study DRI6012) showed that the least-squares mean difference vs. placebo in HbA1c change as the primary efficacy endpoint was dose-dependent. However, the treatment differences from placebo for 30 μg QD, 10 μg BID, and 20 μg BID (-0.57% to -0.59%) were not large relative to that for 20 μg QD (-0.50%) and the incidence of adverse events increased dose-dependently. Hence, it was concluded that there is little additional benefit of doses >20 μg/day, and 20 μg/day was recommended. Furthermore, when 20 μg QD was compared to 10 μg BID, there were no differences in efficacy. Regarding safety, the incidence of events in the SOC of gastrointestinal disorders was 41.8% in the 20 μg QD group, which was higher than 26.8% in the 10 μg BID group, but the incidence of gastrointestinal disorders leading to treatment discontinuation was as low as 1.8% in both groups, suggesting that there were no clinical differences. Also, once-daily dosing (QD) was considered more convenient than twice-daily dosing (BID). For these reasons, 20 μg QD was selected. The results of Foreign Studies 01-016 and ACT6011 assessing different dosage and dose regimens also supported the starting dose of 10 μg QD and the maintenance dose of 20 μg QD.

The dosage and dose regimen for Japanese patients were selected as follows: Based on the results of a multinational phase II study (PDY6797), from a pharmacokinetic point of view, differences in exposure between the Japanese and non-Japanese populations do not imply in the recommendation of different doses in Japanese patients [see “4.(ii).B.(1) Differences in pharmacokinetics and pharmacodynamics between Japanese and non-Japanese populations”] and also from a pharmacodynamic point of view, the applicant concluded that doses exceeding 20 μg/day provide only a small additional benefit in both populations. Moreover, increased efficacy was not observed with BID compared to QD. Also regarding safety, there were no differences in the incidence of adverse events between the Japanese and non-Japanese populations.

Although the overall incidence of nausea with the QD regimen was higher in the Japanese population than in the non-Japanese population, the incidence with 20 μg multiple dosing was similar between the Japanese and non-Japanese populations (Japanese population, 20 μg QD, 25.0% [5 of 20 subjects]; 20 μg BID, 9.1% [2 of 22 subjects]; non-Japanese population, 20 μg QD, 21.1% [4 of 19 subjects]; 20 μg BID, 15.8% [3 of 19 subjects]). The above results support 20 μg QD also for Japanese patients. Furthermore, phase III multinational studies in which Japanese patients participated (EFC6015 and EFC10887) showed similar efficacy of lixisenatide 20 μg QD in the Japanese and overall populations as well as a good safety and tolerability profile. Therefore, the applicant concluded that 10 μg QD as the starting dose and 20 μg QD as the maintenance dose for Japanese patients have been justified.

As to the timing of dosing, Foreign Study EFC6014[135] evaluated not only once daily morning injections but also once daily evening injections. The least-squares mean changes in HbA1c from baseline to endpoint (Week 24) were -0.87% in the morning lixisenatide group and -0.75% in the evening lixisenatide group and clinically meaningful reductions in HbA1c were observed in both groups. Also regarding safety, the incidences of adverse events were 84.7% (216 of 255 subjects) in the morning lixisenatide group and 83.5% (213 of 255 subjects) in the evening lixisenatide group and the incidences of symptomatic hypoglycaemia were 7.1% (18 of 255 subjects) in the morning lixisenatide group and 8.6% (22 of 255 subjects) in the evening lixisenatide group and no meaningful differences between the groups were observed.

PMDA considers as follows:
Concerning the dosage and dose regimen, there is no major problem with selecting the same starting and maintenance doses of lixisenatide as overseas (10 μg QD as the starting dose and 20 μg QD as the maintenance dose) in Japan, based on clinical study results. On the other hand, as to the titration regimen, there is no major problem with selecting a two-step titration regimen, which is different from the titration regimen employed overseas, because this regimen tended to reduce gastrointestinal disorders, etc. As to the timing of dosing, evening injections of lixisenatide (injected prior to evening meal) have not been assessed in clinical studies in which Japanese patients participated. In addition, patients included in Foreign Study EFC6014 that evaluated once-daily morning injections and once-daily evening injections (patients not adequately controlled with metformin) were different from the intended patient population for lixisenatide. If lixisenatide is used in combination with SU or basal insulin, it should be necessary to compare once-daily morning injections and once-daily evening injections, especially for the occurrence of hypoglycaemia. Given that such comparison has not been studied, it is appropriate that “as the usual dosage regimen, lixisenatide should be injected prior to breakfast.” A final conclusion will be made, taking account of comments from the Expert Discussion.

4.(iii).B.(8) Special populations

4.(iii).B.(8).1) Patients with renal impairment

The applicant explained as follows:
According to the pooled data from phase III placebo-controlled studies (the entire treatment period), when the incidences of overall adverse events were examined by renal function at baseline (Table 48), the difference between the placebo and lixisenatide groups was slightly greater in subjects with mild renal

[135] A double-blind, comparative, 24-week study of lixisenatide or placebo as add-on to metformin in non-Japanese patients with type 2 diabetes mellitus not adequately controlled with metformin, followed by an extension.

impairment than in subjects with normal renal function. This was due mainly to differences in the incidences of events in the SOCs of "gastrointestinal disorders" and "metabolism and nutrition disorders." Although the incidence of overall adverse events was higher in subjects with moderate renal impairment compared to subjects with normal renal function and subjects with mild renal impairment, the interpretation of results has a limitation due to the small number of subjects with moderate renal impairment. Only 3 subjects in the placebo group had severe renal impairment and analysis was impossible. The incidence of serious adverse events was similar between the placebo and lixisenatide groups across all subgroups. For the incidence of adverse events leading to treatment discontinuation, the between-treatment difference was slightly greater in subjects with mild renal impairment compared to subjects with normal renal function and subjects with moderate renal impairment.

Table 48. Incidences of adverse events and hypoglycaemia by renal function[a)] at baseline
(Pooled data from phase III placebo-controlled studies, [Entire treatment period], Safety population)

	Normal renal function (n = 2570)		Mild renal impairment (n = 565)		Moderate renal impairment (n = 48)	
	Placebo (n = 839)	Lixisenatide (n = 1731)	Placebo (n = 199)	Lixisenatide (n = 366)	Placebo (n = 20)	Lixisenatide (n = 28)
Any adverse event	74.6 (626)	80.4 (1391)	73.4 (146)	84.7 (310)	75.0 (15)	89.3 (25)
Any serious adverse event	8.6 (72)	8.9 (154)	11.1 (22)	11.7 (43)	10.0 (2)	7.1 (2)
Any adverse event leading to treatment discontinuation	4.9 (41)	8.1 (141)	5.0 (10)	16.4 (60)	15.0 (3)	17.9 (5)
SOC "Gastrointestinal disorders"	26.7 (224)	47.1 (816)	22.6 (45)	53.6 (196)	25.0 (5)	60.7 (17)
SOC "Metabolism and nutrition disorders"	21.7 (182)	24.3 (420)	25.6 (51)	37.2 (136)	45.0 (9)	25.0 (7)
Hypoglycaemia	15.1 (127)	18.0 (311)	21.6 (43)	28.1 (103)	45.0 (9)	21.4 (6)

Incidence % (n)

a) The degree of renal impairment was classified according to creatinine clearance (CL_{CR}) calculated using the Cockcroft-Gault formula as follows: normal (CL_{CR} >80 mL/min), mild (CL_{CR} ≥50 and ≤80 mL/min), moderate (CL_{CR} ≥30 and <50 mL/min), and severe (CL_{CR} <30 mL/min).

In Study EFC6015 of lixisenatide in combination with SU with or without metformin and Study EFC10887 of lixisenatide in combination with basal insulin with or without SU, the incidence of symptomatic hypoglycaemia and the number of symptomatic hypoglycaemic events per 100 patient-years (Table 49) tended to be higher in subjects with mild renal impairment than in subjects with normal renal function across all treatment groups, but there was no relationship between the difference in incidence between the placebo and lixisenatide groups and renal function. The number of subjects with moderate renal impairment was small and no consistent trend was observed.

Table 49. Occurrence of symptomatic hypoglycaemia by renal function at baseline
(EFC6015 and EFC10887 [Entire treatment period], Safety population)

EFC6015	Normal renal function		Mild renal impairment		Moderate renal impairment	
Add on to SU alone	Placebo (n = 25)	Lixisenatide (n = 62)	Placebo (n = 18)	Lixisenatide (n = 26)	Placebo (n = 3)	Lixisenatide (n = 0)
	4.0 (1)	19.4 (12)	22.2 (4)	30.8 (8)	66.7 (2)	—
	15 [39.6]	31 [34.5]	5 [18.4]	46 [129.9]	2 [71.5]	—
Add on to SU and metformin	Placebo (n = 192)	Lixisenatide (n = 389)	Placebo (n = 45)	Lixisenatide (n = 94)	Placebo (n = 2)	Lixisenatide (n = 3)
	16.7 (32)	21.3 (83)	26.7 (12)	24.5 (23)	0.0 (0)	33.3 (1)
	160 [58.8]	199 [34.9]	48 [71.1]	112 [85.3]	0 [0.0]	1 [29.0]
EFC10887	Normal renal function		Mild renal impairment		Moderate renal impairment	
Add on to basal insulin alone	Placebo (n = 25)	Lixisenatide (n = 19)	Placebo (n = 13)	Lixisenatide (n = 24)	Placebo (n = 7)	Lixisenatide (n = 3)
	24.0 (6)	26.3 (5)	23.1 (3)	41.7 (10)	57.1 (4)	0.0 (0)
	9 [78.8]	10 [117.4]	10 [162.7]	19 [196.3]	9 [346.8]	0 [0.0]
Add on to basal insulin and SU	Placebo (n = 65)	Lixisenatide (n = 65)	Placebo (n = 41)	Lixisenatide (n = 38)	Placebo (n = 5)	Lixisenatide (n = 5)
	9.2 (6)	41.5 (27)	39.0 (16)	55.3 (21)	40.0 (2)	60.0 (3)
	16 [54.2]	92 [316.9]	50 [275.3]	60 [380.3]	8 [337.8]	13 [694.2]

Upper row: incidence % (n), Lower row: total number of events [Number of events/100 patient-years], —: Not applicable

a) The degree of renal impairment was classified according to creatinine clearance (CL_{CR}) calculated using the Cockcroft-Gault formula as follows:

normal (CL_{CR} >80 mL/min), mild (CL_{CR} ≥50 and ≤80 mL/min), moderate (CL_{CR} ≥30 and <50 mL/min), and severe (CL_{CR} <30 mL/min).

As there is no clinical experience with lixisenatide in patients with severe renal impairment or end-stage renal disease, careful administration of lixisenatide in these patients will be recommended in the package insert and post-marketing safety information will be collected via spontaneous reporting and literature search.

PMDA considers as follows:
In Japanese and foreign clinical studies, the incidence of hypoglycaemia was higher in subjects with mild renal impairment compared to subjects with normal renal function. The limited number of subjects with moderate or worse renal impairment were studied both in Japan and overseas, and an increase in exposure was suggested especially in patients with severe renal impairment or end-stage renal disease, but no clinical studies in these patients have been conducted. Therefore, it is necessary to continue to collect information on safety in patients with renal impairment via post-marketing surveillance.

4.(iii).B.(8).2) Patients with hepatic impairment

The applicant explained as follows:
According to the pooled data from phase III placebo-controlled studies (the entire treatment period), when the incidences of overall adverse events, serious adverse events, and adverse events leading to treatment discontinuation etc. were examined by the presence or absence of co-morbid conditions related to hepatic disorders[136]at baseline, there were no major differences between the subgroups of either treatment group.

Table 50. Incidences of adverse events by presence or absence of co-morbid conditions related to hepatic disorders at baseline
(Pooled data from phase III placebo-controlled studies, [Entire treatment period], Safety population)

	Co-morbid conditions		No co-morbid conditions	
	Placebo (n = 101)	Lixisenatide (n = 195)	Placebo (n = 960)	Lixisenatide (n = 1932)
Any adverse event	69.3 (70)	78.5 (153)	75.0 (720)	81.5 (1575)
Any serious adverse event	8.9 (9)	8.2 (16)	9.4 (90)	9.5 (183)
Any adverse event leading to treatment discontinuation	6.9 (7)	9.7 (19)	5.0 (48)	9.7 (187)
SOC “Gastrointestinal disorders”	23.8 (24)	49.7 (97)	26.0 (250)	48.2 (932)
SOC “Metabolism and nutrition disorders”	9.9 (10)	31.8 (62)	24.3 (233)	26.0 (502)
Hypoglycaemia	7.9 (8)	22.6 (44)	17.9 (172)	19.5 (376)

Incidence % (n)

As to symptomatic hypoglycaemia (Table 51), in Study EFC6015, though the number of subjects with co-morbid conditions among those receiving add-on therapy with SU alone was small, the difference in incidence between the placebo and lixisenatide groups was greater in subjects with co-morbid conditions related to hepatic disorders than in subjects without co-morbid conditions, either when lixisenatide or placebo was used as add-on to SU alone or as add-on to SU and metformin. In Study EFC10887, either when lixisenatide or placebo was used as add-on to basal insulin or as add-on to basal insulin and SU, the incidence of symptomatic hypoglycaemia was lower and the number of symptomatic hypoglycaemic events per 100 patient-years tended to be lower in subjects with co-morbid conditions related to hepatic disorders in the placebo group, while there were no clear differences according to the presence or absence of co-morbid conditions in the lixisenatide group. Severe symptomatic hypoglycaemia occurred only in 1 subject with

[136] Definition of co-morbid conditions related to hepatic disorders: Sub-SMQs “Cholestasis and jaundice of hepatic origin,” “Congenital, familial, neonatal and genetic disorders of the liver,” “Hepatic disorders specifically reported as alcohol-related,” “Hepatic failure, fibrosis and cirrhosis and other liver damage-related conditions,” “Non-infectious hepatitis,” “Liver infections,” “Liver neoplasms, benign (incl cysts and polyps),” “Liver neoplasms, malignant and unspecified,” “Liver related investigations, signs and symptoms,” “Liver-related coagulation and bleeding disturbances,” and “Pregnancy-related hepatic disorders”

co-morbid conditions (a Japanese subject) in the placebo group and 2 subjects without co-morbid conditions (non-Japanese subjects) in the lixisenatide group in the add-on to SU and metformin setting in Study EFC6015.

Table 51. Occurrence of symptomatic hypoglycaemia by presence or absence of co-morbid conditions related to hepatic disorders at baseline (EFC6015 and EFC10887 [Entire treatment period], Safety population)

EFC6015	Co-morbid conditions		No co-morbid conditions	
Add on to SU alone	Placebo (n = 5)	Lixisenatide (n = 11)	Placebo (n = 41)	Lixisenatide (n = 77)
	0.0 (0)	45.5 (5)	17.1 (7)	19.5 (15)
	0 [0.0]	38 [243.4]	22 [37.2]	39 [35.6]
Add on to SU and metformin	Placebo (n = 35)	Lixisenatide (n = 62)	Placebo (n = 204)	Lixisenatide (n = 424)
	14.3 (5)	27.4 (17)	19.1 (39)	21.2 (90)
	9 [16.3]	36 [38.2]	199 [69.2]	276 [45.2]
EFC10887	Co-morbid conditions		No co-morbid conditions	
Add on to basal insulin alone	Placebo (n = 8)	Lixisenatide (n = 6)	Placebo (n = 38)	Lixisenatide (n = 40)
	25.0 (2)	33.3 (2)	28.9 (11)	32.5 (13)
	2 [53.3]	4 [168.3]	26 [154.0]	25 [145.1]
Add on to basal insulin and SU	Placebo (n = 20)	Lixisenatide (n = 22)	Placebo (n = 91)	Lixisenatide (n = 86)
	5.0 (1)	40.9 (9)	25.3 (23)	48.8 (42)
	5 [55.1]	29 [307.3]	69 [168.3]	136 [365.2]

Upper row: incidence % (n), Lower row: total number of events [Number of events/100 patient-years]

PMDA considers as follows:

Although there is no particular problem with the applicant's explanation, it is necessary to continue to collect information on safety in patients with hepatic impairment via post-marketing surveillance, as the limited number of subjects with hepatic impairment were studied.

4.(iii).B.(8).3) Elderly

The applicant explained as follows:

According to the pooled data from phase III placebo-controlled studies (the entire treatment period), when the incidences of adverse events were examined by age at baseline (<65 years, ≥65 years), generally, the difference between the placebo and lixisenatide groups was slightly greater in subjects aged ≥65 years compared to those aged <65 years. The incidences of events in the SOC of "Metabolism and nutrition disorders," serious adverse events, and adverse events leading to treatment discontinuation were higher in subjects aged ≥65 years compared to those aged <65 years in both treatment groups (Table 52).

Table 52. Incidences of adverse events by age at baseline
(Pooled data from phase III placebo-controlled studies (Entire treatment period), Safety population)

	<65 years (n = 2565)		≥65 years (n = 623)	
	Placebo (n = 817)	Lixisenatide (n = 1748)	Placebo (n = 244)	Lixisenatide (n = 379)
Any adverse event	75.2 (614)	80.7 (1410)	72.1 (176)	83.9 (318)
Any serious adverse event	7.7 (63)	8.1 (142)	14.8 (36)	15.0 (57)
Any adverse event leading to treatment discontinuation	4.3 (35)	8.3 (145)	8.2 (20)	16.1 (61)
SOC "Gastrointestinal disorders"	26.6 (217)	48.1 (840)	23.4 (57)	49.9 (189)
SOC "Metabolism and nutrition disorders"	22.4 (183)	25.5 (446)	24.6 (60)	31.1 (118)
Hypoglycaemia	16.5 (135)	18.4 (321)	18.4 (45)	26.1 (99)

Incidence % (n)

As to symptomatic hypoglycaemia (Table 53), in Study EFC6015, either when lixisenatide was used as add-on to SU alone or as add-on to SU and metformin, the incidence of symptomatic hypoglycaemia in the lixisenatide group was similar between subjects aged <65 and ≥65 years, while the number of symptomatic hypoglycemic events per 100 patient-years tended to be higher in subjects aged ≥65 years compared to those aged <65 years. In Study EFC10887, in the add-on to basal insulin and SU setting, the incidence of symptomatic hypoglycaemia and the number of symptomatic hypoglycemic events per 100 patient-years

tended to be higher in subjects aged ≥65 years in both the lixisenatide and placebo groups. In the add-on to basal insulin alone setting, the incidence of symptomatic hypoglycaemia in the lixisenatide group tended to be higher in subjects aged ≥65 years, but the number of events per 100 patient-years was similar between the categories. However, the number of subjects aged ≥65 years receiving add-on therapy with basal insulin alone was small and the interpretation of results was difficult. Severe symptomatic hypoglycaemia occurred in 1 subject aged ≥65 years (a Japanese subject) in the placebo group and 2 subjects aged <65 years (non-Japanese subjects) in the lixisenatide group in the add-on to SU and metformin setting in Study EFC6015, whereas no severe symptomatic hypoglycaemia occurred in Study EFC10887.

Table 53. Occurrence of symptomatic hypoglycaemia by age at baseline (EFC6015 and EFC10887 [Entire treatment period], Safety population)

EFC6015	<65 years		≥65 years	
Add on to SU alone	Placebo (n = 33)	Lixisenatide (n = 61)	Placebo (n = 13)	Lixisenatide (n = 27)
	9.1 (3)	23.0 (14)	30.8 (4)	22.2 (6)
	17 [38.1]	41 [48.7]	5 [21.5]	36 [87.7]
Add on to SU and metformin	Placebo (n = 178)	Lixisenatide (n = 382)	Placebo (n = 61)	Lixisenatide (n = 104)
	17.4 (31)	21.5 (82)	21.3 (13)	24.0 (25)
	164 [64.0]	209 [37.1]	44 [50.9]	103 [73.3]
EFC10887	<65 years		≥65 years	
Add on to basal insulin alone	Placebo (n = 30)	Lixisenatide (n = 34)	Placebo (n = 16)	Lixisenatide (n = 12)
	30.0 (9)	29.4 (10)	25.0 (4)	41.7 (5)
	18 [134.1]	21 [146.8]	10 [138.7]	8 [150.8]
Add on to basal insulin and SU	Placebo (n = 83)	Lixisenatide (n = 76)	Placebo (n = 28)	Lixisenatide (n = 32)
	18.1 (15)	44.7 (34)	32.1 (9)	53.1 (17)
	40 [104.2]	107 [314.5]	34 [291.2]	58 [458.3]

Upper row: incidence % (n), Lower row: total number of events [Number of events/100 patient-years]

As elderly patients are generally likely to develop hypoglycaemia, careful administration will be recommended in the package insert.

PMDA considers as follows:
In elderly patients, the incidence of serious adverse events or adverse events leading to treatment discontinuation was higher and the number of hypoglycaemic events per 100 patient-years tended to be higher especially with add-on therapy with SU, and the limited number of elderly patients were studied. Therefore, it is necessary to continue to collect information on safety in elderly patients via post-marketing surveillance.

4.(iii).B.(9) Post-marketing surveillance plan

The applicant explained as follows:
A specified drug use-results survey (the planned number of enrolled patients of 3000, a 3-year observation period) is planned to be conducted in order to collect information on the long-term safety and efficacy of lixisenatide in routine clinical settings. The data from ≥800 patients treated with lixisenatide in combination with SU with or without metformin for ≥1 year and ≥800 patients treated with lixisenatide in combination with basal insulin for ≥1 year will be collected. Among the risks listed in a draft risk management plan for lixisenatide, the following information will be collected via the survey: gastrointestinal disorders (nausea and vomiting) and hypoglycaemia as the important identified risks; cardiovascular events, acute pancreatitis, medullary thyroid carcinoma, and malignant neoplasms as the important potential risks; and clinical experience with lixisenatide in elderly patients (≥75 years) as the important missing information.

PMDA considers as follows:
It is necessary to collect the following information via post-marketing surveillance: hypoglycaemia,

gastrointestinal disorders, pancreatitis, injection site reactions, the influence of antibody formation on safety and efficacy, effects on renal function, hypersensitivity reactions, cardiovascular risk, tumor development, safety in patients with renal or hepatic impairment and elderly patients (limited numbers of these patients were included in clinical studies), and the safety and efficacy of lixisenatide in combination with SU and high-dose metformin. As the long-term safety of lixisenatide in combination with basal insulin has not been evaluated in clinical studies in which Japanese patients participated, this information also needs to be collected. The details of post-marketing surveillance will be finalized, taking account of comments from the Expert Discussion.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based inspection and data integrity assessment were conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, there were no particular problems. Thus, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted product application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection took place in accordance with the provisions of the Pharmaceutical Affairs Act for the documents submitted in the new drug application (5.3.5.1-1, 5.3.5.1-3, 5.3.5.1-4, 5.3.5.1-5, 5.3.5.2-1). As a result, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

IV. Overall Evaluation

Based on the submitted data, the efficacy of lixisenatide has been demonstrated and its safety is considered acceptable in view of its observed benefits. It is necessary to investigate the occurrence of hypoglycaemia, gastrointestinal disorders, pancreatitis, and injection site reactions, the influence of antibody formation on safety and efficacy, effects on renal function, hypersensitivity reactions, cardiovascular risk, tumor development, safety in patients with renal or hepatic impairment and elderly patients (limited numbers of these patients were included in clinical studies), the safety and efficacy of lixisenatide in combination with a sulfonylurea and high-dose metformin, and the long-term safety of lixisenatide in combination with basal insulin via post-marketing surveillance.

Lixisenatide may be approved if it can be concluded based on comments from the Expert Discussion that there are no particular problems.

Review Report (2)

May 1, 2013

I. Product Submitted for Registration

[Brand name] Lyxumia Subcutaneous Injection 300 μg
[Non-proprietary name] Lixisenatide
[Name of applicant] Sanofi K.K.
[Date of application] June 11, 2012

II. Content of the Review

The outline of the comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) is described in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

(1) Interpretation of results of multinational studies

(1).1) Add on to SU (with or without metformin) study (EFC6015)

PMDA considered as follows:

There was no clear discrepancy in efficacy between the Japanese and overall populations, thus allowing the interpretation that the trend was consistent between the Japanese and overall populations, since (1) the superiority of lixisenatide over placebo on the primary efficacy endpoint of HbA1c change in the overall population was demonstrated; (2) no major differences between the Japanese and overall populations were observed; and (3) it was confirmed that differences in the demographic characteristics of subjects have no clinically significant impact on HbA1c change. Hence, it may be concluded that the efficacy of lixisenatide in combination with SU (with or without metformin) has been demonstrated.

Regarding safety, there is no particular problem with the applicant's explanation that observed differences in the incidences of some adverse events between the Japanese and overall populations are not considered clinically significant differences in safety and it may be interpreted that there are no safety concerns for Japanese patients. Since the limited number of Japanese patients were treated with lixisenatide in combination with SU and high-dose (>750 mg/day) metformin in the study, it is necessary to continue to collect information on the safety of lixisenatide in combination with SU and high-dose (>750 mg/day) metformin via post-marketing surveillance.

(1).2) Add on to basal insulin (with or without SU) study (EFC10887)

PMDA considered as follows:

There was no clear discrepancy in efficacy between the Japanese and overall populations, thus allowing the interpretation that the trend was consistent between the Japanese and overall populations, since (1) the superiority of lixisenatide over placebo on the primary efficacy endpoint of HbA1c change in the overall population was demonstrated; (2) no major differences between the Japanese and overall populations or

between the Japanese and non-Japanese populations were observed; and (3) it was confirmed that differences in the demographic characteristics of subjects have no clinically significant impact on HbA1c change. Hence, it may be concluded that the efficacy of lixisenatide in combination with basal insulin (with or without SU) has been demonstrated.

Regarding safety, there is no particular problem with the applicant's explanation that observed differences in the incidences of some adverse events between the Japanese and overall populations are not considered clinically significant safety differences and it may be interpreted that there are no safety concerns for Japanese patients.

Based on the above 1) and 2), there is no major problem with the generalization of the results from the overall population in Studies EFC6015 and EFC10887 to Japanese patients with type 2 diabetes mellitus.

The above conclusion by PMDA was supported by the expert advisors.

(2) Safety

The following conclusions by PMDA were supported by the expert advisors:

Based on the occurrence of adverse events and adverse drug reactions in multinational phase III studies (EFC6015 and EFC10887), the safety of lixisenatide is acceptable, provided that appropriate cautions and information will be given. Individual events such as hypoglycaemia were further analyzed. As a result, it is necessary to collect information regarding safety including the occurrence of hypoglycaemia etc., via post-marketing surveillance.

(3) Indication

The following conclusions by PMDA were supported by the expert advisors:

Since the efficacy of lixisenatide in combination with SU (with or without metformin) and lixisenatide in combination with basal insulin (with or without SU) has been demonstrated and the safety is acceptable, there is no major problem with the indication of "Type 2 diabetes mellitus: Lyxumia should be used only when either of the following therapies does not provide adequate glycemic control: (a) diet and exercise plus sulfonylureas (with or without biguanides) or (b) diet and exercise plus soluble prolonged-acting or intermediate-acting insulin (with or without sulfonylureas)." As the efficacy and safety of lixisenatide in combination with basal insulin in insulin-dependent patients with type 2 diabetes mellitus have not been studied, it is necessary to advise physicians to check whether their patient is insulin-dependent and to carefully determine whether or not lixisenatide should be used in combination with basal insulin.

(4) Dosage and administration

The following conclusions by PMDA were supported by the expert advisors:

There is no major problem with selecting the same starting and maintenance doses of lixisenatide as overseas (10 μg QD as the starting dose and 20 μg QD as the maintenance dose) in Japan, based on clinical study results. On the other hand, as to the titration regimen, there is no major problem with selecting a two-step titration regimen, which is different from the titration regimen employed overseas, because this regimen tended to reduce gastrointestinal disorders, etc. As to the timing of dosing, evening injections of lixisenatide (injected prior to evening meal) have not been assessed in clinical studies in which Japanese patients

participated and patients included in Foreign Study EFC6014 that evaluated once-daily morning injections and once-daily evening injections (patients not adequately controlled with metformin) were different from the intended patient population for lixisenatide. If lixisenatide is used in combination with SU or basal insulin, it should be necessary to compare once-daily morning injections and once-daily evening injections, especially for the occurrence of hypoglycaemia. Given that such comparison has not been studied, it is appropriate that "as the usual dosage regimen, lixisenatide should be injected prior to breakfast."

Based on the above, PMDA instructed the applicant to modify the proposed dosage and administration statement.

The applicant responded that the proposed dosage and administration statement will be modified as follows:
The usual adult dosage is 20 μg of Lixisenatide subcutaneously injected once daily prior to breakfast. Lixisenatide should be initiated at 10 μg once daily, which is increased to 15 μg once daily after at least 1 week and then to 20 μg once daily after at least 1 week. The dosage may be adjusted according to the patient's condition. The daily dose should not exceed 20 μg.

PMDA accepted the response.

(5) Post-marketing surveillance plan

The following conclusions by PMDA were supported by the expert advisors:
It is necessary to collect the following information via post-marketing surveillance: hypoglycaemia, gastrointestinal disorders, pancreatitis, injection site reactions, the influence of antibody formation on safety and efficacy, effects on renal function, hypersensitivity reactions, cardiovascular risk, tumor development, safety in patients with renal or hepatic impairment and elderly patients (limited numbers of these patients were included in clinical studies), and the safety and efficacy of lixisenatide in combination with SU and high-dose metformin. As the long-term safety of lixisenatide in combination with basal insulin has not been evaluated in clinical studies in which Japanese patients participated, this information also needs to be collected.

Based on the above, PMDA asked the applicant to present a draft post-marketing surveillance plan, incorporating the above points.

The applicant responded as follows:
In a specified drug use-results survey (a sample size of 3000 patients, a 3-year observation period) in order to collect information on the long-term safety and efficacy of lixisenatide in routine clinical settings, the demographic characteristics of patients, the details of administration of lixisenatide, concomitant medications (the name and dose of each medication, etc.), clinical course (HbA1c, serum creatinine, etc.), laboratory tests, and adverse events (hypoglycaemia, other adverse events) etc. will be recorded and the following information will be collected: hypoglycaemia, gastrointestinal disorders, and hypersensitivity reactions as the important identified risks; and the important potential risks or the important missing information, etc. (pancreatitis, injection site reactions, the influence of antibody formation on safety and efficacy, effects on renal function, cardiovascular risk, tumor development, safety in patients with renal or hepatic impairment and elderly patients, the safety and efficacy of lixisenatide in combination with SU and high-dose metformin,

the long-term safety of lixisenatide in combination with basal insulin).

PMDA accepted the response.

III. Overall Evaluation

As a result of the above review, PMDA concludes that the proposed product may be approved for the following indication and dosage and administration. The re-examination period is 8 years, the drug substance and the drug product are both classified as powerful drugs, and the product is not classified as a biological product or a specified biological product.

[Indication] Type 2 diabetes mellitus:
Lyxumia should be used only when either of the following therapies does not provide adequate glycemic control:
(a) diet and exercise plus sulfonylureas (with or without biguanides) or
(b) diet and exercise plus soluble prolonged-acting or intermediate-acting insulin (with or without sulfonylureas).

[Dosage and administration]
The usual adult dosage is 20 μg of Lixisenatide subcutaneously injected once daily prior to breakfast. Lixisenatide should be initiated at 10 μg once daily, which is increased to 15 μg once daily after at least 1 week and then to 20 μg once daily after at least 1 week. The dosage may be adjusted according to the patient's condition. The daily dose should not exceed 20 μg.