

Report on the Deliberation Results

December 3, 2009

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

[Brand name]	Victoza Subcutaneous Injection 18 mg
[Non-proprietary name]	Liraglutide (Genetical Recombination) (JAN*)
[Applicant]	Novo Nordisk Pharma Ltd.
[Date of application]	July 14, 2008

[Results of deliberation]

In the meeting held on November 27, 2009, the First Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

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

**Japanese Accepted Name (modified INN)*

This English version of the Japanese review report is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail. The PMDA shall not be responsible for any consequence resulting from the use of this English version.

Review Report

November 10, 2009

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] Victoza Subcutaneous Injection 18 mg (changed from Victoza Injection 18 mg)

[Non-proprietary name] Liraglutide (Genetical Recombination)

[Applicant] Novo Nordisk Pharma Ltd.

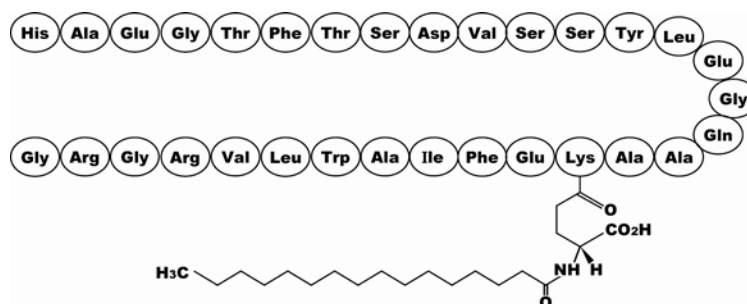
[Date of application] July 14, 2008

[Dosage form/Strength]

A solution for injection in a pre-filled pen. Each pre-filled pen (3 mL) contains 18.0 mg Liraglutide (Genetical Recombination).

[Application classification] Prescription drug (1) Drug with a new active ingredient

[Chemical structure]



Molecular formula: C₁₇₂H₂₆₅N₄₃O₅₁

Molecular weight: 3751.20

Chemical name:

Modified polypeptide of 31 amino acid residues with a covalent linkage at the γ-position of N-palmitoylglutamic acid to the ε-amino group of Lys. The polypeptide is produced in a recombinant cell by expression of DNA encoding amino acid residues at position 7-37 of human glucagon-like peptide-1 with substitution of Arg for Lys at position 34

[Items warranting special mention] None

[Reviewing office] Office of New Drug I

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Review Results

November 10, 2009

[Brand name] Victoza Subcutaneous Injection 18 mg (changed from Victoza Injection 18 mg)

[Non-proprietary name] Liraglutide (Genetical Recombination)

[Applicant] Novo Nordisk Pharma Ltd.

[Date of application] July 14, 2008

[Items warranting special mention] None

[Results of review]

Based on the submitted data, it is concluded that 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 cardiovascular events, pancreatitis, tumors (e.g., thyroid tumors and neuroendocrine tumors), 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;

Victoza should be used only in patients who have not sufficiently responded to either of the following treatments.

(a) Diet and/or exercise therapy alone

(b) Use of sulfonylureas in addition to diet and/or exercise therapy

[Dosage and administration]

The usual adult dosage is 0.9 mg of Liraglutide (Genetical Recombination) subcutaneously injected once daily in the morning or evening. Therapy should be initiated with once-daily doses of 0.3 mg and then the dose should be increased in 0.3 mg increments at intervals of at least 1 week. The dose may be adjusted according to the patient's condition. The daily dose should not exceed 0.9 mg.

Review Report (1)

September 29, 2009

I. Product Submitted for Registration

[Brand name] Victoza Injection 18 mg
[Non-proprietary name] Liraglutide (Genetical Recombination)
[Applicant] Novo Nordisk Pharma Ltd.
[Date of application] July 14, 2008
[Dosage form/Strength] A solution for injection in a pre-filled pen. Each pre-filled pen (3 mL) contains 18.0 mg Liraglutide (Genetical Recombination).

[Proposed indication]

Type 2 diabetes mellitus;

Victoza should be used only in patients who have not sufficiently responded to either of the following treatments.

(a) Diet and/or exercise therapy alone

(b) Use of oral antidiabetic drugs in addition to diet and/or exercise therapy

[Proposed dosage and administration]

The usual adult dosage is 0.9 mg of liraglutide subcutaneously injected once daily. Therapy should be initiated with once-daily doses of 0.3 mg and then the dose should be increased in 0.3 mg increments. Dose increases should occur at intervals of at least 1 week. Victoza should be injected at the same time each day.

[Items warranting special mention] None in particular

II. Summary of the Submitted Data and Outline of Review

A summary of the submitted data 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.

Victoza injection 18 mg is a solution for injection containing an analog of human glucagon-like peptide-1 (GLP-1) (non-proprietary name, Liraglutide [Genetical Recombination], hereinafter referred to as liraglutide) produced by Novo Nordisk (Denmark), as the active ingredient.

GLP-1 is an incretin hormone secreted from L cells in the lower gastrointestinal tract (Nauck MA, *et al.*, *Diabetologia*, 1993; 36: 741-744, Gutniak MK, *et al.*, *Diabetes Care*, 1994; 17: 1039-1044) and is known to lower blood glucose by stimulating glucose-dependent insulin secretion (Holst JJ, *Annu Rev*

Physiol, 1997; 59: 257-271). Since it has been suggested that GLP-1 secretion is impaired in patients with type 2 diabetes mellitus (Toft-Nielsen MB, *et al.*, *J Clin Endocrinol Metab*, 2001; 86: 3717-3723), GLP-1 is considered a drug candidate suitable for treatment of type 2 diabetes mellitus. However, as GLP-1 has a very short elimination half-life (less than 1.5 minutes after intravenous administration) due to rapid degradation by endogenous dipeptidyl peptidase-4 (DPP-4), it is not suitable as a therapeutic drug.

Liraglutide is an intentionally modified GLP-1 molecule with 24-hour durations of blood levels and pharmacologic action and once-daily liraglutide is expected to improve or normalize glycemic control without increasing the risk of hypoglycemia by improving glucose-dependent insulin secretion etc. These effects are expected to be useful for treatment of Japanese patients with type 2 diabetes mellitus especially in view of their decreased insulin secretion (Kiyono H, *et al.*, *Japanese Journal of Clinical Medicine* 1994; 52: 2686-2692, Fukushima M, *et al.*, *Diabetes Res Clin Pract*, 2004; 66S: S37-S43, Fukushima M, *et al.*, *Metabolism*, 2004; 53: 831-835).

In Japan, a clinical trial was initiated in 2002 and as the usefulness of liraglutide in patients with type 2 diabetes mellitus has now been demonstrated, a marketing application has been filed.

Overseas, regulatory applications were submitted in the US and the EU for liraglutide in May 2008 and approval was granted in the EU in June 2009.

2. Data relating to quality

2.A Summary of the submitted data

Liraglutide is a modified polypeptide (C₁₇₂H₂₆₅N₄₃O₅₁; molecular weight, 3751.20) produced using recombinant DNA technology in yeast (*Saccharomyces cerevisiae*) with the expression plasmid pKV308 introduced, and is a fragment of GLP-1 sequence position 7-37 with substitution of the naturally occurring lysine amino acid residue in position 34 by arginine and with addition of a [REDACTED] palmitic acid to the ε-amino group of lysine in position 26. The applicant has filed a new drug application for “Victoza Injection 18 mg,” a 3 ml clear colorless aqueous solution for injection containing 6 mg/ml of liraglutide as the active ingredient, filled in a cartridge assembled into a pen-injector, i.e., a liraglutide pen.

2.A.(1) Drug substance

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

(a) Establishment of cell banking system

The expression vector pKV308 was constructed with the DNA encoding liraglutide precursor and a variant of the DNA for [REDACTED] leader sequence from *S. cerevisiae* inserted into *E. coli*-S.

cerevisiae shuttle vector. The DNA encoding liraglutide precursor was obtained through PCR of the gene for GLP-1 [7-37] from synthetic oligonucleotides.

The pKV308 was transformed into *S. cerevisiae*. After evaluation of transformants by Southern blot and liquid chromatography (HPLC) yields of liraglutide precursor, the liraglutide precursor producing strain was designated as initial cell clone (ICC). The master cell bank (MCB) was derived from a single cell of the ICC. The working cell bank (WCB) was derived from the MCB.

(b) Characterization and control of cell banks

The MCB, the WCB, a fermentation extended beyond the number of generations used in production (LEC; run in pilot scale for [REDACTED] hours from the WCB), and a fermentation in production scale (EPC; run in production scale for [REDACTED] hours from the WCB) were characterized [see Table 1 and Table 2], and all test results complied with the acceptance criteria.

Table 1. Test results for characterization of MCB and WCB

Specification analyses	Acceptance criteria	Test results	
		MCB (Batch number, [REDACTED])	WCB (Batch number, [REDACTED])
Microbial purity	No colonies from organisms other than <i>S. cerevisiae</i>	Complies	Complies
Viability	$\geq 10^6$ CFU/mL	1.5×10^6 CFU/mL ([REDACTED])	1.5×10^6 CFU/mL ([REDACTED])
Phenotype	[REDACTED] immunoblotting must show positive reaction for all colonies measured (at least [REDACTED])	Complies	Complies
Product identity (HPLC)	The product must be [REDACTED] as identified by HPLC	Complies	Complies
Structural context of expression plasmid (Southern blot)	The fragment pattern of the plasmid must be as expected after digestion with appropriate restriction enzymes	Complies	Complies
Supplementary analyses (for MCB only)			
DNA sequencing	Coding DNA sequence identical with expected DNA sequence from ICC	Complies	—
Plasmid copy number	Not applicable. Based on scientific evaluation	1.5×10^6 copies/cell	—
Plasmid loss	Not applicable. Based on scientific evaluation	No plasmid loss	—
Strain identification	Not applicable. Based on scientific evaluation	<i>S. cerevisiae</i> [REDACTED] ex [REDACTED]	—

Table 2. Test results for characterization of LEC and EPC

Specification analyses	Acceptance criteria	Test results	
		LEC	EPC
Microbial purity	No contamination	Complies	Complies
Viability	No acceptance criterion for LEC and EPC	$\times 10^6$ CFU/mL	$\times 10^6$ CFU/mL
Phenotype	% without positive response	% without positive response, positive for all colonies measured	% without positive response, positive for all colonies measured
Product identity (HPLC)	The product must be as identified by HPLC	Complies	Complies
Structural context of expression plasmid (Southern blot)	% with plasmid rearrangements	%- % with plasmid rearrangements	% with plasmid rearrangements
Supplementary analyses (for EPC only)			
DNA sequencing	Coding DNA sequence identical with the coding DNA sequence of MCB ()	—	Complies
Plasmid copy number	Not applicable. Based on scientific evaluation	—	copies/cell
Plasmid loss	Not applicable. Based on scientific evaluation	—	No plasmid loss

To confirm the stability of the cell banks, stability data of the MCB and WCB stored at °C for months were submitted and all results complied with the acceptance criteria. This study will be continued and stability will be monitored on an annual basis. The MCB is expected to last the lifetime of the product. One WCB will theoretically last for years and a new WCB will be generated when the number of vials left reaches between and vials. A new WCB will be characterized as indicated in Table 1 (except for supplementary analyses) and compliance with the acceptance criteria will be checked.

(c) Manufacturing process

The manufacturing process of the drug substance comprises fermentation, recovery, and purification processes and the drug substance will be manufactured at Novo Nordisk A/S .

In the fermentation process of the drug substance, the cell in the WCB vial is inoculated onto agar surface in a fernbach flask (Step 1). After that, the yeast cells are further propagated in the seed tank with fermentation fluid containing carbohydrate source (or) and complex medium (, salt, vitamin) (Step 2, seed fermentation) before propagation in the phase followed by the phase (Step 3, main fermentation).

In the recovery process, is adjusted to after the fermentation to ensure dissolution of the liraglutide precursor and the yeast cells are removed with centrifugations (Step 4, clarification), and capture chromatograph is utilized to capture liraglutide precursor (Step 5, capture). The eluate from Step 5 is precipitated at to (Step 6, precipitation).

In the purification process, the precipitate from Step 6 is dissolved in buffer, and after

pH is adjusted, [REDACTED] buffer and [REDACTED] are added, and [REDACTED] are isolated (Step 7, [REDACTED]). After [REDACTED] from Step 7 are dissolved in a buffer containing [REDACTED], impurities are eluted (elution 1) with [REDACTED] exchange chromatograph and then liraglutide precursor is obtained (elution 2) (Step 8, [REDACTED] exchange chromatography) which is further purified with [REDACTED] chromatograph (Step 9, [REDACTED] chromatography [REDACTED]-HPLC). pH of the eluate from Step 9 is adjusted to [REDACTED] to [REDACTED] and the process solution is cooled and the precipitate is isolated by centrifugation (Step 10, precipitation). The liraglutide precursor from Step 10 is substituted on the ϵ -amino group of lysine in position 26 with [REDACTED] palmitic acid under [REDACTED] acylation conditions to give the target substance, liraglutide (Step 11, acylation). After further purification with [REDACTED] exchange chromatograph (Step 12, [REDACTED] exchange chromatography) and [REDACTED] chromatograph (Step 13, [REDACTED]-HPLC), pH is adjusted to [REDACTED] to [REDACTED] and the precipitate is isolated by centrifugation (Step 14, precipitation). The precipitate from Step 14 is dissolved in [REDACTED] aqueous solution and the liraglutide solution is [REDACTED] at [REDACTED]°C to [REDACTED]°C under pH about [REDACTED] for about [REDACTED] [REDACTED] and then freeze-dried (Step 15, freeze drying) to obtain the drug substance. If the drug substance does not meet the specifications of total viable count and loss on drying and [REDACTED], [REDACTED] by repeating Step [REDACTED]. If it does not meet [REDACTED], [REDACTED] by repeating Step [REDACTED] and Step [REDACTED], and if the acceptance criteria of the in-process control test at Step 10 (test method, RP-HPLC) are not met, [REDACTED] by repeating steps [REDACTED], [REDACTED], and [REDACTED].

For in-process control tests, infection of the remaining broth is set at Step 1, sterility and infection at Step 2, pH, sterility, infection, phenotype, and structural context of expression plasmid at Step 3, pH at Step 4 and Step 5, liraglutide precursor and impurities (rRt [REDACTED]) at Step 6, load and [REDACTED] and [REDACTED] concentration during elution 1 at Step 8, retention volume of the main peak at Step 9, impurities (rRt [REDACTED]) and host cell proteins (HCP) at Step 10, [REDACTED] concentration and pH at Step 11, load and [REDACTED] ([REDACTED]) at Step 12, [REDACTED] concentration in eluate, [REDACTED] for [REDACTED] and [REDACTED] at Step 13, hydrophilic impurities and Peak 4 ([REDACTED]-liraglutide and [REDACTED]-liraglutide) at Step 14 and pH, temperature, and time at Step 15.

For process validation, infection of the remaining broth with foreign microorganisms was evaluated at Step 1 in the fermentation process, sterility and infection with foreign microorganisms at Step 2, sterility, infection with foreign microorganisms, phenotype of fermentation cell, structural context of expression plasmid, and pH (as validation of operational parameter) at Step 3.

At Step 6 in the recovery process, the acceptance criteria for the identity of liraglutide precursor were met and the levels of impurities (rRt [REDACTED] and rRt [REDACTED]) were $\leq$ [REDACTED]% and $\leq$ [REDACTED]%, respectively.

HCP content was evaluated at Step 7 in the purification process, impurity (rRt [REDACTED]), load, and [REDACTED] and [REDACTED] concentration during elution 1 at Step 8, HCP content and retention volume of the main peak at Step 9, impurities (rRt [REDACTED], rRt [REDACTED], rRt [REDACTED]), HCP content, and purity of liraglutide precursor at Step 10, purity of liraglutide, [REDACTED] concentration, and pH at Step 11, Peak 4 ([REDACTED]-liraglutide and [REDACTED]-liraglutide), load, and [REDACTED] for [REDACTED] at Step 12, [REDACTED] concentration in solvent SO24 and SO25, [REDACTED] for [REDACTED] and [REDACTED] at Step 13, hydrophilic impurities, Peak 4 ([REDACTED]-liraglutide and [REDACTED]-liraglutide), and sum of impurities at Step 14 and impurities in [REDACTED], pH and temperature [REDACTED], [REDACTED], [REDACTED], and residual solvents at Step 15.

(d) Controls of critical process steps and intermediates

Main fermentation (Step 3), [REDACTED]-HPLC (Step [REDACTED], Step [REDACTED]), acylation (Step 11), [REDACTED] exchange chromatography (Step [REDACTED]), and freeze drying (Step 15) are regarded as critical process steps.

Critical operational parameters are pH (Steps 3, 4, 5), load, [REDACTED] of elution (impurities), and [REDACTED] concentration during elution (Step 8), retention volume of the main peak (Step 9), [REDACTED] concentration before acylation and pH (Step 11), load and [REDACTED] for [REDACTED] (Step 12), [REDACTED] concentration in elution solvent SO24 and SO25, [REDACTED], and [REDACTED] (Step 13), and pH and temperature [REDACTED], and [REDACTED] (Step 15). There are no critical intermediates.

(e) Manufacturing process development

During development, the drug substance manufacturing process was changed roughly [REDACTED] times (Campaigns 1-7). For cell lines, the expression vector [REDACTED] was changed in Campaign 3 to pKV308 where the ampicillin resistance gene was deleted, and the cell line was changed from [REDACTED] to the currently used [REDACTED]. In Step 3, the fermentation method was changed in Campaign 4 and onwards from [REDACTED] fermentation ([REDACTED] m³) to [REDACTED] fermentation ([REDACTED] m³) which also included a change in medium. In Step 5 where the liraglutide precursor is concentrated, the column material was changed from [REDACTED] exchange column to the present [REDACTED] column [REDACTED] before Campaign 2. Step 6, where precipitation occurs, was introduced for Campaign 2 to obtain a stable intermediate.

In Step 7, for Campaign 4A and onwards, [REDACTED] buffer was used to avoid degradation of the peptide at [REDACTED] [REDACTED] [REDACTED] [REDACTED]. In Campaign 2, the order of Step 8 and 9 was reversed. In Step 11, before Campaign 4A, acylation was undertaken in approximately [REDACTED]% organic solvent ([REDACTED]) but it was changed to the conditions in which a more pure product was

obtained (< 1%). After Step 11, precipitation and isolation by centrifugation were introduced in Campaign 4B, which had no influence on the quality of the product and these steps were omitted for Campaign 6 and onwards. Step 12 was introduced for Campaign 4B and onwards to reduce the loss of liraglutide. In Step 15, for Campaign 3 and onwards, freeze-drying was performed from 100°C instead of from 120°C for Campaign 2. For Campaign 4Ar and onwards, the solvent was changed from 100% ethanol to 50% ethanol to improve stability. In Campaign 4B, the precipitate from Step 14 was dissolved in 100% ethanol ($\leq 10^\circ\text{C}$), which was changed to dissolution at room temperature for Campaign 4Br and onwards as it was found that the precipitate from Step 14 can reliably be dissolved at room temperature. For Campaign 6 and onwards, the solvent under 100% ethanol condition was introduced to minimize the loss, and the solvent before freeze drying was changed to 50% ethanol. Between Campaigns 6 and 7, purification facility was transferred from pilot scale to production scale.

2.A.(1).2) Characterization

For characterization of the drug substance, the structure (N-terminal amino acid sequence, amino acid composition, peptide mapping, circular dichroism [CD], mass spectrometry, bioassay [cAMP determination]) and the physicochemical properties (SDS-PAGE, isoelectric focusing, description [appearance], solubility, pH, ultraviolet [UV] absorbance spectra, water absorption, RP-HPLC, size exclusion chromatography [SE-HPLC]) have been studied.

The N-terminal amino acid sequence determined by Edman degradation agreed with the theoretical one, and amino acid composition analysis (acid hydrolysis, ninhydrin reaction, separation on anion exchange column) showed that the amino acid composition of the primary structure corresponded to theoretical values, except for serine. Serine recovery was low after hydrolysis.

As a result of peptide mapping (Glu-C digestion), though peaks due to incomplete digestion were detected, the retention times of four peptide fragments (P1-P4) were identical to those of secondary reference material.

The CD spectra indicated that the peptide back bone of the drug substance had a secondary structure consisting partly of alpha-helix.

As a result of SDS-PAGE, the molecular size was between 3000 and 4000 Da, and with mass spectrometry (MALDI-TOF MS), the molecular mass was determined to be 3751.1 Da which corresponded to the theoretical mass. According to isoelectric focusing (IEF), the isoelectric point of the drug substance was approximately 4.9.

The drug substance appeared as a white powder. Solubility was ≥ 270 mg/ml at basic pH. The solubility decreased with decreasing pH and reached its lowest value (approximately 0.05 mg/mL) at pH 4 to 5, i.e., around the isoelectric point of the drug substance. The solubility was ≤ 0.8 mg/ml at pH 2.5. The pH of a 1 mg/ml aqueous solution of the drug substance was approximately 9.3. According to UV absorbance spectra, λ_{max} was 282 nm. The increase in water absorption was constant at a relative humidity of 20% to 60% and the average water content at a relative humidity of 50% was around 10%, whereas water absorption was accelerated at a relative humidity of 60% to 100%.

As a result of RP-HPLC, the retention time of the main peak was 16 to 20 minutes. With SE-HPLC, the monomeric liraglutide peak and high molecular weight protein (HMWP) peaks (mainly dimeric and trimeric liraglutide) were detected.

As for biological activity, *in vitro* bioactivity assay was developed based on the fact that liraglutide stimulates BHK cells transfected with human GLP-1 receptors to produce cAMP, which is an intracellular messenger for human GLP-1 receptors, in a dose-dependent manner. “Specific bioactivity” is defined as bioactivity (mg/mg) relative to content by RP-HPLC (mg/mg). The drug substance had a specific bioactivity of about 1.

2.A.(1).3 Product-related substances and impurities

(a) Product-related substances

The product-related substances are [REDACTED]-liraglutide, [REDACTED]-liraglutide, liraglutide [REDACTED], and [REDACTED] liraglutide which are detected with RP-HPLC.

(b) Product-related impurities

The product-related impurities are [REDACTED] liraglutide, [REDACTED]-liraglutide, [REDACTED] liraglutide, liraglutide [REDACTED], [REDACTED]-liraglutide, [REDACTED] liraglutide, [REDACTED] liraglutide, liraglutide [REDACTED] oxygen, liraglutide [REDACTED] oxygen, liraglutide [REDACTED]-liraglutide, [REDACTED] liraglutide, and liraglutide dimer which are detected with RP-HPLC, and HMWP which is detected with [REDACTED]-HPLC.

(a) and (b) are controlled in the drug substance specifications [see 2.A.(1).4) Control of drug substance] and also with in-process limits, as precursor, [REDACTED]-liraglutide at Step 6 and liraglutide [REDACTED], [REDACTED]-liraglutide, and [REDACTED] liraglutide at Step 10, and as liraglutide, [REDACTED]-liraglutide, [REDACTED]-liraglutide, [REDACTED] liraglutide, [REDACTED]-liraglutide, [REDACTED]-liraglutide, and liraglutide [REDACTED] at Step 14 are controlled.

According to the batch analysis of Campaign 7 commercial production scale batches, the total of liraglutide-related impurities was █% to █%, liraglutide-related impurities A which corresponded to Peak 4 (█-liraglutide, █-liraglutide) were ≤ █%, liraglutide-related impurities B (█-liraglutide, liraglutide█, █-liraglutide, █liraglutide) were █% to █%, liraglutide-related impurities C (liraglutide█) were █% to █%, other █ liraglutide-related impurities (█-liraglutide, █-liraglutide, █liraglutide, liraglutide█ oxygen, liraglutide█ oxygen, liraglutide█, █liraglutide, █liraglutide, █-liraglutide) were █% to █%, other █ liraglutide-related impurities (liraglutide dimer) were ≤ █%, and HMWP was ≤ █%.

(c) Process-related impurities

Process-related impurities are HCP and residual solvents and buffer components (ethanol, TEA, NMP, Tris, acetate, NHS [N-hydroxy succinimide]), which have been shown to be adequately removed in the purification process consistently. The content of HCP is controlled below █ ppm with in-process control test (Step █) because HCP in the drug substance will be █ during the █ step.

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

The drug substance specifications have been set for description (appearance), identification (█-HPLC), purity (the total of liraglutide-related impurities, liraglutide-related impurities A, liraglutide-related impurities B, liraglutide-related impurities C, other █ liraglutide-related impurities, other █ liraglutide-related impurities, HMWP, █), loss on drying, bacterial endotoxins, total viable count, specific bioactivity, and assay. The product-related substances and the product-related impurities are controlled as liraglutide-related impurities A (█-liraglutide, █-liraglutide), liraglutide-related impurities B (█-liraglutide, liraglutide█, █liraglutide, █-liraglutide), liraglutide-related impurities C (liraglutide█), other █ liraglutide-related impurities (█-liraglutide, █liraglutide, █-liraglutide, liraglutide█ oxygen, liraglutide█ oxygen, liraglutide█, █liraglutide, █-liraglutide, █-liraglutide), and other █ liraglutide-related impurities (liraglutide dimer).

2.A.(1).5) Reference materials

Primary reference material (PRM) and secondary reference material (SRM) are produced in the same manner as the drug substance manufacturing process, and PRM is used as the standard in identification and liraglutide assay of SRM. The PRM specifications have been set for identification (amino acid sequence, molecular mass), purity (the total of liraglutide-related impurities), homogeneity (SE-HPLC/RP-HPLC), and content. The PRM is stable for █ years when stored below -18°C.

SRM serves for release testing and stability testing of the drug substance and the drug product. The SRM specifications have been set for identification (RP-HPLC), purity (the total of liraglutide-related impurities, HMWP), homogeneity (RP-HPLC), and content. The SRM is filled into glass vials, which is stable for [REDACTED] years when stored below -14°C.

2.A.(1).6) Container closure system

The drug substance is stored in [REDACTED] L and [REDACTED] L [REDACTED] ([REDACTED]) tight containers with caps. [REDACTED] rubber seal ring is mounted in an HDPE cap before closing the container.

2.A.(1).7) Stability

Long-term testing ([REDACTED] ± [REDACTED]°C/[REDACTED] months) and accelerated testing ([REDACTED] ± [REDACTED]°C/12 months) were conducted for three pilot scale drug substance batches, and stress test for one drug substance batch. The containers/closures for samples used in the stability studies are 50 ml [REDACTED] tight containers with closures made of [REDACTED]. On the other hand, the closures for production containers are made from [REDACTED]. The difference was evaluated with respect to hygroscopicity and compatibility of the drug substance with the two containers (25°C ± 2°C, liraglutide-related impurities, HMWP, foreign components [leachables/extractables] after [REDACTED] weeks of storage) was studied, which demonstrated that the stability study condition represents the normal storage condition in the production. In the photostability study, an appropriate amount of the sample was taken into glass containers.

For test items of the long-term testing and the accelerated testing, description (appearance), identification ([REDACTED]-HPLC), assay, the total of liraglutide-related impurities, liraglutide-related impurities A, liraglutide-related impurities B, liraglutide-related impurities C, other [REDACTED] liraglutide-related impurities, other [REDACTED] liraglutide-related impurities, HMWP, loss on drying, specific bioactivity, and total viable count were chosen. As a result, after storage of [REDACTED] months in the long-term testing and [REDACTED] months in the accelerated testing, no significant change was seen for all the test items.

In the stress testing, the drug substance was subjected to degradation under light (25°C, ≥ 1.2 million lx·hr) and heat (50°C, 60°C, 70°C/one week). An amount of 150 mg of the drug substance was filled in each glass vial with a cap and stored after closing the vial. For test items of the stress testing, purity (the total of liraglutide-related impurities, liraglutide-related impurities A, liraglutide-related impurities B, liraglutide-related impurities C, other [REDACTED] liraglutide-related impurities, other [REDACTED] liraglutide-related impurities), HMWP, and assay were chosen. The main peak was identified with mass spectrometry. In the heat condition, the levels of other [REDACTED] liraglutide-related impurities, liraglutide-related impurities B, and other liraglutide [REDACTED]

impurities increased in a temperature-dependent manner. No significant change was seen in the photostability.

Based on the above, a shelf-life of [REDACTED] months was proposed for the drug substance stored in tight containers below [REDACTED]°C. The long-term study of the drug substance will continue up to [REDACTED] months.

2.A.(2) Drug product

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

The proposed commercial formulation is a clear colourless aqueous solution for injection containing 6.0 mg/mL of liraglutide as the active pharmaceutical ingredient (API). Other than the API, it contains, per ml, a buffering agent (1.42 mg disodium phosphate dihydrate), a preservative (5.5 mg phenol), an isotonic agent (14.0 mg propylene glycol), pH adjusters (2 mol/L hydrochloric acid, 2 mol/L sodium hydroxide), and solvent (water for injection). An overage of [REDACTED]% of phenol is used [REDACTED]. The primary packaging of the drug product is a 3 ml colourless glass cartridge. The closure for cartridge consists of a cap with a latex-free laminated rubber disc. The laminated rubber consists of two layers: [REDACTED] and [REDACTED]. The layer of [REDACTED] is in contact with the formulation. The bottom of the cartridge is sealed with the plunger made of [REDACTED]. The proposed drug product is a kit product with the cartridge assembled into a pen-injector which is designed to function with a JIS Type A needle (a pen-injector for Victoza Injection 18 mg; liraglutide pen). The liraglutide pen has been confirmed to fulfill the specification limits for dose accuracy according to JIS T3326-1/ISO 11608-1 and has been certified in Japan (Certification No. 220AABZX00343000).

2.A.(2).2 Pharmaceutical development

In the development process, changes in formulation and manufacturing process for the drug product used in non-clinical and clinical studies have been made. In the early development stage, a mixture of [REDACTED] mg/mL [REDACTED] and [REDACTED] mg/mL [REDACTED] was used as a buffering agent, which has been changed to [REDACTED] mg/ml disodium phosphate dihydrate. As a result, pH has become stable and the physicochemical stability has been increased. In addition, the isotonic agent has been changed from [REDACTED] to propylene glycol, and as pH has been changed to [REDACTED], the concentration of [REDACTED] has been changed from [REDACTED] mg/ml to [REDACTED] mg/ml.

2.A.(2).3 Manufacturing process

For production of the drug product in cartridges, the excipients ([REDACTED] [REDACTED] [REDACTED] [REDACTED]) are dissolved in water for injection (solution I) to which [REDACTED] (solution II) is added. After mixing [REDACTED] and [REDACTED], pH is adjusted to approximately

■ and water for injection is added to make up a constant volume (Step 1). Sterile filtration, filling, and closing of containers (Step 2), assembly (Step 3), and test, inspection, packaging and labeling (Step 4) occur. Batch size ranges from ■ L to ■ L. In Step 1, ■, ■, and ■ are controlled and in Step 2, bioburden, filter integrity before/after use, closure integrity, and filling volume are controlled. After filling, the cartridges are inspected for ■ and readily visible foreign particles. Step ■ is defined as a critical process step.

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

The drug product specifications have been set for description (appearance), identification (■-HPLC), freezing-point depression, pH, purity (1) (the total of liraglutide-related impurities, liraglutide-related impurities A, liraglutide-related impurities B, liraglutide-related impurities C, other ■ liraglutide-related impurities, other ■ liraglutide-related impurities), purity (2) (HMWP), phenol, bacterial endotoxins, foreign insoluble matter, particulate matter, sterility, dose accuracy, and assay. Test for extractable volume is not included in the specification because it is performed as an in-process control.

2.A.(2).5) Stability

(a) Stability studies to establish storage conditions and a shelf life

The stability of the drug product was assessed as follows:

Long-term testing ($5 \pm 3^\circ\text{C}/30$ months), accelerated testing ($25 \pm 2^\circ\text{C}/\text{■}$ months), and stress testing ($\pm \text{■}^\circ\text{C}/\text{■}$ months) were conducted for three batches of the pilot scale drug product (in cartridge). As test items, description (appearance), identification (■-HPLC), pH, purity (1) (the total of liraglutide-related impurities, liraglutide-related impurities A, liraglutide-related impurities B, liraglutide-related impurities C, other ■ liraglutide-related impurities, other ■ liraglutide-related impurities), purity (2) (HMWP), phenol, assay, and specific bioactivity were studied (freezing-point depression, particulate matter, bacterial endotoxins, sterility, and preservative effectiveness tests were performed in long-term testing only).

As a result of long-term testing, the levels of the total liraglutide-related impurities, liraglutide-related impurities B, other ■-related impurities, and HMWP increased. No significant change was seen for the other test items. In the accelerated testing and stress testing, the levels of liraglutide-related impurities and HMWP increased and the liraglutide content decreased.

(b) In-use stability studies

For evaluation of in-use stability, ■ ml and ■ ml of liraglutide 6.0 mg/ml were filled in cartridges and stored for ■ months at $5 \pm 3^\circ\text{C}$ followed by for 32 days at $30 \pm 2^\circ\text{C}$. During the storage of 32 days, the cartridges were turned up and down ■ times per ■ days and each rubber closure was

penetrated with the same needle [REDACTED] times [REDACTED] a week. As test items, description (appearance), pH, phenol, assay, the total of liraglutide-related impurities, liraglutide-related impurities A, liraglutide-related impurities B, liraglutide-related impurities C, other [REDACTED] liraglutide-related impurities, other [REDACTED] liraglutide-related impurities, HMWP, preservative effectiveness, and particulate matter were chosen. As a result, all samples had similar in-use stability and met the specifications for all test items.

(c) Photostability testing

The influence of exposure to light (providing an overall illumination of approximately 1.3 million lx·hr, an integrated near ultraviolet energy of approximately 500 W·h/m², [REDACTED]°C-[REDACTED]°C) was investigated on the drug product in a cartridge and the drug product in a cartridge assembled into a pen-injector (which is considered comparable to the liraglutide pen with regard to stability), and the drug product in a 3-ml cartridge wrapped in aluminium foil as the control. As test items, description (appearance), pH, the total of liraglutide-related impurities, liraglutide-related impurities A, liraglutide-related impurities B, liraglutide-related impurities C, other [REDACTED] liraglutide-related impurities, other [REDACTED] liraglutide-related impurities, HMWP, phenol, assay, and specific bioactivity were chosen. As a result, the drug product in a cartridge failed to meet the specifications for other [REDACTED]-related impurities and HMWP, but the drug product in a cartridge assembled into a pen-injector and the drug product in a cartridge wrapped in aluminium foil met the specifications for all test items.

Based on the above study results, a shelf life of 30 months was proposed for the drug product when stored in sealed containers at 5 ± 3°C (avoid freezing), protected from light. An in-use period of 1 month was proposed when stored at ≤ 30°C, protected from light.

2.B Outline of the review by PMDA

2.B.(1) *In vitro* bioactivity assay

PMDA asked the applicant to explain if the *in vitro* bioactivity assay that was used to evaluate the bioactivity of liraglutide is appropriate to evaluate the bioactivity of GLP-1 and GLP-1 analogs, including its relation to the expected clinical effect.

The applicant responded as follows:

It has been indicated that the primary effects of GLP-1, i.e., insulin secretion from pancreatic β cells, pancreatic β cell proliferation, and glucagonostatic effect, are mediated by increased cAMP. In the bioactivity assay, which is used for quality control of liraglutide drug substance, liraglutide stimulates a BHK cell line expressing the human GLP-1 receptor and intracellular cAMP concentration is measured. Thus, bioactivity assay results reflect physiological and clinical primary effects in the target

cells.

PMDA accepted the response, considering there is no specific problem with the bioactivity assay.

2.B.(2) Prolonged effect of GLP-1 analog

2.B.(2).1) Albumin binding

Since it is assumed that the prolonged action of liraglutide is due to binding to albumin, PMDA asked the applicant to explain how the bioactivity of a GLP-1 derivative has been changed after an amino acid substitution and addition of an acyl side chain and if it is necessary to evaluate the albumin binding capacity of the acyl side chain.

The applicant responded as follows:

Liraglutide and GLP-1 had comparable biological activities on the cloned human GLP-1 receptor when tested without albumin, i.e., 61 ± 7 pM for liraglutide and 55 ± 19 pM for GLP-1. However, when tested in the presence of albumin, GLP-1 remained unaffected by albumin while the receptor binding of liraglutide was affected in an albumin concentration-dependent manner. The decrease in the receptor affinity of liraglutide with increasing albumin concentration was a consequence of the binding of liraglutide to albumin. As it is considered that albumin binding depends on the structure of liraglutide, the structural identity of liraglutide as determined by the drug substance specification test (test for identification) will assure consistent albumin binding properties. Therefore, albumin binding assay has not been performed on individual batches of the drug substance and albumin binding assay is not included in release testing for the drug substance or drug product.

PMDA accepted the response, considering that test for identification of the drug substance and drug product etc. can assure consistent albumin binding properties.

2.B.(2).2) Deacylation

PMDA considers that if deacylation of liraglutide occurs, receptor affinity will be increased and the half-life will be shortened, which can have a big impact on efficacy. Therefore, PMDA asked the applicant if it is possible to detect the deacylated forms with the established specification tests and if the deacylated forms have been detected so far, including those in stress testing of the drug substance and drug product.

The applicant responded as follows:

The non-acylated liraglutide after acylation is controlled as part of hydrophilic impurities by an in-process test in Step 14. In the development process, a consistent absence of the non-acylated liraglutide in any of the drug substance batches has been demonstrated.

PMDA asked whether it is possible that the non-acylated liraglutide is formed after Step 14.

The applicant responded as follows:

Formation of the non-acylated form after Step 14 would require chemical breakdown of amide bond in the structure due to extreme pH values that can not occur in the drug substance or drug product. Thus, in an aqueous environment, such hydrolysis of amide bond does not occur and it is considered that there is no possibility that the non-acylated form is present in the drug substance.

PMDA accepted the response, considering that as the pH in the drug substance after Step 14 and the drug product is controlled, conditions in which amide bond is broken are unlikely to occur.

2.B.(3) pH

PMDA asked if there is any safety problem with the higher pH (8.15) of the proposed commercial formulation, since pH values around 7.4 are generally preferable for subcutaneous injection.

The applicant responded as follows:

A pH of ■■■ was chosen for formulations in early development stage. The pH was changed to 8.15 to ensure physical stability and in-use stability at ■■■°C for patient convenience. In local tolerance studies in pigs, a difference in pH did not affect local reaction. Also, there was no specific problem with regard to injection site adverse events in clinical trials where formulations at pH 8.15 were used [see “3. (iii).A.(6) Local tolerance” and “4.(iii).B. (3). 4) Injection site disorders and immunogenicity”].

PMDA accepted the response since pH is controlled in the drug product specifications and there was no specific problem found in clinical trials.

2.B.(4) HCP specification

PMDA asked for a justification for the specification limit of HCP, in consideration of the amount of HCP present in insulin products produced from yeast based on the marketing experience, the amount of HCP present in the daily dose of liraglutide, and the relation between the dose of liraglutide and HCP content in clinical trials, etc.

The applicant responded as follows:

For insulin products produced from yeast (human insulin [genetical recombination], insulin aspart [genetical recombination], insulin detemir [genetical recombination]), the in-process limit for the amount of HCP present in the drug substance is ≤ ■■■ ppm. Considering that the average daily dose of the insulin products is estimated to be 40 units and the maximum dose ranges from 80 units (insulin

detemir) to 100 units and that 1 unit of human insulin or insulin aspart is 6 nmol and 1 unit of insulin detemir is 24 nmol, the maximal daily HCP exposure is ■ to ■ ng, assuming that HCP content in the drug substance is ■ ppm.

The daily maintenance dose of liraglutide is 0.9 mg in Japan and the daily HCP exposure originating from a drug substance batch containing HCP at the proposed specification limit level (■ ppm) is ■ ng. The overall conclusion from a clinical trial (NN2211-1796) in which a drug product batch manufactured by using a drug substance batch with a high HCP content (HCP content, ■-■ ppm, a weighted average of ■ ppm) was used was that antibody formation was low, efficacy and safety were comparable to those in other phase III trials, and immunogenicity was low. Doses of 0.6, 1.2, and 1.8 mg were used in Trial NN2211-1796 and the 1.8 mg dose corresponds to the daily HCP exposure of ■ ng, which exceeds the daily HCP exposure originating from a drug substance batch containing HCP at the proposed specification limit level of Japan. Therefore, it is considered that the efficacy and safety of the drug product containing HCP at the proposed specification limit level (■ ppm) have been confirmed.

HCP levels in drug substance batches from which the drug product used for Japanese phase III trials (NN2211-1700, NN2211-1701) was produced, were less than ■ ppm. According to batch analysis of individual Campaign 7 batches, HCP content was less than ■ ppm. Based on the above, the proposed specification limit of $\leq$ ■ ppm for HCP is considered to be justified.

PMDA accepted the response, considering that there will be no specific problem even if the drug product containing HCP at the proposed specification limit level is used. This consideration was made because there is no big difference in the maximal daily HCP exposure between insulin products and liraglutide both of which are produced from yeast, there have been no specific problems regarding immunogenicity in the marketing experience with insulin products, and the efficacy and safety of the proposed drug product containing HCP at the proposed specification limit level (■ ppm) have been investigated in Trial NN2211-1796.

3. Non-clinical data

3.(i) Summary of pharmacology studies

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

In primary pharmacodynamic studies, liraglutide activity at the GLP-1 receptor and its mechanism of action *in vitro* and the potency and effects of liraglutide *in vivo* in normal animals and animal models of type 2 diabetes and obesity were investigated. In secondary pharmacology studies, liraglutide was tested for its receptor selectivity. Safety pharmacology studies were performed in accordance with the ICH S7A and ICH S7B guidelines. No pharmacodynamic drug interaction studies have been

performed.

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

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

(a) Activity at human GLP-1 receptor (4.2.1.1.1)

The activities of liraglutide and GLP-1, in the absence of albumin, as measured by cAMP production in membranes from BHK cells expressing the human GLP-1 receptor were determined. As a result, liraglutide and GLP-1 increased cAMP production in a concentration-dependent manner and their EC₅₀ values (mean ± SD) were 61 ± 7.1 and 55 ± 19 pmol/L, respectively.

In addition, the activities of liraglutide and GLP-1 in BHK cells expressing the human GLP-1 receptor and G_s protein, in the presence of pig or human plasma with a high concentration of albumin, were determined in the same manner. As a result, the EC₅₀ values in the presence of pig and human plasma were 127 ± 60 and 100 ± 9 nmol/L, respectively, for liraglutide and 1.4 ± 0.9 and 1.7 ± 0.4 nmol/L, respectively, for GLP-1.

(b) Activity at GLP-1 receptors from different animal species (4.2.1.1.2, 4.2.1.1.3)

The activity of liraglutide were determined by measuring cAMP production in membranes from BHK cells expressing GLP-1 receptors from different animal species. As a result, liraglutide activated GLP-1 receptors from all the animal species and the EC₅₀ value for the monkey receptor (mean and its 95% confidence interval [CI]) was 5.36 [4.46, 6.45] pmol/L and the EC₅₀ values for the pig, rabbit, mouse, and rat receptors (mean ± SD) were 9.28 ± 3.8, 14.9 ± 6.0, 20.0 ± 4.8, and 23.6 ± 2.9 pmol/L, respectively.

(c) Binding to human GLP-1 receptor in the presence of serum albumin (4.2.1.1.4)

The binding affinities of liraglutide and GLP-1 to the GLP-1 receptor were determined by using membranes from BHK cells expressing the cloned human GLP-1 receptor in the presence of human serum albumin (HSA) or bovine serum albumin (BSA). As a result, HSA or BSA did not affect the concentration-response curve for GLP-1, whereas HSA and BSA concentration-dependently shifted the concentration-response curve for liraglutide to the right. The IC₅₀ values of liraglutide were 0.52, 1.2, 5.1, 9.3, and 18 nmol/L at HSA concentrations of 0.05%, 0.1%, 0.5%, 1.0%, and 2.0%, respectively.

(d) Oligomeric structure (4.2.1.1.5)

Liraglutide in the drug product was analyzed by circular dichroism, ¹H-NMR, and ultracentrifugation, which indicates that liraglutide forms micelle-like heptamers with the fatty acid moiety at the core of the complex.

(e) Effect on insulin secretion from perfused islets from mice (4.2.1.1.6)

In the presence of glucose, the effects of liraglutide and GLP-1 (both 1-1000 nmol/L) on insulin secretion from isolated perfused mouse islets, as measured by insulin release (fmol/minute)/100 islets, were determined. As a result, 1 to 100 nmol/L of liraglutide increased insulin secretion to a similar degree as GLP-1. The applicant discussed that decreased insulin secretion at 1000 nmol/L of GLP-1 was due to variability of data.

(f) Effect of a combination of liraglutide and glipizide on insulin secretion from perfused rat pancreas (4.2.1.1.7)

The effect of a combination of liraglutide (300 pmol/L and 3 nmol/L) and an SU, glipizide (30 nmol/L) on insulin secretion from isolated perfused pancreas from overnight fasted male rats was investigated. As a result, the insulin AUC_{28-45 min} values in the glipizide alone group, the glipizide + liraglutide 300 pmol/L group, and the glipizide + liraglutide 3 nmol/L group were 7785, 13892, and 42615 pmol/L·min, respectively [Note by PMDA: Although the study was intended to demonstrate the additive effect of a combination of liraglutide and glipizide, a liraglutide alone group was not set].

(g) Effect on apoptosis of rat pancreatic islet β -cells (4.2.1.1.9)

Using isolated neonatal rat (2-6 days old) pancreatic islets, the effect of liraglutide (1-1000 nmol/L) on β -cell apoptosis was investigated by DNA staining with 7-amino-actinomycin D. As a result, the overall percentage of apoptotic pancreatic islet β -cells was 2.7% in the control (phosphate buffer) group while it was 19.8% in the cytokine treatment group and 55.3% in the free fatty acid treatment group, showing increases in apoptotic cells. Liraglutide concentration-dependently inhibited cytokine- or free fatty acid-induced apoptosis and pretreatment with 1000 nmol/L of liraglutide reduced the overall percentage of apoptotic cells to 3.9% and 26.9%, respectively.

(h) Effect on proliferation of primary cultured rat β -cells (4.2.1.1.8)

Isolated neonatal rat (3-5 days old) pancreatic islets were cultured for 5 to 7 days and the effects of liraglutide, GLP-1, gastric inhibitory polypeptide (GIP), and glucagon (all 10-100 nmol/L) on β -cell proliferation, as measured by the overall percentage of bromodeoxyuridine (BrdU)-positive β -cells, were determined. Liraglutide, GLP-1, and GIP increased the overall percentage of BrdU-positive cells from 3.5% to 4% before treatment to 6% to 7%, whereas glucagon did not cause β -cell proliferation. The β -cell proliferative effect of liraglutide (100 nmol/L) was completely blocked by exendin (9-39) (1 μ mol/L), a GLP-1 receptor antagonist.

Based on the above, the applicant discussed that the β -cell proliferative effect of liraglutide is comparable to those of GLP-1 and GIP, and it is mediated by GLP-1 receptor.

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

(a) Effects in normal animals and animal models of diabetes (single-dose studies)

a) Effects on plasma glucose and food intake in ob/ob mice (4.2.1.1.12)

Female ob/ob mice (5-6 weeks old, n = 9-10 per group) were subcutaneously administered liraglutide (30, 100, 300, 1000 µg/kg). As a result, compared to the control (saline containing 0.2% human serum albumin) group, plasma glucose was decreased at all doses of liraglutide with a peak effect occurring 10 hours after dosing. Within 24 hours after dosing with 1000 µg/kg liraglutide, food intake was reduced by 67%. A dose-dependent body weight reduction associated with reduced food intake was observed.

b) Effects on plasma glucose and food consumption in ZDF rats (4.2.1.1.13)

Male ZDF rats (14 weeks old, n = 5 per group) were subcutaneously administered liraglutide (6.6, 66, 660, 6600 µg/kg). As a result, plasma glucose decreased compared to the control (phosphate buffer containing mannitol) group at 660 and 6600 µg/kg from 2 to 6 hours after dosing. Within 24 hours after dosing, food consumption was reduced dose-dependently at ≥ 66 µg/kg.

c) Effects on plasma insulin and glucagon in normal pigs (4.2.1.1.14)

Conscious, fed female normal pigs (n = 6 per group) were challenged with an intravenous glucose load (0.2 g/kg) 5 hours after a single subcutaneous injection of liraglutide (50, 100, 200 nmol/animal). As a result, compared to the control (phosphate buffer containing mannitol) group, 200 nmol/animal of liraglutide significantly reduced the increase in plasma glucose. Also, compared to the control group, liraglutide significantly reduced the increase in plasma glucagon and the AUC_{glucagon 0-40 min} values (mean ± SEM) in the control and liraglutide (200 nmol/animal) groups were 761 ± 39 and 604 ± 46 pmol/L, respectively. There was no difference in plasma insulin between the liraglutide and control groups.

(b) Effects in animal models of diabetes (repeat-dose studies)

a) Effects on plasma glucose and body weight in ob/ob mice (4.2.1.1.15)

Female ob/ob mice (9 weeks old, n = 10 per group) were subcutaneously administered liraglutide (100 µg/kg) twice daily for 2 weeks. The AUC_{glucose} values (mean ± SEM) in the control (phosphate buffer) and liraglutide groups were 427 ± 17 and 235 ± 17 mmol/L·hr, respectively on Day 1, 556 ± 20 and 417 ± 15 mmol/L·hr, respectively, on Day 8, and 525 ± 14 and 378 ± 24 mmol/L·hr, respectively, on Day 15, showing a significant reduction in AUC_{glucose} in the liraglutide group compared to the control group at all time points. No between-treatment difference in body weight change was observed.

b) Effects on plasma glucose, body weight, food consumption, and β-cell volume in db/db mice

(4.2.1.1.17, 4.2.1.1.18)

Female db/db mice (10-11 weeks old, n = 10 per group) were subcutaneously administered liraglutide (200 µg/kg) twice daily for 2 weeks. As a result, liraglutide significantly lowered AUC_{glucose} compared to vehicle (phosphate buffer) on all measurement days (Days 1, 8, and 15). Compared to vehicle, liraglutide significantly reduced food consumption on Day 1, and significantly increased food consumption on Days 7 to 14. On Day 14, body weight (mean ± SEM) was significantly lower in the liraglutide group (45.1 ± 0.2 g) compared to the vehicle group (46.4 ± 0.4 g). After 2 weeks of dosing, an intraperitoneal dose of BrdU (100 mg/mL) was administered and pancreas sections were prepared, which showed that liraglutide increased β-cell proliferation about 3-fold compared to vehicle. Furthermore, liraglutide significantly increased the β-cell fraction of the total pancreas compared to vehicle.

c) Effect on development of diabetes in ZDF rats (4.2.1.1.19, 4.2.1.1.20)

Male ZDF rats (6 weeks old, n = 7-8 per group) were subcutaneously administered liraglutide (150 µg/kg) twice daily for 6 weeks. As a result, the plasma glucose levels on Days 28 and 38 in the liraglutide group were about half of those in the control (phosphate buffer) group. Furthermore, compared to the control group, liraglutide increased insulin secretion and decreased HbA1c by 3.1% on Day 38. AUC_{glucose} was significantly reduced in the liraglutide group compared to the pair-feeding group receiving the same amount of diet as that consumed by the liraglutide group. In pancreas sections prepared after intraperitoneal administration of BrdU, β-cell volume was increased in the liraglutide group compared to the control group.

d) Effect on plasma glucose in sand rats fed a high energy diet (4.2.1.1.21)

After male and female sand rats (n = 6-7 per group) were fed a high energy diet (3.1 kcal/g) for 3 weeks, the animals that had early morning plasma glucose levels of about 10 mmol/L or higher were treated with liraglutide (12.5, 25, 50, 100, 150, 300 µg/kg) administered subcutaneously once a day for 28 days. As a result, liraglutide dose-dependently decreased plasma glucose levels and liraglutide 300 µg/kg decreased plasma glucose to the levels at the start of a high energy diet. HbA1c (mean ± SEM) was significantly lower in the liraglutide 300 µg/kg group (6.41 ± 0.45%) compared to the control (phosphate buffer) group (10.81 ± 0.40%).

e) Effects on plasma glucose and gastric emptying in minipigs treated with nicotinamide and streptozotocin (4.2.1.1.22)

Male minipigs (n = 6 per group) were intravenously administered nicotinamide (43 mg/kg) and streptozotocin (STZ, 125 mg/kg). Four weeks later, liraglutide (3.3 µg/kg) was subcutaneously administered once a day for 4 weeks and glucose (2 g/kg) and paracetamol (500 mg) were administered in diet after subcutaneous administration of liraglutide at Weeks 2 and 4. As a result,

$AUC_{\text{glucose } 0-120 \text{ min}}$ (mean $\pm$ SD) was lower in the liraglutide group compared to the control (phosphate buffer) group, i.e., the $AUC_{\text{glucose } 0-120 \text{ min}}$ values in the liraglutide group were $74 \pm 12\%$ and $59 \pm 15\%$ of the controls after 2 and 4 weeks of treatment, respectively. The $AUC_{0-120 \text{ min}}$ for paracetamol, a measure of gastric emptying, in the liraglutide group was $80 \pm 14\%$ and $51 \pm 24\%$ of the controls after 2 and 4 weeks of treatment, respectively.

(c) Effects in normal and obese animals

a) Effects on food consumption and body weight in normal rats and rats with deficits in hypothalamic GLP-1 receptor signaling (4.2.1.1.24)

Male normal rats ($n = 10$ per group) and male rats with subcutaneous monosodium glutamate (MSG, 4 mg/kg)-induced deficits in hypothalamic GLP-1 receptor signaling (MSG-treated rats, 12-14 weeks old, $n = 8$ per group) were subcutaneously administered liraglutide (10, 50, 200 $\mu\text{g/kg}$) and vehicle (saline containing 1% BSA) in a crossover fashion. As a result, liraglutide decreased food consumption and water intake at 12 hours after dosing in normal rats at 50 and 200 $\mu\text{g/kg}$ and in MSG-treated rats at 200 $\mu\text{g/kg}$ and 200 $\mu\text{g/kg}$ of liraglutide also increased diuresis.

Male normal rats ($n = 8$ per group) and MSG-treated rats ($n = 8$ per group) were subcutaneously administered liraglutide (100 and 200 $\mu\text{g/kg}$) twice a day for 10 days. As a result, 200 $\mu\text{g/kg}$ of liraglutide significantly decreased body weight in normal rats from Day 7 and in MSG-treated rats from Day 6 through Day 15 compared to vehicle (saline containing 1% BSA). Food consumption was decreased in MSG-treated rats at 200 $\mu\text{g/kg}$. In normal rats, liraglutide increased diuresis and decreased water intake on Day 1, but diuresis on Days 2 and 3 and water intake on Days 3 and 4 were similar to those in the control group. Furthermore, at 200 $\mu\text{g/kg}$ of liraglutide, decreased feces associated with decreased food consumption were observed in normal rats and MSG-treated rats.

The applicant discussed that hypothalamic GLP-1 receptors may not be involved in the decreased food consumption induced by liraglutide since liraglutide decreased food consumption also in MSG-treated rats.

b) Effects on body weight, food consumption, plasma glucose, plasma insulin, and fat mass in obese rats fed a high fat diet (4.2.1.1.25)

High fat diet-induced obese (DIO) male rats (19 weeks old, $n = 10$ per group) were administered liraglutide (200 and 300 $\mu\text{g/kg}$, twice a day, subcutaneous), sibutramine (5 mg/kg, once a day, oral), or rimonabant (Days 0-14, 5 mg/kg; Days 15-28, 10 mg/kg; once a day, oral) for 4 weeks. As a result, compared with vehicle controls (vehicle 1, 0.5% Natrosol [w/v]; vehicle 2, PBS), liraglutide at both doses decreased food consumption, reduced body weight gain, and decreased mass of subcutaneous inguinal, epididymal, mesenteric, and perirenal fat and also compared with sibutramine or rimonabant,

200 µg/kg of liraglutide significantly reduced body weight gain. Although there were no significant differences in plasma glucose, plasma insulin, or HbA1c between the liraglutide and vehicle control groups, when rats were challenged with a 2 g/kg oral glucose load at the end of treatment, liraglutide decreased AUC_{glucose} and AUC_{insulin}.

c) Effects on food consumption and body weight in obese minipigs (4.2.1.1.26)

Obese female minipigs (18-19 months old; body weight at the start of study, 93.7 ± 6.1 kg; n = 6 per group) were subcutaneously administered liraglutide once a day for 7 weeks (after animals received 7 µg/kg for 4 days, 5 µg/kg for 3 days, and 3 µg/kg for 8 days, the dose was escalated by 2 µg/kg on an individual basis and all animals received 7 µg/kg from 3 weeks after the start of treatment until the end of treatment). As a result, food consumption was reduced and reached a steady-state within 3 weeks of treatment, which remained unchanged during the 4 week treatment period after the dose of liraglutide was fixed at 7 µg/kg. Food consumption was reversed to baseline levels within 4 days after treatment was stopped. Food consumption (mean $\pm$ SEM) before and after treatment was 18.4 ± 0.6 and 19.2 ± 0.5 mega joule (MJ)/day, respectively, while it was 7.3 ± 0.3 MJ/day during the 4 week steady state treatment period, which was comparable to that in untreated pigs with normal body weight. Body weight decreased by 4.3 ± 1.2 kg (4%-5%) during the 7 week treatment period and increased by 7.0 ± 1.0 kg during the 7 weeks after treatment.

d) Effects on food consumption and body weight in obese monkeys (4.2.1.1.27)

Obese rhesus monkeys (n = 5; mean body weight, 15.8 ± 1.2 kg; body fat, $\geq 25\%$; overweight and glucose-intolerant, but non-diabetic) were subcutaneously administered vehicle (phosphate buffer containing mannitol) only for 3 weeks and then liraglutide (30 µg/kg) twice a day for 4 days. As a result, liraglutide reduced food consumption (mean $\pm$ SEM) from 722 ± 31 kcal/day at baseline to 44 ± 9.1 kcal/day and body weight by 0.40 ± 0.12 kg from baseline, but food consumption recovered to baseline levels during a washout period.

After a 9-day washout period, liraglutide (10 µg/kg) was subcutaneously administered again twice a day for 16 days. As a result, liraglutide reduced food consumption to 457 ± 52 kcal/day during the treatment period and body weight by 0.24 ± 0.16 kg from baseline, but food consumption recovered to baseline levels after treatment was stopped.

e) Effects on body weight, food consumption, and energy expenditure in candy-fed DIO rats (4.2.1.1.28)

Female rats fed chow and candy (20 g/day) were subcutaneously administered liraglutide (200 µg/kg) twice a day for 12 weeks to determine the effects of liraglutide on body weight, food consumption, and energy expenditure (n = 9 per group). As a result, body weight, body fat mass, and energy expenditure

associated with food consumption were increased in candy fed rats in the control (phosphate buffer containing mannitol) group. Liraglutide decreased body weight to a level similar to normal weight control rats, increased chow intake, and decreased candy consumption and total food consumption.

The applicant discussed that liraglutide decreased body weight, but did not decrease energy expenditure compared to that in candy-fed rats in the control group, suggesting that liraglutide maintains energy expenditure.

f) Effects on antipsychotic olanzapine-induced obesity and impaired glucose tolerance in rats (4.2.1.1.29)

Olanzapine (1.75 mg/24 hr) or vehicle (lactic acid phosphate buffer, pH 6) was continuously dosed subcutaneously for 4 weeks in female rats (n = 20 per group). After 2 week of dosing, each of the olanzapine and vehicle groups was treated with liraglutide or vehicle (phosphate buffer) subcutaneously twice a day for 2 weeks (n = 10 per group). Liraglutide was started at 100 µg/kg with the dose escalated by 50 µg/kg until reaching the final dose of 200 µg/kg 3 days after starting liraglutide. As a result, liraglutide normalized olanzapine-induced elevations of body weight, food intake, mass of subcutaneous inguinal, mesenteric, and retroperitoneal fat, plasma glucose, and total cholesterol. When rats were challenged with a 2 g/kg oral glucose load on Day 25, liraglutide significantly reduced olanzapine-induced elevations of AUC_{glucose} to a level similar to the vehicle group.

(d) Mechanism of action

a) Effects on glucose infusion rate, plasma insulin, and plasma glucagon in minipigs treated with nicotine amide and streptozotocin during glucose clamp (4.2.1.1.30)

Male minipigs (n = 6 per group) were intravenously administered nicotine amide (100 mg/kg) and STZ (125 mg/kg). Four weeks later, liraglutide (2 µg/kg) was intravenously administered under fasting conditions and using the glucose clamp technique, plasma glucose was maintained at 27.0 to 36.0 mg/dL for 80 minutes from 30 minutes after the start of treatment. As a result, in the control (saline containing 0.2% HSA) and liraglutide groups, AUC_{GIR 30-110 min} (mean ± SEM) was 233 ± 59 and 462 ± 68 mg/kg, respectively, AUC_{insulin 30-110 min} (mean ± SEM) was 9014 ± 2952 and 15367 ± 5438 pmol/L·min, respectively, and AUC_{glucagon 70-110 min} (mean ± SEM) was 832 ± 360 and 531 ± 82 mmol/L·min, respectively, and liraglutide increased the glucose infusion rate, glucose-dependently increased insulin secretion, and decreased plasma glucagon.

b) Effects on plasma glucose and food consumption in Zucker obese rats (4.2.1.1.13)

Liraglutide (150 µg/kg) was subcutaneously administered twice a day for 1 week (a total of 15 doses) in male Zucker obese (ZO) rats (8-9 weeks old, n = 5 per group), a model of non-diabetic insulin

resistance. As a result, when rats were challenged with a 2 g/kg oral glucose load after treatment, liraglutide had no effect on AUC_{glucose} , but increased AUC_{insulin} (mean $\pm$ SEM), i.e., 46118 ± 9512 pmol/L·min in the control (phosphate buffer containing mannitol) group and 81885 ± 6889 pmol/L·min in the liraglutide group. During the 1-week study period, food consumption was 43 and 30 g/animal/day in the control and liraglutide groups, respectively, and body weight gain (mean $\pm$ SEM) was lower in the liraglutide group (1 ± 3 g) than in the control group (29 ± 3 g).

c) Effect on β -cell mass in normal rats (4.2.1.1.31)

Male rats (n = 6 per group) were subcutaneously administered liraglutide (200 μ g/kg) twice a day for 6 weeks and β -cells containing insulin were immunostained with an anti-insulin antibody to determine the effect of liraglutide on β -cell mass. As a result, although liraglutide transiently increased β -cell mass after 1 week, there was no significant difference between the liraglutide and control (phosphate buffer containing mannitol) groups after 6 weeks of treatment.

d) Effect on β -cell volume in ZDF rats (4.2.1.1.32, 4.2.1.1.33)

Male ZDF rats (8 weeks old, n = 10 per group) were subcutaneously administered liraglutide (200 μ g/kg) twice a day for 13 days. As a result, during an oral glucose tolerance test (1 g/kg) after the 26th dose, AUC_{glucose} (mean $\pm$ SEM) was 1682 ± 117 mmol/L·min in the control (phosphate buffer containing mannitol) group and 1194 ± 50 mmol/L·min in the liraglutide group.

Male ZDF rats (8 weeks old, n = 10 per group) were subcutaneously administered liraglutide (200 μ g/kg) twice a day for 2 weeks. Pancreas section was immunostained for BrdU and insulin. As a result, liraglutide increased staining intensity of insulin compared to the control group. The β -cell proliferation was 70% lower (control group, $0.46 \pm 0.07\%$; Liraglutide group, $0.13 \pm 0.04\%$) and β -cell volume (mean $\pm$ SEM) was 26% lower in the liraglutide group compared to the control group.

The applicant explained that these results indicate that when plasma glucose levels are normal, β -cell proliferation or β -cell volume is not increased by liraglutide [Note by PMDA: PMDA can not judge that these results show the effects at a normal plasma glucose level because the arm of normal rats was not set in this study,].

e) Effects on body weight, food consumption, and energy expenditure in normal rats (4.2.1.1.34, 4.2.1.1.35)

Male rats (n = 8 per group) were subcutaneously administered liraglutide (150 μ g/kg). Liraglutide had no effect on O_2 consumption, CO_2 production, energy expenditure, or respiratory exchange ratio.

Male rats (n = 8 per group) were subcutaneously administered liraglutide (200 μ g/kg) twice a day for 7

days. As a result, liraglutide reduced body weight by 11% within 2 days of treatment and food consumption by 27% within 3 days of treatment without affecting energy expenditure.

3.(i).A.(1).3) Other pharmacology studies

(a) Effect of combining liraglutide with pioglitazone or atorvastatin (4.2.1.1.23)

Insulin-resistant ZDF rats with blood glucose ≥ 30 mmol/L (14 weeks old, $n = 10$ per group) were treated with liraglutide (200 $\mu\text{g/kg}$, subcutaneous) plus pioglitazone (5 mg/kg, oral) twice a day for 6 weeks. As a result, the combination of liraglutide with pioglitazone decreased plasma glucose, increased plasma insulin, decreased HbA1c, decreased plasma glucose during an oral glucose (2 g/kg) tolerance test, and increased body weight and subcutaneous inguinal and perirenal fat deposits, but the combination decreased food intake and water intake and plasma triglycerides and free fatty acids.

The effect of combining liraglutide with HMG-CoA reductase inhibitor atorvastatin (30 mg/kg, oral) was also studied in the same manner and the combination of liraglutide and atorvastatin increased plasma insulin and decreased HbA1c, total cholesterol, and plasma glucose during an oral glucose tolerance test.

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

3.(i).A.(2).1) Receptor selectivity (4.2.1.2.1-4.2.1.2.4)

The activity of liraglutide as measured by cAMP production in membranes from BHK cells expressing the human glucagon receptor was determined. As a result, liraglutide did not activate the glucagon receptor. Using radioligands, the binding affinity of liraglutide at a broad panel of receptors was determined. As a result, liraglutide did not crossreact with 39 and 75 different receptors or ion channels in the first and second sets of screening assays, respectively and liraglutide at 10 $\mu\text{mol/L}$ inhibited radioligand binding to bombesin receptor by 58%, but the effect was not observed in the third set of screening assays.

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

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

(a) Effects on central nervous system in mice (modified Irwin's test) (4.2.1.3.1, 4.2.1.3.2)

Male mice ($n = 6$ per group) were subcutaneously administered liraglutide (0.02, 0.2, 2.0 mg/kg) or vehicle (phosphate buffer containing mannitol) to assess the neurobehavioral effects of liraglutide. As a result, the incidence of behavioral changes was 1 to 2 of 6 mice treated with liraglutide, which was similar to those in the negative control (saline) and vehicle groups and there were no liraglutide-related effects. The C_{max} at 2.0 mg/kg was 592.5 nmol/L, which was about 33-fold the C_{max} at the maximum recommended human dose (MRHD).

(b) Effect on hexobarbital-induced sleeping time in mice (4.2.1.3.3)

Male mice (n = 6 per group) were subcutaneously administered liraglutide (0.02, 0.2, 2.0 mg/kg) or vehicle control (phosphate buffer containing mannitol) 2 hours prior to intraperitoneal administration of hexobarbital (70 mg/kg). Liraglutide had no effect on hexobarbital-induced sleeping time.

(c) Effect on ethanol-induced sleeping time in mice (4.2.1.3.4)

Male mice (n = 6 per group) were subcutaneously administered liraglutide (0.02, 0.2, 2.0 mg/kg) or vehicle control (phosphate buffer containing mannitol) 2 hours prior to intraperitoneal administration of ethanol (4.5 mg/kg). Liraglutide had no effect on ethanol-induced sleeping time.

3.(i).A.(3).2) Pulmonary effects in rats (4.2.1.3.5)

Conscious male rats (n = 10 per group) were subcutaneously administered liraglutide (0.02, 0.2, 2.0 mg/kg) or vehicle control (phosphate buffer containing mannitol). Liraglutide did not affect pulmonary airway resistance, respiratory rate, tidal volume, or minute volume.

3.(i).A.(3).3) Cardiovascular effects

(a) Effect on hERG tail current in transfected HEK293 cells (4.2.1.3.6)

The effect of liraglutide (0.14, 0.29, 1.43 $\mu\text{mol/L}$) on the tail current in HEK293 cells stably transfected with the hERG cDNA was investigated. Liraglutide at any of the tested concentrations had no effect on hERG tail current. A concentration of 1.43 $\mu\text{mol/L}$ is about 78-fold the human C_{max} at the MRHD.

(b) Effects on QT interval and MAP duration in isolated perfused rabbit hearts (4.2.1.3.7)

Whether liraglutide (0.14, 0.29, 1.43 $\mu\text{mol/L}$) prolongs the QT interval and MAP duration in isolated perfused rabbit hearts was assessed. Liraglutide at any of the tested concentrations did not cause arrhythmia or abnormalities in the ECG or MAP waveform. Liraglutide caused a slight, concentration-dependent increase in heart rate.

(c) Cardiovascular effects in rats (telemetry) (4.2.1.3.8, 4.2.1.3.9)

Male rats (n = 4) were subcutaneously administered liraglutide (0.02, 0.2, 2.0 mg/kg). Liraglutide at 0.02 mg/kg had no effect on systolic, diastolic, or mean blood pressure, heart rate, body temperature, or locomotor activity for up to 24 hour after dosing. Compared to vehicle control (phosphate buffer containing mannitol), liraglutide at 0.2 mg/kg significantly increased systolic, diastolic, and mean blood pressure at 7.5 to 16.5 hours after dosing, slightly increased heart rate at 7.5 to 24 hours after dosing, and significantly decreased body temperature at 5 to 12 hours after dosing. Liraglutide at 2 mg/kg increased systolic, diastolic, and mean blood pressure at 0.5 to 22.5 hours after dosing and significantly decreased body temperature at 1.5 to 16 hours after dosing. Effects on locomotor activity

were sporadically observed, but there was no definitive relationship to liraglutide. Plasma levels of liraglutide increased dose-dependently and $C_{\max}$ was reached at 8 hours (0.02 mg/kg) or 4 hours (0.2 and 2.0 mg/kg) after dosing. The $C_{\max}$ values at 0.02, 0.2, and 2.0 mg/kg were 6009, 74333, and 1044993 pmol/L, respectively, which were about 0.33-, 4.04-, and 56.9-fold the human $C_{\max}$ at the MRHD, respectively.

(d) Cardiovascular effects in conscious cynomolgus monkeys (telemetry) (4.2.1.3.10)

Conscious male cynomolgus monkeys (n = 6 per group) were subcutaneously administered liraglutide (0.02, 0.2, 2.0 mg/kg). Liraglutide had no effect on systolic, diastolic, or mean blood pressure, heart rate, ECG (QRS, PR, and QT intervals), body temperature, or locomotor activity for up to 22 hours after dosing. The $C_{\max}$ calculated from the results of a monkey 4-week toxicity study (4.2.3.2.9) was approximately 600 nmol/L, which was about 33-fold the human $C_{\max}$ at the MRHD.

3.(i).A.(3).4) Renal effects (4.2.1.3.11)

Water-loaded, male rats (n = 6 per group) were subcutaneously administered liraglutide (0.02, 0.2, 2.0 mg/kg) and urine was collected at 2, 6, and 24 hours after dosing to assess the effects of liraglutide on renal function, e.g., urine volume, urinary excretion of electrolytes, and enzyme activity. Compared to vehicle control (phosphate buffer containing mannitol), liraglutide significantly reduced urine specific gravity and osmolality at 6 hours after dosing. At 2.0 mg/kg, γ -GTP activity significantly decreased. Furthermore, at 0.2 and 2.0 mg/kg, urine volume at 2 to 6 hours after dosing and excretions of Na^+ , K^+ , and Cl^- increased. Excretions of Na^+ , K^+ , and Cl^- (mean $\pm$ SD) at 0 to 2 hours after dosing in the control and liraglutide 0.2 and 2.0 mg/kg groups were 0.203 ± 0.125 , 0.513 ± 0.213 , and 1.277 ± 0.255 mmol, respectively, for Na^+ , 0.275 ± 0.175 , 0.435 ± 0.091 , and 0.472 ± 0.094 mmol, respectively, for K^+ , and 0.200 ± 0.140 , 0.512 ± 0.202 , and 1.128 ± 0.175 mmol, respectively, for Cl^- . Also at 24 hours after dosing, increased excretions of Na^+ and Cl^- and reduced specific gravity at 0.2 mg/kg and significant increases in urine specific gravity, osmolality, and total protein, decreased excretions of Na^+ and Cl^- , and decreased urine pH at 2.0 mg/kg were observed.

3.(i).A.(3).5) Effects on autonomic nervous system (isolated guinea pig ileum) (4.2.1.3.12)

Liraglutide (0.14, 0.29, 1.43 $\mu\text{mol/L}$) had no effect on isolated guinea pig ileum and furthermore, liraglutide had no effect on the contraction of the isolated guinea pig ileum elicited by histamine or barium chloride, but 1.43 $\mu\text{mol/L}$ liraglutide slightly and reversibly reduced acetylcholine-induced contraction.

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

3.(i).B.(1) Mechanism of action

The applicant explained as follows:

In vitro studies showed that liraglutide stimulated insulin secretion from rat and mouse pancreatic islets, increased β -cell proliferation, and inhibited apoptosis. In *in vivo* studies in normal animals and animal models of diabetes and obesity, liraglutide decreased fasting and fed plasma glucose levels, plasma glucose AUC, and HbA1c. Furthermore, results from a glucose clamp study in pigs treated with STZ suggested that like GLP-1, liraglutide increases plasma insulin and decreases plasma glucagon, resulting in decreased plasma glucose. It is considered that the primary mechanisms of action are to stimulate glucose-dependent insulin secretion from pancreatic β cells, decrease glucagon secretion, increase β -cell proliferation and β -cell volume, and slow gastric emptying etc. via pancreatic GLP-1 receptors.

PMDA considers that the applicant's explanation (i.e., a human GLP-1 analog, liraglutide stimulates glucose-dependent insulin secretion, decreases glucagon secretion, and lowers blood glucose by acting on GLP-1 receptors) is reasonable.

3.(i).B.(2) Prolonged pharmacological action

The applicant explained that the prolonged pharmacological action and the longer $t_{1/2}$ of subcutaneously administered liraglutide compared to GLP-1 is a result of self-association that delays absorption, binding to serum albumin, and stability against degradation by DPP-4 and neutral endopeptidase (NEP).

PMDA asked the applicant to explain the prolonged pharmacological action of liraglutide in comparison with GLP-1.

The applicant responded as follows:

Liraglutide is highly bound to serum albumin. Liraglutide forms micelle-like heptamers in a solution for injection, which is considered to contribute to slow absorption. Liraglutide molecule itself has been shown to be stable against metabolic enzymes DPP-4 and NEP. Therefore, liraglutide is considered highly stable against metabolic enzymes. *In vitro* GLP-1 receptor binding assay showed that HSA concentrations did not affect the concentration-response curve for GLP-1 while HSA shifted the concentration-response curve for liraglutide to the right.

PMDA asked the applicant to explain the prolonged pharmacological action of liraglutide, presenting the results of comparison of liraglutide and GLP-1 in the same *in vivo* test system, if available.

The applicant responded as follows:

As GLP-1 and liraglutide have different pharmacokinetic profiles (Knudsen LB, *et al.*, *J Med Chem*, 2000; 43: 1664-1669), it is difficult to directly compare the pharmacodynamic profiles of GLP-1 and liraglutide in the same test system. As GLP-1 has a short $t_{1/2}$ of about 1.5 minutes after intravenous administration (Knudsen LB, *et al.*, *J Med Chem*, 2000; 43: 1664-1669), normal pigs were treated with continuous intravenous infusion of GLP-1 in a preliminary study. As a result, blood glucose decreased from hyperglycemic state after glucose loading (10 nmol/L) to less than baseline levels and then recovered to baseline levels (50 minutes after the end of GLP-1 continuous infusion). The results of this study indicated that as with liraglutide with a long $t_{1/2}$ (2.6.4.1) administered subcutaneously 5 hours prior to glucose loading (4.2.1.1.18), GLP-1 lowers blood glucose, but continuous infusion needs to be started before glucose loading. In conclusion, in order to exert as comparable a blood glucose lowering effect as liraglutide in an *in vivo* study in normal pigs, GLP-1 needs to be continuously infused.

Although the prolonged pharmacological action of liraglutide has not been adequately explained pharmacologically based on data, as the explanation from both pharmacodynamic and pharmacokinetic point of view is understandable, PMDA accepted the response [see “3.(ii).B. Outline of the review by PMDA”].

3.(i).B.(3) Cardiovascular effects

The applicant explained the effects of liraglutide observed in safety pharmacology studies as follows: Although the cardiovascular and renal effects of liraglutide in rats were those mediated by GLP-1 receptors, these effects were not observed in monkeys at about 33-fold the MRHD. Therefore, none of these effects observed in safety pharmacology studies are considered relevant to human safety.

In rats, subcutaneous liraglutide at 0.2 mg/kg significantly increased mean blood pressure at some time points between 7.5 and 16.5 hours after dosing and slightly increased heart rate up to 24 hours after dosing. Considering that liraglutide at a dose around 0.2 mg/kg decreased HbA1c in ZDF rats, the $t_{1/2}$ of liraglutide is 3.6 hours in rats while it is longer in humans, i.e., 14 to 15 hours, and increased heart rate in rats persisted for 24 hours, PMDA asked the applicant to discuss possible cardiovascular effects of liraglutide in humans.

The applicant responded as follows:

GLP-1 and GLP-1 agonists are known to affect blood pressure and heart rate in rats (Barragán JM, *et al.*, *Am J Physiol*, 1994; 226: E459-E466, Barragán JM, *et al.*, *Regulatory Peptides*, 1996; 67: 63-68, Barragán JM, *et al.*, *Am J Physiol*, 1999; 277: E784-E791, Bojanowska E & Stempniak B, *Regulatory Peptides*, 2000; 91: 75-81, Yamamoto H, *et al.*, *J Clin Invest*, 2002; 110: 43-52, Gardiner SM, *et al.*, *J*

Pharm Exp Ther, 2006; 316: 852-859, Mack C, *et al.*, *Drug Development Research*, 2006; 67: 553-558). However, increased blood pressure associated with a GLP-1 agonist has been observed only in rats. Even when the same doses of liraglutide as those in rats (0.02-2.0 mg/kg) were administered to telemetered unrestrained, conscious cynomolgus monkeys, there were no changes in blood pressure or heart rate (4.2.1.3.10). The exposure in cynomolgus monkeys based on C_{max} was about 33-fold higher than the human exposure at the maximum clinical dose of 0.9 mg/day for Japanese patients.

In humans, the change from baseline in systolic blood pressure at 52 weeks of treatment was -3.0 to -2.6 mmHg in the liraglutide 0.9 mg group in a Japanese long-term treatment trial and systolic blood pressure tended to decrease in the liraglutide group compared to the control group. On the other hand, liraglutide caused an increase in heart rate of 4.7 to 4.8 beats/minute compared to the control group in a Japanese phase III trial. The possible mechanisms include a compensatory response to decreased systolic blood pressure, a compensatory response to the vasodilating action of GLP-1, and alterations in the sympathetic and parasympathetic nervous system balance. However, the increase in heart rate was slight, which occurred only in the early phase of treatment (within 4 weeks) and there were no increases thereafter. Based on the data from all intermediate- and long-term treatment trials, the incidence rate of serious arrhythmic adverse events was 2.8/1000 subject-years in the liraglutide group, 2.2/1000 subject-years in the placebo control group, and 2.9/1000 subject-years in the active control group and liraglutide was not associated with an increased incidence rate of serious arrhythmic adverse events. The above results indicate that the cardiovascular effects observed in rats at a therapeutic/pharmacological dose are unlikely to be relevant to human safety.

PMDA considers that as long as the increases in blood pressure and heart rate are effects mediated by GLP-1 receptors, the possibility of species differences in receptor binding affinity or signaling etc. should also be taken into account for discussion of human relevance and there is no sufficient evidence to conclude that these effects are specific to rats. However, PMDA accepted the response, considering that these effects are unlikely to become a pharmacologically relevant problem because there were no changes in cardiovascular parameters in conscious cynomolgus monkeys at 33-fold the MRHD [see “4.(iii).B.(3).9 Cardiovascular effects” for cardiovascular effects in humans].

3.(ii) Summary of pharmacokinetic studies

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

Pharmacokinetics were determined after single intravenous or subcutaneous injections of liraglutide or radiolabeled liraglutide (^{14}C , ^{125}I , ^3H) in mice, rats, rabbits, monkeys, and pigs. Repeat-dose pharmacokinetics were determined based on toxicokinetics in toxicity studies. Plasma liraglutide concentrations were measured using a radioimmunoassay (RIA) or an enzyme-linked immunosorbent assay (ELISA) and the ELISA was used in chronic repeat-dose toxicity studies and all clinical studies.

The RIA detected both liraglutide and GLP-1 and the detection limit of the RIA was [REDACTED] pmol/L for mouse plasma, [REDACTED] pmol/L for rat and monkey plasma, and [REDACTED] pmol/L for rabbit plasma. The ELISA did not cross-react with endogenous GLP-1 and the lower limit of quantification of the ELISA was [REDACTED] pmol/L for plasma samples from animals other than rats and [REDACTED] pmol/L for rat plasma. Liraglutide exposures determined using RIA and ELISA methods on the same plasma samples showed that exposures were comparable at low doses (low plasma concentrations) and there was up to [REDACTED]-fold difference between exposures at high doses (the exposures determined by the ELISA method were higher.)

3.(ii).A.(1) Absorption (4.2.2.2.4-6, 4.2.3.1.5, 4.2.3.2.1-11, 4.2.3.4.1.1, 4.2.3.4.1.2, 4.2.3.5.2.1, 4.2.3.7.3.23, 4.2.3.7.3.26)

Single-dose pharmacokinetic parameters of liraglutide after intravenous or subcutaneous dosing in mice, rats, rabbits, monkeys, and pigs were as shown in Table 3.

Liraglutide was well-absorbed from the subcutaneous injection site and bioavailability (BA) after subcutaneous injection was 53% in monkeys and 76% in pigs. The apparent volume of distribution was small and close to the plasma volume, which indicates that a high fraction of liraglutide is circulating in the blood. Pharmacokinetics were similar between males and females and it was considered that there are no marked dose-dependent changes.

Table 3. Single-dose pharmacokinetic parameters of liraglutide

Animal species	Route of administration	Dose (mg/kg)	Sex	N	$t_{\max}$ (h)	$C_{\max}$ (nmol/L)	AUC ^{a)} (nmol/L·h)	F (%)	CL ^{b)} (L/h/kg)	V_z ^{c)} (L/kg)	$t_{1/2}$ (h)
Mouse	s.c.	0.1	♂	2	8	75.1	1155		0.0231	0.229	6.9
		0.5	♂	2	6	522.5	6891		0.0193	0.178	6.4
		1.0	♂	2	4	1246.0	15554		0.0171	0.138	5.6
		5.0	♂	2	4	6505.5	100125		0.0133	0.153	7.9
		0.1	♀	2	4	74.7	990		0.0269	0.256	6.6
		0.5	♀	2	4	666.5	4888		0.0273	0.148	3.8
		1.0	♀	2	4	1132.0	12345		0.0216	0.200	6.4
		5.0	♀	2	6	5145.5	64378		0.0207	0.144	4.8
Rat	s.c.	0.1	♂	2	6	69.7	868		0.0308	0.189	4.3
		0.25	♂	2	4	180	2130		0.0313	0.219	4.8
		1.0	♂	2	6	675	9460		0.0282	0.251	6.2
		0.1	♀	2	6	84.3	875		0.0305	0.129	2.9
		0.25	♀	2	6	196	2130		0.0313	0.199	4.4
		1.0	♀	2	8	560	7290		0.0365	0.274	5.2
Rabbit	s.c.	0.01 ^{d)}	♀	2	4, 8	9, 11	158, 159				6.3, 7.4
		0.02 ^{d)}	♀	2	8, 8	15, 19	265, 296				7.0, 7.1
Pig	i.v.	0.00188 ^{d)}	♀	4			35 ± 13		0.016 ± 0.006	0.23 ± 0.05	10 ± 2
	s.c.	0.00188 ^{d)}	♀	4	7 ± 3	1.1 ± 0.1	27 ± 12	76 ± 18			14 ± 2
Monkey	i.v.	5 ^{d)}	♂	2			112835, 121540		0.0108, 0.0120	0.10, 0.20	6.1, 12.9
		5 ^{d)}	♀	2			174273, 242094		0.0054, 0.0078	0.05, 0.06	5.7, 6.3
	s.c.	0.05	♂	4	7 ± 1	24.5 ± 4.2	407 ± 21				6.2-8.3
		0.5	♂	4	8 ± 1	384 ± 54.5	6550 ± 927				6.6-10.1
		5	♂	6	8 ± 2	4900 ± 628.2	87800 ± 8325				6.3-12.7
		5 ^{d)}	♂	2	6, 12	2601, 4056	68719, 718250	61, 60			7.9, 17.7
		0.05	♀	4	8 ± 1	35.1 ± 7.2	560 ± 111				5.7-8.8
		0.5	♀	4	7 ± 1	450 ± 91.3	7870 ± 1369				8.4-9.7
		5	♀	6	9 ± 2	5160 ± 808.9	118000 ± 22601				7.6-17.0
		5 ^{d)}	♀	2	6, 8	4099, 7197	78372, 115138	45, 48			6.2, 7.0

$t_{\max}$: Time to maximum plasma concentration, $C_{\max}$: Maximum plasma concentration, AUC: Area under the plasma concentration-time curve, F: Absolute bioavailability, CL: Clearance, V_z : Volume of distribution, $t_{1/2}$: Elimination half-life

a) AUC_{0-24 h} for pigs, AUC_{0-inf} for other animal species

b) CL/f for mice and rats

c) V_z /f for mice and rats

d) RIA method was used.

PK parameters in mice and rats were calculated from means at different sampling points. For PK parameters in rabbits, each value of 2 rabbits is presented. PK parameters in pigs are expressed as mean ± SD. For monkeys, PK parameters are expressed as mean ± SD, $t_{1/2}$ is expressed as range, and each value of 2 monkeys is presented when RIA method was used.

Repeat-dose pharmacokinetics were determined based on toxicokinetics in long-term toxicity studies (4 studies up to 104 weeks in mice; 6 studies up to 104 weeks in rats; a 16-day study in rabbits; 6 studies up to 87 weeks in monkeys; a 5-day study in pigs). After once-daily repeated subcutaneous dosing, plasma accumulation did not occur or was minimal in mice, rats, and monkeys while the steady-state trough concentration increased 1.3-fold in pigs. Although anti-liraglutide antibodies were detected in 52-week and 87-week repeat-dose toxicity studies in monkeys, there was no correlation between liraglutide exposure and antibody formation. Anti-liraglutide antibodies were not detected in

rats or mice.

3.(ii).A.(2) Distribution (4.2.2.3.1-9, 4.2.2.5.1, 4.2.2.5.2, 5.3.2.1.3)

Tissue distribution after a single subcutaneous dose of 0.1, 0.15, or 1 mg/kg of radiolabeled liraglutide (^3H -[Pal], ^{14}C or ^{125}I) or repeated subcutaneous doses once daily for 7 days in rats ($n = 1$ or 3/sex/time point), a single intravenous dose of 1 mg/kg of radiolabeled liraglutide (^3H -[Tyr]) in male rats ($n = 1$ /time point), and a single subcutaneous dose of 0.15 mg/kg of radiolabeled liraglutide (^3H -[Pal]) in pigmented male rats ($n = 1$ /time point) was determined. At earlier time points (≤ 4 h after dosing) that are considered to represent liraglutide distribution, regardless of the liraglutide-labeling site, high levels of radioactivity were found in tissues with high blood flow including the liver, lungs, kidneys, and adrenal gland; radioactivity levels in the brain, skeletal muscle, thymus, eyes, and prostate gland were low; the tissue/plasma radioactivity ratio was less than 1 in all tissues. It was considered that distribution of liraglutide represents blood flow and does not show specific uptake or distribution. Tissue distribution pattern ≥ 4 hours after dosing was different, depending on the liraglutide-labeling site. Liraglutide is metabolized to amino acids and a fatty acid and radioactivity distribution ≥ 24 hours after dosing was considered to represent distribution of low molecular weight degradation products (peptides etc.). Tissue distribution of radioactivity was similar between males and females and between pigmented and albino rats.

Tissue distribution after a single subcutaneous dose of 0.1 or 1 mg/kg of radiolabeled liraglutide (^{14}C or ^{125}I) in pregnant rats ($n = 3$ /time point), repeated subcutaneous doses of 1 mg/kg of liraglutide once daily for 5 days in pregnant rats, and repeated subcutaneous doses of 0.05 mg/kg of liraglutide once daily for 5 days in pregnant rabbits was determined. Although liraglutide crossed the placenta, the level of uptake of liraglutide or related substances into fetuses or amniotic fluid was considered to be low.

The plasma protein binding of liraglutide (0.1 nmol/L to 0.1 mmol/L, *in vitro*) in mice, rats, rabbits, monkeys, and pigs was 95.8% to 99.8%.

3.(ii).A.(3) Metabolism (4.2.2.4.2-12, 5.3.2.2.1, 5.3.2.2.2, 5.3.2.3.1-4)

Liraglutide consists of a peptide chain and a fatty acid with addition of [REDACTED]. *In vitro* and *in vivo* studies and a study using perfused liver and kidney from rats in the presence of human serum albumin (6%) indicated that liraglutide is sequentially cleaved into peptide fragments and amino acids by DPP-4 and NEP in different organs/tissues and the cleavage sites are the same as those reported for GLP-1. The metabolic profile of liraglutide was similar among animal species.

Liraglutide did not inhibit human CYPs ($IC_{50} > 100 \mu\text{mol/L}$) and the effects of 4-week treatment with up to 1 mg/kg of liraglutide in rats on the total CYP amount and CYP activity were small.

3.(ii).A.(4) Excretion (4.2.2.5.1-6)

Excretion of radioactivity was determined after single subcutaneous dosing or 7 days of once daily repeated subcutaneous dosing with 0.1 or 1 mg/kg of radiolabeled liraglutide (^{14}C or ^{125}I) in rats (3 males and 3 females per group) and after single subcutaneous dosing with 0.05 or 5 mg/kg of radiolabeled liraglutide (^3H -[Pal]) in male and female monkeys (3 males and 3 females per group). In rats, cumulative recovery of ^{125}I was 94% to 101% and radioactivity was mainly excreted in urine. Cumulative recovery of ^{14}C was 92% to 93% and the majority was excreted as $^{14}\text{CO}_2$ in expired air. At 168 hours after dosing, 7% to 10% of the administered radioactivity was recovered in the carcass. In monkeys, within 168 hours after dosing with ^3H -[Pal]-liraglutide, cumulative recovery of radioactivity was low at about 30% with most being excreted in urine and it was considered that a portion of $^3\text{H}_2\text{O}$ formed is excreted in expired air or sweat and the other portion remains in the body as water.

After a single subcutaneous 1 mg/kg dose of radiolabeled liraglutide (^3H -[Pal], ^{14}C or ^{125}I) in lactating rats ($n = 3$ per group/time point), the maximum amount of liraglutide and its metabolites in milk consumed by a pup was estimated to be about 0.3% of the maternal daily dose.

3.(ii).A.(5) Pharmacokinetic drug interactions (4.2.1.1.26)

In pigs, coadministration of paracetamol and liraglutide reduced the paracetamol AUC up to 2 hours after dosing to 80% of that with paracetamol alone at Week 2 and 50% at the end of study. The result demonstrated that liraglutide delays gastric emptying.

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

PMDA asked the applicant to explain the distribution of liraglutide into tissues where GLP-1 receptors are present.

The applicant responded as follows:

GLP-1 receptors are widely distributed throughout the body, including the brain, pancreatic islets, and gastrointestinal tract, and though its functions are not defined, GLP-1 receptors are located also in the lung, pituitary gland, heart, kidney, and liver (Kiefer TJ & Habener JF, *Endocrine Reviews*, 1999; 20: 876-913, Holst JJ, *Physiol Rev*, 2007; 87: 1409-1439). Quantitative tissue distribution studies and whole body autoradiography using radiolabeled liraglutide showed that radioactivity was mainly present in plasma and there was no trend towards distribution of radioactivity into specific tissues, which is considered to be attributed to the high plasma protein binding of liraglutide. Therefore, it is

considered that liraglutide does not distribute into or accumulate in any specific tissue expressing or not expressing GLP-1 receptors.

PMDA asked the applicant to explain the characteristics of liraglutide compared with GLP-1 with the data relating to a difference in albumin binding and the $t_{1/2}$ or the duration of pharmacological action between liraglutide and GLP-1.

The applicant responded as follows:

Liraglutide is a long-acting human GLP-1 analog, i.e., a GLP-1 derivative with a fatty acid attached to the molecule, and has a pharmacokinetic profile suitable for once-daily injection. Elimination of GLP-1 occurs via degradation in the gastrointestinal tract and circulation, and renal and hepatic clearance. Liraglutide was designed to bind to albumin in order to increase $t_{1/2}$ and prolong pharmacological activity. Liraglutide is highly bound to protein and human GLP-1 receptor activation assay based on cAMP formation also showed that albumin shifted the concentration-response curve for liraglutide to the right, suggesting that unbound liraglutide only is involved in the exertion of its pharmacological action. On the other hand, increases in albumin concentration did not affect the receptor binding profile of GLP-1 and its protein binding is considered very low compared to liraglutide. Liraglutide forms micelle-like heptamers in a solution for injection and this self-association is considered to contribute to slow release of liraglutide from the injection site. Although the cleavage sites of liraglutide identified based on *in vitro* studies using DPP-4 and NEP and *in vivo* studies were the same as those identified for GLP-1 using DPP-4 and NEP, it was considered that liraglutide is stable against metabolic degradation by DPP-4 and NEP due to high plasma protein binding and the formation of heptamers. In conclusion, the characteristics of liraglutide compared to GLP-1 are attributed to the above-mentioned multiple factors.

PMDA accepted the response [see “4.(ii).B.(1).1) Characteristics of liraglutide”].

3.(iii) Summary of toxicology studies

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

Liraglutide toxicity studies conducted include single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and local tolerance studies and a study on impurities.

3.(iii).A.(1) Single-dose toxicity (4.2.3.1.1-4.2.3.1.5)

Single subcutaneous and intravenous dose studies in mice, rats, and cynomolgus monkeys were conducted. Regardless of the route of administration, the approximate lethal dose was determined to be > 10 mg/kg in mice and rats and > 5 mg/kg in cynomolgus monkeys. The dose for mice and rats (10

mg/kg) was selected so that animal exposures would be 100-fold the human exposure. The dose for cynomolgus monkeys (5 mg/kg) was selected as a subcutaneous tolerated dose based on the results from a preceding maximum tolerated dose study in cynomolgus monkeys (4.2.3.2.7) and a preceding 14-day repeated subcutaneous dose toxicity study in cynomolgus monkeys (4.2.3.2.8). As symptoms after dosing, decreased food consumption and lower body weight were observed in mice and rats. Decreased food consumption and lower body weight are considered pharmacologic effects relating to suppression of the appetite center and inhibition of gastrointestinal motility (Drucker DJ, *Endocrinol*, 2001; 142: 521-527). In cynomolgus monkeys, there were no changes in food consumption and reduced body weight gain only was noted.

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

Repeated subcutaneous dose studies in mice (1 and 3 months), rats (1, 3, 6 months), and cynomolgus monkeys (1, 3, 12 months) were conducted. In all animal species, decreased food consumption and lower body weight, which are considered the pharmacologic effects of liraglutide, were observed. In mice, thyroid C-cell hyperplasia occurred. The no observed adverse effect level (NOAEL) was determined to be < 0.2 mg/kg/day in a mouse 3-month study, 1.0 mg/kg/day in a rat 6-month study, and 5.0 mg/kg/day in a cynomolgus monkey 12-month study and the exposures at the NOAELs ($AUC_{0-24\text{ h}}$) in rats and cynomolgus monkeys were 14-fold and 132-fold the human exposure at the intended clinical dose (15 µg/kg equivalent to the maximum dose of 0.9 mg administered to a human weighing 60 kg), respectively.

3.(iii).A.(2).1) Mouse 1-month subcutaneous dose study (4.2.3.2.1)

Mice (n = 10/sex/dose) were subcutaneously injected with 0 (control), 0.1, 0.5, 1.0, or 5.0 mg/kg/day of liraglutide for 1 month. Decreased food consumption at ≥ 0.1 mg/kg/day and lower body weight at ≥ 0.5 mg/kg/day were observed, which were of no toxicological significance and the NOAEL was determined to be 5.0 mg/kg/day. The highest dose was selected based on a single-dose toxicity study in which 10 mg/kg/day was tolerated and an exploratory 7-day study in which body weight was slightly decreased at 5 mg/kg/day.

3.(iii).A.(2).2) Mouse 3-month subcutaneous dose study (4.2.3.2.2)

Mice (n = 10/sex/dose) were subcutaneously injected with 0 (control), 0.2, 1.0, or 5.0 mg/kg/day of liraglutide for 3 months. Decreased food consumption and lower body weight were observed at ≥ 0.2 mg/kg/day. Although decreased red blood cell count and decreased hematocrit occurred at ≥ 0.2 mg/kg/day, as no correlative pathology was observed, their toxicological significance was unknown.

As thyroid C-cell hyperplasia occurred at ≥ 0.2 mg/kg/day, separate mechanistic studies (4.2.3.7.3) have been conducted. Among 23 to 24 mice per dose group (46-48 mice when combining males and

females) including 14 mice in a satellite group for toxicokinetic assessment at Week 13, C-cell hyperplasia occurred in 0 of 46 mice in the 0 mg/kg/day group, 17 of 47 mice in the 0.2 mg/kg/day group, 18 of 48 mice in the 1.0 mg/kg/day group, and 23 of 48 mice in the 5.0 mg/kg/day group. There were no differences between males and females. As C-cell hyperplasia occurred in all liraglutide groups, the NOAEL was determined to be < 0.2 mg/kg/day.

3.(iii).A.(2).3 Rat 1-, 3-, and 6-month subcutaneous dose studies (4.2.3.2.4-4.2.3.2.6)

Rats (1- and 3-month studies, n=10/sex/dose; 6-month study, 15/sex/dose; the 3-month study only included 1-month recovery groups [5/sex/group]) were subcutaneously injected with 0 (control), 0.1, 0.25, or 1.0 mg/kg/day of liraglutide. As a result, decreased food consumption and lower body weight occurred at ≥ 0.1 mg/kg/day in all of 1-, 3-, and 6-month studies, which were not considered findings of toxicological significance. In the 1.0 mg/kg/day group of the 3-month study, hunched posture, rolling gait and high stepping gait, and piloerection were noted during the first or second week of treatment. These clinical signs have been reported to be general signs of discomfort, which have been observed also with a drug of the same class, exenatide (unapproved in Japan) (Mack C, *et al.*, *Drug Dev Res*, 2006; 67: 553-558) and were not considered toxicity effects. At ≥ 0.1 mg/kg/day in the 6-month study, relative heart weight decreased $\leq 10\%$, which was not dose-dependent and no correlative histopathology was observed. Thus, this finding was not considered a toxicity effect. The NOAEL was determined to be 1.0 mg/kg/day for all studies. The exposure at the NOAEL was 20-fold (1-month study), 24-fold (3-month study), and 14-fold (6-month study) the human exposure at the intended clinical dose.

3.(iii).A.(2).4 Cynomolgus monkey 1-month subcutaneous dose study (4.2.3.2.9)

Cynomolgus monkeys (n = 3/sex/dose) were subcutaneously injected with 0 (control), 0.05, 0.5, or 5.0 mg/kg/day of liraglutide for 1 month. As a result, decreased food consumption at ≥ 0.05 mg/kg/day and lower body weight at ≥ 0.5 mg/kg/day occurred. Pancreas weight was higher in males at ≥ 0.05 mg/kg/day, but there was no significant difference and both absolute weight (3.68 g at 5 mg/kg/day) and relative weight (0.207 g at 5 mg/kg/day) of pancreas were within the laboratory background values in males obtained within about 2 years before and after this study (1996-2000, mean absolute weight, 3.52 g; maximum absolute weight, 4.31 g; mean relative weight, 0.168 g; maximum relative weight, 0.216 g). Also, as pathological changes of the pancreas including histopathology suggestive of pancreatitis were not observed, these findings were not considered of toxicological significance. Anti-liraglutide antibodies were not detected in any animal. The NOAEL was determined to be 5.0 mg/kg/day.

3.(iii).A.(2).5) Cynomolgus monkey 3-month subcutaneous dose study with 2-week recovery period (4.2.3.2.10)

Cynomolgus monkeys (n = 4/sex/dose, n = 2/sex/recovery group for 0 and 5.0 mg/kg/day only) were subcutaneously injected with 0 (control), 0.05, 0.5, or 5.0 mg/kg/day of liraglutide for 3 months. As a result, decreased food consumption at ≥ 0.05 mg/kg/day and lower body weight at ≥ 0.5 mg/kg/day occurred, which were not considered findings of toxicological significance. Anti-liraglutide antibodies were not detected in any animal. The NOAEL was determined to be 5.0 mg/kg/day.

3.(iii).A.(2).6) Cynomolgus monkey 12-month subcutaneous dose study with 4-week recovery period (4.2.3.2.11)

Cynomolgus monkeys (n = 4/sex/dose, n = 2/sex/recovery group for 0 and 5.0 mg/kg/day only) were subcutaneously injected with 0 (control), 0.05, 0.5, or 5.0 mg/kg/day of liraglutide for 12 months. As a result, at ≥ 0.5 mg/kg/day, decreased food consumption and lower body weight occurred, which were not considered findings of toxicological significance. At ≥ 0.5 mg/kg/day, increased pancreas weight occurred. However, as pathological changes of the pancreas including histopathology suggestive of pancreatitis were not observed, this finding was not considered of toxicological significance. The NOAEL was determined to be 5.0 mg/kg/day.

3.(iii).A.(3) Genotoxicity (4.2.3.3.1.1, 4.2.3.3.1.2, 4.2.3.3.2.1, 4.2.3.3.2.2)

Liraglutide was negative for genotoxicity in a bacterial reverse mutation assay, a chromosomal aberration assay using human lymphocytes, a rat bone marrow micronucleus assay, and a micronucleus assay using peripheral blood and bone marrow from rats.

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

Carcinogenicity studies in mice and rats were conducted and thyroid C-cell neoplastic lesions occurred in both mice and rats. Plasma calcitonin levels increased in mice. It has been discussed that mechanistic studies of liraglutide-induced rodent thyroid C-cell tumors suggested that calcitonin release from thyroid C-cells mediated by persistent activation of GLP-1 receptors on C-cells by liraglutide leads to C-cell tumors. It has also been discussed that this phenomenon is specific to rodents and is not relevant to humans.

3.(iii).A.(4).1) Mouse 2-year carcinogenicity study (4.2.3.4.1.1)

CD-1 mice (n = 67/sex/dose, or n = 79/sex/dose [in the 0 or 3.0 mg/kg/day groups]) were subcutaneously injected with 0 (control), 0.03, 0.2, 1.0, or 3.0 mg/kg/day of liraglutide for 104 weeks. Thyroid C-cell focal hyperplasia at ≥ 0.2 mg/kg/day (males, 0 of 79 mice, 0 of 66 mice, 1 of 65 mice, 11 of 67 mice, and 30 of 79 mice, respectively; females, 0 of 75 mice, 0 of 66 mice, 7 of 67 mice, 10 of 66 mice, and 22 of 76 mice, respectively), C-cell adenomas at ≥ 1.0 mg/kg/day (males, 0 of 79 mice,

0 of 66 mice, 0 of 65 mice, 9 of 67 mice, and 15 of 79 mice, respectively; females, 0 of 75 mice, 0 of 66 mice, 0 of 67 mice, 4 of 66 mice, and 15 of 76 mice, respectively), and C-cell carcinomas at 3.0 mg/kg/day (males, 0 of 79 mice, 0 of 66 mice, 0 of 65 mice, 0 of 67 mice, and 0 of 79 mice, respectively; females, 0 of 75 mice, 0 of 66 mice, 0 of 67 mice, 0 of 66 mice, and 2 of 76 mice, respectively) occurred. All findings were dose-related. There were no definitive differences in these findings between males and females. A small number of males had thyroid follicular cell adenomas (1 of 79 mice, 1 of 66 mice, 0 of 65 mice, 2 of 67 mice, and 1 of 79 mice, respectively) and 1 male in the 3.0 mg/kg/day group had thyroid follicular cell carcinoma. At ≥ 0.03 mg/kg/day, plasma calcitonin levels increased.

Pancreatic inflammatory cell infiltration occurred in 7 of 50 female decedents treated with the highest dose in the mouse carcinogenicity study (only 1 of 56 mice in the control group) (4.2.3.4.1).

Table 4. Pancreatic findings in mouse carcinogenicity study (pancreatitis and pancreatic inflammatory cell infiltration)

	Male										Female									
	Survivors					Decedents					Survivors					Decedents				
Dose (mg/kg/day)	0	0.03	0.2	1	3	0	0.03	0.2	1	3	0	0.03	0.2	1	3	0	0.03	0.2	1	3
No. of mice analyzed	39	34	37	38	32	40	33	30	29	47	23	14	23	23	26	56	53	43	43	50
Pancreatitis	1	1	0	0	1	2	1	1	1	0	0	1	1	1	2	0	2	2	2	1
Pancreatic inflammatory cell infiltration	0	3	3	2	6	2	1	0	1	3	3	4	5	3	2	1	4	2	0	7

Samples for anti-liraglutide antibody assay were taken at Treatment Weeks 0, 26, 52, 78, and 104. Anti-liraglutide antibodies were not detected.

3.(iii).A.(4).2) Rat 2-year carcinogenicity study (4.2.3.4.1.2)

SD rats (n = 50/sex/dose) were subcutaneously injected with 0 (control), 0.075, 0.25, or 0.75 mg/kg/day of liraglutide for 104 weeks. As a result, necropsy revealed enlarged thyroid at ≥ 0.75 mg/kg/day (males, 0 of 50 rats, 0 of 50 rats, 5 of 50 rats, and 7 of 50 rats, respectively; females, 0 of 50 rats, 0 of 50 rats, 1 of 50 rats, and 2 of 50 rats, respectively). Enlarged thyroid tended to occur more frequently in males. Histopathological examination revealed thyroid C-cell focal hyperplasia (males, 11 of 50 rats, 14 of 49 rats, 20 of 50 rats, and 24 of 50 rats, respectively; females, 14 of 50 rats, 14 of 49 rats, 27 of 49 rats, and 24 of 50 rats, respectively), C-cell adenomas (males, 6 of 50 rats, 8 of 49 rats, 21 of 50 rats, and 23 of 50 rats, respectively; females, 5 of 50 rats, 13 of 49 rats, 16 of 49 rats, and 28 of 50 rats, respectively), and C-cell carcinomas (males, 1 of 50 rats, 4 of 49 rats, 3 of 50 rats, and 7 of 50 rats, respectively; females, 0 of 50 rats, 0 of 49 rats, 2 of 49 rats, and 3 of 50 rats, respectively) in all groups and all findings were dose-dependent. For focal hyperplasia and C-cell adenomas, there were no differences between males and females, whereas C-cell carcinoma tended to occur more

frequently in males. Thyroid follicular cell adenoma or thyroid follicular cell carcinoma were reported in 0 to 2 rats in each group. No other treatment-related tumors were observed. Anti-liraglutide antibody assay was not performed.

3.(iii).A.(4).3) Mechanistic studies of liraglutide-induced C-cell tumors in mice and rats (4.2.3.7.3.1)

As thyroid C-cell tumors were induced by liraglutide in mice and rats, the following (a) to (e) studies were performed to analyze the mode of action to induce tumors.

(a) Investigation of GLP-1 receptor expression in mouse, rat, cynomolgus monkey, and human C-cells

The following studies were performed to compare the levels of GLP-1 receptor expression in animal species used in toxicity studies and humans.

Immunohistochemical staining (4.2.3.7.3.2) identified GLP-1 receptor expression in CD-1 mouse, SD rat, cynomolgus monkey, and human thyroid C-cells. Quantitative assessment was not performed by this method.

Next, using an *in vitro* test system with C-cell lines (rat, rMTC6-23 and CA77; human, TT, derived from human C-cell carcinoma), RT-PCR quantification was performed (4.2.3.7.3.7). As a result, it was shown that relative levels of GLP-1 receptor mRNA were ≥ 14 -fold higher in rat C-cell lines compared to human TT cells. GLP-1 receptor mRNA was undetectable in cynomolgus monkey and human thyroid tissues by *in situ* hybridization (4.2.3.7.3.3).

The above results indicated that the level of GLP-1 receptor expression is remarkable in rat C-cells and very low in cynomolgus monkey and human C-cells.

(b) *In vitro* studies on C-cell GLP-1 receptor activation and calcitonin release

The following studies were performed to elucidate differences in the biological properties at a cellular level after liraglutide administration, as a result of the difference in the level of GLP-1 receptor expression between rat and human C-cells as demonstrated in (a).

Using *in vitro* C-cell lines (rat, rMTC6-23 and CA77; human, TT; cell lines derived from human C-cell carcinoma, SINJ, SHER-1, and MTC-SK), the receptor bioactivity as measured by cAMP accumulation and calcitonin release after administration of liraglutide and GLP-1 receptor agonists (GLP-1 and exenatide) (4.2.3.7.3.8 and 4.2.3.7.3.9) was determined. As a result, remarkable cAMP accumulation and calcitonin release in rat C-cells were observed and were blocked by a GLP-1

receptor antagonist, exendin (9-39), demonstrating that these effects were GLP-1 receptor mediated. On the other hand, cAMP activity and calcitonin release in human C-cells were both near the detection limit (liraglutide and GLP-1 receptor agonists increased cAMP about 40-fold in the rat C-cell line MTC6-23 and about 2-fold in the human C-cell line TT) (4.2.3.7.3.8).

The above results revealed that liraglutide causes remarkable calcitonin release from rat C-cells via intracellular cAMP accumulation while the response in human C-cells is weaker than that in rat C-cells. These findings support the results from (a) in terms of biological events occurring in the downstream of GLP-1 receptors.

(c) *In vivo* studies on calcitonin synthesis and C-cell hyperplasia

In order to determine how the events at a cellular level, i.e., high expression of GLP-1 receptors on rat C-cells and calcitonin secretion from rat C-cells as demonstrated in (a) and (b), are associated with C-cell tumors observed in the carcinogenicity studies and furthermore, determine whether these phenomena occur also in cynomolgus monkeys, the following studies were performed.

Calcitonin secretion and mRNA transcriptional activity, and C-cell proliferation were investigated after 9-week subcutaneous administration of 0 (control), 0.2, and 5.0 mg/kg/day of liraglutide in 80 mice/sex/dose in “a mouse 9-week exploratory study - investigations of calcitonin secretion and mRNA transcriptional activity and pathological examination of thyroid C-cells” (4.2.3.7.3.15) and after 6-week subcutaneous administration of 0 (control) and 0.75 mg/kg/day of liraglutide in 64 male rats/group in “Effects on calcium homeostasis and relative volume of thyroid C-cells and follicular cells after 6-week daily subcutaneous administration followed by a 2-week recovery period in male rats” (4.2.3.7.3.20). As a result, it was shown that following liraglutide administration to mice and rats, plasma calcitonin was elevated before C-cell proliferative changes were observed. In mice, calcitonin mRNA levels increased 2.0-fold and 4.0-fold at 0.2 and 5 mg/kg/day, respectively over concurrent controls after 2 weeks of treatment. In rats, calcitonin mRNA levels in the 0.75 mg/kg/day group increased 1.5-fold compared to the control group after 4 weeks of treatment. Then, in “a cynomolgus monkey 20-month mechanistic study” (4.2.3.7.3.23), cynomolgus monkeys (n = 5/sex/dose) were subcutaneously injected with 0 (control), 0.25, or 5.0 mg/kg/day of liraglutide and plasma calcitonin was determined every 4 weeks and C-cell proliferation was assessed by quantitative analysis of immunohistochemical staining. As a result, even at 5.0 mg/kg/day, which was $\geq$ 100-fold higher than the intended clinical dose in humans (exposure ratio), plasma calcitonin was not elevated and C-cell tumors also did not occur.

The above results indicated that in light of the time course for the development of C-cell hyperplasia, C-cell hyperplasia may be caused by calcitonin secretion from C-cells after liraglutide administration

as observed in mice and rats and these phenomena did not occur in the 20-month cynomolgus monkey study.

In summary, regarding thyroid C-cell hyperplasia after liraglutide administration in mice and rats, it has been inferred from the results from (a) (b) (c) that liraglutide activates GLP-1 receptors on C-cells, which stimulates calcitonin release from C-cells via intracellular cAMP accumulation, and furthermore, chronic C-cell stimulation due to persistent calcitonin secretion from C-cells causes C-cell hyperplasia. It has been discussed that species differences in the development of C-cell tumors are associated with high expression of GLP-1 receptors, which are involved in tumor induction, in rodents.

Then, concerning the mechanism of development of C-cell tumors after liraglutide administration in mice and rats, the following (d) and (e) studies were performed to address the possibility that an additional mechanism other than the above (a) (b) (c) may exist.

(d) Study on the mitogenic potential of liraglutide in rat and human C-cell lines

In “a study using rat thyroid C-cell lines rMTC6-23 and CA77, human thyroid C-cell line TT, and rat insulinoma INS1E cells” (4.2.3.7.3.10), the direct mitogenic potential of GLP-1, exenatide, and liraglutide in human and rat C-cells was assessed. As GLP-1 stimulates rat insulinoma cell proliferation in a dose-dependent manner (Buteau J, *et al.*, *Diabetologia*, 1999; 42: 856-864), rat insulinoma cells were used as a positive control in this study. As a result, rat insulinoma cells proliferated in response to GLP-1 and liraglutide, but rat or human C-cells did not proliferate. Thus, liraglutide is not a mitogen in C-cells.

(e) Studies on crossreactivity to receptors other than GLP-1 receptor

In a study using BHK cells expressing the human calcitonin receptor (4.2.3.7.3.11), a calcitonin receptor binding study was performed to exclude a feedback loop mechanism where liraglutide could crossreact with the calcitonin receptor. In “a study using AR42J rat pancreatic cells expressing CCK2 and BB2 receptors” (4.2.3.7.3.12), binding studies to bombesin (BB2), neuromedin, and cholecystokinin (CCK) receptors expressed in cells were performed to investigate the possibility that liraglutide is involved in the initial stage of C-cell proliferation via these receptors. As a result, as no crossreactivity of liraglutide to any of these receptors was found, the possibility of a mechanism of tumor development via receptors other than the GLP-1 receptor on C-cells is considered low.

It is considered that the conclusions of (a) (b) (c) are supported by the results from (d) and (e).

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

A rat study of fertility and embryo-fetal development, a rabbit embryo-fetal development study, and a

rat study for effects on pre- and postnatal development, including maternal function were conducted.

Liraglutide decreased the weight of testes and prostate in paternal rats and increased the incidence of early embryonic deaths in maternal rats. In rabbits, liraglutide caused minor fetal skeletal abnormalities, which were inferred to be associated with decreased food consumption in maternal animals.

Liraglutide has been shown to cross the placenta and be excreted in milk, though in low levels (4.2.2.3.8, 4.2.2.3.9, 4.2.2.5.2-5).

3.(iii).A.(5).1) Rat study of fertility and embryo-fetal development (4.2.3.5.1.2)

SD rats (n = 24/sex/dose) were subcutaneously injected with liraglutide at doses of 0 (control), 0.1, 0.25, and 1.0 mg/kg/day. Males were dosed from 4 weeks prior to mating until females were necropsied (gestation day 20) and females were dosed from 2 weeks prior to mating until gestation day 17. In both male and female rats at ≥ 0.1 mg/kg/day, decreased food consumption and lower body weight occurred, which were considered pharmacologic effects of liraglutide and were not considered findings of toxicological significance. In male rats, decreased seminal vesicle weight at ≥ 0.25 mg/kg/day and decreased prostate weight at 1.0 mg/kg/day were observed. There were no histopathological abnormalities in the testes or ovaries. Liraglutide had no effect on mating or fertility indices in males. As embryo-fetal effects, the incidence of early embryonic deaths and the incidence of kinked ribs increased at 1.0 mg/kg/day. The NOAELs were determined to be 0.1 mg/kg/day (2 times the MRHD exposure in Japanese patients) for paternal general toxicity, 1.0 mg/kg/day (21 times the MRHD exposure in Japanese patients) for maternal general toxicity, 1.0 mg/kg/day (21 times the MRHD exposure in Japanese patients) for paternal reproductive toxicity, and 0.25 mg/kg/day (6 times the MRHD exposure in Japanese patients) for maternal reproductive toxicity and for fetal toxicity. Decreased seminal vesicle weight observed in paternal rats was likely to be associated with decreased food consumption occurring most notably in the first week of treatment and as eating and nutritional status can be controlled in patients treated with liraglutide, there should practically be no clinical concerns.

3.(iii).A.(5).2) Rabbit embryo-fetal development study (4.2.3.5.2.2)

Pregnant rabbits (n = 20/dose) were subcutaneously injected with liraglutide at doses of 0 (control), 0.01, 0.025, and 0.05 mg/kg/day from gestation day 6 to gestation day 18. The highest dose (0.05 mg/kg/day) was selected based on findings of sharp decreases in food consumption and reduced body weight at 0.1 mg/kg/day in an exploratory dose-range finding study. At ≥ 0.01 mg/kg/day, decreased food consumption and lower body weight occurred, which were considered pharmacologic effects of liraglutide and were not considered toxicity effects. The incidence of fetuses with skeletal variations of

jugals connected or fused to maxilla increased at 0.05 mg/kg/day. The incidence of fetuses with supernumerary ribs increased at 0.025 and 0.05 mg/kg/day, which was considered associated with decreased food consumption and lower body weight in maternal animals in the early phase of treatment (Rogers JM, *et al.*, *Birth Defects Res*, 2004; 71: 17-25). The NOAEL for maternal general and reproductive toxicity was determined to be 0.05 mg/kg/day (1.7 times the MRHD exposure in Japanese patients) and the NOAEL for developmental toxicity was determined to be 0.01 mg/kg/day (0.3 times the MRHD exposure in Japanese patients) based on minor skeletal abnormalities.

3.(iii).A.(5).3) Rat study for effects on pre- and postnatal development, including maternal function (4.2.3.5.3.1)

Pregnant rats (n = 24/dose) were subcutaneously injected with liraglutide at doses of 0 (control), 0.1, 0.25, and 1.0 mg/kg/day from gestation day 6 to lactation day 24. In F0 dams at ≥ 0.1 mg/kg/day, decreased food consumption and lower body weight occurred, which were not considered findings of toxicological significance. The NOAEL for F1 pups was based on lower body weight in F1 pups from F0 rats at ≥ 0.1 mg/kg/day. In male pups from F0 rats at 1.0 mg/kg/day, about 10% lower body weight compared to controls persisted during the postweaning period, but there were no treatment-related effects on sexual maturity or physical development, development assessed by behavioral/functional/developmental tests after weaning, or reproductive function and no teratogenic effects were observed. In the F2 generation, mean litter weight was decreased at 1.0 mg/kg/day, which was considered attributed to lower body weight in the F1 generation. The F0 maternal NOAEL for general and reproductive toxicity was determined to be 1.0 mg/kg/day (21 times the MRHD exposure in Japanese patients) and the F1 pup NOAEL was determined to be < 0.1 mg/kg/day based on lower pup body weight throughout the lactation period.

3.(iii).A.(6) Local tolerance (4.2.3.6.1, 4.2.3.6.2, 4.2.3.6.3)

Local tolerability of three formulations of liraglutide (Formulations 1, 2, and 3) in pigs was evaluated. At Day 2 and Day 5 after single subcutaneous injection, slight to moderate injection site inflammation occurred with all formulations. There were no differences in injection site reactions among formulations.

3.(iii).A.(7) Study on impurities (4.2.3.7.6.1)

A 1-month repeated subcutaneous dose toxicity study in rats was conducted to assess degradation products and impurities of liraglutide. Rats (n = 10/sex/group) were administered 0 mg/kg/day of liraglutide (control), 1.0 mg/kg/day of liraglutide in Formulation 3, or 1.0 mg/kg/day of liraglutide in Formulation 4 (the to-be-marketed formulation) that had undergone forced degradation (stored at 37°C for 2 months). As a result, both formulations of liraglutide decreased body weight and food consumption, which were considered pharmacologic effects of liraglutide. Decreased heart and

salivary gland weights occurred with both formulations and especially rats treated with liraglutide in Formulation 4 that had undergone forced degradation had slightly lower weights, but the decrease in the relative weight of heart or salivary gland was about 10%. Increased ALT and AST occurred in the both formulation groups. However, due to a lack of correlative histopathology, the applicant explained that all of the above findings were of little toxicological significance.

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

3.(iii).B.(1) Thyroid C-cell tumors

As increased calcitonin secretion leading to C-cell adenomas observed in rodent repeat-dose toxicity and carcinogenicity studies of liraglutide have also been reported with a drug of the same class, exenatide (US Food and Drug Administration [FDA]. Byetta [exenatide], FDA pharmacology review, 2005), PMDA asked the applicant to explain whether thyroid tumors have been reported in exenatide-treated patients, with a view to assessment of the human relevance of C-cell adenomas.

The applicant responded as follows:

A search of adverse events reported from exenatide clinical trials and post-marketing surveillance (FDA review report 2005, FDA SRS-AERS post-marketing surveillance database, European public assessment report [EPAR, December 2005]) was conducted. As a result, thyroid tumors (C-cell adenoma/C-cell carcinoma) assessed as causally related to the test drug were not identified.

Furthermore, PMDA asked the applicant to explain whether GLP-1 receptors were seen on human thyroid follicular cells, with a view to assessing the possibility that liraglutide was involved in the development of papillary thyroid carcinomas reported in liraglutide clinical trials (NN2211-1334, 1436, 1573, 1574).

The applicant responded as follows:

Immunohistochemical staining of normal thyroid tissues from humans showed that GLP-1 receptor was not found on follicular cells in the thyroid (4.2.3.7.3.2) or other cells than C-cells. In mouse and rat carcinogenicity studies, the incidence of follicular cell tumors did not increase and there was no relationship between the incidence of C-cell tumors and that of follicular cell tumors.

PMDA considers as follows:

Papillary thyroid carcinomas reported in liraglutide clinical trials were unrelated to liraglutide as the mechanism of its development is different from that of liraglutide-induced C-cell tumors in rodents. Based on the aforementioned response of the applicant, liraglutide is unlikely to cause medullary carcinoma (C-cell carcinoma) in humans. However, the risk of C-cell tumors including medullary carcinoma associated with the clinical use of liraglutide should be noted for the following reasons: the

exenatide post-marketing surveillance cited by the applicant covered a limited period (e.g., about 2 years in the UK); and C-cell carcinoma occurred dose-dependently in mouse and rat carcinogenicity studies of liraglutide, whereas for exenatide, C-cell tumors still at the stage of adenoma occurred only in rats without dose-dependency. In addition, as the liraglutide mechanistic studies investigating receptors other than GLP-1 receptor tested only limited molecules reported to be involved in C-cell proliferation in the literature, the possibility that liraglutide contributes to the development of C-cell tumors via other unreported molecules can not be excluded.

Based on the above, PMDA highlighted the findings of thyroid C-cell tumors observed in the liraglutide carcinogenicity studies and asked the applicant to consider whether the findings of thyroid C-cell tumors should be listed in the draft package insert.

The applicant responded that they agreed to list these findings in the draft package insert.

PMDA is asking the applicant to develop a specific statement in the draft package insert [See “4.(iii).B.(3).3 Thyroid effects” for thyroid effects in humans].

3.(iii).B.(2) Pancreatic inflammatory cell infiltration

Pancreatic inflammatory cell infiltration occurred at the highest dose in a mouse carcinogenicity study of liraglutide (4.2.3.4.1.1). Two cases of pancreatitis assessed as “possibly related” to liraglutide have been reported in foreign clinical trials (1 case each in NN2211-1572 and NN2211-1797) and there are post-marketing adverse event reports of pancreatitis also with a drug of the same class, exenatide. PMDA asked the applicant to explain the risk of pancreatitis associated with liraglutide in humans.

The applicant responded that pancreatic inflammatory cell infiltration observed in the mouse carcinogenicity study was localized and sporadically found also in untreated controls and is irrelevant to the cases of pancreatitis reported in the foreign clinical trials.

PMDA accepted the applicant’s response that localized inflammatory cell infiltration in mice is irrelevant to human pancreatitis, because Table 4 shows no correlation between the incidence of pancreatic inflammatory cell infiltration and that of pancreatitis in each treatment group, there is a literature that reaches a similar conclusion (Greaves P, Chapter 9: *Histopathology of preclinical toxicity studies*. Third edition, 2007; 457-569), and furthermore, there is a literature questioning if rodent pancreatitis is an appropriate model of human pancreatitis (Case RM, *Pancreatology*, 2006; 6: 180-190).

4. Clinical data

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

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

In clinical trials of liraglutide, four formulations (Formulations 1-4), which are different in the drug substance manufacturing process and the drug product formulation and manufacturing process, were used. The to-be-marketed formulation is Formulation 4, which was used in Japanese phase II/III and phase III clinical trials.

Both protein bound and unbound liraglutide in human biological samples were measured by an ELISA (lower limit of quantification, ■ pmol/L). Anti-liraglutide antibodies were measured using a RIA and samples considered positive were assayed for neutralizing activity against liraglutide using cultured cells.

The results from the following biopharmaceutic studies were submitted: foreign clinical trials to assess the bioavailability of liraglutide after subcutaneous administration and the bioequivalence of four formulations of liraglutide (NN2211-1149, 1331, 1636, 1692, 1693, 1745). The results from the main studies are described below.

4.(i).A.(1) Bioavailability study (5.3.1.1.1, Trial NN2211-1149 [March to December 1999], Reference data)

A randomized, double-blind, placebo-controlled, dose escalation study of single doses of liraglutide to assess tolerability, pharmacokinetics, pharmacodynamics, and bioavailability in foreign healthy adult male subjects was conducted.

Liraglutide was administered as single subcutaneous doses of 1.25, 2.5, 5, 10, 12.5, 15, 17.5, and 20 µg/kg of liraglutide (Formulation 1) (6 subjects receiving liraglutide and 2 subjects receiving placebo in each dose group), and the subjects receiving the subcutaneous dose level 5 µg/kg additionally received, after a washout of at least 7 days, a single intravenous dose of 5 µg/kg liraglutide or placebo. Three subjects in each dose group (2 liraglutide, 1 placebo) underwent an intravenous glucose tolerance test (IVGTT) for 2 hours, starting approximately 9 hours after study drug administration. The 1.25 µg/kg dose level was repeated.

All of 72 treated subjects (54 subjects treated with liraglutide, 18 subjects treated with placebo) were

included in the safety and pharmacodynamic analyses¹ and among the 54 subjects treated with liraglutide, 5 subjects did not receive the specified dose (3 subjects in the 1.25 µg/kg group, 2 subjects in the 5 µg/kg group) and 49 subjects excluding these 5 subjects were included in the pharmacokinetic analysis.

Pharmacokinetic analysis showed that the absorption of liraglutide following subcutaneous administration was slow, the $t_{\max}$ occurred approximately 9 to 12 hours post-dosing, and there was a dose-proportional increase in $C_{\max}$ and $AUC_{0-\text{inf}}$ for doses between 2.5 and 20 µg/kg. The bioavailability for the 5 µg/kg subcutaneous dose (equivalent to 0.29-0.49 mg based on body weight of subjects in this study) was $55 \pm 37\%$.

As to pharmacodynamics, in 61 subjects included in the analysis, there was a trend towards lower average glucose levels following liraglutide administration compared to placebo. However, this trend was not seen in subjects undergoing IVGTT (20 subjects). For insulin, in subjects undergoing IVGTT (20 subjects), there was a trend towards higher insulin levels following liraglutide administration, but this trend was not seen in 61 subjects included in the analysis. Glucagon (61 subjects included in the analysis, 20 subjects undergoing IVGTT), serum leptin levels (69 subjects), or diuresis (72 subjects) were not affected by liraglutide compared to placebo.

Regarding safety, adverse events occurred in 9 subjects of the 1.25 µg/kg group (17 events), 5 subjects of the 2.5 µg/kg group (5 events), 3 subjects of the subcutaneous 5 µg/kg group (4 events), 4 subjects of the intravenous 5 µg/kg group (7 events), 4 subjects of the 10 µg/kg group (18 events), 4 subjects of the 12.5 µg/kg group (9 events), 5 subjects of the 15 µg/kg group (10 events), 5 subjects of the 17.5 µg/kg group (15 events), 6 subjects of the 20 µg/kg group (17 events), 7 subjects of the subcutaneous placebo group (17 events), and 1 subject of the intravenous placebo group (1 event), most of which were dizziness and headache. All 6 subjects in the 20 µg/kg group experienced a total of 13 gastrointestinal adverse events, including 1 severe event (vomiting).

4.(i).A.(2) Relative bioavailability study of liraglutide administered at different injection sites (5.3.1.2.5, Trial NN2211-1745 [February to May 2007], Reference data)

A randomized, open-label, three period cross-over study in foreign healthy adult male and female subjects was conducted to compare the pharmacokinetic profiles after single dose administration of

¹ All of 72 treated subjects were included in analysis for diuresis and 69 subjects excluding 3 subjects (2 subjects in the 5 µg/kg group who did not receive the specified dose, 1 subject in the 17.5 µg/kg group with high blood glucose [7.3 mmol/L, it was likely that the subject had a meal before IVGTT]) were included in analysis for leptin levels. For analyses for glucose, glucagon, and insulin in all subjects, 61 subjects excluding 11 subjects (all of 8 subjects in the initial 1.25 µg/kg group due to different timing of IVGTT, the aforementioned 2 subjects in the 5 µg/kg group and 1 subject in the 17.5 µg/kg group) were included. For analyses for glucose, glucagon, and insulin in subjects undergoing IVGTT, 20 subjects excluding 7 subjects (all of 3 subjects in the initial 1.25 µg/kg group due to different timing of IVGTT, all of 3 subjects in the 17.5 µg/kg group due to the exclusion of the aforementioned 1 subject in the 17.5 µg/kg group who served as a control, the aforementioned 1 subject in the 5 µg/kg group) were included.

liraglutide at three different injection sites (administration in the abdomen, thigh, or upper arm).

Single subcutaneous doses of 0.6 mg of liraglutide (Formulation 4) were administered and the wash-out period was 1 to 3 weeks.

All of 21 treated subjects (22-49 years old) were included in the pharmacokinetic and safety analyses.

Pharmacokinetic analysis showed that the relative bioavailability ($AUC_{0-\infty}$ ratio [90% CI]) was 0.81 [0.76, 0.86] after subcutaneous injection of liraglutide in the thigh versus the abdomen, 0.90 [0.83, 0.96] after subcutaneous injection of liraglutide in the upper arm versus the abdomen, and 1.11 [1.03, 1.19] after subcutaneous injection of liraglutide in the upper arm versus the thigh and the 90% CI of the relative bioavailability between injection of liraglutide in the thigh and the abdomen did not fall within the pre-defined limits of 0.80 to 1.25.

Regarding safety, adverse events occurred in 2 subjects after injection in the abdomen (3 events), 2 subjects after injection in the thigh (2 events), and 3 subjects after injection in the upper arm (5 events), of which 7 events (nausea [6], head fullness [1]) were those for which a causal relationship to study drug could not be denied (adverse drug reactions).

4.(i).A.(3) Bioequivalence studies (5.3.1.2.1-4, Trials NN2211-1331, 1636, 1692, 1693 [May to July 2004, January to March 2005, January to April 2007, April to June 2006], Reference data)

Randomized, double-blind (NN2211-1331 was a single-blind trial), cross-over studies in foreign healthy adult male and female subjects (NN2211-1331, 19-43 years old; NN2211-1636, 18-45 years old; NN2211-1692, 19-27 years old; NN2211-1693, 19-28 years old) were conducted to investigate the bioequivalence of four formulations of liraglutide used in clinical development. The results of these studies were as shown in Table 5.

Table 5. Results of bioequivalence studies

Trial Number	Formulation	Dose	C_{max} ratio [90% CI]	$AUC_{0-\infty}$ ratio [90% CI]
NN2211-1331	Formulation 2 and Formulation 3 (pH 4.5)	1 mg	0.96 [0.89, 1.04]	0.98 [0.92, 1.04]
NN2211-1636	Formulation 3 at pH 4.5 and at pH 5.5	0.75 mg	0.93 [0.88, 1.00]	0.93 [0.89, 0.98]
	Formulation 3 at pH 4.5 and at pH 6.5	0.75 mg	1.02 [0.96, 1.09]	1.02 [0.97, 1.07]
NN2211-1692	Formulation 4 and Final formulation 4	0.72 mg	1.02 [0.91, 1.14]	0.99 [0.92, 1.06]
NN2211-1693	Formulation 3 (pH 4.5) and Formulation 4	0.71 mg	1.04 [0.95, 1.13]	1.06 [1.00, 1.13]

The 90% CIs for the ratios of C_{max} and $AUC_{0-\infty}$ between the pre-change and post-change formulations were contained within the pre-defined limits of 0.80 to 1.25. As Formulation 1 is identical to Formulation 2 (5 mg/mL), the above results demonstrated the bioequivalence between the pre-change and post-change formulations for all formulations used in clinical development.

Regarding safety, there were no clinically relevant adverse events except for 1 serious adverse event (moderate vomiting; causally related to study drug; the subject was withdrawn) after administration of Formulation 3 at pH [REDACTED] reported in Trial NN2211-1636.

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

PMDA asked the applicant to explain the mechanism of absorption of subcutaneously injected liraglutide, the factors affecting absorption, and the basis for concluding that a lower bioavailability after injection in the thigh versus the abdomen in Trial NN2211-1745 is not clinically relevant.

The applicant responded as follows:

Following subcutaneous administration, a drug can be transported to the systemic circulation either by the blood capillaries or by the lymphatics. For small molecules up to 1000 Da, the blood capillary wall diffusivity is very high and represents a small barrier to drug transport. However, permeability of macromolecules through the blood capillary is low, and therefore, direct movement into the blood is restricted. Hence, soluble macromolecules were found to enter the bloodstream indirectly by way of lymphatic vessels (Supersaxo A, *et al.*, *Pharmaceutical Research*, 1990; 7: 167-169). Liraglutide self-associates into heptamers in an aqueous solution or in the drug product and after subcutaneous administration, liraglutide monomers are slowly released from the heptamers and absorbed into the systemic circulation via the blood capillaries or the lymphatics, or the heptamers enter the bloodstream via the lymphatics and dissociate into monomers. Based on the above, as subcutaneous tissues and lymphatics are involved in the absorption of liraglutide, skin conditions affecting the skin anatomy at or near the injection site may potentially influence the absorption of liraglutide.

The basis for concluding that a lower bioavailability after injection in the thigh versus the abdomen is not clinically relevant is as follows:

It is considered that after injection in the thigh compared to the abdomen, absorption was delayed, resulting in decreased AUC. It has been reported that the bioavailability of insulin products etc. also differs between different injection sites (Frid A & Linde B, *Diabet Med*, 1992; 9: 236-239, Clauson PG & Linde B, *Diabetes Care*, 1995; 18: 986-991) and although the mechanism of differences in the absorption rate has not completely been elucidated, it is considered that the absorption rate is affected by the density of blood capillaries, membrane permeability, and blood flow at the injection site. As liraglutide stimulates glucose-dependent insulin secretion, the recommended dose (0.9 mg) of liraglutide should lower blood glucose without increasing the risk of hypoglycemia. Therefore, even if blood concentrations differ by injection site, there will be no clinical problem from a safety and efficacy point of view and the same dose of liraglutide may be administered for three different injection sites (the upper arm, the abdomen, or the thigh).

PMDA accepted the response.

4.(ii) Summary of clinical pharmacology studies

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

The results from 10 *in vitro* studies using human biomaterials (2 plasma protein binding assays, 5 metabolism studies, 3 drug interaction studies, all reference data), Japanese clinical studies (NN2211-1326, 1551, 1694, 1591, 1334, all evaluation data), and foreign clinical studies (NN2211-1224, 1327-1330, 1332, 1573, 1589, 1608, 1698, 1699, 2063, all reference data) were submitted. The results from the main studies are described below.

4.(ii).A.(1) *In vitro* studies using human biomaterials (5.3.2.1.1-4, 5.3.2.2.1, 5.3.2.2.2, 5.3.2.3.1-4)

Liraglutide protein binding in human plasma (0.1 nmol/L to 1 μ mol/L, *in vitro*) was 98.8% to 99.2% in male subjects and 98.7% to 99.1% in female subjects, and liraglutide binding (10 nmol/L, *in vitro*) to human serum albumin (HSA) or α -acid glycoprotein was 99.4% or 99.3%, respectively. Warfarin, furosemide, tolbutamide, diazepam, glibenclamide, nicardipine, repaglinide, aspirin, valproic acid, acenocoumarol, phenprocoumon, metformin, pioglitazone, rosiglitazone, myristic acid, and palmitic acid did not affect liraglutide (10 nmol/L) protein binding in human plasma *in vitro*.

Liraglutide was sequentially cleaved into peptide fragments and amino acids by DPP-4 and NEP, which are involved in the metabolism of GLP-1. The initial cleavage occurred at amino acids 18-19, 19-20, 27-28, and 28-29 of the peptide backbone, and further cleavage resulted in shorter peptides (that include lysine to which a fatty acid is attached via a glutamic acid linker). Although the cleavage sites were the same as those reported for GLP-1, metabolism of liraglutide in human hepatocytes was slower compared to GLP-1. The rate of liraglutide metabolism depends on HSA concentration and the rate of liraglutide metabolism increased in the presence of a low concentration of HSA, but the rate of metabolism changed to a lesser extent over the HSA concentration range of 1% to 5% and liraglutide was stable in human plasma. On the other hand, GLP-1 metabolism in human plasma was rapid and extensive. Liraglutide metabolites formed from incubation with DPP-4 and NEP corresponded to metabolites identified *in vivo*. Liraglutide did not inhibit human CYPs ($IC_{50} > 100 \mu$ mol/L).

The above results indicated that liraglutide is unlikely to cause drug-drug interactions related to CYP inhibition or plasma protein binding.

4.(ii).A.(2) Human pharmacokinetics

4.(ii).A.(2).1 Japanese clinical studies

(a) Phase I single-dose study (5.3.3.1.1, Trial NN2211-1326 [December 2002 to March 2003])

A randomized, placebo-controlled, double-blind study was conducted to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics after single subcutaneous doses of liraglutide from 2.5 to 17.5 μ g/kg in Japanese healthy adult male subjects (target number of cases, 40; 8 cases per group).

Eight subjects per group (6 subjects receiving liraglutide and 2 subjects receiving placebo per group) were to receive a single subcutaneous dose of liraglutide (2.5, 5, 10, 15, or 17.5 µg/kg) or placebo. The 17.5 µg/kg dose group was cancelled due to the occurrence of adverse events (nausea and/or vomiting, “related to study drug”) at the 15 µg/kg dose level.

All of 32 treated subjects (22.1 ± 1.7 years, 24 subjects treated with liraglutide, 8 subjects treated with placebo) were included in the pharmacokinetic and safety analyses and 2 subjects in the 15 µg/kg dose group who consumed a very small meal due to gastrointestinal adverse events were excluded from the pharmacodynamic analysis. The pharmacokinetic parameters of liraglutide were as shown in Table 6.

Table 6. Pharmacokinetic parameters after a single subcutaneous dose of liraglutide

Dose (µg/kg)	AUC _{0-inf} (h·pmol/L)	C _{max} (pmol/L)	t _{max} (h)	t _{1/2} (h)	λ _z (1/h)	CL/F (L/h/kg)	V _z /F (L/kg)	MRT (h)
2.5	64005.2 (11724.3)	3129.0 (834.6)	7.50 (4.5-10.0)	10.13 (0.81)	0.06865 (0.00504)	0.01070 (0.00185)	0.1573 (0.0363)	19.32 (1.94)
5	134242.7 (14563.8)	4857.8 (720.6)	11.00 (8.0-12.0)	11.03 (1.18)	0.06335 (0.00676)	0.01002 (0.00105)	0.1590 (0.0175)	22.27 (2.60)
10	295248.0 (74748.6)	12267.3 (2601.8)	10.00 (5.5-12.0)	11.35 (0.87)	0.06135 (0.00497)	0.00961 (0.00281)	0.1558 (0.0380)	21.18 (2.59)
15	447940.0 (83133.1)	18378.0 (2937.9)	10.00 (8.0-12.0)	10.88 (0.90)	0.06402 (0.00527)	0.00921 (0.00181)	0.1440 (0.0270)	21.23 (2.94)

λ_z: terminal elimination rate constant, MRT: mean residence time
Mean (SD). Median (min.-max.) for t_{max} only.

Absorption of liraglutide was slow and C_{max} was reached 7.5 to 11 hours (median) after administration. Then, liraglutide was eliminated monophasically with a t_{1/2} of 10 to 11 hours (mean). AUC_{0-inf} and C_{max} were dose-proportional.

According to pharmacodynamic analysis, with respect to insulin release after a meal stimulus, being served 11 hours after administration of liraglutide, no difference between dose groups was observed for average serum insulin concentrations during the fasting period (until 11 hours post-dose) and after the meal. While no dose effect was seen for serum glucose concentrations during the fasting period, attenuation in peak glucose concentrations after the meal occurred in a dose-dependent fashion and a dose effect was seen also for the AUC for serum glucose.Suppressions in postprandial glucagon levels were most pronounced in the 10 and 15 µg/kg dose groups.

Regarding safety, 9 adverse events were reported from 4 subjects (2 events in 1 subject exposed to 2.5 µg/kg of liraglutide, 7 events in 3 subjects exposed to 15 µg/kg of liraglutide) and 50.0% of the 15 µg/kg dose group (3 of 6 subjects) experienced gastrointestinal disorders (nausea and/or vomiting). There were no clinically relevant abnormalities in clinical laboratory tests, ECG, or vital signs.

(b) Phase I multiple-dose study (5.3.3.1.2, Trial NN2211-1551 [September 2003 to March 2004])

A randomized, placebo-controlled, double-blind study was conducted to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics after multiple subcutaneous doses of 5 to 15 µg/kg of liraglutide in Japanese healthy adult male subjects (target number of cases, 24; 8 cases per group).

Eight subjects per group (6 subjects receiving liraglutide and 2 subjects receiving placebo per group) were to receive multiple subcutaneous doses of liraglutide (5, 10, or 15 µg/kg) or placebo once daily (5 µg/kg group, 5 µg/kg treatment for 21 days; 10 µg/kg group, 5 µg/kg for 7 days followed by 10 µg/kg for 14 days; 15 µg/kg group, 5 µg/kg for 7 days, 10 µg/kg for 7 days, and then 15 µg/kg for 7 days).

All of 24 treated subjects (24.7 ± 2.1 years, 18 subjects treated with liraglutide, 6 subjects treated with placebo) were included in the pharmacokinetic, pharmacodynamic, and safety analyses. The pharmacokinetic parameters of liraglutide were as shown in Table 7.

Table 7. Pharmacokinetic parameters after multiple subcutaneous doses of liraglutide

Dose (µg/kg)	AUC ₀₋₂₄ (h·pmol/L)	C _{max} (pmol/L)	t _{max} (h)	t _{1/2} (h)	λ _z (1/h)	CL/F (L/h/kg)	V _z /F (L/kg)	MRT (h)
5	138671.3 (16181.2)	7691.0 (794.0)	9.00 (4.0-24.0)	13.98 (1.09)	0.04987 (0.00397)	0.009732 (0.001193)	0.1955 (0.0238)	25.18 (2.77)
10	269333.2 (45464.4)	15269.2 (2793.7)	8.00 (5.0-10.0)	13.67 (0.97)	0.05102 (0.00362)	0.010128 (0.001608)	0.1998 (0.0376)	23.90 (2.20)
15	397468.7 (97412.0)	22414.3 (5041.6)	8.50 (5.0-10.0)	13.38 (0.78)	0.05195 (0.00313)	0.010610 (0.002733)	0.2023 (0.0410)	22.97 (3.47)

Mean (SD). Median (min.-max.) for t_{max} only.

Trough concentrations over time during the treatment period indicated that plasma concentrations reached a steady state relatively fast and after the last dose, the t_{max} was 8 to 9 hours (median) and the t_{1/2} was 13 to 14 hours (mean). AUC₀₋₂₄ and C_{max} were dose-proportional and the accumulation indices after multiple doses were 1.4 to 1.6.

As to pharmacodynamics, compared to a 24-hour baseline profile of serum glucose, the meal-induced increases in glucose levels after the 21-day liraglutide treatment were reduced and compared to placebo, the liraglutide treatment effect on glucose levels was found.

Regarding safety, 3 adverse events were reported by 2 subjects (1 event in 1 subject of the 5 µg/kg group [nasopharyngitis]; 2 events in 1 subject of the placebo group [alanine aminotransferase (ALT) increased and rash]). None of the subjects was withdrawn due to an adverse event and there were no clinically relevant abnormalities in ECG or vital signs. No anti-liraglutide antibodies were detected.

(c) Phase I multiple-dose study (5.3.3.1.3, Trial NN2211-1694 [January to April 2006])

A randomized, placebo-controlled, double-blind study was conducted to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics after multiple subcutaneous doses of 15 to 25 µg/kg of

liraglutide in Japanese healthy adult male subjects (target number of cases, 24; 8 cases per group).

Eight subjects per group (6 subjects receiving liraglutide and 2 subjects receiving placebo per group) were to receive multiple subcutaneous doses of liraglutide (15, 20, or 25 µg/kg) or placebo once daily (15 µg/kg group, 5 µg/kg for 7 days + 10 µg/kg for 7 days + 15 µg/kg for 21 days; 20 µg/kg group, 5 µg/kg for 7 days + 10 µg/kg for 7 days + 15 µg/kg for 7 days + 20 µg/kg for 14 days; 25 µg/kg group, 5 µg/kg for 7 days + 10 µg/kg for 7 days + 15 µg/kg for 7 days + 20 µg/kg for 7 days + 25 µg/kg for 7 days).

All of 24 treated subjects (aged 23.1 ± 2.4 years, 18 subjects treated with liraglutide, 6 subjects treated with placebo) were included in the pharmacokinetic, pharmacodynamic, and safety analyses. The pharmacokinetic parameters of liraglutide were as shown in Table 8.

Table 8. Pharmacokinetic parameters after multiple subcutaneous doses of liraglutide

Dose (µg/kg)	AUC ₀₋₂₄ (h·pmol/L)	C _{max} (pmol/L)	t _{max} (h)	t _{1/2} (h)	λ _z (1/h)	CL/F (L/h/kg)	V _z /F (L/kg)	MRT (h)	C _{trough} (pmol/L)
15	217412.7 (45412.8)	13767.5 (3065.8)	7.0 (5-12)	15.00 (1.32)	0.04647 (0.00443)	0.019020 (0.003685)	0.4117 (0.0871)	22.72 (2.75)	5475.7 (1549.9)
20	356999.2 (93532.6)	19463.5 (5256.8)	8.0 (7-14)	15.38 (1.63)	0.04547 (0.00458)	0.015777 (0.003952)	0.3483 (0.0913)	24.68 (3.38)	10075.7 (3252.8)
25	397752.8 (146950.6)	21253.3 (7998.1)	7.0 (1-10)	15.02 (1.24)	0.04637 (0.00367)	0.018047 (0.004207)	0.3903 (0.0946)	23.95 (3.66)	11503.3 (4438.1)

C_{trough}: trough concentration

Mean (SD). Median (Min.-Max.) for t_{max} only.

After the last dose, the t_{max} was 7 to 8 hours (median) and the t_{1/2} was 15 hours (mean). Although the AUC₀₋₂₄, C_{max}, and trough concentration increased dose-dependently, the inter-subject variability was large at 20 and 25 µg/kg. The estimated slope of the regression line [95% CI] was 1.16 [0.54, 1.78], 0.82 [0.18, 1.45], and 1.46 [0.71, 2.21], respectively.

Pharmacodynamic analysis showed that the plasma glucose and serum insulin concentrations after the last dose decreased with increasing dose of liraglutide.

Regarding safety, no adverse events were reported and there were no clinically relevant abnormalities in clinical laboratory tests, thyroid ultrasound, ECG, or vital signs. Formation of anti-liraglutide antibodies was not suggested.

(d) Phase I multiple-dose study (5.3.3.2.1, Trial NN2211-1591 [December 2003 to March 2004])

A randomized, placebo-controlled, double-blind study was conducted to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics after multiple subcutaneous doses of 5 and 10 µg/kg of liraglutide in Japanese male and female patients with type 2 diabetes mellitus (target number of cases, 16; 8 cases per dose group).

Eight subjects per group (6 subjects receiving liraglutide and 2 subjects receiving placebo per group) were to receive multiple subcutaneous doses of liraglutide (5 or 10 µg/kg) or placebo once daily in a dose-escalation regimen (5 µg/kg group, 5 µg/kg for 14 days; 10 µg/kg group, 5 µg/kg for 7 days followed by 10 µg/kg for 7 days).

Among 16 randomized subjects, 1 subject in the 10 µg/kg group was withdrawn from the study before the start of study treatment for a personal reason and all of the 15 treated subjects (aged 57.0 ± 6.1 years, 11 subjects treated with liraglutide, 4 subjects treated with placebo) were included in the pharmacokinetic, pharmacodynamic, and safety analyses. The pharmacokinetic parameters of liraglutide were as shown in Table 9.

Table 9. Pharmacokinetic parameters after multiple subcutaneous doses of liraglutide

Dose (µg/kg)	AUC ₀₋₂₄ (h·pmol/L)	C _{max} (pmol/L)	t _{max} (h)	t _{1/2} (h)	λ _z (1/h)	CL/F (L/h/kg)	V _z /F (L/kg)	MRT (h)	C _{trough} Day7 (pmol/L)	C _{trough} Day14 (pmol/L)
5	119024.8 (26978.2)	6113.3 (1062.9)	9.0 (7-14)	14.00 (1.58)	0.050 (0.006)	0.0141 (0.0052)	0.289 (0.112)	25.67 (1.97)	3618.7 (1118.2)	3636.8 (561.1)
10	237346.2 (63093.7)	13202.4 (4438.3)	12.0 (10-16)	14.54 (1.90)	0.048 (0.006)	0.0107 (0.0056)	0.230 (0.126)	27.78 (4.11)	4085.4 (820.0)	8190.0 (1716.2)

Mean (SD). Median (Min.-Max.) for t_{max} only.

Trough concentrations over time during the treatment period indicated that plasma concentrations reached a steady state relatively fast and after the last dose, the t_{max} was 9 to 12 hours (median) and the t_{1/2} was 14 to 15 hours (mean). AUC₀₋₂₄ and C_{max} were dose-proportional and the accumulation indices after multiple doses were 1.6 to 1.8.

As to pharmacodynamics, a decrease in fasting glucose concentrations from baseline during the 14 days of treatment was found and the decrease following the administration of 5 or 10 µg/kg of liraglutide was faster and greater compared to placebo. The average glucose level in the liraglutide 5 or 10 µg/kg group was significantly lower than the placebo group ($P < 0.001$). The postprandial glucose peak was reached 2 hours after a meal at baseline and following the administration of 5 or 10 µg/kg of liraglutide, the peak occurred 1 hour after a meal. The average insulin level remained higher in the 10 µg/kg group compared to the placebo group.

Regarding safety, 3 adverse events were reported by 3 subjects (1 event in 1 subject of the 5 µg/kg group [constipation]; 2 events in 2 subjects of the 10 µg/kg group [constipation, skin depigmentation]). The 2 reports of constipation from 2 subjects (1 event in 1 subject of the 5 µg/kg group, 1 event in 1 subject of the 10 µg/kg group following the administration of 5 µg/kg) were assessed as related to study drug, both of which were mild in severity. There were no treatment discontinuations due to adverse events and there were no clinically relevant abnormalities in ECG or vital signs. No anti-liraglutide antibodies were detected.

(e) Phase II clinical study (5.3.5.1.1, Trial NN2211-1334 [March 2005 to May 2006])

A randomized, double-blind, parallel-group, comparative study was conducted to evaluate the dose-response relationship for four doses of liraglutide (0.1-0.9 mg) and placebo with respect to glycemic control in Japanese patients with type 2 diabetes mellitus (aged 57.3 ± 8.2 years) [see “4. (iii).A.(1) Phase II clinical trial” for study design and safety data].

Plasma concentrations of liraglutide in each treatment group were as shown in Table 10.

Table 10. Plasma concentrations of liraglutide

Treatment group	Week 0	Week 2	Week 6	Week 10	Week 14 (LOCF)
Placebo	9.0 ± 0.0 (45)	10.3 ± 8.9 (45)	10.3 ± 8.2 (40)	11.4 ± 9.4 (40)	9.5 ± 3.4 (46)
Liraglutide 0.1 mg	9.0 ± 0.0 (45)	2112.9 ± 976.5 (44)	2048.1 ± 930.4 (41)	1924.3 ± 916.6 (41)	1997.5 ± 909.0 (45)
Liraglutide 0.3 mg	9.0 ± 0.0 (46)	5032.4 ± 1932.6 (46)	4862.8 ± 2023.5 (45)	4587.4 ± 2715.8 (43)	4230.7 ± 1794.3 (46)
Liraglutide 0.6 mg	9.3 ± 2.3 (44)	11076.2 ± 4129.8 (42)	9559.6 ± 4734.4 (42)	8243.2 ± 4660.6 (43)	9210.6 ± 4180.4 (45)
Liraglutide 0.9 mg	9.0 ± 0.0 (43)	9419.7 ± 4334.3 (42)	12442.3 ± 4888.7 (41)	10752.5 ± 5232.2 (41)	9676.0 ± 4519.4 (44)

mean $\pm$ SD (N)

4.(ii).A.(2).2) Foreign clinical studies (reference data)

(a) Phase I single-dose study (5.3.3.1.5, Trial NN2211-1699 [November to December 2006])

An open-label study was conducted to investigate the metabolites in plasma, urine, and feces after a single subcutaneous dose of ^3H -liraglutide in foreign healthy adult male subjects.

A single subcutaneous dose of 0.75 mg of liraglutide (a mixture of ^3H -liraglutide and unlabeled compound) was administered and blood was collected for 60 hours after dosing and urine and feces were collected until the excretion end criteria² were fulfilled or until 14 days post-dose, whichever came first.

All of 7 treated subjects (aged 47-60 years) were included in the pharmacokinetic and safety analyses.

Pharmacokinetic analysis showed that unchanged liraglutide represented as high as 89% to 100% of plasma radioactivity at 2 to 24 hours post-dose. Two metabolites were detected and comparison with the results from an *in vitro* study using DPP-4 and NEP suggested that DPP-4 and NEP are involved in the *in vivo* metabolism of liraglutide in humans. Twenty percent of the administered radioactivity was detected as tritiated water and three metabolites in urine and 6% of the administered radioactivity was detected as three metabolites in feces. No unchanged liraglutide was detected in urine or feces.

² Excreted levels of radioactivity reached 1000 dpm/g in pooled 24-hour samples.

Regarding safety, 7 adverse events were reported by 5 subjects and the most commonly reported event was dizziness.

(b) Single-dose study in elderly (5.3.3.3.1, Trial NN2211-1327 [April to June 2004])

An open-label trial was conducted to compare the pharmacokinetics and safety of liraglutide in young versus elderly and male versus female foreign healthy adult subjects.

A single dose of 1 mg liraglutide was administered as a subcutaneous injection in the abdomen.

All of 32 treated subjects (young subjects aged 21-45 years, 8 males and 8 females; elderly subjects aged 65-83 years, 8 males and 8 females) were included in the pharmacokinetic, pharmacodynamic, and safety analyses.

Pharmacokinetic analysis showed that the AUC_{0-t} ratio (elderly/young) and its 90% CI were 0.94 [0.84, 1.06] and no statistically significant differences were found also for other pharmacokinetic parameters between young and elderly subjects. Plasma concentrations were higher in female subjects compared to male subjects and when adjusting for body weight, the AUC_{0-t} or C_{max} ratio (female/male) with its 90% CI was 1.08 [0.93, 1.26] or 0.96 [0.83, 1.11], respectively. Thus, the differences in the AUC_{0-t} and C_{max} were considered due to an effect of body weight.

Regarding safety, 16 adverse events were reported by 8 subjects (3 events in 1 young male subject, 11 events in 6 young female subjects, 2 events in 1 elderly female subject) and the most frequently reported adverse events were headache (4 events), vomiting (4 events), and nausea (3 events).

(c) Pharmacokinetic study in subjects with renal impairment (5.3.3.3.2, Trial NN2211-1329 [September 2005 to March 2006])

An open-label study in foreign adult male and female subjects was conducted to investigate the pharmacokinetics, safety, and tolerability of liraglutide in subjects with normal renal function³ and in subjects with various degrees of renal impairment.

A single dose of 0.75 mg liraglutide was administered as a subcutaneous injection in the thigh.

All of 30 treated subjects (aged 31-82 years; 6 subjects with normal renal function [$C_{CR} > 80$ mL/min, “normal group”], 6 subjects with mild renal impairment [$50 < C_{CR} \leq 80$ mL/min, “mild group”], 7 subjects with moderate renal impairment [$30 < C_{CR} \leq 50$ mL/min, “moderate group”], 5 subjects with

³ The classification of renal impairment was based on creatinine clearance (C_{CR}) estimated with the Cockcroft & Gault formula. Subjects' health status and their renal impairment were assessed at screening, including physical examination, vital signs, medical history, ECG, and clinical laboratory tests.

severe renal impairment [$C_{CR} \leq 30$ mL/min, “severe group”], 6 subjects with end-stage renal disease [on continuous ambulatory peritoneal dialysis (CAPD), “end stage group”], 22 males and 8 females) were included in the pharmacokinetic and safety analyses. The ratios of AUC_{0-inf} and C_{max} for subjects with renal impairment relative to subjects with normal renal function were as shown in Table 11.

Table 11. Relationship between renal impairment and exposure

Renal function	AUC_{0-inf} ratio [90% CI]	C_{max} ratio [90% CI]
Mild/Normal	0.67 [0.54, 0.85]	0.75 [0.57, 0.98]
Moderate/Normal	0.86 [0.70, 1.07]	0.96 [0.74, 1.23]
Severe/Normal	0.73 [0.57, 0.94]	0.77 [0.57, 1.03]
End stage/Normal	0.74 [0.56, 0.97]	0.92 [0.67, 1.27]

n = 6 for normal group, n = 6 for mild group, n = 7 for moderate group, n = 5 for severe group, n = 6 for end stage group
Adjusted for age and body weight

The AUC_{0-inf} and C_{max} in the renal impairment groups were lower than in the normal group. However, no clear association with the degree of renal impairment was observed. Based on an analysis of AUC_{0-inf} adjusted for age, body weight, and C_{CR} , the degree of renal impairment according to C_{CR} was not considered to have an impact on the AUC_{0-inf} of liraglutide.

Regarding safety, 41 treatment emergent adverse events were reported by 20 subjects (100% of the normal group [6 of 6 subjects], 14 events; 67% of the mild group [4 of 6 subjects], 7 events; 43% of the moderate group [3 of 7 subjects], 6 events; 40% of the severe group [2 of 5 subjects], 5 events; 83% of the end stage group [5 of 6 subjects], 9 events). Commonly reported adverse events were headache (8 events reported by 6 subjects), vomiting (5 events reported by 4 subjects), and nausea (4 events reported by 4 subjects). Vomiting was reported from 20% of the severe group (1 of 5 subjects) (1 event) and 50% of the end stage group (3 of 6 subjects) (4 events) and was not reported from other groups. Gastrointestinal adverse events were all mild or moderate in severity and resolved within 1 to 2 days. No deaths or serious adverse events were reported.

The above results indicated that no dosage adjustment of liraglutide is required for patients with renal impairment.

(d) Pharmacokinetic study in subjects with hepatic impairment (5.3.3.3.3, Trial NN2211-1328 [March to June 2006])

An open-label study in foreign adult male and female subjects was conducted to investigate the pharmacokinetics, safety, and tolerability of liraglutide in subjects with normal hepatic function⁴ and in subjects with various degrees of hepatic impairment.

⁴ The classification of hepatic impairment was based on the Child-Pugh scores. Subjects' health status and their hepatic impairment were assessed at screening, including physical examination, vital signs, medical history, ECG, and clinical laboratory tests.

A single dose of 0.75 mg liraglutide was administered as a subcutaneous injection in the thigh.

All of 24 treated subjects (aged 21-61 years; normal group, mild group [Child-Pugh grade A], moderate group [Child-Pugh grade B], severe group [Child-Pugh grade C]; 6 subjects per group, 14 males and 10 females) were included in the pharmacokinetic and safety analyses. One subject did not attend a study visit after study drug administration and was withdrawn from the study. The ratios of AUC_{0-inf} and C_{max} for subjects with hepatic impairment relative to subjects with normal hepatic function were as shown in Table 12.

Table 12. Relationship between hepatic impairment and exposure

Hepatic function	AUC_{0-inf} ratio [90% CI]	C_{max} ratio [90% CI]
Mild/Normal	0.77 [0.53, 1.11]	0.89 [0.65, 1.21]
Moderate/Normal	0.87 [0.60, 1.25]	0.80 [0.59, 1.09]
Severe/Normal	0.56 [0.39, 0.81]	0.71 [0.52, 0.97]

n = 6 for normal group, n = 6 for mild group, n = 6 for moderate group, n = 6 for severe group
Adjusted for age, gender, and body weight

The AUC_{0-inf} and C_{max} in the hepatic impairment groups were lower than in the normal group. A statistically significant positive relationship ($P = 0.041$) between albumin concentration and AUC_{0-inf} was observed. However, there was no statistically significant effect of hepatic impairment or albumin levels on AUC_{0-inf} in an analysis where both variables were included. Therefore, it is unknown whether the observed relationship between hepatic impairment and AUC_{0-inf} is attributed to lower albumin levels or other aspects of hepatic impairment.

Regarding safety, 3 adverse events were reported by 3 subjects in the moderate group (nausea, bronchitis, headache), of which 2 events reported by 2 subjects (nausea, headache) were classified as adverse drug reactions and were both moderate in severity, but resolved within 1 day.

The above results indicated that no dosage adjustment of liraglutide is required for patients with hepatic impairment.

(e) Drug-drug interaction study (5.3.3.4.1, Trial NN2211-1330 [November 2006 to April 2007])

A double-blind, two period cross-over study in postmenopausal foreign healthy female subjects was conducted to investigate the influence of multiple-dose administration of liraglutide on the pharmacokinetics of an oral contraceptive drug.

Liraglutide at 1.8 mg (with weekly dose increases in increments of 0.6 mg) or placebo was subcutaneously administered once daily for 3 weeks and then at the steady state after multiple-dose administration, a single oral dose of one tablet of an oral contraceptive drug (ethinyl estradiol 0.03 mg, levonorgestrel 0.15 mg) was administered 7 hours after the administration of liraglutide or placebo.

All of 21 treated subjects (aged 58.3 ± 4.9 years) were included in the pharmacokinetic and safety analyses.

As to pharmacokinetics, the ratios of $AUC_{0-\infty}$, $C_{\max}$, and $t_{\max}$ were as shown in Table 13.

Table 13. Pharmacokinetics of oral contraceptive drug when given during liraglutide treatment

Oral contraceptive drug	$AUC_{0-\infty}$ ratio ^{a)} [90% CI]	$C_{\max}$ ratio ^{a)} [90% CI]	$t_{\max}$ difference ^{b)} [90% CI]
Ethinyl estradiol	1.06 [0.99, 1.13]	0.88 [0.79, 0.97]	1.50 [1.00, 2.50]
Levonorgestrel	1.18 [1.04, 1.34] ^{c)}	0.87 [0.75, 1.00]	1.50 [0.50, 2.00]

a) Liraglutide/Placebo

b) Liraglutide – Placebo

c) Equivalence was demonstrated with respect to AUC_{0-t} (1.15 [1.06, 1.24]).

Regarding safety, adverse events and adverse drug reactions occurred more frequently with liraglutide compared to placebo and the main events were nausea, decreased appetite, fatigue, headache, and dizziness.

The above results indicated that liraglutide does not affect the pharmacokinetics of an oral contraceptive drug and no safety concerns arise from the concomitant use of the two drugs.

(f) Drug-drug interaction study (5.3.3.4.2, Trial NN2211-1608 [May 2006 to April 2007])

A placebo-controlled, two-way cross-over study with two parts in foreign healthy adult male and female subjects was conducted to investigate the potential influence of multiple-dose administration of liraglutide on the absorption pharmacokinetics of lisinopril, atorvastatin, griseofulvin, and digoxin and on the intragastric pH.

Liraglutide at 1.8 mg (with weekly dose increases in increments of 0.6 mg) or placebo was subcutaneously administered once daily for 5 weeks and at the steady state after multiple-dose administration, a single oral dose of lisinopril 20 mg and of atorvastatin 40 mg in Part A and a single oral dose of griseofulvin 500 mg and of digoxin 1 mg in Part B were administered.

In Part A, all of 42 treated subjects (29 males, 13 females) were included in the pharmacokinetic and safety analyses. In Part B, all of 28 treated subjects (27 males, 1 female) were included in the safety analysis, of whom 27 subjects excluding 1 subject who was withdrawn from the trial due to a positive cannabis test were included in the pharmacokinetic analysis.

As to pharmacokinetics, the ratios of $AUC_{0-\infty}$, $C_{\max}$, and $t_{\max}$ were as shown in Table 14.

Table 14. Pharmacokinetics of each drug when coadministered with liraglutide

Coadministered drug	BCS ^{a)} class	AUC _{0-inf} ratio ^{b)} [90% CI]	C _{max} ratio ^{b)} [90% CI]	t _{max} difference ^{c)} [90% CI]
Atorvastatin	II	0.95 [0.89, 1.01]	0.62 [0.53, 0.72]	1.25 [1.00, 1.50]
Griseofulvin	II	1.10 [1.01, 1.19]	1.37 [1.24, 1.51]	0.00 [-7.0, 2.00]
Lisinopril	III	0.85 [0.75, 0.97]	0.73 [0.63, 0.85]	2.00 [2.00, 3.00]
Digoxin	IV	0.84 [0.72, 0.98] ^{d)}	0.69 [0.60, 0.79]	1.125 [0.50, 1.25]

a) Biopharmaceutics Classification System (Class II, Low solubility/High permeability; Class III, High solubility/Low permeability; Class IV, Low solubility/Low permeability)⁵

b) Liraglutide/Placebo

c) Liraglutide—Placebo

d) AUC₀₋₇₂ ratio [90%CI]

For griseofulvin and atorvastatin, the 90% CI for the ratio of the single-dose AUC_{0-∞} (at liraglutide steady state conditions/during placebo treatment) was within the pre-specified limits for equivalence, i.e., 0.80 to 1.25. Coadministration with liraglutide decreased the AUCs of lisinopril and digoxin. The lower C_{max} and delayed t_{max} for the oral drugs when given concomitantly with steady-state liraglutide were considered to reflect a delay in gastric emptying.

Regarding safety, adverse events and adverse drug reactions occurred more frequently with liraglutide compared to placebo and the main events were nausea, diarrhoea, vomiting, decreased appetite, weight decreased, fatigue, and feeling cold. The number of adverse events following co-administration was similar between liraglutide and placebo. One serious adverse event in the placebo group (pelvic fracture, unrelated to study treatment) and 22 events of hypoglycemia during the study period (8 events during placebo treatment) were reported.

(g) Drug-drug interaction study (5.3.4.2.5, Trial NN2211-1698 [October 2006 to May 2007])

A randomized, placebo-controlled, double-blind, two-period cross-over study in foreign male and female patients with type 2 diabetes mellitus was conducted to investigate drug-drug interactions between liraglutide and paracetamol and the effects of multiple-dose administration of liraglutide on post-prandial plasma glucose and insulin concentrations and gastric emptying etc.

Liraglutide at 1.8 mg (with weekly dose increases in increments of 0.6 mg) or placebo was subcutaneously administered once daily for 3 weeks. For an investigation of drug-drug interactions, a single oral dose of paracetamol 1 g was administered at the steady state after multiple-dose administration of liraglutide. For an investigation of the effects on post-prandial plasma glucose concentrations etc., a single oral dose of paracetamol 1.5 g was administered after 1 week of treatment at each dose level.

All of 18 treated subjects (aged 48-70 years, 14 males and 4 females) were included in the

⁵ FDA 2000. Guidance for Industry: Waiver of in vivo bioavailability and bioequivalence studies for intermediate release solid oral dosage forms containing certain active moieties/active ingredients based on a biopharmaceutics classification system. Rockville, MD: CDER. 2007.

pharmacokinetic, pharmacodynamic, and safety analyses.

As to pharmacokinetics, paracetamol AUC_{0-inf} and C_{max} ratios (liraglutide/placebo) and t_{max} difference (liraglutide—placebo) and their 90% CIs were 1.04 [0.97, 1.10], 0.69 [0.56, 0.85], and 0.25 [0.00, 1.54], respectively. The lower C_{max} and delayed t_{max} were considered attributed to a delay in gastric emptying by liraglutide.

Pharmacodynamic analysis showed that after multiple-dose administration of liraglutide compared to placebo treatment, post-prandial glucose $AUC_{0-300min}$ and fasting blood glucose were lower, increases in post-prandial blood glucose concentrations were suppressed, and fasting and post-prandial insulin secretion were increased. When the effect on gastric emptying was assessed by plasma paracetamol levels, the C_{max} and t_{max} values of paracetamol suggested that liraglutide delayed gastric emptying up to 1 hour after meal.

Regarding safety, 18 adverse events were reported by 9 subjects and the most commonly reported adverse events were nasopharyngitis and headache.

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

4.(ii).B.(1) Pharmacokinetic and pharmacodynamic profiles of liraglutide

4.(ii).B.(1).1 Characteristics of liraglutide

PMDA asked the applicant to explain the clinical pharmacologic characteristics of liraglutide compared with GLP-1, presenting clinical study data supporting the applicant's claim that liraglutide is characterized by 24-hour coverage of blood levels and pharmacologic action following once-daily administration.

The applicant responded as follows:

The $t_{1/2}$ of GLP-1 is 1.5 minutes after intravenous administration and 1.5 hours after subcutaneous administration (Gutniak MK, *et al.*, *Diabetes Care*, 1994; 17: 1039-1044). In Japanese subjects, the $t_{1/2}$ (mean) after a single subcutaneous dose of liraglutide was 10 to 11 hours (Trial NN2211-1326, healthy adult subjects) and the $t_{1/2}$ after multiple subcutaneous doses of liraglutide was 13 to 14 hours, 15 hours, and 14 to 15 hours in Trial NN2211-1551 (healthy adult subjects), Trial NN2211-1694 (healthy adult subjects), and Trial NN2211-1591 (patients with type 2 diabetes mellitus), respectively. The t_{max} after a single subcutaneous dose of liraglutide was 7.5 to 11 hours and the t_{max} after multiple subcutaneous doses was 8 to 9 hours, 7 to 8 hours, and 9 to 12 hours, respectively, and absorption of liraglutide from the injection site was slow. The trough concentrations of liraglutide after once daily administration were about 1/2 of the peak concentrations and there were small fluctuations in plasma concentrations during the 24-hour dosing interval. It has been reported that when GLP-1 infusion was

given for 16 or 24 hours per day for 7 days, as nocturnal and fasting glucose levels increased in the 16-hour groups during the night (when GLP-1 infusion was not given), GLP-1 should be infused continuously for 24 hours per day to obtain the most optimal glycemic control (Larsen J, *et al.*, *Diabetes Care*, 2001; 24: 1416-1421). Once-daily administration of liraglutide provided 24-hour glycemic control in patients with type 2 diabetes mellitus (NN2211-1591). Furthermore, in Trials NN2211-1334, 1701, and 1700, low glucose levels were maintained and when the glucose concentration was increased in a stepwise manner, liraglutide glucose-dependently improved the insulin response to the level of healthy adults and when the fasting glucose concentration was within the normal range, no effect on insulin secretion was observed.

Based on the submitted data from multiple clinical pharmacology studies, PMDA accepted the applicant's response that unlike GLP-1, the absorption and elimination of liraglutide are slow and once-daily subcutaneous administration of liraglutide can maintain blood levels for 24 hours, and such pharmacokinetic profile enables glucose-dependent 24-hour glycemic control. [see "3.(ii).B.Outline of the review by PMDA"].

4.(ii).B.(2) Dose proportionality of pharmacokinetics

PMDA asked the applicant to explain why pharmacokinetics were not dose-proportional with an even larger inter-subject variability in plasma levels at doses ≥ 20 $\mu\text{g/kg}$ in Trial NN2211-1694 and ≥ 0.6 mg/day in Trial NN2211-1334 (10 $\mu\text{g/kg}$ for a 60 kg person).

The applicant responded as follows:

In Trial NN2211-1694, the coefficients of variation (CV %) of plasma levels at different time points were similar between the 20 and 25 $\mu\text{g/kg}$ groups. However, in the 20 $\mu\text{g/kg}$ group, the plasma levels in 6 subjects were distributed over a wide range, while in the 25 $\mu\text{g/kg}$ group, the plasma levels in 1 subject were higher compared to other subjects. In Trial NN2211-1334, the plasma levels at Week 14 (LOCF) were slightly higher in the 0.9 mg group than in the 0.6 mg group and the CV (%) of plasma levels was comparable across all dose groups (around 40%). Among about 45 subjects in each dose group, the proportion of subjects aged ≥ 65 years and the mean age were slightly higher in the 0.6 mg group compared to other dose groups, but the subject demographics were generally comparable across the dose groups. The dose-plasma concentration relationship was different between male and female subjects and while dose proportionality was seen in female subjects, the plasma levels in the 0.9 mg group were similar to those in the 0.6 mg group in male subjects. Although study drug compliance was good, the time from the last dose to blood sampling was not specified, which is considered related to a lack of dose proportionality of pharmacokinetics, but the details are unknown. Based on Japanese clinical trials that assessed the pharmacokinetics of liraglutide (Trials NN2211-1326 and 1551), it is considered that the pharmacokinetics of liraglutide are almost dose-proportional over a dose range up

to at least 15 µg/kg (equivalent to 0.9 mg in Japanese subjects). On the other hand, in Trial NN2211-1334, the efficacy endpoints of HbA1c and fasting blood glucose were dose-proportional over the dose range of 0.1 to 0.9 mg/day in both male and female subjects. The larger change in HbA1c seen in the 0.9 mg group in spite of similar plasma levels at Week 14 (LOCF) was considered to be due to the differences in plasma levels throughout the treatment period (14 weeks) influencing the change in HbA1c.

PMDA accepts the response, but considers that it is necessary to assess the usefulness of a 0.6 mg/day dose from a pharmacokinetic and clinical standpoint [See “4.(iii).B.(6).1) Dose”].

4.(ii).B.(3) Patients with hepatic or renal impairment

PMDA asked the applicant to sort out liver and renal function tests, albumin levels, and α_1 -acid glycoprotein, and liraglutide exposure in subjects from foreign clinical studies (Trials NN2211-1573, 1328, and 1329) and explain the mechanism of decreased liraglutide exposure in subjects with hepatic or renal impairment.

The applicant responded as follows:

In Trial NN2211-1573, there was a slight trend towards decreased daily exposure to liraglutide (AUC per dosing interval estimated from a model) with higher creatinine and BUN. However, a regression analysis of AUC_{0-inf} adjusted for age, body weight, and C_{CR} showed that the degree of renal impairment according to C_{CR} did not affect liraglutide exposure, and the estimated AUC_{0-inf} ratio between a subject with the lowest C_{CR} (severe group, 14 mL/min) and a subject with the highest C_{CR} (normal group, 132 mL/min) and its 95% CI were 0.88 [0.58, 1.34], which was similar to the trend observed for BUN etc. Albumin levels in Trial NN2211-1329 were higher than those in Trial NN2211-1328 involving subjects with hepatic impairment and no clear association between albumin levels and liraglutide exposure was found. As to the relationship between clinical laboratory tests and the daily exposure to liraglutide in Trial NN2211-1573, liraglutide exposure tended to decrease with lower albumin and total protein, and higher liver function enzyme levels. When the rate of liraglutide metabolism in the presence of varying concentrations of albumin was investigated *in vitro*, the rate of liraglutide metabolism increased at a low concentration of albumin, whereas 1% to 5% of albumin, including the albumin concentration range of healthy adults and of the severe hepatic impairment group in Trial NN2211-1328, changed the rate of liraglutide metabolism up to 2-fold, which is consistent with the results of Trial NN2211-1328. At least, the decreased exposure in subjects with severe hepatic impairment is considered attributed to lower albumin levels, but the mechanism behind the decreased liraglutide exposure in subjects with hepatic impairment is unclear as to whether it was attributed to lower albumin levels or other aspects of hepatic impairment.

PMDA asked the applicant to sort out the timing of the onset of gastrointestinal disorders (adverse drug reactions) and plasma levels of liraglutide in Trials NN2211-1328 and 1329, and plasma levels and adverse events in patients with mild to moderate hepatic or renal impairment in Japanese trial NN2211-1334, and explain liraglutide exposure and safety.

The applicant responded as follows:

In Trials NN2211-1328 and 1329, 1 gastrointestinal adverse event from 1 subject and 11 gastrointestinal adverse events from 9 subjects, respectively, were reported. Nausea and vomiting occurred mostly at plasma concentrations near the C_{max} , which was similar to the trend observed in other single-dose trials. The plasma concentrations at the onset of gastrointestinal disorders varied from < 3000 pmol/L to 18577 pmol/L and there was no consistent trend of dependence on the degree of renal or hepatic impairment. On the other hand, the trough concentrations obtained from Trial NN2211-1334 showed no trend towards decreased liraglutide exposure in subjects with mild renal or hepatic impairment, and the liraglutide plasma concentration after multiple-dose administration using a gradual dose escalation regimen was not clearly associated with the occurrence of all/gastrointestinal adverse events within the dose range up to 0.9 mg/day. It was concluded that patients with hepatic or renal impairment tend to have lower exposure, and therefore are unlikely to produce safety problems, but it may lead to reduced efficacy. Thus, the level of glycemic control needs to be monitored carefully.

Although the submitted study data show a trend towards decreased liraglutide exposure in subjects with hepatic or renal impairment, no clear association between the degree of impairment and decreased exposure was found and there seems no major safety problem. Therefore, PMDA accepted the response.

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

Trials NN2211-1608 and 1698 showed lower C_{max} and delayed t_{max} for the coadministered drugs except for griseofulvin with low solubility. Thus, there is a concern about the decreased absorption rate of a coadministered drug due to a delay in gastric emptying by liraglutide. PMDA asked the applicant to explain (a) the influences of decreased C_{max} (30%) of digoxin with a narrow therapeutic window on its efficacy and (b) the influences of coadministration with an enteric-coated or extended-release formulation of a drug.

The applicant responded as follows:

(a) When a single dose of 1 mg digoxin was given concomitantly with liraglutide, absorption of digoxin was delayed and the plasma C_{max} was lowered compared to placebo, but the time course of plasma levels after 6 hours was similar between liraglutide and placebo treatment. Based on this data,

the steady-state concentration at a maintenance dose of 0.25 mg/day of digoxin was estimated. As a result, although plasma concentrations up to several hours post-dose were different, trough concentrations were similar between liraglutide and placebo treatment. Also, in Japanese and foreign long-term treatment trials, no cardiovascular adverse events suggestive of attenuated cardiotonic action were reported when liraglutide was used with digoxin or metildigoxin. Based on the above, it was concluded that no dose adjustment of digoxin when used with liraglutide is required.

(b) An enteric-coated or extended-release formulation of a drug used with liraglutide may stay in the stomach longer and its absorption from the gastrointestinal tract may be delayed. Although the effects of liraglutide on the cyclical pattern of interdigestive gastrointestinal motility (migrating motor complex) were not studied, the occurrence of adverse events with liraglutide when used with omeprazole enteric-coated tablets in Trials NN2211-1700 and 1701 indicates that the effect of liraglutide on gastric emptying is not significant enough to affect the efficacy and safety of an enteric-coated or extended-release formulation of a drug. The influences of coadministration with an enteric-coated or extended-release formulation of a drug on safety will be investigated by assessing the trend of adverse drug reactions etc. with or without an enteric-coated or extended-release formulation of a drug via post-marketing surveillance.

PMDA accepts the above response, but considers that it is necessary to collect post-marketing safety and efficacy information regarding coadministration with an enteric-coated or extended-release formulation of a drug as the submitted trials did not investigate drug-formulation interactions.

4.(iii) Summary of clinical efficacy and safety

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

As the efficacy and safety evaluation data, the results from Japanese phase I clinical pharmacology trials (NN2211-1326,1551,1591,1694), a Japanese phase II clinical trial (NN2211-1334), a Japanese phase II/III clinical trial (NN2211-1701), and a Japanese phase III clinical trial (NN2211-1700) were submitted. In Trials NN2211-1334, NN2211-1700, and 2211-1701, liraglutide was administered by self-injection [See “4.(ii) Summary of clinical pharmacology studies” for phase I clinical pharmacology data].

4.(iii).A.(1) Phase II clinical trial (5.3.5.1.1, Trial NN2211-1334 [March 2005 to May 2006])

A randomized, parallel-group, comparative trial⁶ was conducted to evaluate the dose-response relationship for four doses of liraglutide and placebo with respect to glycemic control in Japanese

⁶ As the administration regimen was different among the different doses, the different doses of liraglutide were distinguishable, but randomization to liraglutide or placebo at each dose level was double-blinded.

patients with type 2 diabetes mellitus⁷ (target number of subjects, 50 subjects at each dose level [40 subjects receiving liraglutide and 10 subjects receiving placebo], a total of 200 subjects).

Following an 8-week run-in period (subjects on one oral antidiabetic drug underwent an 8-week wash-out period), placebo or liraglutide 0.1 mg (0.1 mg for 14 weeks), 0.3 mg (0.3 mg for 14 weeks), 0.6 mg (0.3 mg for 1 week → 0.6 mg for 13 weeks), or 0.9 mg (0.3 mg for 1 week → 0.6 mg for 1 week → 0.9 mg for 12 weeks) was administered once daily in the evening (at the same time of each day wherever possible) as a subcutaneous injection in the abdomen (self-injection).

All of 226 treated subjects (46 subjects in the placebo group, 45 subjects in the liraglutide 0.1 mg group, 46 subjects in the liraglutide 0.3 mg group, 45 subjects in the liraglutide 0.6 mg group, 44 subjects in the 0.9 mg group) were included in the safety analysis population and in the efficacy analysis population (FAS: Full Analysis Set). There were 16 withdrawals from the trial, including 8 withdrawals in the placebo group (1 withdrawal due to an adverse event, 1 withdrawal due to ineffective therapy, 6 withdrawals for other reasons), 2 withdrawals in the liraglutide 0.1 mg group (1 withdrawal due to non-compliance with the protocol, 1 withdrawal due to ineffective therapy), 3 withdrawals in the liraglutide 0.3 mg group (1 withdrawal due to non-compliance with the protocol, 1 withdrawal due to ineffective therapy, 1 withdrawal for other reasons), 1 withdrawal in the liraglutide 0.6 mg group (1 withdrawal due to ineffective therapy), and 2 withdrawals in the liraglutide 0.9 mg group (1 withdrawal due to an adverse event, 1 withdrawal for other reasons).

The efficacy analysis showed that there was a statistically significant monotonic reduction in the primary endpoint of HbA1c at Week 14 (LOCF) with increasing dose of liraglutide, as shown in Table 15 ($P < 0.001$, F-test for the linear contrast with a contrast coefficient of [-2, -1, 0, 1, 2] assigned to the placebo, liraglutide 0.1 mg, liraglutide 0.3 mg, liraglutide 0.6 mg, and liraglutide 0.9 mg groups based on the ANCOVA model including treatment group and previous antidiabetic treatment [dietary therapy, one oral antidiabetic drug] as fixed effects and baseline HbA1c as a covariate).

⁷ Patients with type 2 diabetes mellitus on dietary therapy with or without one oral antidiabetic drug who were aged ≥ 20 and < 75 years and had BMI < 30.0 kg/m² and HbA1c $\geq 7.0\%$ and $< 10.0\%$ at Visit 1. Oral antidiabetic drugs were biguanides, sulfonamides, sulfonylureas (SU, patients treated with up to half maximal approved dose), α -glucosidase inhibitors (α -GI), and insulin secretagogues.

Table 15. Results of HbA1c analysis (FAS)

Treatment group	Baseline ^{a)}	Week 14 ^{a)}	P-value ^{b) c)}	Difference from placebo ^{d)} [95% CI] ^{b)}	Change from baseline ^{a)}
Placebo (n = 46)	8.43 (1.02)	8.52 (1.23)	P < 0.001	—	0.10 (0.72)
Liraglutide 0.1 mg (n = 45)	8.50 (0.84)	7.78 (0.91)		-0.79 [-1.08, -0.50]	-0.72 (0.74)
Liraglutide 0.3 mg (n = 46)	8.24 (0.92)	7.17 (1.01)		-1.22 [-1.50, -0.93]	-1.07 (0.60)
Liraglutide 0.6 mg (n = 45)	8.21 (0.83)	6.71 (0.92)		-1.64 [-1.93, -1.35]	-1.50 (0.84)
Liraglutide 0.9 mg (n = 44)	8.12 (0.98)	6.45 (0.77)		-1.85 [-2.14, -1.56]	-1.67 (0.81)

a) Mean (SD) %

b) ANCOVA model including treatment group and previous antidiabetic treatment (dietary therapy, one oral antidiabetic drug) as fixed effects and baseline HbA1c as a covariate

c) F-test for the linear contrast with a contrast coefficient of [-2, -1, 0, 1, 2] assigned to the placebo, liraglutide 0.1 mg, liraglutide 0.3 mg, liraglutide 0.6 mg, and liraglutide 0.9 mg groups

d) Least squares mean %

As for the secondary endpoints, fasting blood glucose at Week 14 (LOCF) (least squares mean $\pm$ SE) was 173.8 ± 4.2 mg/dL in the placebo group, 160.2 ± 4.2 mg/dL in the liraglutide 0.1 mg group, 151.3 ± 4.1 mg/dL in the liraglutide 0.3 mg group, 132.9 ± 4.2 mg/dL in the liraglutide 0.6 mg group, and 129.2 ± 4.3 mg/dL in the liraglutide 0.9 mg group. Post-prandial blood glucose AUC (glucose, 0-3 hours) at Week 14 (LOCF) was 741.83 ± 17.21 mg/dL·h in the placebo group, 682.37 ± 16.16 mg/dL·h in the liraglutide 0.1 mg group, 626.22 ± 16.10 mg/dL·h in the liraglutide 0.3 mg group, 556.41 ± 15.82 mg/dL·h in the liraglutide 0.6 mg group, and 511.41 ± 16.23 mg/dL·h in the liraglutide 0.9 mg group. Body weight at Week 14 (LOCF) was 61.21 ± 0.24 kg in the placebo group, 62.08 ± 0.24 kg in the liraglutide 0.1 mg group, 62.30 ± 0.24 kg in the liraglutide 0.3 mg group, 62.05 ± 0.24 kg in the liraglutide 0.6 mg group, and 61.68 ± 0.25 kg in the liraglutide 0.9 mg group.

Regarding safety, the incidence of adverse events was 67.4% (31 of 46 subjects) (65 events) in the placebo group, 55.6% (25 of 45 subjects) (62 events) in the liraglutide 0.1 mg group, 69.6% (32 of 46 subjects) (67 events) in the liraglutide 0.3 mg group, 73.3% (33 of 45 subjects) (78 events) in the liraglutide 0.6 mg group, and 75.0% (33 of 44 subjects) (63 events) in the liraglutide 0.9 mg group and the incidence of adverse drug reactions was 19.6% (9 of 46 subjects) (10 events) in the placebo group, 24.4% (11 of 45 subjects) (16 events) in the liraglutide 0.1 mg group, 23.9% (11 of 46 subjects) (19 events) in the liraglutide 0.3 mg group, 40.0% (18 of 45 subjects) (28 events) in the liraglutide 0.6 mg group, and 27.3% (12 of 44 subjects) (12 events) in the liraglutide 0.9 mg group. No deaths were reported and serious adverse events occurred in 1 subject of the liraglutide 0.6 mg group (papillary thyroid carcinoma) and 1 subject of the liraglutide 0.9 mg group (fall). Adverse events leading to treatment discontinuation occurred in 1 subject of the placebo group (abdominal discomfort/viral gastroenteritis) and 1 subject of the liraglutide 0.9 mg group (abdominal pain upper). Adverse events

reported by $\geq 5\%$ of subjects in any group were as shown in Table 16.

Table 16. Adverse events reported by $\geq 5\%$ of subjects in any group

	Placebo (n = 46)	Liraglutide 0.1 mg (n = 45)	Liraglutide 0.3 mg (n = 46)	Liraglutide 0.6 mg (n = 45)	Liraglutide 0.9 mg (n = 44)
Any event	31 (67.4) 65	25 (55.6) 62	32 (69.6) 67	33 (73.3) 78	33 (75.0) 63
Nasopharyngitis	13 (28.3) 14	10 (22.2) 12	16 (34.8) 18	14 (31.1) 15	13 (29.5) 15
Constipation	4 (8.7) 4	1 (2.2) 1	1 (2.2) 1	5 (11.1) 6	3 (6.8) 3
Diarrhoea	2 (4.3) 2	3 (6.7) 5	2 (4.3) 4	1 (2.2) 1	4 (9.1) 4
Nausea	1 (2.2) 1	1 (2.2) 1	5 (10.9) 6	2 (4.4) 2	3 (6.8) 3
Stomach discomfort	1 (2.2) 1	3 (6.7) 3	0 (0.0) 0	2 (4.4) 3	1 (2.3) 1
Gastritis	0 (0.0) 0	1 (2.2) 1	1 (2.2) 1	0 (0.0) 0	3 (6.8) 3
Thyroid neoplasm	3 (6.5) 3	7 (15.6) 8	1 (2.2) 1	2 (4.4) 2	1 (2.3) 1
Upper respiratory tract inflammation	0 (0.0) 0	4 (8.9) 4	2 (4.3) 2	0 (0.0) 0	1 (2.3) 1

No. of subjects with events (Incidence %) No. of events

The incidence of hypoglycemia⁸ was 4.3% (2 of 46 subjects) (2 events) in the placebo group, 4.4% (2 of 45 subjects) (3 events) in the liraglutide 0.1 mg group, 6.5% (3 of 46 subjects) (6 events) in the liraglutide 0.3 mg group, 4.4% (2 of 45 subjects) (3 events) in the liraglutide 0.6 mg group, and 9.1% (4 of 44 subjects) (7 events) in the liraglutide 0.9 mg group. As to clinical laboratory tests, eosinophils, serum creatinine, Na, and free T3 tended to increase with increasing dose of liraglutide during the treatment period, which were not clinically relevant changes. There were no clinically relevant changes in ECG. The proportion of subjects with positive anti-liraglutide antibodies at the last visit (Week 15, LOCF) was 2.2% (1 of 46 subjects) in the placebo group, 6.7% (3 of 45 subjects) in the liraglutide 0.1 mg group, 0.0% (0 of 46 subjects) in the liraglutide 0.3 mg group, 4.4% (2 of 45 subjects) in the liraglutide 0.6 mg group, and 13.6% (6 of 44 subjects) in the liraglutide 0.9 mg group and none of the subjects had antibody levels exceeding 3.1% B/T (percent bound radioactivity of the total amount of radioactivity), i.e., the maximum % B/T at screening.

4.(iii).A.(2) Phase II/III clinical trial (5.3.5.1.3, Trial NN2211-1701 [November 2006 to April 2008])

A randomized, parallel-group, comparative trial in Japanese patients with type 2 diabetes mellitus on SU therapy⁹ was conducted to evaluate the efficacy and safety of liraglutide as an add-on to SU therapy (target number of subjects, 114 subjects at each dose level [76 subjects receiving liraglutide and 38 subjects receiving placebo], a total of 228 subjects). This trial consisted of a 4-week run-in

⁸ Classification of hypoglycemia: Hypoglycemia with subjective symptoms was classified as follows:

(a) If severe neuroglycopenia, e.g., lowering of consciousness, was suspected and the subject required assistance of another person for treatment → “Major hypoglycemia”

(b) If the subject was able to self-treat symptoms and a blood glucose measurement was not available or a measured plasma glucose concentration was ≥ 56 mg/dL → “Hypoglycemic symptoms” and if the subject was able to self-treat symptoms and a measured plasma glucose concentration was ≤ 56 mg/dL → “Minor hypoglycemia”

A low blood glucose value without subjective symptoms (a measured plasma glucose concentration ≤ 55 mg/dL) was defined as “Biochemical hypoglycemia.”

⁹ Patients with type 2 diabetes mellitus treated with dietary therapy plus glibenclamide (1.25-10 mg/day), gliclazide (40-160 mg/day), or glimepiride (1-6 mg/day) for at least 8 weeks who were aged ≥ 20 years and had BMI < 35.0 kg/m² and HbA1c $\geq 7.0\%$ and $< 10.0\%$ at Visit 1.

period, a 24-week double-blind phase,¹⁰ and a 28-week open-label phase.

Using a prefilled pen fitted with a needle, liraglutide or placebo was administered once daily in the morning or evening (at the same time of each day throughout the treatment period wherever possible) as a subcutaneous injection (self-injection) in the upper arm, abdomen, or thigh. The dose of liraglutide was increased in weekly 0.3 mg increments and as a rule, subjects continued on the same dosage of SU that they had been taking before the baseline visit. However, only when major or unacceptable hypoglycemia occurred, the dose of SU was allowed to be reduced to its lowest Japanese approved dose.

All of 264 treated subjects (88 subjects in the liraglutide 0.6 mg + SU group, 88 subjects in the liraglutide 0.9 mg + SU group, 88 subjects in the SU monotherapy group) were included in the FAS for efficacy and safety analyses. There were 36 withdrawals from the trial, including 10 withdrawals in the liraglutide 0.6 mg + SU group (5 withdrawals due to adverse events, 2 withdrawals due to ineffective therapy, 3 withdrawals for other reasons), 4 withdrawals in the liraglutide 0.9 mg + SU group (2 withdrawals due to adverse events, 1 withdrawal due to non-compliance with the protocol, 1 withdrawal for other reasons), and 22 withdrawals in the SU monotherapy group (2 withdrawals due to adverse events, 17 withdrawals due to ineffective therapy, 3 withdrawals for other reasons).

The efficacy analysis demonstrated the superiority of liraglutide 0.9 mg + SU over SU monotherapy for the primary endpoint of HbA1c at Week 24 (LOCF) (Table 17). In this trial, if there was a significant difference between the liraglutide 0.9 mg + SU and SU monotherapy groups, comparison between the liraglutide 0.6 mg + SU and SU monotherapy groups was to be made.

Table 17. Results of analysis of HbA1c at Week 24 (LOCF) (FAS)

Treatment group	Baseline ^{a)}	Week 24 ^{a)}	Difference from SU monotherapy ^{c)} [95% CI] ^{b)}	P-value ^{b)}	Change from baseline ^{a)}
Liraglutide 0.6 mg + SU (n = 86 ^{d)})	8.60 (0.92)	7.14 (0.89)	-1.00 [-1.24, -0.75]	<i>P</i> < 0.001	-1.46 (0.95)
Liraglutide 0.9 mg + SU (n = 87 ^{d)})	8.23 (0.78)	6.67 (0.83)	-1.27 [-1.51, -1.02]	<i>P</i> < 0.001	-1.56 (0.84)
SU monotherapy (n = 88)	8.45 (0.99)	8.06 (1.13)	—	—	-0.40 (0.93)

a) Mean (SD) %

b) ANCOVA model including treatment group and previous SU therapy (glibenclamide, gliclazide, glimepiride) as fixed effects and baseline HbA1c as a covariate

c) Least squares mean %

d) Subjects with missing HbA1c at baseline or at Week 24 (LOCF) (at the end of treatment/withdrawal) were excluded from the FAS.

As for the secondary endpoints, the proportion of subjects achieving HbA1c < 6.5% at Week 24 was 23.9% (21 of 88 subjects) in the liraglutide 0.6 mg + SU group, 46.6% (41 of 88 subjects) in the

¹⁰ As the administration regimen was different between the two doses, the two doses of liraglutide were distinguishable, but randomization to liraglutide or placebo at each dose level was double-blinded.

liraglutide 0.9 mg + SU group, and 4.5% (4 of 88 subjects) in the SU monotherapy group. Fasting blood glucose (least squares mean $\pm$ SE) at Week 24 (LOCF) was 132.2 ± 3.5 mg/dL in the liraglutide 0.6 mg + SU group, 126.2 ± 3.5 mg/dL in the liraglutide 0.9 mg + SU group, and 158.5 ± 3.5 mg/dL in the SU monotherapy group and 2-hour post-prandial blood glucose after a meal tolerance test was 217.0 ± 6.3 mg/dL in the liraglutide 0.6 mg + SU group, 202.8 ± 6.4 mg/dL in the liraglutide 0.9 mg + SU group, and 265.9 ± 6.7 mg/dL in the SU monotherapy group.

Changes in HbA1c from baseline to Week 52 (LOCF) were as shown in Table 18. HbA1c values over time during the treatment period were as shown in Figure 1.

Table 18. Changes in HbA1c from baseline to Week 52 (LOCF)

Treatment group	Baseline	Week 52	Change from baseline
Liraglutide 0.6 mg + SU (n = 86 ^a)	8.60 (0.92)	7.51 (0.98)	-1.09 (0.84)
Liraglutide 0.9 mg + SU (n = 87 ^a)	8.23 (0.78)	6.93 (0.99)	-1.30 (0.91)
SU monotherapy (n = 88)	8.45 (0.99)	8.39 (1.32)	-0.06 (1.29)

Mean (SD) %

a) Subjects with missing HbA1c at baseline or at the end of treatment/withdrawal were excluded from the FAS.

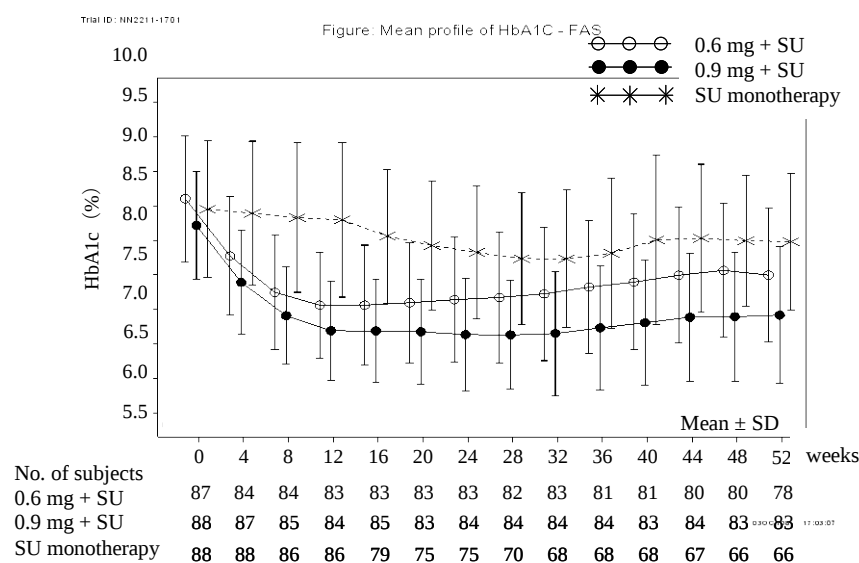


Figure 1. HbA1c values over time during treatment period

The change in body weight from baseline to Week 52 (LOCF) (mean $\pm$ SD) was 0.07 ± 1.87 kg in the liraglutide 0.6 mg + SU group, -0.03 ± 2.16 kg in the liraglutide 0.9 mg + SU group, and -1.07 ± 2.16 kg in the SU monotherapy group and the difference between the liraglutide and placebo groups for body weight at Week 52 (LOCF) [95% CI] was 1.13 [0.51, 1.75] for liraglutide 0.6 mg + SU and 1.04 [0.42, 1.66] for liraglutide 0.9 mg + SU.

Regarding safety, the incidence of adverse events was 95.5% (84 of 88 subjects) (417 events) in the

liraglutide 0.6 mg + SU group, 89.8% (79 of 88 subjects) (364 events) in the liraglutide 0.9 mg + SU group, and 94.3% (83 of 88 subjects) (352 events) in the SU monotherapy group. The incidence of adverse drug reactions was 38.6% (34 of 88 subjects) (75 events) in the liraglutide 0.6 mg + SU group, 35.2% (31 of 88 subjects) (68 events) in the liraglutide 0.9 mg + SU group, and 38.6% (34 of 88 subjects) (57 events) in the SU monotherapy group. The most commonly reported system organ classes of adverse events were “gastrointestinal disorders” and “infections and infestations” and the incidence of adverse events classified as “gastrointestinal disorders” was higher in the liraglutide groups (48.9% [43 of 88 subjects] in the liraglutide 0.6 mg + SU group, 47.7% [42 of 88 subjects] in the liraglutide 0.9 mg + SU group) compared to the SU monotherapy group (38.6% [34 of 88 subjects]). Adverse events reported by $\geq 5\%$ of subjects in any group were as shown in Table 19.

Table 19. Adverse events reported by $\geq 5\%$ of subjects in any group

	Liraglutide 0.6 mg + SU (n = 88)	Liraglutide 0.9 mg + SU (n = 88)	SU monotherapy (n = 88)
Any event	84 (95.5) 417	79 (89.8) 364	83 (94.3) 352
Nasopharyngitis	37 (42.0) 63	38 (43.2) 51	35 (39.8) 61
Diarrhoea	7 (8.0) 8	14 (15.9) 17	7 (8.0) 10
Constipation	8 (9.1) 9	11 (12.5) 12	4 (4.5) 7
Diabetic retinopathy	8 (9.1) 8	11 (12.5) 11	7 (8.0) 7
Back pain	10 (11.4) 10	7 (8.0) 8	6 (6.8) 6
Upper respiratory tract inflammation	9 (10.2) 12	6 (6.8) 8	5 (5.7) 5
Dizziness	5 (5.7) 7	6 (6.8) 7	2 (2.3) 2
Dental caries	5 (5.7) 5	5 (5.7) 5	1 (1.1) 1
Headache	10 (11.4) 11	4 (4.5) 4	8 (9.1) 9
Gastritis	3 (3.4) 3	4 (4.5) 4	8 (9.1) 8
Arthralgia	6 (6.8) 6	3 (3.4) 3	1 (1.1) 1
Hypertension	5 (5.7) 5	3 (3.4) 3	4 (4.5) 5
ALT increased	6 (6.8) 7	2 (2.3) 2	1 (1.1) 1
Fall/Fall from high level	5 (5.7) 5	2 (2.3) 3	2 (2.3) 2
Malaise	5 (5.7) 6	1 (1.1) 2	5 (5.7) 6
Hypoaesthesia	5 (5.7) 6	0 (0.0) 0	5 (5.7) 5

No. of subjects with events (Incidence %) No. of events

No deaths were reported. Serious adverse events were reported by 4 subjects in the liraglutide 0.6 mg + SU group (oesophageal carcinoma, sudden hearing loss, spinal osteoarthritis, pneumonia), 3 subjects in the liraglutide 0.9 mg + SU group (epiglottic cyst, ischemic colitis, bursitis infective), and 5 subjects in the SU monotherapy group (hyperglycaemia, cataract operation, benign neoplasm of skin, cholecystitis, colon adenoma). Adverse events leading to trial discontinuation were reported by 5 subjects in the liraglutide 0.6 mg + SU group (stomach discomfort, oesophageal carcinoma, AST increased/ALT increased, sudden hearing loss, gastroenteritis), 2 subjects in the liraglutide 0.9 mg + SU group (drug eruption, headache/photophobia/myalgia/dizziness), and 2 subjects in the SU monotherapy group (hyperglycaemia, cholecystitis).

The incidence of hypoglycemia¹¹ was 53.4% (47 of 88 subjects) (257 events) in the liraglutide 0.6 mg + SU group, 64.8% (57 of 88 subjects) (313 events) in the liraglutide 0.9 mg + SU group, and 31.8% (28 of 88 subjects) (221 events) in the SU monotherapy group. Hypoglycemia meeting the definition of a serious adverse event or major hypoglycemia was not reported. The incidence of adverse events classified as “eye disorders” including diabetic retinopathy was 20.5% (18 of 88 subjects) in the liraglutide 0.6 mg + SU group, 26.1% (23 of 88 subjects) in the liraglutide 0.9 mg + SU group, and 19.3% (17 of 88 subjects) in the SU monotherapy group. Anti-liraglutide antibodies were negative in all subjects at baseline, but were positive in 14.8% (13 of 88 subjects) of the liraglutide 0.6 mg + SU group and 15.9% (14 of 88 subjects) of the liraglutide 0.9 mg + SU group at Week 53¹² (LOCF), of whom 11 subjects in the liraglutide 0.6 mg + SU group and 2 subjects in the liraglutide 0.9 mg + SU group were also positive for antibodies cross-reacting with GLP-1.¹³ No positive results were reported in the SU monotherapy group.

4.(iii).A.(3) Phase III clinical trial (5.3.5.1.2, Trial NN2211-1700 [December 2006 to May 2008])

A randomized, parallel-group, comparative trial was conducted to demonstrate the non-inferiority of liraglutide monotherapy to glibenclamide monotherapy in Japanese patients with type 2 diabetes mellitus¹⁴ (target number of subjects of 378 [252 subjects in the liraglutide 0.9 mg group, 126 subjects in the glibenclamide group]). This trial consisted of a 4- to 6-week run-in period for washout of previous oral antidiabetic drugs, a 24-week double-blind phase, and a 28-week open-label phase.

Using a prefilled pen fitted with a needle, liraglutide or liraglutide placebo was administered once daily in the morning or evening (at the same time of day throughout the treatment period wherever possible) as a subcutaneous injection (self-injection) in the upper arm, abdomen, or thigh. The dose was increased in weekly 0.3 mg increments to 0.9 mg over 2 weeks. Glibenclamide or glibenclamide placebo was orally administered once daily (before or after breakfast) or twice daily (before or after breakfast and evening meal) and the dose of glibenclamide was started at 1.25 mg and as a rule, increased to 2.5 mg at Week 4, and then maintained at this dose. If major or unacceptable hypoglycemia occurred, the dose of glibenclamide was allowed to be reduced to 1.25 mg.

¹¹ Classification of hypoglycemia: Hypoglycemia with subjective symptoms was classified as follows:

(a) If severe neuroglycopenia, e.g., lowering of consciousness, was suspected and the subject required assistance of another person for treatment → “Major hypoglycemia”

(b) If the subject was able to self-treat symptoms and a blood glucose measurement was not available or a measured plasma glucose concentration was ≥ 56 mg/dL → “Hypoglycemic symptoms” and if the subject was able to self-treat symptoms and a measured plasma glucose concentration was ≤ 56 mg/dL → “Minor hypoglycemia”

A low blood glucose value without subjective symptoms (a measured plasma glucose concentration ≤ 55 mg/dL) was defined as “Minor hypoglycemia.”

¹² Anti-liraglutide antibodies measured at Week 53 when plasma liraglutide concentrations were considered low enough not to interfere with the assay, i.e., 4 to 10 days after the last dose of liraglutide, were used as the primary results.

¹³ Anti-liraglutide antibody-positive samples were further analyzed for antibodies cross-reacting with GLP-1.

¹⁴ Patients with type 2 diabetes mellitus on dietary therapy alone or dietary therapy plus one oral antidiabetic drug who were aged ≥ 20 years and had BMI < 35.0 kg/m² and HbA1c $\geq 7.0\%$ and $< 10.0\%$ at Visit 1. The oral antidiabetic drugs were biguanides, sulfonamides, sulfonylureas (SU, patients treated with up to half maximal approved dose), α -glucosidase inhibitors (α -GI), insulin secretagogues, and insulin-sensitizing agents.

All of 400 treated subjects (268 subjects in the liraglutide 0.9 mg group, 132 subjects in the glibenclamide group) were included in the FAS for efficacy and safety analyses. There were 65 withdrawals from the trial, including 43 withdrawals in the liraglutide 0.9 mg group (20 withdrawals due to adverse events, 1 withdrawal due to non-compliance with the protocol, 10 withdrawals due to ineffective therapy, 12 withdrawals for other reasons) and 22 withdrawals in the glibenclamide group (8 withdrawals due to adverse events, 2 withdrawals due to non-compliance with the protocol, 9 withdrawals due to ineffective therapy, 3 withdrawals for other reasons).

For the primary efficacy endpoint of HbA1c at Week 24 (LOCF), the difference between the liraglutide 0.9 mg and glibenclamide groups [95% CI] was -0.50 [-0.70, -0.30] and the upper limit of the 95% CI fell below the pre-specified non-inferiority margin of 0.4%. Thus, the non-inferiority of liraglutide 0.9 mg to glibenclamide was demonstrated (Table 20).

Table 20. Results of analysis of HbA1c at Week 24 (LOCF) (FAS)

Treatment group	Baseline ^{a)}	Week 24 ^{a)}	Difference from glibenclamide ^{b)} [95% CI]	Change from baseline ^{a)}
Liraglutide 0.9 mg (n = 263 ^{c)})	8.91 (1.08)	7.17 (1.18)	-0.50 [-0.70, -0.30]	-1.74 (1.06)
Glibenclamide (n = 130 ^{c)})	8.80 (0.97)	7.61 (1.13)		-1.18 (1.03)

a) Mean (SD) %

b) Least squares mean %, ANCOVA model including treatment group and previous antidiabetic treatment (dietary therapy, one oral antidiabetic drug) as fixed effects and baseline HbA1c as a covariate

c) Subjects with missing HbA1c at baseline or at the end of treatment/withdrawal or who used prohibited concomitant medications were excluded from the FAS.

As for the secondary efficacy endpoints, the proportion of subjects achieving HbA1c < 6.5% at Week 24 was 26.9% (72 of 268 subjects) in the liraglutide 0.9 mg group and 10.6% (14 of 132 subjects) in the glibenclamide group. Fasting blood glucose (least squares mean $\pm$ SE) at Week 24 (LOCF) was 137.2 $\pm$ 1.9 mg/dL in the liraglutide 0.9 mg group and 150.1 $\pm$ 2.5 mg/dL in the glibenclamide group. Two-hour post-prandial blood glucose after a meal tolerance test was 196.3 $\pm$ 4.2 mg/dL in the liraglutide 0.9 mg group and 238.0 $\pm$ 5.5 mg/dL in the glibenclamide group. Changes in HbA1c from baseline to Week 52 (LOCF) were as shown in Table 21 and HbA1c values over time during the treatment period were as shown in Figure 2.

Table 21. Changes in HbA1c from baseline to Week 52 (LOCF)

Treatment group	Baseline	Week 52	Change from baseline
Liraglutide 0.9 mg (n = 263 ^{a)})	8.91 (1.08)	7.43 (1.32)	-1.48 (1.12)
Glibenclamide (n = 130 ^{a)})	8.80 (0.97)	7.84 (1.21)	-0.95 (1.06)

Mean (SD) %

a) Subjects with missing HbA1c at baseline or at the end of treatment/withdrawal or who used prohibited concomitant medications were excluded from the FAS.

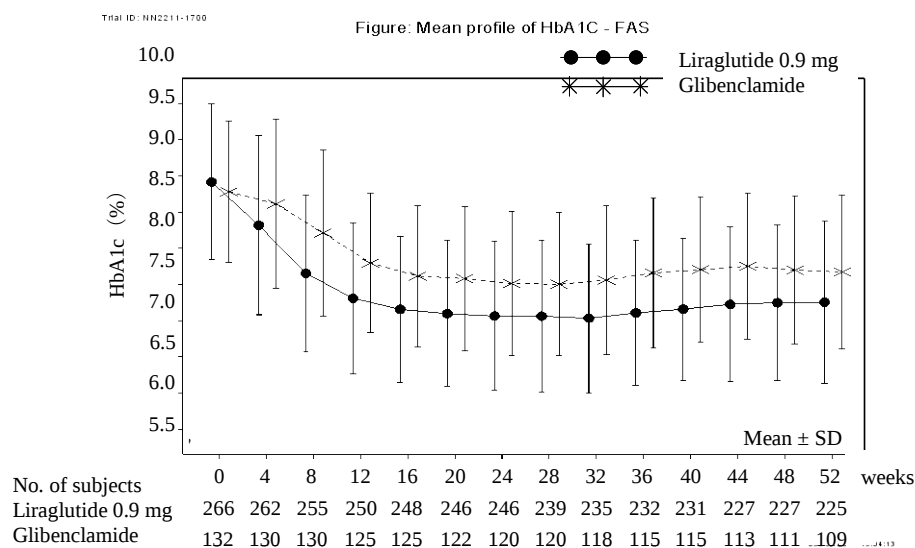


Figure 2. HbA1c values over time during treatment period

The change in body weight from baseline to Week 52 (LOCF) (mean $\pm$ SD) was -0.75 ± 2.67 kg in the liraglutide 0.9 mg group and 0.96 ± 2.26 kg in the glibenclamide group and the between-treatment difference in body weight at Week 52 (LOCF) [95% CI] was -1.71 [-2.25 , -1.18].

Regarding safety, the incidence of adverse events was 91.4% (245 of 268 subjects) (1098 events) in the liraglutide 0.9 mg group and 91.7% (121 of 132 subjects) (511 events) in the glibenclamide group and the incidence of adverse drug reactions was 35.4% (95 of 268 subjects) (184 events) in the liraglutide 0.9 mg group and 25.0% (33 of 132 subjects) (60 events) in the glibenclamide group. The commonly reported system organ classes of adverse events were “gastrointestinal disorders” and “infections and infestations” and the incidence of adverse events classified as “gastrointestinal disorders” was higher in the liraglutide 0.9 mg group (45.1% [121 of 268 subjects]) compared to the glibenclamide group (36.4% [48 of 132 subjects]). Adverse events reported by $\geq 5\%$ of subjects in either group were as shown in Table 22.

Table 22. Adverse events reported by $\geq 5\%$ of subjects in either group

	Liraglutide 0.9 mg (n = 268)	Glibenclamide (n = 132)
Any event	245 (91.4) 1098	121 (91.7) 511
Nasopharyngitis	100 (37.3) 183	57 (43.2) 94
Diarrhoea	26 (9.7) 31	9 (6.8) 12
Upper respiratory tract inflammation	25 (9.3) 48	9 (6.8) 10
Constipation	22 (8.2) 24	7 (5.3) 8
Back pain	17 (6.3) 17	9 (6.8) 9
Diabetic retinopathy	16 (6.0) 16	9 (6.8) 10
Headache	15 (5.6) 22	6 (4.5) 11
Nausea	14 (5.2) 24	2 (1.5) 3
Stomach discomfort	14 (5.2) 21	3 (2.3) 5
Bronchitis	11 (4.1) 13	8 (6.1) 11
Hypertension	11 (4.1) 11	7 (5.3) 7
Arthralgia	8 (3.0) 9	11 (8.3) 11
Pharyngolaryngeal pain	2 (0.7) 2	7 (5.3) 7

No. of subjects with events (Incidence %) No. of events

One death was reported in the liraglutide 0.9 mg group (gastroenteritis), which was judged by the investigator to be unrelated to study drug. Serious adverse events occurred in 7.5% of the liraglutide 0.9 mg group (20 of 268 subjects; myocardial infarction [2 subjects], gastroenteritis, spinal ligament ossification, multiple myeloma, renal cell carcinoma, acute myocardial infarction, rectal cancer, ventriculoperitoneal shunt malfunction, gastric cancer, vomiting, constipation/prostate cancer, neurogenic bladder, pneumonia, sleep apnoea syndrome, colon polypectomy, blood CK increased/blood CK-MB increased/AST increased/LDH increased, arthritis, endometriosis, thyroid neoplasm [1 subject each]) and 10.6% of the glibenclamide group (14 of 132 subjects; cerebral infarction [2 subjects], gangrene, arthropod sting, metastatic neoplasm, colonic polyp, dizziness/vomiting, cholelithiasis, inguinal hernia, suicide attempt, road traffic accident, Bowen's disease, myocardial infarction, laryngeal cancer [1 subject each]). Adverse events leading to trial discontinuation occurred in 7.5% of the liraglutide 0.9 mg group (20 of 268 subjects; myocardial infarction [2 subjects], vomiting [2 subjects], gastroenteritis, hepatic function abnormal, constipation/diarrhoea, stomach discomfort/diarrhoea, gastrointestinal disorder, multiple myeloma, renal cell carcinoma, acute myocardial infarction, rectal cancer, gastric cancer, prostate cancer, dizziness, C-reactive protein increased, endometriosis, thyroid neoplasm, injection site rash [1 subject each]) and 6.1% of the glibenclamide group (8 of 132 subjects; gangrene, cerebral infarction, colonic polyp, dizziness/vomiting, inguinal hernia, road traffic accident, myocardial infarction, laryngeal cancer).

The incidence of hypoglycemia¹⁵ was 24.6% (66 of 268 subjects) (167 events) in the liraglutide 0.9 mg group and 41.7% (55 of 132 subjects) (460 events) in the glibenclamide group. Hypoglycemia meeting the definition of a serious adverse event or major hypoglycemia was not reported. Blood calcitonin increased occurred in 0.7% (2 of 268 subjects) of the liraglutide 0.9 mg group, but not in the

¹⁵ See the Trial NN2211-1701 section for the definition of hypoglycemia.

glibenclamide group. Adverse events classified as “eye disorders” including diabetic retinopathy were reported by 17.5% (47 of 268 subjects) of the liraglutide 0.9 mg group (53 events) and 21.2% (28 of 132 subjects) of the glibenclamide group (33 events). Anti-liraglutide antibodies were negative in all subjects at baseline, but were positive in 12.3% (33 of 268 subjects) of the liraglutide 0.9 mg group at Week 53 (LOCF), of whom 16 subjects were also positive for antibodies crossreacting with GLP-1. One subject in the glibenclamide group was positive for anti-liraglutide antibodies, but was negative for antibodies crossreacting with GLP-1.

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

4.(iii).B.(1) Clinical positioning

PMDA asked the applicant to explain the clinical positioning of liraglutide, in light of liraglutide clinical trial data and its injectable dosage form.

The applicant responded as follows:

Liraglutide is a GLP-1 analog with a novel mode of action. A phase III clinical trial in patients with relatively early type 2 diabetes mellitus (NN2211-1700) has demonstrated that liraglutide 0.9 mg is not inferior to an SU, glibenclamide, and has a lower incidence of hypoglycemia and suppresses body weight gain compared to glibenclamide. Furthermore, a phase II/III trial (NN2211-1701) has demonstrated the superiority of liraglutide as an add-on to an SU over SU monotherapy in patients with more advanced type 2 diabetes mellitus with inadequate glycemic control on SU monotherapy. The above results indicate that liraglutide is useful for a broad range of type 2 diabetes mellitus patients from early to more advanced disease. On the other hand, considering that liraglutide is an injection, due to a reluctance to self-inject every day, the liraglutide product will be used only in limited number of patients especially when they have early disease. Therefore, the clinical benefits of liraglutide are considered particularly great for patients with more advanced disease with inadequate glycemic control on one oral antidiabetic drug. Patients with advanced insulin-dependent type 2 diabetes mellitus will require intensive insulin therapy.

PMDA considers that although liraglutide has a novel mode of action and can serve as a treatment option for patients with type 2 diabetes mellitus, as liraglutide is an injection, like insulin products, liraglutide is more likely to be used in patients with more advanced disease rather than in patients with early diabetes. In medical practice these days, combination therapy of oral antidiabetics with insulin has been used even in patients who have a non-insulin-dependent condition of type 2 diabetes mellitus but are unable to achieve adequate glycemic control on oral antidiabetic therapy alone. Given this, PMDA asked the applicant to explain how differently liraglutide and insulin should be used.

The applicant responded as follows:

Foreign trial NN2211-1697 that evaluated liraglutide in combination with oral antidiabetic drugs has demonstrated the non-inferiority of liraglutide combination therapy (liraglutide + metformin + glimepiride) to insulin glargine combination therapy (insulin glargine + metformin + glimepiride). In Japan, a comparative study of liraglutide vs. insulin has not been conducted. However, since insulin requires complicated dose adjustments and frequent blood glucose monitoring, induces body weight gain, and is associated with the risk of hypoglycemia, it is hard to say that insulin therapy is widely accepted by both physicians and patients. On the other hand, liraglutide requires no complicated dose adjustment, is associated with a low risk of hypoglycemia despite a potent blood glucose lowering effect, and does not increase body weight. Thus, liraglutide as part of oral antidiabetic combination therapy is more likely to be accepted also by patients who are reluctant to use an insulin product.

PMDA accepted the response, considering that liraglutide as monotherapy or in combination with an SU can serve as a treatment option in Japan since Japanese clinical trials have demonstrated the efficacy and safety of liraglutide monotherapy and liraglutide combination therapy with an SU [see “4.(iii).B.(2) Efficacy” and “4.(iii).B.(3) Safety”].

4.(iii).B.(2) Efficacy

4.(iii).B.(2).1 Monotherapy

PMDA asked the applicant to explain the basis for choosing glibenclamide (an SU) as a comparator and to justify the maintenance dose of 2.5 mg/day of glibenclamide in the Japanese phase III clinical trial (NN2211-1700, liraglutide monotherapy).

The applicant responded as follows:

This trial included patients with relatively early type 2 diabetes mellitus on dietary therapy alone or dietary therapy plus one oral antidiabetic drug. Due to reports that Japanese patients with type 2 diabetes mellitus are characterized by a remarkable decrease in insulin secretion (Seino Y, *et al.*, *Japanese Journal of Clinical Medicine* 1994; 52: 2686-2692, Fukushima M, *et al.*, *Diabetes Res Clin Pract*, 2004; 66S:S37-S43, Fukushima M, *et al.*, *Metabolism*, 2004; 53: 831-835), SUs are widely used in patients with early type 2 diabetes mellitus in Japan. As glibenclamide is regarded as the most potent drug among SUs available in Japan and the safety of its long-term use has been established, it was chosen as a comparator. Although the approved dose range of glibenclamide is 1.25 to 10 mg/day, the usual maintenance dose is thought to be 2.5 mg/day due to the risk of hypoglycemia (Japan Diabetes Society ed. *Advances in Diabetology* 2004; 38; 71-75). Thus, a maintenance dose of 2.5 mg/day of glibenclamide was specified for this study.

PMDA considers as follows:

There are no major problems using glibenclamide as a comparator with the specified maintenance dose of 2.5 mg/day in Trial NN2211-1700, in light of the usage of SUs in medical practice. This trial has demonstrated the non-inferiority of liraglutide 0.9 mg to glibenclamide for the primary endpoint of HbA1c at Week 24 (LOCF) and the change in HbA1c from baseline to Week 24 (LOCF) (mean $\pm$ SD) was $-1.74 \pm 1.06\%$ in the liraglutide 0.9 mg group and $-1.18 \pm 1.03\%$ in the glibenclamide group. Also, in this trial, the efficacy of liraglutide was confirmed to be maintained until Week 52. Thus, the efficacy of liraglutide monotherapy has been demonstrated.

4.(iii).B.(2).2) Combination therapy with SU

PMDA asked the applicant to discuss the efficacy of liraglutide by dose of coadministered SU in the phase II/III trial (NN2211-1701).

The applicant responded as follows:

Subjects were stratified into a high-dose SU group (glibenclamide $\geq$ 5 mg/day, glimepiride $\geq$ 4 mg/day, gliclazide $\geq$ 80 mg/day) and a low-dose SU group (glibenclamide $<$ 5 mg/day, glimepiride $<$ 4 mg/day, gliclazide $<$ 80 mg/day) to discuss the efficacy of liraglutide in terms of change in HbA1c from baseline to Week 24 (LOCF). As a result, the change in HbA1c was greater in the high-dose group than in the low-dose group for all treatment groups, which was considered attributed to higher HbA1c values at baseline in subjects treated with high-dose SU compared to subjects treated with low-dose SU (Table 23). There was no statistically significant interaction between the dose of SU (high-dose group or low-dose group) and treatment group with respect to the primary efficacy endpoint of HbA1c at Week 24 (LOCF) ($P = 0.8175$, ANCOVA model). The above results indicate that the effect of the dose of SU on the efficacy of liraglutide in terms of HbA1c is small.

Table 23. Change in HbA1c by dose of SU (FAS) (Trial NN2211-1701)

Timing of endpoint	Liraglutide 0.6 mg + SU		Liraglutide 0.9 mg + SU		SU monotherapy	
	Low-dose (n = 47)	High-dose (n = 39)	Low-dose (n = 53)	High-dose (n = 34)	Low-dose (n = 55)	High-dose (n = 33)
Baseline	8.32 (0.72)	8.94 (1.02)	8.07 (0.74)	8.47 (0.78)	8.28 (0.92)	8.75 (1.04)
Week 24	7.03 (0.84)	7.27 (0.94)	6.58 (0.78)	6.81 (0.89)	8.02 (1.02)	8.12 (1.32)
Change from baseline to Week 24	-1.29 (0.90)	-1.67 (0.98)	-1.49 (0.96)	-1.67 (0.61)	-0.26 (0.73)	-0.63 (1.15)
Mean (SD) %						

PMDA considers as follows:

Trial NN2211-1701 has demonstrated the superiority of liraglutide 0.9 mg + SU over SU monotherapy for the primary endpoint of HbA1c at Week 24 (LOCF), the change in HbA1c from baseline to Week 24 (LOCF) (mean $\pm$ SD) was $-1.56 \pm 0.84\%$ in the liraglutide 0.9 mg + SU group and $-0.40 \pm 0.93\%$ in the SU monotherapy group, and the efficacy of liraglutide combination therapy with an SU was confirmed to be maintained until Week 52. Thus, the efficacy of liraglutide combination therapy with

an SU has been demonstrated.

4.(iii).B.(2).3 Effect on body weight

Body weight at Week 24 (LOCF) was significantly lower in the liraglutide group than in the glibenclamide group in Trial NN2211-1700 ($P < 0.001$, ANCOVA model) while it was significantly higher in the liraglutide groups (0.6 mg + SU group, 0.9 mg + SU group) compared to the SU monotherapy group in Trial NN2211-1701 ($P < 0.001$, $P = 0.007$, ANCOVA model). Taking account of these findings, PMDA asked the applicant to explain the effect of liraglutide on body weight.

The applicant responded as follows:

The change in body weight from baseline to Week 24 (LOCF) (mean) was -0.92 kg in the liraglutide 0.9 mg group in Trial NN2211-1700 and 0.06 kg in the liraglutide 0.6 mg + SU group and -0.37 kg in the liraglutide 0.9 mg + SU group in Trial NN2211-1701, i.e., there was little increase in body weight with liraglutide in both trials. However, there was a 0.99 kg increase in body weight in the glibenclamide group in Trial NN2211-1700. In foreign confirmatory trials: NN2211-1436 (liraglutide at doses of 0.6, 1.2, and 1.8 mg, combination therapy with glimepiride, 26-week treatment), NN2211-1572 (liraglutide at doses of 0.6, 1.2, and 1.8 mg, combination therapy with metformin, 26-week treatment), NN2211-1573 (liraglutide at doses of 1.2 and 1.8 mg, liraglutide monotherapy, 52-week treatment), NN2211-1574 (liraglutide at doses of 1.2 and 1.8 mg, combination therapy with rosiglitazone and metformin, 26-week treatment), and NN2211-1697 (liraglutide at a dose of 1.8 mg, combination therapy with glimepiride and metformin, 26-week treatment), the mean change in body weight from baseline to the endpoint (estimate) was 0.7 kg in the liraglutide 0.6 mg + SU group and 0.3 kg in the liraglutide 1.2 mg + SU group, showing increases in body weight compared to the SU monotherapy group (-0.1 kg) in Trial NN2211-1436, whereas in other trials, body weight decreased by 1.0 to 2.8 kg with liraglutide and there were 0% to 5% decreases in 40% to 60% of subjects and $\geq 5\%$ decreases in 20% to 30% of subjects. In Trial NN8022-1807 involving foreign non-diabetic obese subjects, the proportion of subjects with a $> 5\%$ or $> 10\%$ decrease in body weight following treatment with liraglutide 3.0 mg/day was 75% or 37%, respectively, and the decrease in body weight at Week 52 (mean) was 7.8 kg in the liraglutide 3.0 mg group and the difference from placebo was 5.8 kg.

In Japanese clinical trials, there was no similar body weight decrease with liraglutide as in foreign clinical trials, but liraglutide did not induce body weight gain. Since other therapeutic drugs that improve glycemic control to a similar degree as liraglutide (insulin, SUs) induce body weight gain etc., this result that liraglutide does not induce body weight gain is considered clinically meaningful.

PMDA considers as follows:

The applicant's view that liraglutide does not induce body weight gain is understood. However, the

liraglutide 0.6 mg + SU group in Japanese trial NN2211-1701 and the liraglutide 0.6 mg + SU group and the liraglutide 1.2 mg + SU group in foreign trial NN2211-1436 showed a tendency towards increased body weight compared to the SU monotherapy group and no consistent results have been obtained. Therefore, the effect of liraglutide on body weight should be confirmed via post-marketing surveillance.

4.(iii).B.(3) Safety

4.(iii).B.(3).1 Hypoglycemia

The applicant explained as follows:

In the Japanese long-term treatment trials NN2211-1700 (liraglutide monotherapy) and NN2211-1701 (liraglutide combination therapy with an SU), no major or serious hypoglycemia was not reported. When the incidence and rate of hypoglycemia by duration of treatment were analyzed (Table 24), the incidence of hypoglycemia was lower with liraglutide 0.9 mg compared to glibenclamide in all strata in Trial NN2211-1700. In Trial NN2211-1701, more events of hypoglycemia were reported in both liraglutide groups than in the SU monotherapy group during the first 24 weeks and especially during the first 4 weeks, more events of hypoglycemia occurred in the liraglutide 0.9 mg + SU group than in the liraglutide 0.6 mg + SU group.

Table 24. Incidence and rate of any hypoglycemia (by duration of treatment)

Duration of treatment	Trial NN2211-1700		Trial NN2211-1701		
	Liraglutide 0.9 mg	Glibenclamide	Liraglutide 0.6 mg + SU	Liraglutide 0.9 mg + SU	SU monotherapy
Overall	66/268 (24.6) 167 [0.694]	55/132 (41.7) 460 [3.843]	47/88 (53.4) 257 [3.131]	57/88 (64.8) 313 [3.715]	28/88 (31.8) 221 [2.990]
< 4 weeks	26/268 (9.7) 36 [1.779]	31/132 (23.5) 76 [7.539]	23/88 (26.1) 56 [8.456]	32/88 (36.4) 99 [14.899]	11/88 (12.5) 22 [3.289]
≥ 4 weeks and ≤ 24 weeks	32/258 (12.4) 56 [0.585]	45/130 (34.6) 243 [5.083]	36/85 (42.4) 134 [4.202]	44/86 (51.2) 133 [4.094]	22/87 (25.3) 92 [2.985]
> 24 weeks	32/244 (13.1) 75 [0.602]	30/120 (25.0) 141 [2.281]	21/83 (25.3) 67 [1.538]	32/84 (38.1) 81 [1.795]	20/70 (28.6) 107 [2.939]

No. of subjects with events/No. of subjects included in the analysis set (Incidence %)

No. of events [Incidence rate, events per subject-year]

The influence of previous treatment on the incidence and rate of hypoglycemia in Trial NN2211-1700 was investigated. As a result, in the liraglutide 0.9 mg group, the incidences of “any hypoglycemia” and “minor hypoglycemia” were similar between subjects previously treated with dietary therapy only and subjects previously treated with oral antidiabetic drugs while the incidence rate of “hypoglycemic symptoms” tended to be higher in subjects previously treated with dietary therapy only (Table 25).

Table 25. Incidence and rate of hypoglycemia (by type of previous treatment) (Trial NN2211-1700)

Hypoglycemia classification	Previous treatment	Liraglutide 0.9 mg (n = 268)	Glibenclamide (n = 132)
Any hypoglycemia	Dietary therapy only	13/50 (26.0) 56 [1.237]	12/23 (52.2) 107 [5.322]
	With oral antidiabetic drugs	53/218 (24.3) 111 [0.568]	43/109 (39.4) 353 [3.544]
Minor hypoglycemia	Dietary therapy only	6/50 (12.0) 7 [0.155]	8/23 (34.8) 35 [1.741]
	With oral antidiabetic drugs	22/218 (10.1) 38 [0.195]	28/109 (25.7) 97 [0.974]
Hypoglycemic symptoms	Dietary therapy only	11/50 (22.0) 49 [1.083]	10/23 (43.5) 72 [3.581]
	With oral antidiabetic drugs	42/218 (19.3) 73 [0.374]	40/109 (36.7) 256 [2.570]

No. of subjects with events/No. of subjects included in the analysis set (Incidence %)

No. of events [Incidence rate, events per subject-year]

PMDA asked the applicant to explain the possibility that the time of day of liraglutide injection (morning or evening) specified in clinical trials affects the occurrence of hypoglycemia.

The applicant responded as follows:

The incidence and rate of hypoglycemia were similar between morning and evening injections in Trial NN2211-1700 (liraglutide monotherapy), but were higher for morning injection regardless of hypoglycemia classification in Trial NN2211-1701 (liraglutide combination therapy with an SU) (Table 26). Although the cause for this finding could not be identified, it is considered that when liraglutide and an SU are administered in the morning, the blood concentrations of both drugs increase simultaneously, which may lead to the occurrence of hypoglycemia.

Table 26. Incidence and rate of hypoglycemia by time of day of liraglutide injection (morning or evening)

Hypoglycemia classification	Time of day	Trial NN2211-1700		Trial NN2211-1701		
		Liraglutide 0.9 mg (n = 268)	Glibenclamide (n = 132)	Liraglutide 0.6 mg + SU (n = 88)	Liraglutide 0.9 mg + SU (n = 88)	SU monotherapy (n = 88)
Any hypoglycemia	Morning	38/161 (23.6) 104 [0.720]	33/82 (40.2) 305 [4.236]	32/57 (56.1) 204 [3.909]	42/62 (67.7) 263 [4.385]	19/62 (30.6) 146 [2.745]
	Evening	28/107 (26.2) 63 [0.655]	22/50 (44.0) 155 [3.249]	15/31 (48.4) 53 [1.773]	15/26 (57.7) 50 [2.060]	9/26 (34.6) 75 [3.618]
Minor hypoglycemia	Morning	12/161 (7.5) 12 [0.083]	22/82 (26.8) 81 [1.125]	19/57 (33.3) 95 [1.821]	30/62 (48.4) 99 [1.651]	8/62 (12.9) 65 [1.222]
	Evening	16/107 (15.0) 33 [0.343]	14/50 (28.0) 51 [1.069]	9/31 (29.0) 23 [0.769]	9/26 (34.6) 16 [0.659]	7/26 (26.9) 30 [1.447]
Hypoglycemic symptoms	Morning	34/161 (21.1) 92 [0.637]	30/82 (36.6) 224 [3.111]	26/57 (45.6) 109 [2.089]	35/62 (56.5) 164 [2.734]	18/62 (29.0) 81 [1.523]
	Evening	19/107 (17.8) 30 [0.312]	20/50 (40.0) 104 [2.180]	11/31 (35.5) 30 [1.003]	10/26 (38.5) 34 [1.401]	8/26 (30.8) 45 [2.171]

No. of subjects with events/No. of subjects included in the analysis set (Incidence %)

No. of events [Incidence rate, events per subject-year]

PMDA considers as follows:

Regarding the following findings from the Japanese long-term treatment trials, caution statements should be included in the package insert etc.:

The incidence of hypoglycemia in the liraglutide 0.9 mg group was lower compared to the glibenclamide group; the incidence rate of hypoglycemia (events per subject-year) during the initial

phase of treatment (Weeks 0-4) tended to be higher than those after Week 4; the incidence rate of hypoglycemia tended to be higher in subjects previously untreated with oral antidiabetic drugs than in subjects previously treated with oral antidiabetic drugs; in the combination therapy study, the incidence rate of hypoglycemia tended to be higher with liraglutide combination therapy with an SU compared to SU monotherapy during the first 24 weeks of treatment; and the incidence rate of hypoglycemia tended to be higher for morning injection compared to evening injection, regardless of the dose of liraglutide.

4.(iii).B.(3).2) Gastrointestinal disorders

The applicant explained as follows:

The incidences of gastrointestinal adverse events in Trials NN2211-1700 and 1701 are shown in Table 27. The incidence of gastrointestinal events during the first 4 weeks of treatment was 19.4% (52 of 268 subjects) in the liraglutide 0.9 mg group and 9.8% (13 of 132 subjects) in the glibenclamide group in Trial NN2211-1700, and 14.8% (13 of 88 subjects) in the liraglutide 0.6 mg + SU group, 20.5% (18 of 88 subjects) in the liraglutide 0.9 mg + SU group, and 11.4% (10 of 88 subjects) in the SU monotherapy group in Trial NN2211-1701, showing a higher incidence in the liraglutide group compared to the control group in both trials and a trend towards a dose-dependent incidence in Trial NN2211-1701 (Figure 3).

Table 27. Incidence of gastrointestinal adverse events

		Trial NN2211-1700 (Liraglutide monotherapy)		Trial NN2211-1701 (Liraglutide combination therapy with an SU)		
		Liraglutide 0.9 mg (n = 268)	Glibenclamide (n = 132)	Liraglutide 0.6 mg + SU (n = 88)	Liraglutide 0.9 mg + SU (n = 88)	SU monotherapy (n = 88)
Overall adverse events (gastrointestinal disorders)		121 (45.1) 224	48 (36.4) 81	43 (48.9) 57	42 (47.7) 76	34 (38.6) 60
Serious adverse events		2 (0.7) 2	3 (2.3) 3	0 (0.0) 0	1 (1.1) 1	0 (0.0) 0
Severity	Mild	116 (43.3) 215	47 (35.6) 77	42 (47.7) 52	41 (46.6) 73	34 (38.6) 58
	Moderate	8 (3.0) 9	3 (2.3) 4	5 (5.7) 5	3 (3.4) 3	1 (1.1) 2
	Severe	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Adverse drug reactions		52 (19.4) 86	16 (12.1) 26	18 (20.5) 21	21 (23.9) 34	15 (17.0) 22
Adverse events leading to discontinuation		5 (1.9) 7	3 (2.3) 3	1 (1.1) 1	0 (0.0) 0	0 (0.0) 0

No. of subjects with events (Incidence %) No. of events

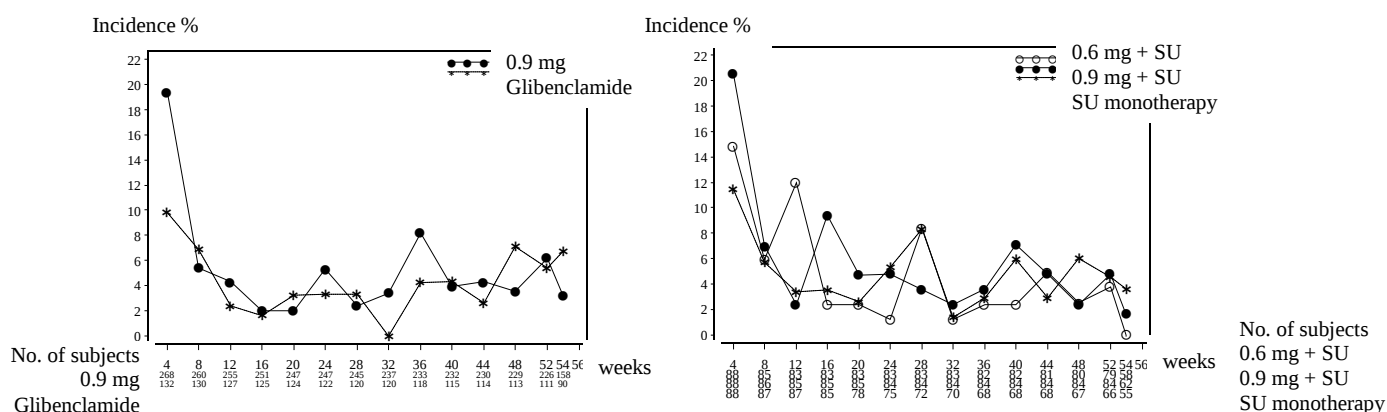


Figure 3. Incidence of gastrointestinal disorders by treatment week (Left, Trial NN2211-1700; Right, Trial NN2211-1701)

PMDA asked the applicant to explain actions to be taken with the drug if gastrointestinal disorders occur following the start of treatment with liraglutide.

The applicant responded as follows:

Most of gastrointestinal disorders reported during treatment with liraglutide in the Japanese clinical trials occurred within 4 to 6 weeks after the start of treatment and these events were mild to moderate in severity and generally transient. Thus, even if gastrointestinal disorders occur, usually, liraglutide treatment can be continued without changing the timing of dosing or the dose. On the other hand, if constipation, diarrhoea, or nausea etc. persists for more than 1 month, temporary dose reduction to 0.6 mg or interruption may be considered. This action is based on results from foreign clinical trials where some subjects had their dose temporarily reduced to reduce gastrointestinal symptoms and in most cases, the dose was reduced by 30% to 50%. (In Japanese confirmatory trials, treatment was to be discontinued if liraglutide was not tolerated.) If symptoms resolve within 1 to 2 days following a dose reduction or interruption, liraglutide at the recommended dose (0.9 mg) can be resumed. If treatment is interrupted for ≥ 2 days, the dose should be titrated in weekly 0.3 mg increments to the recommended dose of 0.9 mg.

PMDA accepts the response, but considers that actions to be taken (dose reduction, interruption, treatment resumption etc.) in the event of gastrointestinal disorders should be advised in the package insert etc. and it is necessary to continue to collect post-marketing information on gastrointestinal disorders [see “4.(iii).B.(6).1) Dose” for the usefulness of 0.6 mg].

4.(iii).B.(3).3) Thyroid effects

The applicant explained as follows:

In Japanese and foreign clinical trials, the incidence rate of thyroid-related adverse events in the liraglutide group was similar to that in the placebo group, but was higher than that in the active

comparator group (Table 28). The event with the highest incidence in the liraglutide group was blood calcitonin increased, but the incidence of blood calcitonin increased was similar between the liraglutide and placebo groups. The incidence of thyroid neoplasm in the liraglutide group was higher than that in the active comparator group, but was similar to that in the placebo group. Most of thyroid neoplasms were benign thyroid nodules and about 50% of nodules (preferred term, thyroid neoplasm) reported as adverse events in the liraglutide group were reported from Trial NN2211-1334 in which thyroid ultrasound was performed at baseline and at the end of trial.

Table 28. Incidence and rate of thyroid-related adverse events
All Japanese and foreign intermediate-term* and long-term treatment trials** (Safety Analysis Set)

System Organ Class Preferred term	Liraglutide	Placebo	Active comparator	Total comparators
No. of subjects (Total exposure [subject-years])	n = 4505 (3671.5)	n = 907 (466.6)	n = 1474 (1195.2)	n = 2381 (1661.8)
All thyroid-related adverse events	95 (2.1) 123 [33.5]	13 (1.4) 14 [30.0]	21 (1.4) 22 [18.4]	34 (1.4) 36 [21.7]
Investigations	46 (1.0) 51 [13.9]	6 (0.7) 6 [12.9]	7 (0.5) 7 [5.9]	13 (0.5) 13 [7.8]
Blood calcitonin increased	37 (0.8) 40 [10.9]	5 (0.6) 5 [10.7]	5 (0.3) 5 [4.2]	10 (0.4) 10 [6.0]
Blood thyroid stimulating hormone increased	6 (0.1) 8 [2.2]	1 (0.1) 1 [2.1]	1 (0.1) 1 [0.8]	2 (0.1) 2 [1.2]
Ultrasound thyroid abnormal	1 (0.0) 1 [0.3]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]
Thyroxine decreased	1 (0.0) 1 [0.3]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]
Blood thyroid stimulating hormone decreased	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	1 (0.1) 1 [0.8]	1 (0.0) 1 [0.6]
Blood calcitonin abnormal	1 (0.0) 1 [0.3]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]
Endocrine disorders	34 (0.8) 39 [10.6]	4 (0.4) 4 [8.6]	13 (0.9) 13 [10.9]	17 (0.7) 17 [10.2]
Goitre	19 (0.4) 20 [5.4]	1 (0.1) 1 [2.1]	2 (0.1) 2 [1.7]	3 (0.1) 3 [1.8]
Hypothyroidism	5 (0.1) 5 [1.4]	1 (0.1) 1 [2.1]	5 (0.3) 5 [4.2]	6 (0.3) 6 [3.6]
Hyperthyroidism	4 (0.1) 4 [1.1]	1 (0.1) 1 [2.1]	2 (0.1) 2 [1.7]	3 (0.1) 3 [1.8]
Thyroid cyst	4 (0.1) 4 [1.1]	0 (0.0) 0 [0.0]	1 (0.1) 1 [0.8]	1 (0.0) 1 [0.6]
Autoimmune thyroiditis	3 (0.1) 3 [0.8]	1 (0.1) 1 [2.1]	1 (0.1) 1 [0.8]	2 (0.1) 2 [1.2]
Thyroid disorder	2 (0.0) 2 [0.5]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]
Toxic nodular goitre	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	1 (0.1) 1 [0.8]	1 (0.0) 1 [0.6]
Thyroiditis chronic	1 (0.0) 1 [0.3]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]
Thyroid pain	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	1 (0.1) 1 [0.8]	1 (0.0) 1 [0.6]
Neoplasms benign, malignant and unspecified	30 (0.7) 33 [9.0]	4 (0.4) 4 [8.6]	2 (0.1) 2 [1.7]	6 (0.3) 6 [3.6]
Thyroid neoplasm	24 (0.5) 25 [6.8]	3 (0.3) 3 [6.4]	1 (0.1) 1 [0.8]	4 (0.2) 4 [2.4]
Papillary thyroid carcinoma	3 (0.1) 3 [0.8]	1 (0.1) 1 [2.1]	0 (0.0) 0 [0.0]	1 (0.0) 1 [0.6]
Thyroid cancer	2 (0.0) 2 [0.5]	0 (0.0) 0 [0.0]	1 (0.1) 1 [0.8]	1 (0.0) 1 [0.6]
Parathyroid tumour benign	1 (0.0) 1 [0.3]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]
Benign neoplasm of thyroid gland	2 (0.0) 2 [0.5]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]	0 (0.0) 0 [0.0]

No. of subjects with events (Incidence %) No. of events [Incidence rate, events per 1000 subject-years]

*: Exploratory trials with a treatment duration of > 5 weeks and < 24 weeks. Japanese trial NN2211-1334 and foreign trials NN2211-1571, 1310, 1333, 2072, and 1499, and NN8022-1807 (excluding extension periods)

**: Confirmatory trials with a treatment duration of ≥ 24 weeks. Japanese trials NN2211-1700 and 1701 and foreign trials NN2211-1573, 1572, 1436, 1574, 1697, and 1797 and NN8022-1807 (including an extension period)

As for calcitonin, in the Japanese long-term treatment trials NN2211-1700 and 1701, 3 events of blood calcitonin increased were reported by 2 subjects in the liraglutide 0.9 mg group in Trial NN2211-1700, of which 2 events reported by 2 subjects were classified as adverse drug reactions, but both resolved after the end of treatment. The changes in blood calcitonin from baseline to Week 52 (LOCF) were as follows: in Trial NN2211-1700, the difference between the liraglutide 0.9 mg and glibenclamide groups [95% CI] (pg/mL) was 0.157 [0.013, 0.302], and in Trial NN2211-1701, the difference between the liraglutide combination therapy with an SU and SU monotherapy groups [95% CI] (pg/mL) was 0.052 [-0.109, 0.213] for the liraglutide 0.6 mg + SU group and -0.013 [-0.174, 0.148]

for the liraglutide 0.9 mg + SU group, and none of these differences was considered clinically meaningful. Furthermore, as many subjects had calcitonin levels below the lower limit of quantitation of 2.0 pg/mL, changes in blood calcitonin were analyzed by dividing calcitonin levels into four categories of “below the lower limit of quantitation,” “above the lower limit of quantitation and below the upper limit of reference range,” “above the upper limit of reference range and below 2 times the upper limit of reference range,” and “above 2 times the upper limit of reference range.” As a result, there were upward shifts in calcitonin category from baseline to Week 52 (LOCF) in 7 subjects in the liraglutide 0.9 mg group and 3 subjects in the glibenclamide group in Trial NN2211-1700 and 5 subjects in the liraglutide 0.6 mg + SU group, 2 subjects in the liraglutide 0.9 mg + SU group, and 2 subjects in the SU monotherapy group in Trial NN2211-1701, and there were no significant changes in all treatment groups (Trial NN2211-1700, $P = 0.3197$ for the liraglutide 0.9 mg group, $P = 0.1046$ for the glibenclamide group; Trial NN2211-1701, $P = 0.3690$ for the liraglutide 0.6 mg + SU group, $P = 0.5630$ for the liraglutide 0.9 mg + SU group, $P = 0.5773$ for the SU monotherapy group, Agresti’s test [Agresti A. *Biometrics*, 1983; 39: 505-10]).

In foreign phase III clinical trials (foreign trials NN2211-1436, 1572, 1573, 1574, 1697, and 1797), blood calcitonin levels (mean) were between the lower limit of quantitation of 0.7 ng/L (expressed as pg/mL in Japanese trials) and < 1.0 ng/L in all treatment groups throughout the trial period. In foreign trials NN2211-1573 and 1572 with a treatment duration of 2 years, the proportion of subjects with a blood calcitonin level exceeding 20 ng/L at least once was similar between the liraglutide (1.67%) and active comparator (1.88%) groups. Based on pooled data from the foreign long-term treatment trials (NN2211-1572, 1573, 1436, 1574, and 1697), the incidence rate of adverse events of blood calcitonin increased (events per 1000 subject-years) was 23.2 in the liraglutide 0.6 mg group, 8.3 in the liraglutide 1.2 mg group, 13.3 in the liraglutide 1.8 mg group, 18.9 in the placebo group, and 6.8 in the active comparator group, showing no relationship between the dose of liraglutide and the incidence rate, and all events were nonserious except for 1 case in the liraglutide 0.6 mg + metformin group in Trial NN2211-1572. The lower limit of quantitation of a standard commercial assay is 2 ng/L and generally, patients with a blood calcitonin level of 15 to 20 ng/L are considered to require a follow-up test unless they are smokers or are receiving H₂-blockers or proton pump inhibitors (Costante G, *et al.*, *Nat Clin Pract Endocrinol Metab*, 2009; 5: 35-44, Rink T, *et al.*, *Thyroid*. 2009; 19: 327-332). Therefore, the changes in blood calcitonin observed in clinical trials were not considered clinically significant.

PMDA asked the applicant to explain whether thyroid tests are needed, taking into account that the development of thyroid neoplasms has been observed with liraglutide in Japanese and foreign clinical trials and thyroid cancer for which a causal relationship to liraglutide can not be denied has been reported in foreign clinical trials.

The applicant responded as follows:

Since it has been reported that thyroid ultrasound does not have sufficient sensitivity or specificity to assess changes in human thyroid C-cells (Schwerk WB, *et al.*, *Cancer*, 1985; 55: 624-630) and it is known that in the general population, the prevalence of thyroid nodules is high and increases with age (Gough J *et al.*, *World J Sur*, 2008; 32: 1264-1268), performing thyroid ultrasound in all type 2 diabetic patients in medical practice is of little significance. Although neck palpation is considered useful for determining whether a follow-up test is needed, if neck palpation is mandatory, an unnecessary follow-up test might be performed. As for calcitonin, as mentioned above, in all the Japanese and foreign long-term treatment clinical trials, blood calcitonin levels were around the lower limit of quantitation of a high-sensitivity calcitonin assay in many subjects and there were no differences in blood calcitonin levels over time between the liraglutide and control groups. Thus, periodic monitoring of blood calcitonin is of little significance. The incidence of thyroid-related adverse events reported in all clinical trials will be listed in the package insert and the occurrence of thyroid-related adverse events will be carefully monitored via post-marketing surveillance.

PMDA considers as follows:

There is no particular problem with the applicant's view that the results from Japanese and foreign clinical trials suggested no relationship between liraglutide and thyroid abnormalities including blood calcitonin increased. However, C-cell tumors were induced in mice and rats in non-clinical studies; considering its target disease, liraglutide is supposed to be administered for long periods; and thyroid tumors often remain asymptomatic until they grow. Given these, the package insert should advise that the presence or absence of thyroid signs and symptoms should be checked by neck palpation etc. during treatment with liraglutide and if abnormalities are detected, thyroid tests, e.g., blood calcitonin should be performed. The above conclusion will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(3).4) Injection site disorders and immunogenicity

The applicant explained as follows:

In Japanese long-term treatment clinical trials, the proportion of subjects with injection site disorders was as follows: in Trial NN2211-1700 (liraglutide monotherapy), 6.3% (17 of 268 subjects) in the liraglutide 0.9 mg group and 0.0% (0 of 132 subjects) in the glibenclamide group, and in Trial NN2211-1701 (liraglutide combination therapy with an SU) 6.8% (6 of 88 subjects) in the liraglutide 0.6 mg + SU group, 5.7% (5 of 88 subjects) in the liraglutide 0.9 mg + SU group, and 3.4% (3 of 88 subjects) in the SU monotherapy group, showing a higher incidence in the liraglutide group compared to the control group (treatment with liraglutide placebo). The events with a high incidence were injection site erythema and injection site bruising. There were only 2 moderate events (injection site

rash) reported in the liraglutide 0.9 mg group in Trial NN2211-1700 and other events were all mild in severity. The one subject with an event of moderate injection site rash was withdrawn from the trial due to this event. In foreign long-term treatment trials,¹⁶ the incidence rate of injection site disorders (events per 1000 subject-years) was 15.5 in the liraglutide 0.6 mg group, 23.6 in the liraglutide 1.2 mg group, 35.5 in the liraglutide 1.8 mg group, 32.0 in the placebo group, and 18.9 in the active comparator group, showing a trend towards a higher incidence rate with increasing dose of liraglutide. As the incidence rate of injection site disorders was similar between the liraglutide 1.8 mg group (the maximal dose of liraglutide approved overseas) and the placebo group, this dose dependence was considered possibly related to the increasing volume injected with increasing dose.

The occurrence of immunogenicity-related adverse events in the Japanese long-term treatment trials NN2211-1700 and 1701 was analyzed for “anaphylactic reaction,” “angioedema,” and “severe skin adverse drug reactions.” As a result, 7 events of urticaria (in the liraglutide group only), 3 events of gingival swelling (2 events in the liraglutide group, 1 event in the control group), and 1 event of oedema mouth (in the liraglutide group) were reported and there was a trend towards a higher incidence in the liraglutide group compared to the control group (Trial NN2211-1700, 2.2% [6 of 268 subjects] in the liraglutide 0.9 mg group, 0.8% [1 of 132 subjects] in the glibenclamide group; Trial NN2211-1701, 4.5% [4 of 88 subjects] in the liraglutide 0.6 mg + SU group, 0% [0 of 88 subjects] in the liraglutide 0.9 mg + SU group, 0.0% [0 of 88 subjects] in the SU monotherapy group). Three of the 7 events of urticaria observed in the liraglutide group only were assessed as “possibly related” to study drug. The reported events were all mild or moderate in severity and there were no serious adverse events or events leading to treatment discontinuation. In all the Japanese and foreign intermediate-term and long-term treatment studies [see the footnote of Table 28], the incidence rate of immunogenicity-related adverse events was 13.3 per 1000 subject-years in the liraglutide group, which tended to be higher than 6.0 per 1000 subject-years in the control group, but most events were mild in severity. Based on the above, urticaria will be listed in the adverse reactions section of the draft package insert to alert physicians.

As the incidences of injection site and immunogenicity adverse events with liraglutide in Japanese and foreign clinical trials are not substantially different from those with approved insulin products, PMDA accepts the applicant’s response that urticaria will be listed in the adverse reactions section of the package insert (draft), and considers that it is necessary to continue to collect information on injection site disorders and immunogenicity via post-marketing surveillance.

¹⁶ In all the foreign long-term treatment trials excluding Trials NN2211-1697 and NN2211-1797, subjects in the placebo and active comparator groups received liraglutide placebo. In Trials NN2211-1697 and NN2211-1797, open-label insulin glargine and exenatide, respectively, was administered as an active comparator.

4.(iii).B.(3).5) Formation of anti-liraglutide antibody

PMDA asked the applicant to explain the impact of the formation of anti-liraglutide antibody on the safety and efficacy of liraglutide.

The applicant responded as follows:

As the criteria for anti-liraglutide antibody positivity (cutoff values) defined in the protocol for Trials NN2211-1700 and 1701 were inappropriate,¹⁷ the data were reanalyzed using new cutoff values. As a result, in Trial NN2211-1700, 14.7% (33 of 225 subjects) of the liraglutide group were tested positive for anti-liraglutide antibodies at Week 53 (LOCF) and the change in HbA1c from baseline to Week 52 (LOCF) (mean $\pm$ SD) in these antibody-positive subjects was $-1.76 \pm 0.80\%$, which was not substantially different from $-1.48 \pm 1.12\%$ in the overall liraglutide group. Also in Trial NN2211-1701, 16.9% (13 of 77 subjects) of the liraglutide 0.6 mg + SU group and 16.9% (14 of 83 subjects) of the liraglutide 0.9 mg + SU group were tested positive for anti-liraglutide antibodies at Week 53 (LOCF) and the change in HbA1c from baseline to Week 52 (LOCF) in these antibody-positive subjects was $-1.36 \pm 0.70\%$ for the liraglutide 0.6 mg + SU group and $-1.51 \pm 0.88\%$ for the liraglutide 0.9 mg + SU group, which were not substantially different from the changes in the respective overall groups ($-1.09 \pm 0.84\%$ for the liraglutide 0.6 mg + SU group, $-1.30 \pm 0.91\%$ for the liraglutide 0.9 mg + SU group). Commonly reported adverse events (system organ classes) in liraglutide-treated subjects tested positive for anti-liraglutide antibodies at Week 53 (LOCF) in Trials NN2211-1700 and 1701 were “infections and infestations,” “gastrointestinal disorders,” and “musculoskeletal and connective tissue disorders” and the incidences and rates of these events in these subjects were similar to those in the overall subject groups (Table 29). Immunogenicity-related adverse events reported by antibody-positive subjects treated with liraglutide were 1 event of gingival swelling in Trial NN2211-1700 and 1 event of urticaria (liraglutide 0.6 mg + SU group) in Trial NN2211-1701 and urticaria was judged possibly related to study drug, but both events were mild in severity. The above results indicate that the formation of anti-liraglutide antibody has no major impact on safety.

¹⁷ Two cutoff values for determining anti-liraglutide antibody positivity were used: one was calculated from values from 100 samples at baseline and the other was calculated as $1.96 \times \text{sqrt}(2) \times \text{SD}$. However, a large variability among batches of these samples analyzed resulted in low cutoff values and many subjects in the control group were determined to be positive for anti-liraglutide antibodies. Therefore, it was decided to use the cutoff value calculated from values from all samples at baseline and the cutoff value calculated as the 97.5th percentile of all samples at baseline.

Table 29. Incidence of adverse events in subjects tested positive for anti-liraglutide antibodies at Week 53 (LOCF)

Adverse event (System organ class)		Trial NN2211-1700	Trial NN2211-1701	
		Liraglutide 0.9 mg	Liraglutide 0.6 mg + SU	Liraglutide 0.9 mg + SU
Infections and infestations	Overall	132/268 (49.3) 267	47/88 (53.4) 86	51/88 (58.0) 83
	Antibody -positive subjects	19/33 (57.6) 33	7/13 (53.8) 13	6/14 (42.9) 10
Gastrointestinal disorders	Overall	121/268 (45.1) 224	43/88 (48.9) 57	42/88 (47.7) 76
	Antibody -positive subjects	14/33 (42.4) 19	6/13 (46.2) 7	6/14 (42.9) 18
Musculoskeletal and connective tissue disorders	Overall	65/268 (24.3) 85	28/88 (31.8) 38	23/88 (26.1) 30
	Antibody -positive subjects	12/33 (36.4) 15	5/13 (38.5) 6	2/14 (14.3) 2

No. of subjects with events/No. of subjects included in the analysis set (Incidence %) No. of events

PMDA accepts the response, but considers that it is necessary to continue to collect information on the formation of anti-liraglutide antibody via post-marketing surveillance.

4.(iii).B.(3).6 Pancreatitis

The applicant explained as follows:

While pancreatitis was not reported in Japanese clinical studies, 9 events of pancreatitis were reported by 9 subjects (acute pancreatitis [7 subjects, 7 events], chronic pancreatitis [2 subjects, 2 events]) in foreign clinical studies. A breakdown of these cases by treatment was as follows: 8 events reported by 8 subjects in the liraglutide group (incidence rate, 2.2 per 1000 subject-years) and 1 event reported by 1 subject in the control group (incidence rate, 0.6 per 1000 subject-years). Of which, 2 events reported by 2 subjects were judged “possibly related” to study drug (1 event reported by 1 subject in the liraglutide 1.2 mg + metformin group, 1 event reported by 1 subject in the liraglutide 1.8 mg + OAD group). All the events were classified as serious adverse events except for 1 event of chronic pancreatitis reported by 1 subject (Trial NN2211-1797). One subject in the liraglutide 1.8 mg group who developed acute pancreatitis on the 669th day of study treatment (Trial NN2211-1573, a woman in her 60s) died, but its causal relationship to liraglutide was denied. Of the remaining 6 subjects with acute pancreatitis, 1 subject had study treatment interrupted (outcome, resolved) and 5 subjects were discontinued from study treatment (outcome, resolved in 4 subjects, unknown in 1 subject). The 2 subjects with chronic pancreatitis continued liraglutide treatment. In all the Japanese and foreign intermediate-term and long-term treatment trials [see the footnote of Table 28], the incidence rate of pancreatitis was 2.2 per 1000 subject-years in the liraglutide group and 0.6 per 1000 subject-years in the control group, showing a difference. Meanwhile, the incidence rate of pancreatitis (events per 1000 subject-years) by dose of liraglutide was 0.0 for < 0.6 mg, 1.8 for 0.6 mg, 0.0 for 0.6 to < 1.2 mg, 2.3 for 1.2 mg, 0.0 for 1.2 to 1.8 mg, 3.0 for 1.8 mg, and 2.1 for 1.8 to 3.0 mg, showing no relationship between the dose of liraglutide and the incidence rate of pancreatitis. Since most of the subjects with pancreatitis had risk factors for pancreatitis and the incidence rate of pancreatitis in patients with type 2 diabetes mellitus has been reported to be 3-fold higher compared to age- and gender-matched

general population and has been estimated to be 1.5 to 4.5 per 1000 patient-years (Yadav D & Lowenfels AB, *Pancreas*, 2006; 33: 323-330, Noel R, *et al.*, *Diabetes Care*, 2009; 32: 834-838), a definitive causal relationship between liraglutide and the development of pancreatitis has not been established at present.

Since gastrointestinal disorders have been reported following liraglutide treatment, PMDA asked the applicant to explain distinction between acute pancreatitis and gastrointestinal disorders and how to manage acute pancreatitis.

The applicant responded as follows:

As acute pancreatitis is an acute abdomen, it is generally difficult to distinguish between acute pancreatitis and other gastrointestinal disorders based only on symptoms. Regarding the management of acute pancreatitis following liraglutide treatment, written information for patients and for medical institutions (draft) will be developed to advise that if persistent severe abdominal pain, usually accompanied by vomiting, suspected of acute pancreatitis occurs, prompt and appropriate work-up (imaging, measurement of pancreas-specific enzymes, etc.) will be important.

PMDA asked the applicant to explain the clinical course of 1 subject in the liraglutide 0.9 mg group who died (cause of death, gastroenteritis) in Trial NN2211-1700 and the basis for denying its causal relationship to liraglutide.

The applicant responded as follows:

This subject was a woman in her 60s who also had hypertension, hyperlipidaemia, tinea unguium, tinea pedis, arrhythmia, cataract, diabetic peripheral neuropathy, and diabetic nephropathy. From 34 days after starting liraglutide treatment, the subject experienced bloating, diarrhoea, vomiting, and pyrexia etc. On the 36th day of liraglutide treatment, the subject was admitted to the hospital (the trial site) (findings at admission, body temperature, 37.5°C; blood pressure, 91/49 mmHg; casual blood glucose, 254 mg/dL; WBC, 29700/ μ L; CPK, 400 IU; creatinine, 1.82 mg/dL; CRP, 15.75 mg/dL; a negative influenza test and a negative troponin T test; normal resting ECG; and normal chest and abdominal X-ray). The subject was diagnosed with acute gastroenteritis and treatment was initiated without discontinuing study drug. Ten and several hours after admission, the subject developed hyperthermia, chills, and shivering and died on the following day of admission (on the 37th day of liraglutide treatment). The investigator reported that the cause of death was “gastroenteritis” and the direct cause of death was “airway obstruction due to vomitus.” However, an external gastroenterologist assessed the cause of death as follows: “Because of high WBC count, high CRP, and high body temperature, the cause of death is not ordinary gastroenteritis, but is likely to be septic shock due to gastrointestinal bacterial infection. As the subject may have had a low sensitivity to pain

due to neuropathy, the possibility of a disease with pain, e.g., appendicitis, cholangitis, cholelithiasis, or pancreatitis can not be ruled out. However, as ultrasound or CT was not performed, further diagnosis is not possible.” The applicant agreed with the external gastroenterologist’s opinion and considers that as a cause of death for this subject, the possibility of pancreatitis can not be excluded, but can not determine the diagnosis even based on all available data. Based on the above, it is considered necessary to continue to investigate the occurrence of pancreatitis via post-marketing surveillance and the occurrence of pancreatitis will be investigated in a foreign post-marketing long-term cardiovascular outcome study under planning (a randomized, placebo-controlled, double-blind study; planned number of cases of 6000-10000; planned duration of study of up to 5 years).

PMDA considers as follows:

As the subject who died in Trial NN2211-1700 did not receive adequate work-up with imaging etc. and had neuropathy, the possibility of the development of pancreatitis can not be excluded. In the Japanese clinical trials, pancreatitis was not reported, but gastrointestinal disorders occurred frequently following treatment with liraglutide and the aforementioned death was reported. In the foreign clinical trials, adverse events of pancreatitis occurred in liraglutide-treated subjects and a death was also reported. Adverse drug reactions of pancreatitis have been reported with another GLP-1 analog (FDA Safety information, Aug 18, 2008). Also considering the difficulty in distinguishing between pancreatitis and other gastrointestinal disorders, the package insert (draft) should advise that gastrointestinal disorders occurring during treatment with liraglutide should be managed carefully, for example, by considering diagnostic work-up with imaging etc. as appropriate, in view of the possibility of pancreatitis, and specific advice should also be included, e.g., liraglutide should not be readministered to patients who have developed pancreatitis during treatment with liraglutide. Furthermore, it is necessary to continue to investigate the occurrence of gastrointestinal disorders including pancreatitis following treatment with liraglutide via post-marketing surveillance and a post-marketing long-term cardiovascular outcome study and provide the information on the results of investigations to the medical institutions promptly. The above conclusion will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(3).7) Diabetic retinopathy

As the proportion of subjects with abnormal fundal findings was higher in the liraglutide group compared to the control group in a Japanese long-term treatment trial NN2211-1701, PMDA asked the applicant to explain the association between liraglutide treatment and worsening of diabetic retinopathy.

The applicant responded as follows:

In Japanese trial NN2211-1700, the proportion of subjects with diabetic retinopathy during the treatment period (52 weeks) (including not only subjects whose funduscopy finding changed from “normal” to “abnormal”, but also worsening of “abnormal” findings) was 6.0% (16 of 268 subjects) in the liraglutide 0.9 mg group, which was similar to 6.8% (9 of 132 subjects) in the glibenclamide group. In Trial NN2211-1701, the proportion was 9.1% (8 of 88 subjects) in the liraglutide 0.6 mg + SU group, 12.5% (11 of 88 subjects) in the liraglutide 0.9 mg + SU group, and 8.0% (7 of 88 subjects) in the SU monotherapy group, showing a higher incidence in the liraglutide 0.9 mg + SU group. It is known that generally, in patients with poor glycemic control over a long period of time, acute improvement in glycemic control within a short period of time may lead to transient worsening of diabetic retinopathy. For the liraglutide 0.6 mg + SU group in Trial NN2211-1701, subjects with diabetic retinopathy had higher HbA1c and fasting blood glucose at baseline and tended to show greater changes in HbA1c and fasting blood glucose from baseline to Week 4, Week 24, and Week 52. For the other liraglutide groups, there were no major differences between subjects with diabetic retinopathy and the overall treatment group and there was no consistent trend between glycemic control improvement by liraglutide and the incidence of diabetic retinopathy (Table 30). No association between the presence or absence of concurrent diabetic retinopathy at baseline and the incidence of diabetic retinopathy was found (Table 31) and a higher incidence of diabetic retinopathy in the liraglutide 0.9 mg + SU group is considered attributed to a longer duration of diabetes (mean) in the liraglutide 0.9 mg + SU group.

Table 30. Glycemic control over time in subjects with diabetic retinopathy

Timing of endpoint ^{a)}		Trial NN2211-1700		Trial NN2211-1701			
		Liraglutide 0.9 mg		Liraglutide 0.6 mg + SU		Liraglutide 0.9 mg + SU	
		N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
HbA1c (%)							
Baseline	Overall	266	8.92 (1.08)	87	8.60 (0.91)	88	8.21 (0.78)
	Subjects with diabetic retinopathy	16	8.76 (0.72)	8	9.35 (1.11)	11	8.12 (0.46)
Week 4	Overall	261	-0.59 (0.53)	83	-0.87 (0.40)	87	-0.83 (0.35)
	Subjects with diabetic retinopathy	16	-0.74 (0.27)	8	-1.18 (0.56)	11	-0.79 (0.28)
Week 24	Overall	245	-1.83 (1.01)	82	-1.49 (0.96)	84	-1.59 (0.84)
	Subjects with diabetic retinopathy	16	-1.51 (0.84)	8	-2.43 (1.14)	11	-1.43 (0.78)
Week 52 (LOCF)	Overall	263	-1.48 (1.12)	86	-1.09 (0.84)	87	-1.30 (0.91)
	Subjects with diabetic retinopathy	16	-1.39 (1.03)	8	-1.99 (0.80)	11	-1.32 (0.78)
Fasting blood glucose (mg/dL)							
Baseline	Overall	268	204.2 (51.1)	88	177.3 (40.4)	88	164.8 (37.3)
	Subjects with diabetic retinopathy	16	198.1 (48.7)	8	206.3 (60.7)	11	158.5 (44.1)
Week 4	Overall	256	-66.6 (38.1)	83	-51.4 (41.1)	86	-50.7 (33.9)
	Subjects with diabetic retinopathy	16	-64.8 (36.2)	8	-85.4 (58.3)	11	-43.2 (39.9)
Week 24	Overall	246	-65.5 (41.2)	83	-41.0 (36.1)	84	-41.0 (33.8)
	Subjects with diabetic retinopathy	16	-64.1 (38.7)	8	-78.3 (49.1)	11	-38.0 (30.2)
Week 52 (LOCF)	Overall	261	-57.5 (42.5)	85	-33.8 (37.7)	86	-32.8 (35.2)
	Subjects with diabetic retinopathy	16	-53.1 (34.6)	8	-67.0 (61.9)	11	-30.8 (41.8)

a) Data at all time points excluding baseline represent changes from baseline.

Table 31. Incidence of diabetic retinopathy (by presence or absence of concurrent diabetic retinopathy)

		Trial NN2211-1700		Trial NN2211-1701		
		Liraglutide 0.9 mg (n = 268)	Glibenclamide (n = 132)	Liraglutide 0.6 mg + SU (n = 88)	Liraglutide 0.9 mg + SU (n = 88)	SU monotherapy (n = 88)
Duration of diabetes ^{a)} (years)		8.13 (6.68)	8.48 (6.84)	9.33 (5.77)	11.61 (7.68)	10.06 (7.28)
Overall		16/268 (6.0) 16	9/132 (6.8) 10	8/88 (9.1) 8	11/88 (12.5) 11	7/88 (8.0) 7
Concurrent diabetic retinopathy ^b	Present	6/62 (9.7) 6	4/36 (11.1) 5	4/23 (17.4) 4	2/27 (7.4) 2	1/18 (5.6) 1
	Absent	10/206 (4.9) 10	5/96 (5.2) 5	4/65 (6.2) 4	9/61 (14.8) 9	6/70 (8.6) 6

No. of subjects with events/No. of subjects included in the analysis set (Incidence %) No. of events

a) Mean duration of diabetes at screening (SD)

PMDA asked the applicant to explain the association between the incidence rate of hypoglycemia and the occurrence of diabetic retinopathy in Trials NN2211-1700 and 1701.

The applicant responded as follows:

The incidence rate of any hypoglycemia (events per subject-year) was higher in subjects with diabetic retinopathy than in the overall treatment group for the liraglutide 0.9 mg + SU group in Trial NN2211-1701 (7.36 versus 3.71) and a similar trend was observed also for the glibenclamide group in Trial NN2211-1700 (7.67 versus 3.84). On the other hand, for the liraglutide 0.9 mg group in Trial NN2211-1700 and the liraglutide 0.6 mg + SU group in Trial NN2211-1701, the incidence rate of any hypoglycemia was lower in subjects with diabetic retinopathy than in the overall treatment group (0.26 versus 0.69 and 0.88 versus 3.13, respectively). Thus, there was no consistent trend associated with liraglutide treatment.

PMDA considers as follows:

There is no major problem with the applicant's discussion that the Japanese clinical trials suggested no association between liraglutide treatment and worsening of diabetic retinopathy. However, acute improvement in glycemic control, especially the development of hypoglycemia may worsen diabetic retinopathy. Thus, when liraglutide is used in patients with poor glycemic control over a long period of time, glycemic control should be improved, watching for the possible occurrence of hypoglycemia and it is necessary to pay attention to the fundus condition.

4.(iii).B.(3).8) Tumor development

The applicant explained as follows:

In long-term treatment trials NN2211-1700 and 1701, the incidence of adverse events classified under the system organ class "neoplasms benign, malignant and unspecified (incl cysts and polyps)" (hereinafter referred to as neoplasms) was similar between the liraglutide and control groups (liraglutide monotherapy trial, 4.1% [11 of 268 subjects] in the liraglutide 0.9 mg group, 4.5% [6 of 132 subjects] in the glibenclamide group; liraglutide combination therapy trial with an SU, 1.1% [1 of 88 subjects] in the liraglutide 0.6 mg + SU group, 0.0% [0 of 88 subjects] in the liraglutide 0.9 mg +

SU group, 4.5% [4 of 88 subjects] in the SU monotherapy group). Serious adverse events of neoplasms were reported by 7 subjects in the liraglutide group (7 events) and 5 subjects in the control group (5 events), of which 9 events (6 events in the liraglutide group, 3 events in the control group) were malignant tumors. In foreign long-term treatment trials, the incidence rate of neoplasms (events per 1000 subject-years) was 15.5 in the liraglutide 0.6 mg group, 31.9 in the liraglutide 1.2 mg group, 26.0 in the liraglutide 1.8 mg group, 10.7 in the placebo group, and 12.6 in the active comparator group, showing a higher incidence rate with liraglutide compared to the active comparator. The event with the highest incidence rate was thyroid neoplasm.

As the mechanism of liraglutide action is mediated by GLP-1 receptors and GLP-1 receptors are expressed at high levels in some of human neuroendocrine tumors, PMDA asked the applicant to explain the impact of long-term treatment with liraglutide on the development of neuroendocrine tumors.

The applicant responded as follows:

Although high levels of GLP-1 receptor expression in human pheochromocytomas, insulinomas, gastrinomas, brain tumors, and embryonal tumors have been reported, the occurrence of neuroendocrine tumors has not been reported during the clinical development of liraglutide. On the other hand, the incidence of neuroendocrine tumors in the general population has been reported to be less than 2 in 100000 (Taal BG, *et al.*, *Neuroendocrinology*, 2004; 80 [Suppl 1]: 3-7) and the possibility that GLP-1 receptor stimulation is involved in neuroendocrine tumor proliferation or poses a risk to humans with tumors could not be confirmed by the literature. Thus, there is little need to include a warning regarding the development of neuroendocrine tumors in the package insert.

PMDA considers that as liraglutide has a novel mechanism of action and there are also no adequate foreign post-marketing clinical data, it is necessary to collect information on tumors including thyroid and neuroendocrine tumors via post-marketing surveillance.

4.(iii).B.(3).9 Cardiovascular effects

PMDA asked the applicant to explain cardiovascular effects of liraglutide.

The applicant responded as follows:

Cardiovascular effects were investigated by identifying the occurrence of adverse events classified under the system organ classes “cardiac disorders” and “vascular disorders.” The incidence of “cardiac disorders” in Trials NN2211-1700 and 1701 (both 52-week treatment) was as follows: in Trial NN2211-1700, 6.3% (17 of 268 subjects) in the liraglutide 0.9 mg group and 10.6% (14 of 132 subjects) in the glibenclamide group; and in Trial NN2211-1701, 5.7% (5 of 88 subjects) in the

liraglutide 0.6 mg + SU group, 10.2% (9 of 88 subjects) in the liraglutide 0.9 mg + SU group, and 9.1% (8 of 88 subjects) in the SU monotherapy group. The incidence of “vascular disorders” was 6.3% (17 of 268 subjects) in the liraglutide 0.9 mg group and 7.6% (10 of 132 subjects) in the glibenclamide group in Trial NN2211-1700 and 5.7% (5 of 88 subjects) in the liraglutide 0.6 mg + SU group, 3.4% (3 of 88 subjects) in the liraglutide 0.9 mg + SU group, and 6.8% (6 of 88 subjects) in the SU monotherapy group in Trial NN2211-1701. There were no major differences between the liraglutide and control groups.

In the discussion on cardiovascular risk with the US FDA, although the clinical development program for liraglutide was not designed to prospectively evaluate cardiovascular risk in the new antidiabetic therapy to treat type 2 diabetes, as a considerable number of major adverse cardiovascular events (MACE) were reported throughout the clinical development program, it was possible to retrospectively evaluate cardiovascular risk. The broad MACE analyses showed that the point estimates for the incidence ratio of liraglutide to the total comparator (placebo and active comparator groups) were < 1 and the upper 95% CI bounds were < 1.8 , showing a lower incidence with liraglutide compared to the total comparator (Table 32). Moreover, in order to evaluate the cardiovascular safety of long-term treatment with liraglutide in patients with a high cardiovascular risk, e.g., elderly patients, patients with renal impairment, and patients with relatively advanced disease, a foreign post-marketing long-term cardiovascular outcome study (a randomized, placebo-controlled, double-blind study; planned number of subjects of 6000-10000; planned duration of study of up to 5 years) is planned to be conducted and the point estimate for MACE defined as a composite endpoint of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke is to be calculated. The protocol for this study is under discussion with the European Medicines Agency and the FDA and a final report is expected to be submitted in the 1st quarter of 2016.

Table 32. Results of MACE* analyses in Japanese and foreign clinical trials

		Treatment group		Incidence ratio for liraglutide vs. total comparator ^{a)} [95% CI] ^{b)}
Population A1		Liraglutide (n = 4022)	Total comparator (n = 1760)	
	Exposure (subject-years)	1771.8	787.6	
	All MACE	48 (1.2) 49 [27.7]	24 (1.4) 27 [34.3]	0.92 [0.56, 1.50]
	Serious MACE	15 (0.4) 15 [8.5]	8 (0.5) 8 [10.2]	0.80 [0.34, 1.90]
Population A2		Liraglutide (n = 4257)	Total comparator (n = 2381)	
	Exposure (subject-years)	1879.5	1045.7	
	All MACE	51 (1.2) 52 [27.7]	35 (1.5) 39 [37.3]	0.87 [0.57, 1.34]
	Serious MACE	16 (0.4) 16 [8.5]	16 (0.7) 16 [15.3]	0.67 [0.32, 1.41]
Population B		Liraglutide (n = 4257)	Total comparator (n = 2381)	
	Exposure (subject-years)	2882	1486	
	All MACE	(1.6) 71 [24.6]	(1.9) 50 [33.6]	0.88 [0.61, 1.28]
	Serious MACE	(0.6) 25 [8.7]	(0.8) 19 [12.8]	0.83 [0.44, 1.56]

No. of subjects with events (Incidence %) No. of events [Incidence rate, events per 1000 subject-years]

Population A1 includes all the phase II and phase III randomized, double-blind, controlled studies

Population A2 includes population A1 plus active-controlled open-label studies

Population B includes population A2 plus open-label extension periods

*: MACE endpoint was composed of cardiovascular death and Standardized MedDRA Queries for “Myocardial infarction” and “Central Nervous System Hemorrhages and Cerebrovascular Accidents.”

a) Placebo and active comparator

b) Cochran-Mantel-Haenszel estimates stratified by study

PMDA considers that there is no major problem with the applicant’s response that no cardiovascular effects of liraglutide were observed in Japanese and foreign clinical trials. However, liraglutide has a novel mechanism of action, there are no adequate foreign post-marketing clinical data, and it can not be concluded that cardiovascular risk is similar between Japanese and foreign patients with type 2 diabetes mellitus. Thus, the necessity of cardiovascular risk evaluation in Japanese patients after the market launch, and the evaluation method if necessary, will be determined, taking account of comments from the Expert Discussion.

4.(iii).B.(4) Special populations

4.(iii).B.(4).1 Patients with renal impairment

Although the applicant explained that no dose adjustment is required for patients with renal impairment, a single dose of 0.75 mg, which was lower than the recommended clinical dose of 0.9 mg, was administered in Trial NN2211-1329 involving subjects with renal impairment. PMDA asked the applicant to explain the safety of 0.9 mg of liraglutide in patients with renal impairment.

The applicant responded as follows:

In this trial, the exposure after a single dose of 0.75 mg was lower in subjects with renal impairment than in subjects with normal renal function, but no clear association between the degree of renal impairment and liraglutide exposure was found. As the pharmacokinetics of liraglutide are almost linear and are not time-dependent, it was considered possible to use pharmacokinetic data after a single dose of 0.75 mg obtained in this trial to predict multiple-dose pharmacokinetics. Regarding safety, when subjects were stratified according to the degree of renal impairment, there were no major

differences in the incidence of all adverse events or gastrointestinal disorders reported in this trial among different strata. Thus, it was considered that there is no association between the safety of liraglutide and the degree of renal impairment. The incidences and rates of all adverse events, gastrointestinal disorders, and any hypoglycemia by the degree of renal impairment in the Japanese long-term treatment trials NN2211-1700 and 1701 are shown in Table 33. The incidence rate of all adverse events (events per subject-year) tended to be slightly higher for mild renal impairment in the liraglutide 0.6 mg + SU group in Trial NN2211-1701 than in other treatment groups, but the incidence rate was generally similar between the liraglutide and control groups (SU monotherapy and glibenclamide groups). The incidence rate of gastrointestinal disorders was higher in subjects with mild renal impairment than in subjects with normal renal function in the liraglutide 0.6 mg + SU group in Trial NN2211-1701 while the incidence rate tended to be higher in subjects with normal renal function than in subjects with mild renal impairment in the control group. In Trial NN2211-1700 (monotherapy), the incidence rate of any hypoglycemia was similar between subjects with normal renal function and subjects with mild renal impairment in the liraglutide 0.9 mg group while the incidence rate was higher in subjects with mild renal impairment than in subjects with normal renal function in the glibenclamide group. In Trial NN2211-1701 (combination therapy with an SU), the incidence rate of any hypoglycemia was higher in subjects with mild renal impairment than in subjects with normal renal function in the liraglutide 0.6 mg + SU and SU monotherapy groups while there were no differences according to the degree of renal impairment in the liraglutide 0.9 mg + SU group.

Table 33. Incidences and rates of all adverse events, gastrointestinal disorders, and any hypoglycemia (by baseline renal function)

Degree of renal impairment ^{a)}	Trial NN2211-1700 (Liraglutide monotherapy)		Trial NN2211-1701 (Liraglutide combination therapy with SU)		
	Liraglutide 0.9 mg (n = 268)	Glibenclamide (n = 132)	Liraglutide 0.6 mg + SU (n = 88)	Liraglutide 0.9 mg + SU (n = 88)	SU monotherapy (n = 88)
All adverse events					
Normal	198/216 (91.7) 922 [4.69]	89/98 (90.8) 378 [4.15]	64/68 (94.1) 297 [4.57]	58/66 (87.9) 275 [4.30]	67/71 (94.4) 281 [4.76]
Mild	43/48 (89.6) 172 [4.19]	29/31 (93.5) 112 [4.21]	19/19 (100.0) 115 [7.12]	18/19 (94.7) 76 [4.39]	14/15 (93.3) 49 [3.78]
Moderate	4/4 (100.0) 4 [1.31]	3/3 (100.0) 21 [9.85]	1/1 (100.0) 5 [5.02]	3/3 (100.0) 13 [4.39]	2/2 (100.0) 22 [11.04]
Gastrointestinal disorders					
Normal	97/216 (44.9) 176 [0.90]	39/98 (39.8) 64 [0.70]	31/68 (45.6) 40 [0.62]	30/66 (45.5) 55 [0.86]	29/71 (40.8) 52 [0.88]
Mild	23/48 (47.9) 47 [1.14]	7/31 (22.6) 10 [0.38]	12/19 (63.2) 17 [1.05]	10/19 (52.6) 17 [0.98]	4/15 (26.7) 6 [0.46]
Moderate	1/4 (25.0) 1 [0.33]	2/3 (66.7) 7 [3.28]	0/1 (0.0) 0 [0.00]	2/3 (66.7) 4 [1.35]	1/2 (50.0) 2 [1.00]
Any hypoglycemia					
Normal	56/216 (25.9) 150 [0.76]	37/98 (37.8) 292 [3.21]	34/68 (50.0) 149 [2.29]	39/66 (59.1) 243 [3.80]	20/71 (28.2) 134 [2.27]
Mild	9/48 (18.8) 16 [0.39]	17/31 (54.8) 158 [5.94]	12/19 (63.2) 94 [5.82]	15/19 (78.9) 59 [3.41]	6/15 (40.0) 78 [6.02]
Moderate	1/4 (25.0) 1 [0.33]	1/3 (33.3) 10 [4.69]	1/1 (100.0) 14 [14.05]	3/3 (100.0) 11 [3.71]	2/2 (100.0) 9 [4.52]

No. of subjects with events/No. of subjects in subgroup (Incidence %) No. of events [Incidence rate (events per subject-year)]

a) The degree of renal impairment was classified according to the creatinine clearance (C_{CR}) calculated using the Cockcroft & Gault formula: normal ($C_{CR} > 80$ mL/min), mild ($50 < C_{CR} \leq 80$ mL/min), moderate ($30 < C_{CR} \leq 50$ mL/min), and severe ($C_{CR} \leq 30$ mL/min).

In the foreign long-term treatment trials, the incidence and rate of adverse events were assessed according to the degree of renal impairment (Table 34). As a result, the incidence rate of all adverse events (events per subject-year) tended to be higher in subjects with mild renal impairment compared to subjects with normal renal function in the liraglutide 1.2 mg and 1.8 mg groups but a similar trend was seen also for the active comparator group. The incidence rate of gastrointestinal adverse events tended to be higher in subjects with mild renal impairment compared to subjects with normal renal function in the liraglutide 1.2 mg and 1.8 mg groups but a similar trend was seen also for the active comparator group. The incidence rate of any hypoglycemia (events per subject-year) tended to be higher in subjects with mild renal impairment compared to subjects with normal renal function in the liraglutide group in Trial NN2211-1436 (combination therapy with glimepiride, 26-week treatment) (1.2 mg group, 2.50 in subjects with normal renal function, 3.38 in subjects with mild renal impairment; 1.8 mg group, 2.34 in subjects with normal renal function, 3.90 in subjects with mild renal impairment). On the other hand, there were no differences according to the degree of renal impairment in the liraglutide group in Trial NN2211-1573 (monotherapy, 52-week double-blind phase, 52-week open-label extension phase) (1.2 mg group, 0.7 in subjects with normal renal function, 0.3 in subjects with mild renal impairment; 1.8 mg group, 0.5 in subjects with normal renal function, 0.4 in subjects with mild renal impairment).

Table 34. Incidences and rates of all adverse events and gastrointestinal disorders (by baseline renal function)
Foreign long-term treatment trials* (Safety Analysis Set)

Degree of renal impairment ^{a)}	Liraglutide 0.6 mg n = 475 (2 subjects with missing data)	Liraglutide 1.2 mg n = 896 (1 subject with missing data)	Liraglutide 1.8 mg n = 1551 (6 subjects with missing data)	Placebo n = 524 (4 subjects with missing data)	Active comparator n = 1185 (5 subjects with missing data)
All adverse events					
Normal	293/383 (76.5) 1110 [3.059]	595/749 (79.4) 2695 [3.686]	956/1330 (71.9) 3966 [3.888]	307/452 (67.9) 845 [3.544]	695/992 (70.1) 2575 [3.111]
Mild	59/82 (72.0) 212 [2.619]	117/141 (83.0) 583 [5.218]	158/200 (79.0) 620 [5.024]	35/64 (54.7) 104 [2.601]	129/170 (75.9) 462 [4.041]
Moderate or severe ^{b)}	5/8 (62.5) 31 [5.293]	4/5 (80.0) 9 [2.564]	11/15 (73.3) 49 [6.449]	2/4 (50.0) 13 [12.0]	10/18 (55.6) 21 [3.211]
Gastrointestinal disorders					
Normal	143/383 (37.3) 246 [0.678]	332/749 (44.3) 680 [0.930]	540/1330 (40.6) 1235 [1.211]	82/452 (18.1) 124 [0.520]	237/992 (23.9) 449 [0.542]
Mild	31/82 (37.8) 56 [0.692]	76/141 (53.9) 179 [1.602]	105/200 (52.5) 235 [1.904]	9/64 (14.1) 15 [0.375]	40/170 (23.5) 90 [0.787]
Moderate or severe ^{b)}	2/8 (25.0) 5 [0.854]	1/5 (20.0) 2 [0.570]	7/15 (46.7) 14 [1.843]	1/4 (25.0) 3 [2.788]	3/18 (16.7) 8 [1.223]

No. of subjects with events/No. of subjects in subgroup (Incidence %) No. of events [Incidence rate (events per subject-year)]

*: Foreign confirmatory trials with a treatment duration of ≥ 24 weeks (Trials NN2211-1573,1572,1436,1574,1697, and 1797)

a) The degree of renal impairment was classified according to the creatinine clearance (C_{CR}) calculated using the Cockcroft & Gault formula: normal ($C_{CR} > 80$ mL/min), mild ($50 < C_{CR} \leq 80$ mL/min), moderate ($30 < C_{CR} \leq 50$ mL/min), and severe ($C_{CR} \leq 30$ mL/min).

b) 2 subjects were classified as severe renal impairment (1 subject in the 0.6 mg group, 1 subject in the 1.8 mg group)

The results from these Japanese and foreign long-term treatment trials indicated a trend towards a higher incidence rate of gastrointestinal disorders associated with liraglutide in subjects with mild renal impairment compared to subjects with normal renal function. However, the difference in the

incidence rate at the Japanese clinical dose (0.9 mg) between subjects with normal renal function and subjects with mild renal impairment was small and was not clinically relevant.

PMDA considers as follows:

The applicant's view that no clear association between the degree of renal impairment and liraglutide exposure was found is understood. However, patients with advanced renal impairment are generally less tolerable to a body burden and might experience gastrointestinal disorders, i.e., the main adverse drug reaction associated with liraglutide. As the incidence rate of gastrointestinal disorders associated with liraglutide tended to be higher in subjects with mild renal impairment than in subjects with normal renal function in Japanese and foreign long-term treatment trials and there is little experience with long-term use of liraglutide in type 2 diabetes patients with moderate or severe renal impairment, liraglutide should be used with caution in patients with renal impairment and furthermore, it is necessary to continue to collect information on the safety of liraglutide in patients with renal impairment via post-marketing surveillance. The above conclusion will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(4).2) Patients with hepatic impairment

Although the applicant explained that no dose adjustment is required for patients with hepatic impairment, a single dose of 0.75 mg, which was lower than the recommended clinical dose of 0.9 mg, was administered in Trial NN2211-1328 involving subjects with hepatic impairment. PMDA asked the applicant to explain the safety of 0.9 mg of liraglutide in patients with hepatic impairment.

The applicant responded as follows:

In this trial, liraglutide metabolism or excretion was not delayed in subjects with hepatic impairment, the exposure was lower in subjects with hepatic impairment compared to subjects with normal hepatic function, and the exposure in the severe group was 56% of that in the normal group. Adverse events occurred in only 3 subjects in the moderate group (nausea, bronchitis, and headache, one case each), but not in the normal, mild, or severe group and these events were not considered due to high liraglutide exposure.

As subjects with transaminase values (AST/ALT) > 80 IU/L were excluded from Japanese phase II and later trials, the incidences and rates of all adverse events, gastrointestinal disorders, hepatobiliary disorders (system organ class), and hypoglycemia reported in long-term treatment trials NN2211-1700 and 1701 were assessed by dividing subjects into category 1 (both AST and ALT at baseline ≤ the upper limit of normal) and category 2 (at least either AST or ALT > the upper limit of normal) (Table 35). As a result, the incidence rates of all adverse events and hypoglycemia were similar between subjects in the two categories. The incidence rate of gastrointestinal disorders was slightly lower in

subjects in the category 2 compared to subjects in the category 1, and the incidence rate of hepatobiliary disorders tended to be higher in subjects in the category 2 compared to subjects in the category 1 across all treatment groups.

Table 35. Incidence and rate of adverse events (by category of baseline AST/ALT)

AST/ALT category ^{a)}	Trial NN2211-1700		Trial NN2211-1701		
	Liraglutide 0.9 mg	Glibenclamide	Liraglutide 0.6 mg + SU	Liraglutide 0.9 mg + SU	SU monotherapy
	n = 268	n = 132	n = 88	n = 88	n = 88
All adverse events					
1	223/243 (91.8) 1012 [4.66]	109/120 (90.8) 471 [4.29]	72/76 (94.7) 353 [4.96]	66/72 (91.7) 295 [4.32]	68/73 (93.2) 297 [4.90]
2	22/25 (88.0) 86 [3.70]	12/12 (100.0) 40 [4.00]	12/12 (100.0) 64 [5.89]	13/16 (81.3) 69 [4.33]	15/15 (100.0) 55 [4.12]
Gastrointestinal adverse events					
1	112/243 (46.1) 206 [0.95]	44/120 (36.7) 76 [0.69]	39/76 (51.3) 51 [0.72]	35/72 (48.6) 64 [0.94]	30/73 (41.1) 51 [0.84]
2	9/25 (36.0) 18 [0.78]	4/12 (33.3) 5 [0.50]	4/12 (33.3) 6 [0.55]	7/16 (43.8) 12 [0.75]	4/15 (26.7) 9 [0.67]
Hepatobiliary adverse events					
1	9/243 (3.7) 10 [0.05]	6/120 (5.0) 7 [0.06]	2/76 (2.6) 2 [0.03]	3/72 (4.2) 3 [0.04]	7/73 (9.6) 7 [0.12]
2	2/25 (8.0) 2 [0.09]	1/12 (8.3) 1 [0.10]	2/12 (16.7) 2 [0.18]	4/16 (25.0) 4 [0.25]	2/15 (13.3) 2 [0.15]
Any hypoglycemia					
1	57/243 (23.5) 153 [0.70]	52/120 (43.3) 455 [4.15]	43/76 (56.6) 248 [3.48]	47/72 (65.3) 254 [3.72]	24/73 (32.9) 183 [3.02]
2	9/25 (36.0) 14 [0.60]	3/12 (25.0) 5 [0.50]	4/12 (33.3) 9 [0.83]	10/16 (62.5) 59 [3.70]	4/15 (26.7) 38 [2.85]

No. of subjects with adverse events or hypoglycemia/No. of subjects in category (Incidence of adverse events or hypoglycemia %)
No. of events [Incidence rate (events per subject-year)]

a) Category 1: Subjects with both AST and ALT ≤ the upper limit of normal, Category 2: Subjects with at least either AST or ALT > the upper limit of normal

As shown above, as there was no specific trend between the degree of hepatic impairment as classified by baseline AST/ALT and the occurrence of adverse events in the Japanese clinical trials, liraglutide at doses up to 0.9 mg is unlikely to cause safety problems in patients with hepatic impairment. On the other hand, as a decrease in liraglutide exposure in patients with hepatic impairment may lead to reduced efficacy, the level of glycemic control in these patients needs to be monitored carefully.

PMDA considers as follows:

Generally, patients with hepatic impairment are considered to have a low tolerance to adverse events. In the Japanese clinical trials, there are limited data on long-term treatment with liraglutide in subjects with hepatic impairment, and subjects with severe hepatic impairment have not been studied. Furthermore, as the possibility of a decrease in liraglutide exposure leading to reduced efficacy can not be ruled out, liraglutide should be used with caution in patients with hepatic impairment and it is necessary to continue to collect information on the safety of liraglutide in patients with hepatic impairment via post-marketing surveillance. The above conclusion will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(4).3) Elderly

PMDA asked the applicant to explain the efficacy and safety of liraglutide in elderly patients.

The applicant responded as follows:

The effect of age on the efficacy of liraglutide (HbA1c reduction) is considered small as the interaction between age and treatment group with respect to HbA1c at Week 24 was not statistically significant in long-term treatment trials NN2211-1700 and 1701 ($P = 0.9427$ in Trial NN2211-1700, $P = 0.6113$ in Trial NN2211-1701, ANCOVA model). Regarding safety, when the incidences of hypoglycemia and gastrointestinal disorders at Week 52 in both trials were compared between non-elderly subjects aged < 65 years and elderly subjects aged ≥ 65 years, the incidence of gastrointestinal disorders tended to be slightly higher in the elderly compared to the non-elderly (Table 36). This trend was observed not only for the liraglutide groups but also for the SU monotherapy group (Trial NN2211-1701). The incidence of any hypoglycemia also tended to be generally higher in the elderly compared to the non-elderly for the glibenclamide group (Trial NN2211-1700) and the SU monotherapy group (Trial NN2211-1701) as well as for the liraglutide groups. Therefore, higher incidences of gastrointestinal disorders and hypoglycemia were considered an overall trend in subjects aged ≥ 65 years including the control group. Concerning the development of gastrointestinal disorders, the package insert will advise that if 0.9 mg of liraglutide is not well-tolerated, a dose reduction to 0.6 mg should be considered, which will be applied to all patients. Therefore, a precaution for use in elderly patients is considered unnecessary.

Table 36. Incidence of adverse events by age category

Age category	Trial NN2211-1700		Trial NN2211-1701		
	Liraglutide 0.9 mg	Glibenclamide	Liraglutide 0.6 mg + SU	Liraglutide 0.9 mg + SU	SU monotherapy
	n = 268	n = 132	n = 88	n = 88	n = 88
All adverse events					
< 65 years	171/188 (91.0) 784	78/88 (88.6) 326	52/55 (94.5) 243	44/49 (89.8) 195	54/58 (93.1) 207
≥ 65 years	74/80 (92.5) 314	43/44 (97.7) 185	32/33 (97.0) 174	35/39 (89.7) 169	29/30 (96.7) 145
Adverse events for which a causal relationship to study drug could not be denied					
< 65 years	62/188 (33.0) 126	20/88 (22.7) 39	25/55 (45.5) 56	14/49 (28.6) 26	21/58 (36.2) 40
≥ 65 years	33/80 (41.3) 58	13/44 (29.5) 21	9/33 (27.3) 19	17/39 (43.6) 42	13/30 (43.3) 17
Gastrointestinal adverse events					
< 65 years	80/188 (42.6) 148	32/88 (36.4) 52	24/55 (43.6) 32	20/49 (40.8) 26	21/58 (36.2) 33
≥ 65 years	41/80 (51.3) 76	16/44 (36.4) 29	19/33 (57.6) 25	22/39 (56.4) 50	13/30 (43.3) 27
Any hypoglycemia ^{a)}					
< 65 years	45/188 (23.9) 98 [0.573]	34/88 (38.6) 245 [3.023]	27/55 (49.1) 104 [2.002]	29/49 (59.2) 145 [3.016]	17/58 (29.3) 122 [2.495]
≥ 65 years	21/80 (26.3) 69 [0.994]	21/44 (47.7) 215 [5.563]	20/33 (60.6) 153 [5.075]	28/39 (71.8) 168 [4.643]	11/30 (36.7) 99 [3.958]

No. of subjects with events/No. of subjects included in the analysis set (Incidence %) No. of events

a) No. of subjects with events/No. of subjects included in the analysis set (Incidence %) No. of events, [Incidence rate (events per subject-year)]

PMDA considers as follows:

Although the effect of age on the efficacy of liraglutide is small, as the incidences of gastrointestinal disorders and hypoglycemia associated with liraglutide tended to be higher in elderly subjects aged ≥ 65 years, it is necessary to advise in the package insert (draft) etc. that elderly patients treated with

liraglutide are at greater risk of gastrointestinal disorders and hypoglycemia and especially, liraglutide combination therapy with an SU is associated with an increased risk of hypoglycemia.

4.(iii).B.(5) Indication

In Japan, liraglutide combination therapy with oral antidiabetic drugs has been studied for SUs only and the efficacy and safety of liraglutide in combination with oral antidiabetic drugs other than SUs have not been evaluated. Thus, the proposed indication statement that liraglutide should be used only in patients who inadequately respond to oral antidiabetic drugs in addition to dietary and/or exercise therapy is not appropriate. PMDA instructed the applicant to modify this statement.

The applicant responded that the statement regarding combination therapy with oral antidiabetic drugs will be modified as follows: “liraglutide should be used only in patients who inadequately respond to sulfonylureas (SUs) in addition to dietary and/or exercise therapy.”

PMDA considers that the wording “use of sulfonylureas in addition to dietary and/or exercise therapy” should be used in accordance with the indication statements for already approved oral antidiabetic drugs.

4.(iii).B.(6) Dosage and administration

4.(iii).B.(6).1) Dose

PMDA asked the applicant to explain the reason for a gradual dose escalation regimen specified in the dosage and administration section (Therapy should be initiated with once-daily doses of 0.3 mg and then the dose should be increased in 0.3 mg increments. Dose increases should occur at intervals of at least 1 week.) to reduce gastrointestinal disorders.

The applicant responded as follows:

A gradual dose escalation is accepted as a method for reducing the risk of transient adverse events. Metformin and another GLP-1 analog, exenatide whose main adverse effects are gastrointestinal disorders, have been administered using a gradual dose escalation regimen in clinical practice. In Trial NN2211-1326 in Japanese healthy adult male subjects, 3 of 6 subjects treated with a single dose of 15 µg/kg (equivalent to 0.9 mg dose based on 60 kg body weight) experienced dose-limiting nausea and vomiting. For this reason, phase I multiple-dose trials in Japanese healthy adult male subjects (Trial NN2211-1551, Trial NN2211-1694), a phase II clinical trial (Trial NN2211-1334), and a phase I multiple-dose trial in patients with type 2 diabetes mellitus (Trial NN2211-1591) used a gradual dose escalation regimen, where the starting dose of liraglutide was 0.3 mg (5 µg/kg in the phase I multiple-dose trials) and the dose was increased in 0.3 mg (5 µg/kg in the phase I multiple-dose trials) increments at intervals of at least 1 week. As a result, no gastrointestinal disorders were reported in

Trial NN2211-1694 in which 20 µg/kg and 25 µg/kg, which were higher than the doses used in Trial NN2211-1326, were administered. In the phase II trial (Trial NN2211-1334, 14-week treatment) in which the same doses as in Trial NN2211-1326 were administered, the incidence of gastrointestinal disorders was 17.8% (8 of 45 subjects) in the liraglutide 0.1 mg group, 15.2% (7 of 46 subjects) in the liraglutide 0.3 mg group, 31.1% (14 of 45 subjects) in the liraglutide 0.6 mg group, 29.5% (13 of 44 subjects) in the liraglutide 0.9 mg group, and 23.9% (11 of 46 subjects) in the placebo group and there was no increase in the incidence of gastrointestinal disorders with increasing dose of liraglutide.

PMDA accepted the applicant's response about a gradual dose escalation regimen, but asked the applicant to explain the necessity of a clinical dose less than 0.9 mg/day.

The applicant responded as follows:

The clinical dose of liraglutide either as a monotherapy or in combination with an SU is considered 0.9 mg/day. However, as it is inferred that there are patients who do not tolerate 0.9 mg/day, the package insert will advise that if 0.9 mg of liraglutide is not well-tolerated, a dose reduction to 0.6 mg should be considered.

PMDA considers as follows:

As 0.9 mg/day was chosen as the recommended dose of liraglutide based on the results from a phase II clinical trial (Trial NN2211-1334), a phase III clinical trial (Trial NN2211-1700) has demonstrated the non-inferiority of liraglutide 0.9 mg/day to glibenclamide, and furthermore, the efficacy of liraglutide was maintained until Week 52 in this trial, the usual dose of 0.9 mg/day is justified. Trial NN2211-1334 showed that the difference from placebo for change in HbA1c from baseline to Week 14 (%) (mean [95% CI]) was -1.64 [-1.93, -1.35] for 0.6 mg/day of liraglutide, which was not substantially inferior to that for 0.9 mg/day of liraglutide (-1.85 [-2.14, -1.56]) and Trial NN2211-1701 has demonstrated the efficacy of liraglutide 0.6 mg in combination with an SU. Thus, although the safety and efficacy of long-term treatment with 0.6 mg have not been evaluated, the use of 0.6 mg/day may be permitted if a safety problem arises with 0.9 mg and a dose reduction is required, if glycemic control is achieved with 0.6 mg during dose escalation from 0.3 mg and a further dose increase to 0.9 mg is not required, or if it is considered difficult to increase the dose from 0.6 mg to 0.9 mg due to safety concerns. The above conclusion will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(6).2) Time of day of injection

PMDA asked the applicant to explain the basis for the proposed dosing instruction regarding the time of day of injection, i.e., "liraglutide should be injected at the same time each day," despite that liraglutide was to be injected in the morning or evening in Japanese trials NN2211-1700 and 1701.

The applicant responded as follows:

The pharmacokinetic model of liraglutide administered in the morning or evening has indicated that the difference between $C_{\max}$ and $C_{\min}$ is small relative to the blood concentrations regardless of whether liraglutide is administered in the morning or evening, and the impact of the time of day of injection on the effectiveness of liraglutide is small. Also, there were no differences in the efficacy endpoints of HbA1c and fasting blood glucose at Week 24 according to the time of day of injection (injection in the morning or evening) in trials NN2211-1700 and 1701 and similar results were obtained also from foreign trial NN2211-1573. As shown in the above, although clinical data on liraglutide administered in the morning (from waking up until after breakfast) or evening (from before evening meal until bedtime) only have been obtained from trials NN2211-1700 and 1701, as liraglutide may be administered at any time of day theoretically, it has been concluded that liraglutide may be administered at a time of day best suited to the patient's lifestyle.

PMDA considers as follows:

As the incidence and rate of hypoglycemia tended to be different between morning and evening injections in Japanese trials NN2211-1700 and 1701, liraglutide should be injected in the morning or evening (at the same time each day, wherever possible) as investigated in the clinical trials. The following information should be provided: when both an SU and liraglutide are administered in the morning in patients on liraglutide combination therapy with an SU, the risk of hypoglycemia is increased.

4.(iii).B.(7) Post-marketing surveillance

PMDA asked the applicant to present a post-marketing surveillance plan (draft).

The applicant responded as follows:

A specified drug use-results survey on long-term treatment, with a target number of cases of 1800 and a 24-month observation period, is planned to be conducted. If any problem is identified via specified drug use-results survey etc., the conduct of a post-marketing clinical trial will be considered.

PMDA considers as follows:

As liraglutide has a novel mechanism of action and there are also no adequate foreign post-marketing data, taking account of the results from non-clinical and clinical studies, it is necessary to continue to collect information on the development of gastrointestinal disorders including pancreatitis, thyroid effects, tumor development, effects of formation of anti-liraglutide antibody, the safety of long-term treatment in patients with renal impairment, patients with hepatic impairment, and elderly patients, effect on body weight, and concomitant medications via post-marketing surveillance. As described in

“4.(iii).B.(3).9) Cardiovascular effects,” as it can not be judged that the cardiovascular risk in type 2 diabetes mellitus is similar between Japanese and foreign patients, etc., the necessity of cardiovascular risk evaluation in Japanese patients after the market launch and if so, the evaluation method etc., will be determined, 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 compliance inspection and data integrity assessment was 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 application dossier.

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 data submitted in the new drug application (5.3.5.1.1, 5.3.5.1.2, 5.3.5.1.3). As a result, inconsistencies between the source document and the CRF (an adverse event was undocumented), a failure of the head of the medical institution to conclude a contract with the organizer of the Institutional Review Board for examination and deliberation, and protocol deviations (noncompliance with the procedure for provisional registration) were found at some clinical trial sites, but PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application dossier.

IV. Overall Evaluation

Based on the submitted data, it is concluded that the efficacy of liraglutide in patients with type 2 diabetes mellitus has been demonstrated and the safety of liraglutide is acceptable in view of its observed benefits.

PMDA considers that liraglutide may be approved for the indication of type 2 diabetes mellitus if it can be concluded based on comments from the Expert Discussion that there are no particular problems.

Review Report (2)

November 10, 2009

1. Product Submitted for Registration

[Brand name] Victoza Subcutaneous Injection 18 mg (changed from Victoza Injection 18 mg¹⁸)
[Non-proprietary name] Liraglutide (Genetical Recombination)
[Applicant] Novo Nordisk Pharma Ltd.
[Date of application] July 14, 2008

2. Content of the Review

The Pharmaceuticals and Medical Devices Agency (PMDA)'s conclusions were supported at the Expert Discussion. Taking account of comments from the Expert Discussion, PMDA conducted an additional review of the following points and took necessary actions.

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) Safety

1) Thyroid effects

The following conclusion by PMDA was supported by the expert advisors:

There is no particular problem with the applicant's view that the results from Japanese and foreign clinical trials suggested no relationship between liraglutide and thyroid abnormalities including blood calcitonin increased. However, as C-cell tumors were induced in mice and rats in non-clinical studies, liraglutide is intended to be administered for long periods, and thyroid tumors often remain asymptomatic until they grow, the package insert should advise that the presence or absence of thyroid signs and symptoms should be checked by neck palpation etc. during treatment with liraglutide and if abnormalities are detected, thyroid tests, e.g., blood calcitonin should be performed.

The following comment was raised from some of the expert advisors:

Considering that GLP-1 analogs may promote the growth of medullary thyroid carcinoma derived from C-cells and hereditary medullary thyroid carcinoma is common, liraglutide should be used with

¹⁸ The proposed Japanese brand name was changed based on "Handling of labelings and brand names of drugs for prevention of medical accidents" (PMSB Notification No. 935 dated September 19, 2000).

caution in patients with medullary thyroid carcinoma (including those with a previous history of medullary thyroid carcinoma) and patients with a family history of medullary thyroid carcinoma.

Based on the above, PMDA instructed the applicant to caution users as follows: the presence or absence of thyroid signs and symptoms should be checked by neck palpation etc. during treatment with liraglutide and if abnormalities are detected, a work-up should be performed. In addition, PMDA instructed the applicant to consider whether a precaution should be raised regarding the use of liraglutide in patients with a previous history or family history of medullary thyroid carcinoma, because patients with current or previous malignant tumors were excluded from the Japanese clinical trials (Trials NN2211-1334, NN2211-1700, NN2211-1701) and furthermore, patients with a family history or previous history of thyroidal malignant tumors were excluded from Trial NN2211-1334.

The applicant responded as follows:

The package insert will advise that the presence or absence of thyroid signs and symptoms should be checked during treatment with liraglutide and if abnormalities are detected, the patient should be instructed to see a specialist. There is no clinical experience with liraglutide in patients with a previous history of medullary thyroid carcinoma and there is also no information on the use of liraglutide in patients with a family history of medullary thyroid carcinoma. Although thyroid C-cell tumors observed in rat and mouse carcinogenicity studies are unlikely to be relevant to humans, human relevance can not be entirely ruled out. Therefore, a precaution for use in high-risk patients is considered necessary and it will be stated in the package insert that the safety of liraglutide in patients with a previous history of medullary thyroid carcinoma or patients with a family history of medullary thyroid carcinoma or multiple endocrine neoplasia type 2 has not been established.

PMDA accepted the response.

2) Pancreatitis

The following conclusion by PMDA was supported by the expert advisors:

In the Japanese clinical trials, pancreatitis was not reported, but gastrointestinal disorders occurred frequently following treatment with liraglutide and a death case in which the possibility of the development of pancreatitis could not be ruled out was reported. In foreign clinical trials, adverse events of pancreatitis occurred in liraglutide-treated subjects and a death was also reported. Adverse drug reactions of pancreatitis have been reported with another GLP-1 receptor agonist (FDA Safety Information, Aug 18, 2008). Also considering the difficulty in distinguishing pancreatitis from other gastrointestinal disorders, specific advice should be included in the package insert, including (a) If gastrointestinal disorders occur during treatment with liraglutide, diagnostic work-up with imaging etc. should be considered as appropriate, in view of the possibility of pancreatitis and (b) liraglutide should

not be readministered to patients who have developed pancreatitis during treatment with liraglutide. Furthermore, it is necessary to continue to investigate the occurrence of gastrointestinal disorders including pancreatitis following treatment with liraglutide via post-marketing surveillance and a foreign post-marketing long-term cardiovascular outcome study and provide the information on the results of investigations to the medical institutions promptly.

Some of the expert advisors commented that it is necessary to thoroughly consider whether the package insert should include a precaution regarding the use of liraglutide in patients with pancreatitis.

Based on the above, PMDA instructed the applicant to include the following advice in the important precautions section of the package insert: Gastrointestinal disorders should be managed carefully, for example, by considering diagnostic work-up with imaging etc. as appropriate, in view of the possibility of pancreatitis; and if pancreatitis occurs, liraglutide should be discontinued and should not be readministered. PMDA also instructed the applicant to consider whether the package insert should include a precaution for use in patients with a history of pancreatitis.

The applicant responded as follows:

The following precautions will be included in the package insert: Gastrointestinal disorders should be managed carefully, for example, by considering diagnostic work-up with imaging etc. as appropriate, in view of the possibility of pancreatitis; if acute pancreatitis occurs, liraglutide should be discontinued and should not be readministered; and if initial symptoms of acute pancreatitis (persistent severe abdominal pain accompanied by vomiting) occur, treatment should be discontinued and the patient should be instructed to see a physician for diagnosis as soon as possible. Since the association between the development of pancreatitis and liraglutide can not be excluded, and there is limited clinical experience with liraglutide in patients with a history of pancreatitis, liraglutide should be used with caution in these patients, paying attention to a possible relapse of pancreatitis. Thus, careful administration of liraglutide will be recommended in patients with a history of pancreatitis.

PMDA accepted the response.

3) Cardiovascular effects

PMDA considered that there was no major problem with the applicant's response that no cardiovascular effects of liraglutide were observed in Japanese and foreign clinical trials, which was supported by the expert advisors. However, liraglutide has a novel mechanism of action, there are no adequate foreign post-marketing clinical data at present, and it can not be concluded that cardiovascular risk is similar between Japanese and foreign patients with type 2 diabetes mellitus. PMDA sought the expert advisors' comments on the necessity of cardiovascular risk evaluation in

Japanese patients after the market launch and the evaluation method if necessary. The following comments were raised from the expert advisors:

- The need for prospective evaluation of cardiovascular risk is high. According to a Japanese type 2 diabetes mellitus cohort study “a study on the prevention of the development and progression of vascular complications in diabetes mellitus (JDCStudy [Sone H., *et al.*, BIO Clinica 2007; 22: 353-360]),” the incidence of cardiovascular events is 15 to 18 patients per 1000 patient-years. In order to determine the cardiovascular effects of liraglutide, it is recommended that a survey in which at least 2000 patients are observed for ≥ 3 years should be conducted. MACE should be used as an endpoint.
- The only way to evaluate cardiovascular risk in Japanese patients is to conduct a randomized comparative trial similar to that in foreign countries. However, as the incidence of cardiovascular events is lower in Japanese patients than in Caucasian patients, a larger number of cases is needed compared to a foreign trial under planning. Therefore, such a trial seems not feasible.
- Cardiovascular risk evaluation should also include an investigation of the effects of liraglutide on serum lipids and pulse rate.

Based on the above, PMDA instructed the applicant to design a survey of cardiovascular events in Japanese patients (in terms of the number of subjects, the observation period etc.) so that a comparative discussion with the foreign post-marketing long-term cardiovascular outcome study can be allowed as much as possible.

The applicant presented a post-marketing surveillance protocol (draft) and then responded as follows: A specified drug use-results survey on long-term treatment will be conducted to identify safety and efficacy problems with long-term use. The observation period will be 3 years and the target number of survey completers will be 3000 so that a comparison of the incidence of cardiovascular events between the survey and the foreign post-marketing long-term cardiovascular outcome study/JDC Study can be discussed. In this survey, cardiovascular events (acute myocardial infarction, unstable angina, vascular recanalization, cerebrovascular events, heart failure requiring hospitalization) will be one of the priority items [See “(6) Post-marketing surveillance” for other priority items] and if a potential cardiovascular event occurs, the cardiovascular event will be identified and the potential risk factors (lipids, blood pressure, pulse rate, body weight, etc.) and test results of ECG etc. will be investigated.

PMDA accepted the response, considering that there is no major problem with the post-marketing surveillance protocol for cardiovascular risk evaluation (draft).

(2) Use of liraglutide in patients with renal impairment

The following conclusion by PMDA was supported by the expert advisors:

As the incidence of gastrointestinal disorders associated with liraglutide tended to be higher in subjects with mild renal impairment than in subjects with normal renal function in Japanese and foreign long-term treatment trials and there is little experience with long-term use of liraglutide in type 2 diabetes patients with moderate or severe renal impairment, liraglutide should be used with caution in patients with renal impairment and furthermore, it is necessary to continue to collect information on the safety of liraglutide in patients with renal impairment via post-marketing surveillance.

Based on the above, PMDA instructed the applicant to recommend careful administration in patients with renal impairment and include a statement in the package insert to the effect that the use of liraglutide in patients with renal impairment has not adequately been studied.

The applicant responded that the package insert will recommend careful administration of liraglutide in patients with renal impairment and state that there is limited clinical experience with liraglutide in patients with renal impairment.

PMDA accepted the response.

(3) Use of liraglutide in patients with gastrointestinal disorders

At the Expert Discussion, some of the expert advisors commented that as there is a concern about a higher incidence of gastrointestinal disorders in patients with advanced diabetic neuropathy, it may be necessary to administer liraglutide with caution to patients having gastrointestinal disorders.

Based on the above, PMDA instructed the applicant to discuss the incidence of gastrointestinal disorders associated with the use of liraglutide in patients with concomitant chronic gastrointestinal disorders due to diabetic neuropathy etc. in Japanese clinical trials (Trials NN2211-1334, NN2211-1700, NN2211-1701) and consider whether the package insert should include a precaution for use in these patients.

The applicant responded as follows:

Patients with both diabetic neuropathy and any disease classified as gastrointestinal disorders (system organ class) as a background factor were identified and the incidence of gastrointestinal adverse events in these patients was assessed. In the liraglutide group, the proportion of patients with at least one gastrointestinal adverse event was slightly higher in patients having both diabetic neuropathy and gastrointestinal disorders at baseline than in other patients (liraglutide 0.9 mg group in Trial NN2211-1700 [61.9% (13 of 21 patients)] of patients having both diabetic neuropathy and

gastrointestinal disorders at baseline, 43.7% (108 of 247 patients) of other patients], liraglutide 0.6 mg + SU group in Trial NN2211-1701 [62.5% (5 of 8 patients) of patients having both diabetic neuropathy and gastrointestinal disorders at baseline, 47.5% (38 of 80 patients) of other patients], liraglutide 0.9 mg + SU group in Trial NN2211-1701 [77.8% (7 of 9 patients) of patients having both diabetic neuropathy and gastrointestinal disorders at baseline, 44.3% (35 of 79 patients) of other patients]). A similar trend was observed also in the SU monotherapy group in Trial NN2211-1701 (50.0% [4 of 8 patients] of patients having both diabetic neuropathy and gastrointestinal disorders at baseline, 37.5% [30 of 80 patients] of other patients), but not in the glibenclamide group in Trial NN2211-1700 (25.0% [3 of 12 patients] of patients having both diabetic neuropathy and gastrointestinal disorders at baseline, 37.5% [45 of 120 patients] of other patients). Patients having both diabetic neuropathy and gastrointestinal disorders at baseline reported various types of gastrointestinal disorders during the study period, with no consistent trend. In Trials NN2211-1700 and NN2211-1701, the incidence of gastrointestinal adverse events was assessed by the presence or absence of gastrointestinal disorders and of diabetic neuropathy at baseline [see the table below]. As a result, the incidence of gastrointestinal adverse events tended to be higher in patients having gastrointestinal disorders at baseline, regardless of whether they had diabetic neuropathy. On the other hand, in Trial NN2211-1334, due to the small number of relevant patients in each group, it was difficult to compare the incidence of gastrointestinal disorders between patients having both diabetic neuropathy and gastrointestinal disorders at baseline and other patients, but generally there was no trend towards a higher incidence of gastrointestinal disorders in subjects having both diabetic neuropathy and gastrointestinal disorders at baseline.

As described in the above, as no definitive causal relationship between liraglutide and gastrointestinal adverse events in patients having both diabetic neuropathy and gastrointestinal disorders at baseline has been suggested, a specific precaution for use in these patients is considered unnecessary.

Table. Incidence of gastrointestinal adverse events
by the presence or absence of gastrointestinal disorders and of diabetic neuropathy at baseline
(Trials NN2211-1700 and NN2211-1701)

Gastrointestinal disorders	Diabetic neuropathy	Trial NN2211-1700 (Liraglutide monotherapy)		Trial NN2211-1701 (Liraglutide combination therapy with SU)		
		Liraglutide 0.9 mg (n = 268)	Glibenclamide (n = 132)	Liraglutide 0.6 mg+SU (n = 88)	Liraglutide 0.9 mg+SU (n = 88)	SU monotherapy (n = 88)
Present	Present	13/21 (61.9)	3/12 (25.0)	5/8 (62.5)	7/9 (77.8)	4/8 (50.0)
	Absent	37/76 (48.7)	17/33 (51.5)	16/26 (61.5)	12/23 (52.2)	8/21 (38.1)
	Overall	50/97 (51.5)	20/45 (44.4)	21/34 (61.8)	19/32 (59.4)	12/29 (41.4)
Absent	Present	9/30 (30.0)	7/17 (41.2)	3/9 (33.3)	8/16 (50.0)	3/8 (37.5)
	Absent	62/141 (44.0)	21/70 (30.0)	19/45 (42.2)	15/40 (37.5)	19/51 (37.3)
	Overall	71/171 (41.5)	28/87 (32.2)	22/54 (40.7)	23/56 (41.1)	22/59 (37.3)

No. of subjects with events/No. of subjects in subgroup (Incidence %)

Adapted by PMDA from the response to inquiries after the Expert Discussion, 091020-C-007 Table C-007-3

PMDA considers that there is no specific problem with the applicant's view that the incidence of gastrointestinal adverse events tended to be higher in patients having concomitant gastrointestinal disorders, regardless of whether they had diabetic neuropathy in the Japanese clinical trials (Trials NN2211-1700 and NN2211-1701) and no definitive causal relationship between liraglutide and gastrointestinal adverse events in patients having both diabetic neuropathy and gastrointestinal disorders at baseline has been suggested. However, considering that there is limited clinical experience with liraglutide in patients having concomitant gastrointestinal disorders and the safety of liraglutide in patients having concomitant severe gastrointestinal disorders has not been established, PMDA instructed the applicant to recommend careful administration in patients with severe gastrointestinal disorders.

The applicant responded that the package insert will recommend careful administration in patients with gastrointestinal disorders such as diabetic gastroparesis and inflammatory bowel disease and state that the safety of liraglutide has not adequately been evaluated in these patients.

PMDA accepted the response.

(4) Use of liraglutide in patients with hepatic impairment

The following conclusion by PMDA was supported by the expert advisors:

Generally, patients with hepatic impairment are considered to have a low tolerance to adverse events. In Japanese clinical studies, there are limited data on long-term treatment with liraglutide in subjects with hepatic impairment and the safety of liraglutide in subjects with severe hepatic impairment has not been evaluated. From these considerations, liraglutide should be used with caution in patients with hepatic impairment and it is necessary to continue to collect information on the safety of liraglutide in patients with hepatic impairment via post-marketing surveillance.

Based on the above, PMDA instructed the applicant to recommend careful administration of liraglutide in patients with hepatic impairment and include a statement in the package insert to the effect that the use of liraglutide in patients with hepatic impairment has not adequately been studied.

The applicant responded that the package insert will recommend careful administration in patients with hepatic impairment and state that there is limited clinical experience with liraglutide in patients with hepatic impairment.

PMDA accepted the response.

(5) Dosage and administration

The following conclusion by PMDA was supported by the expert advisors:

The usual dose of 0.9 mg/day selected based on the Japanese clinical study results is justified. Since Trial NN2211-1334 showed that 0.6 mg/day of liraglutide was not substantially inferior to 0.9 mg/day and Trial NN2211-1701 has demonstrated the efficacy of liraglutide 0.6 mg in combination with an SU, although the safety and efficacy of long-term treatment with 0.6 mg have not been evaluated, the use of 0.6 mg/day may be permitted if, for example, a safety problem arises with 0.9 mg and a dose reduction is required. With respect to the time of day of injection, as the incidence and rate of hypoglycemia tended to be different between morning and evening injections in Trial NN2211-1701 (combination therapy with an SU), liraglutide should be injected in the morning or evening (at the same time each day, wherever possible) as investigated in clinical trials and it should be stated in the package insert that when both an SU and liraglutide are administered in the morning in patients on liraglutide combination therapy with an SU, the risk of hypoglycemia is increased.

Based on the above, PMDA instructed the applicant to modify the proposed dosage and administration statement as shown below and include the statements in the package insert to the effect that liraglutide should be injected at the same time each day wherever possible; and when both an SU and liraglutide are administered in the morning in patients on liraglutide combination therapy with an SU, the risk of hypoglycemia may be increased.

(Proposed dosage and administration)

The usual adult dosage is 0.9 mg of liraglutide subcutaneously injected once daily. Therapy should be initiated with once-daily doses of 0.3 mg and then the dose should be increased in 0.3 mg increments. Dose increases should occur at intervals of at least 1 week. Victoza should be injected at the same time each day.

(After modification: the underlined parts are the changes)

The usual adult dosage is 0.9 mg of Liraglutide (Genetical Recombination) subcutaneously injected once daily in the morning or evening. Therapy should be initiated with once-daily doses of 0.3 mg and then the dose should be increased in 0.3 mg increments at intervals of at least 1 week. The dose may be adjusted according to the patient's condition. The daily dose should not exceed 0.9 mg.

The applicant responded as follows:

The dosage and administration statement will be modified as indicated above. In addition, the package insert will include a caution statement to the effect that Victoza should be administered once daily in the morning or evening, at the same time each day wherever possible; and when both an SU and Victoza are administered in the morning in patients on Victoza combination therapy with an SU, the

risk of hypoglycemia may be increased.

PMDA accepted the response.

(6) Post-marketing surveillance

The following conclusion by PMDA was supported by the expert advisors:

As liraglutide has a novel mechanism of action and there are also no adequate foreign post-marketing data, taking account of the results from non-clinical and clinical studies, it is necessary to continue to collect information on the development of gastrointestinal disorders including pancreatitis, thyroid effects, tumor development, effects of formation of anti-liraglutide antibody, the safety of long-term treatment in patients with renal impairment, patients with hepatic impairment, and elderly patients, effect on body weight, and concomitant medications via post-marketing surveillance.

Based on the above, PMDA instructed the applicant to present a post-marketing surveillance protocol (draft).

The applicant presented a post-marketing surveillance protocol (draft) and then responded as follows: A specified drug use-results survey on long-term treatment, with an observation period of 3 years and a target number of survey completers of 3000 will be conducted to identify safety and efficacy problems with long-term use of liraglutide. The priority investigation items will be the development of gastrointestinal disorders, thyroid effects, tumors, pancreatitis, and cardiovascular events [see “(1) Safety 3) Cardiovascular effects” for details]. If gastrointestinal disorders occur, treatment status at onset and other relevant information will be investigated. If calcitonin has been measured in patients with thyroid-related events, calcitonin levels before and after the start of treatment with liraglutide will be obtained and if palpation, thyroid ultrasound, and biopsy etc. have been performed, their results will be obtained. If tumor events occur, the background factors will be investigated and if relevant tests have been performed, the test results will be evaluated. If amylase and lipase have been measured in patients with pancreatitis events, amylase and lipase levels before and after the start of treatment with liraglutide will be obtained and if imaging etc. have been performed, its details will be investigated. If antibody formation is suspected to be involved in the occurrence of adverse events and the investigator considers it necessary to measure antibody titers, anti-liraglutide antibody titers will be measured. Furthermore, effect on body weight, effects of concomitant medications, and liraglutide treatment status etc. will be evaluated and the safety and efficacy of liraglutide in patients with renal or hepatic impairment etc. will also be investigated.

PMDA accepted the response, considering that there is no major problem with the post-marketing surveillance protocol (draft).

3. Overall Evaluation

As a result of the above review, PMDA concludes that the product may be approved after modifying the indication and the dosage and administration as shown below. 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;

Victoza should be used only in patients who have not sufficiently responded to either of the following treatments.

- (a) Diet and/or exercise therapy alone
- (b) Use of sulfonylureas in addition to diet and/or exercise therapy

[Dosage and administration]

The usual adult dosage is 0.9 mg of Liraglutide (Genetical Recombination) subcutaneously injected once daily in the morning or evening. Therapy should be initiated with once-daily doses of 0.3 mg and then the dose should be increased in 0.3 mg increments at intervals of at least 1 week. The dose may be adjusted according to the patient's condition. The daily dose should not exceed 0.9 mg.