Discovery and Development of Semaglutide

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Abstract: Diabetes Mellitus is a universal disease around the world. There are many different types of drugs for treatment, among which the glucagon-like peptide 1 receptor agonists (GLP-1 RAs) have attached much attention in recent years. Semaglutide, one of the GLP-1 RAs, is a long-acting diabetes drug developed by Novo Nordisk. Compared with traditional diabetes drugs, semaglutide possesses a longer biological half-life, which conduces to reduce the dosing frequency. And based on the general preparation of semaglutide, Novo Nordisk made changes to the dosage form and made it into the oral drug. In 2019, FDA launched the new drug, oral semaglutide. Oral semaglutide has been proved that it still maintains good effectiveness, and oral semaglutide has better safety and can reduce the occurrence of hypoglycemia through pre-clinical and clinical trials. This review introduces the mechanism, structure, pre-clinical and clinical trials of semaglutide and discusses the entire process from the research and development background to the market.

1 INTRODUCTION

1.1 Diabetes

Diabetes is a metabolic disease characterized by high blood sugar, and insulin secretion defects are the main cause of diabetes.

Long-term diabetes can damage various tissues, such as the eyes, heart, blood vessels, and kidneys. Diabetes can also cause symptoms such as polydipsia, polyuria, polyphagia, and weight loss.

Table 1 shows the comparison of some indexes between healthy people and diabetic patients. The highest fasting blood glucose (FBG) of healthy people is only 6.1 mmol/L, while the free blood glucose index of diabetic patients is at least 7 mmol/L. The glycosylated hemoglobin (HbA1c) and body mass index (BMI) of healthy people are also lower than those of diabetic patients. The 2-hour postprandial blood glucose of diabetic patients is even about twice that of healthy people.

Table 1: Comparison between healthy and diabetes patients. (American Diabetes Association 2010, *World Health Organization*, Vijan 2010).

Indexes	Healthy people	Patients with diabetes mellitus
FBG	4.4 – 6.1 mmol/L	≥7.0 mmol/L
HbA1c	4 % – 6 %	≥6.5 %
(OGTT) 2hPBG	4.6 – 7.8 mmol/L	≥11.1 mmol/L
BMI	18.50 – 24.99	Overweight: 25.00-29.99 Obese Class I: 30.00-34.99 Obese Class II: 35.00-39.99 Obese Class III: ≥40.00
C-peptide (Kong 2016)	0.8 – 4.2 ng/ml	Extremely-low (T1D)

As shown in Table 2, diabetes can be divided into type 1 diabetes and type 2 diabetes.

Type 1 diabetes is an autoimmune disease caused by the effects of external environmental factors based on genetic susceptibility, leading to the damage, even destruction of pancreatic islet cells, and finally, the

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failure of their function. Type 1 diabetes is mainly caused by insulin deficiency, and its onset is sudden. The patient's body is mainly thin or normal, and this type of diabetes occurs mostly in children.

Type 2 diabetes is the most common one among diabetes, which is due to insulin resistance. Insulin resistance is the inability of cells to respond adequately to normal levels of insulin. The onset of Type 2 is gradual, and patients are usually obese. This type of diabetes occurs mostly in adults.

Table 2: Comparison between type 1 and 2 diabetes. (*Williams textbook of endocrinology*).

Feature	Type 1 diabetes	Type 2 diabetes
Cause	Insulin deficiency	Insulin resistance
Onset	Sudden	Gradual
Age at onset	Mostly in children	Mostly in adults
Body shape	Thin or normal	Mostly obese
Ketoacidosis	Common	Rare
Autoantibodies	Usually present	Absent
Endogenous insulin	Low or absent	Normal, decreased, or increased
Concordance in identical twins	50%	90%
Prevalence	~10%	~90%

1.2 Glucagon-like Peptide 1

Glucagon-like peptide 1 receptor agonist drugs are an effective option for treatment for patients with type 2 diabetes, which is used as a single treatment or supplement to other antihyperglycemic therapies to reduce glycosylated hemoglobin and body weight.

Glucagon-like peptide 1 (GLP-1) works by binding to GLP-1 related receptors. GLP-1 receptors are distributed in various tissues throughout the body, including the pancreas, lungs, kidneys, and cardiovascular system (Andersen 2018). GLP-1 regulates blood sugar in many ways, including increasing blood sugar and suppressing the source of blood sugar. The effects of GLP-1 on the pancreas include regulating the secretion of insulin by lifetime control on β-cells, inhibiting the secretion of glucagon from α-cells, acting on δ-cells, and promoting somatostatin synthesis (Smits 2016).

It is currently believed that the cAMP/Epac2/PKA pathway is a classic activation pathway for the regulation of insulin secretion by GLP-1 (Shigeto 2017). The protective effect of GLP-1 on β-cells is reflected in the reduction of the damage caused by endoplasmic reticulum stress to β-cells through TUM1-Ex4 (Son 2018). The regulation of glucagon may be achieved indirectly through δ cells to promote the synthesis of somatostatin. Studies suggest that GLP-1 can directly act on α-cells to inhibit the secretion of glucagon (Davis 2020). The control of GLP-1 on blood sugar is also affected by the basic condition of patients and the type of medication. For diabetic patients, the ones with a lower BMI can achieve better results than those with a higher BMI (Meier 2015).

In general, the regulation of blood sugar by GLP-1 is a complex process, which is affected by blood sugar levels, GLP-1 levels, receptor distribution, and diversity (Wang 2020).

1.3 GLP-1RAs

Intravenous injection of exogenous GLP-1 to patients with type 2 diabetes can reduce the blood glucose concentration to the normal fasting range. However, the short half-life and fast degradation speed limit the further therapeutic application of GLP-1 (Gupta 2013). To overcome this problem, the development of glucagon-like peptide 1 receptor agonists (GLP-1RAs) was proposed.

GLP-1RAs are a class of anti-diabetic drugs with unique characteristics. Although there are intra-class differences in clinical efficacy due to different biochemical structures and pharmacokinetic characteristics, a significant hypoglycemic effect was shown in all members of the GLP1-RA class, such as liraglutide, abiglutide, exenatide, and semaglutide. In addition, the safety of these drugs is generally satisfactory (Christina 2019). Table 3 is the comparison between liraglutide, exenatide and semaglutide.

Table 3: Comparison of liraglutide, exenatide and semaglutide.

	Liraglutide	Exenatide	Semaglutide
Chemical Structure	H-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys (Pal-γ-Glu)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-	H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-	H-His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys (AEEAc-AEEAc-γ-Glu-17-carboxyheptadecanoyl)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

	Liraglutide	Exenatide	Semaglutide
	Arg-Gly-OH	Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH2 acetate salt	
Pancreatic β-cell	Improve pancreatic β-cell function	Protect pancreatic β-cells	Protect pancreatic β-cells
DPP-IV	Antagonize the degration of DPP-IV	Antagonize the degration of DPP-IV	Cover the hydrolysis site of DPP-IV encyme
Half-life	Long half-life	Long half-life	Long half-life
Cardiovascular system	Improve cardiovascular risk factors	Protect the cardiovascular system	Protect the cardiovascular system

1.3.1 Liraglutide

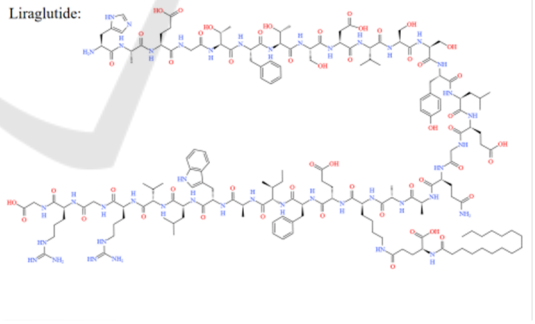
As shown in Figure 1, liraglutide is a GLP-1 analogue formed by adding a 16-carbon acyl chain to the 26th lysine residue of GLP-1 and substituting arginine for the 34th lysine of GLP-1. The plasma forms a non-covalent binding with albumin and releases slowly, antagonizing the degradation of DPP-IV (Bock 2003). The hypoglycemic effect of liraglutide is glucose concentration-dependent, so the incidence of hypoglycemia with long-term use of liraglutide is very low. Liraglutide can also reduce patient weight, improve cardiovascular risk factors, and at the same time improve pancreatic β-cell function (Astrup 2012).

1.3.2 Exenatide

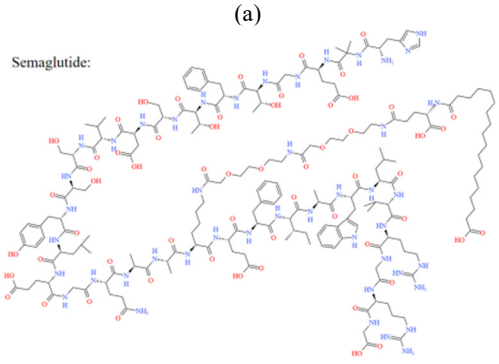
As shown in Figure 1, Exenatide is a GLP-1 analog isolated from the salivary glands of the blunt-tailed lizard distributed in the southwestern United States and northern Mexico. It consists of 39 amino acid residues and has a molecular mass of 4186.6, which has 53% homology to GLP-1., And GLP-1 acts on G-protein coupled receptors with higher affinity. The second-to-last amino acid at the N-terminal of exenatide is glycine (His-Gly-Glu), which is different from the N-terminal sequence of GLP-1 (His-Ala-Glu) and is not decomposed by DPP-IV, so it has a longer half-life, can be more effective (HANSEN 1999, Zhou 2010). Dramatical reduction of blood sugar and body weight was found by dosing twice a day and protecting pancreatic β-cells and the cardiovascular system (Bunck 2009). The main adverse reaction of Exenatide is mild to moderate nausea, which mostly occurs in the early stage of medication, and its severity decreases with the passage of time (Yoo 2006).

1.3.3 Semaglutide

As shown in Figure 1, Semaglutide is a long-acting dosage developed from the structure of liraglutide (Lau 2015). A1a at position 8 on the GLP-1 (7-37) chain is replaced with Aib, and Lys at position 34 is replaced with Arg. The Lys at position 26 is connected to the fatty acid chain of octadecanoic acid. Compared with Liraglutide, semaglutide has a longer aliphatic chain with higher hydrophobicity. However, semaglutide has been modified with a short chain of PEG to greatly increase its hydrophilicity. The modified semaglutide can bind tightly to albumin, cover up the DPP-4 enzymatic hydrolysis site, and reduce renal excretion, prolong the biological half-life, and achieve the effect of long circulation (Kapitza 2012).



(a)



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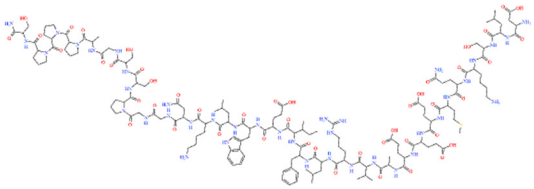

(c)

Figure 1: Structure of liraglutide (a), exenatide (b) and semaglutide (c).

2 DEVELOPMENT OF ORAL SEMAGLUTIDE

Their main disadvantage is that the subcutaneous route of administration can cause malabsorption. Therefore, the development of oral GLP1-RA preparations will further consolidate its beneficial effects in clinical practice. Oral semaglutide is a modified form of semaglutide with the addition of a carrier sodium N-(8-[2-hydroxybenzoyl] amino) caprylate.

2.1 The Mode of Action of Oral Semaglutide

Figure 2 shows the mode of action of oral semaglutide. Oral semaglutide is limited to extensive degradation by proteolytic enzymes in the gastrointestinal tract and poor absorption across the gastrointestinal epithelium (Mahato 2003). To achieve adequate bioavailability of semaglutide after oral administration, oral semaglutide has been co-formulated with a 300 mg concentration of the absorption enhancer called SNAC (Rasmussen 2020). One disadvantage of using SNAC is the fast absorption rate. Because of its low potency as a type of permeation enhancer, it is difficult to maintain a threshold concentration in the intestinal wall for a long enough time. Therefore, semaglutide tablets must use a 300 mg concentration of SNAC. SNAC protects semaglutide against enzymatic degradation via a local pH buffering effect (Rasmussen 2020). As the oral semaglutide tablet rapidly erodes, SNAC causes a local increase in pH, leading to the higher solubility of semaglutide and protection from proteolytic degradation (Rasmussen 2020). SNAC also promotes the absorption of semaglutide across the gastric epithelium in a concentration-dependent manner by effects on transcellular pathways, which are transient and fully reversible (Rasmussen 2020). This absorption of semaglutide is highly localized and depends on the spatial proximity of semaglutide and SNAC (Buckley 2018).

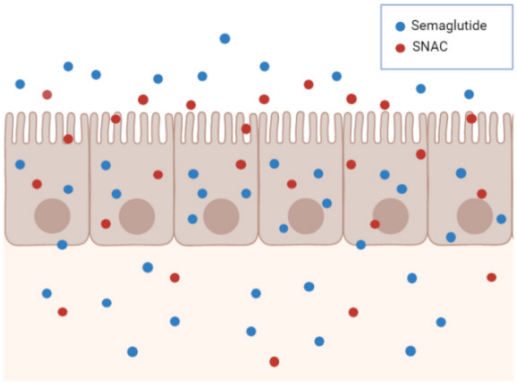

Figure 2: Mode of action of oral semaglutide. SNAC, sodium N-(8-hydroxybenzoyl] amino) caprylate.

2.2 Dosing and Medical Condition

Now researchers face another question, which is the appropriate dosing condition.

Clinically, they found out food intake can have a negative impact on the absorption of oral semaglutide, so when oral semaglutide is taken in a fasting sate, sufficient exposure can be reached. After the dosage condition was figured out, the clinical pharmacology of oral semaglutide becomes the focal point of the research. Through several studies, researchers get deep insight into how the exposure of semaglutide following oral administration is influenced by comorbidities or medication and how oral semaglutide might impact the exposure of concomitant medications (Rasmussen 2020).

Besides, some special medical conditions, such as hepatic or renal impairment, might have a strong impact on the pharmacokinetics of oral semaglutide. To assess this, researchers clinically assessed how oral semaglutide changes in its efficacy or absorption under those medical conditions. From the results, there is no apparent effect observed on the pharmacokinetics or tolerability of oral semaglutide, which indicates the dose redesign is not necessary at this point.

Last but not the least, they investigated the effect of oral semaglutide on exposure to various medications normally taken by T2D patients. Based on their results, when co-administered, oral semaglutide had no clinically relevant effect on the exposure of lisinopril, warfarin, and digoxin in healthy subjects (Rasmussen 2020). When co-administrated with metforminm furosemide, and rosuvastatin, it only resulted in small change of oral semaglutide. Similarly, they observed that there was

an increase in thyroxine exposure when co-administered with levothyroxine. Until now, they still keep doing studies about drug-drug interaction for oral semaglutide. When enough information is gathered, it will be more lucid and straightforward for researchers to give more useful suggestions when oral semaglutide is co-administered with other medications by T2D patients.

3 PRE-CLINICAL TRAILS OF SEMAGLUTIDE

3.1 AME Test

Semaglutide is an analogue of human glucagon-like peptide 1 and is in clinical development to treat type 2 diabetes. In Lene Jensen's study, the radioactivity in blood, plasma, urine, and feces was measured in rats and monkeys; the radioactivity in exhaled air was measured in rats. The researchers quantified metabolites in plasma, urine, and feces after analysis and radiological testing. The blood-to-plasma ratio and pharmacokinetics of both radiolabelled semaglutide-related materials were assessed (Lene 2017).

This trial studied 0.5 mg of Semaglutide, providing sufficient exposure to estimate the relevant PK endpoint. Semaglutide was radiolabelled in the octadecanedioic acid moiety in the side chain of lysine 26 to characterise the metabolism of the most modified part of the molecule (Lene 2017).

3.1.1 Blood-to-Plasma Ratio

The researchers collected blood and plasma samples for 24 hours in rats to assess the ratio of blood to plasma. The average blood-to-plasma ratio of the rats was found to be 0.44-0.46 (Lene 2017). For monkeys, in the absence of a small amount of tritium water, the average blood-to-plasma ratio of samples collected 48 hours after dosing is in the range of 0.54-0.60 (Lene 2017).

3.1.2 Excretion of [^{3}H]-semaglutide-related Material

Table 4 shows the excretion of radioactivity in rats and monkeys. In rats, the recovery of total excretion of [^{3}H]-semaglutide related substances after 168 hours showed that urine and feces are important excretion pathways (Table 4). Radioactivity was still detectable in urine 168h after dose administration, and 22.4% was recovered in the carcasses. Less than 0.5% of the radioactivity was detected in expired air (Lene 2017).

For monkeys, the total recovery of [^{3}H]-semaglutide-related material after 336 h showed that similar to rats, urine and faeces were important routes of excretion. When the collection is stopped, the recovery of urine and feces is not complete. This can be seen from the slightly positive slope of the cumulative excretion versus the time curve. Unlike mice, the radioactivity in the carcass has not been determined. The total recovery rates of intact and dry urine and stool samples were 12.6% and 7.0%, respectively (Lene 2017).

Table 4: Excretion of radioactivity. (Lene 2017).

Species	Rat (% of dose [% coefficient of variation]) n=3	Monkey (% of dose [% coefficient of variation]) n=3
Dose	0.3 mg/kg 10 MBq/kg	0.03 mg/kg 14 MBq/kg
Time	0-1week	0-2weeks
Gender	Male	Male
Urine	35.6 (27.7)	30.3 (25.3)
Faeces	32.6 (9.7)	20.7 (3.0)
Expired air	<0.2	Not applicable
Carcass	22.4 (27.0)	Not applicable
Cage wash and debris	3.7 (22.2)	7.2 (28.4)
Total excretion	72.1 (8.8)	58.3 (10.2)
Total recovery	94.5 (0.5)	58.2

3.1.3 Metabolite Profiling

(1) Plasma

As shown in Figure 3, twelve components were detected in the plasma of rats. At all time points of the analysis, [^{3}H]-semaglutide is the main component in plasma, and the retention time of [^{3}H]-semaglutide reference substance is similar to the main peak in plasma chromatogram. [^{3}H]-semaglutide accounts for 69% of the total semaglutide related substances. As shown in Table 5, another 10 metabolites were detected in the plasma, each accounting for <1-7% of the total $AUC_{0\text{-last}}$ (Lene 2017).

Figure 3 shows that 6 components were detected in plasma of monkeys. Semaglutide was the primary component in plasma at all time-point analysed. As shown in Table 5, in the other peak areas, 4 metabolites are eluting close to semaglutide, each accounting for <1-9% of the total $AUC_{0\text{-last}}$ (Lene 2017).

The retention time for the first eluting peak was characteristic of tritiated water for both rats and monkeys, and this was confirmed by data from freeze-dried samples (Lene 2017).

Table 5: Exposure of semaglutide and metabolites in plasma across species. (Lene 2017).

AUC_{total}	Timepoint of samples profiled	% semaglutide	Total number of metabolites	% metabolites
Rat	2-72 hours	69	10	<1-7
Monkey	0.5-168 hours	71	5	<1-9

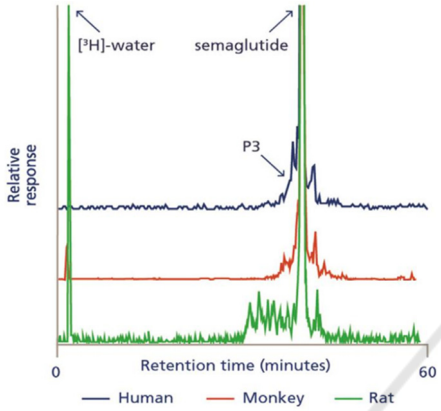

Figure 3: HPLC analysis of metabolite profile in plasma from rat, monkey, and human (Lene 2017).

(2) Urine and feces

For rats, the radioactive content in urine accounted for about 89% of the total excreted radioactivity (0-168 hours) from 0-120 hours after administration, and a total of six components were detected. The total amount of radioactivity in the feces accounted for 89% of the total excreted radioactivity (0-168 hours) from 0-120 hours after the dose, and 14 ingredients were detected (Lene 2017).

In monkeys, the total amount of radioactivity in urine from 0-216 hours after administration accounted for 63% of the total excreted radioactivity (0-336 hours). A total of 9 components were detected; none of them had a retention time similar to [^{3}H]-semaglutide. The total amount of radioactivity in the feces from 0-216 hours after the dose accounts for 74% of the total excreted radioactivity (0-336 hours), and 15 ingredients were detected in monkeys (Lene 2017).

For both rats and monkeys, the retention time for the first eluting peak was characteristic of tritiated water (Lene 2017).

3.2 Nonclinical Toxicology

Carcinogenesis, mutagenesis, impairment of fertility. In a 2-year carcinogenicity study in CD-1 mice, subcutaneous doses of 0.3, 1, and 3 mg/kg/day of semaglutide were administered to the males, and 0.1, 0.3, and 1 mg/kg/day were administered to the females. A statistically significant increase in thyroid C-cell adenomas and a numerical increase in C-cell carcinomas were observed in males and females at all dose levels (http://www.novonordisk-us.com/products/product-patents.html.).

In a 2-year carcinogenicity study in Sprague Dawley rats, subcutaneous doses of 0.0025, 0.01, 0.025, and 0.1 mg/kg/day of semaglutide were administered. A statistically significant increase in thyroid C-cell adenomas was observed in males and females at all dose levels, and a statistically significant increase in thyroid C-cell carcinomas was observed in males at ≥0.01 mg/kg/day, at clinically relevant exposures (http://www.novonordisk-us.com/products/product-patents.html.).

In a combined fertility and embryo-fetal development study in rats, subcutaneous doses of 0.01, 0.03, and 0.09 mg/kg/day of semaglutide were administered to male and female rats. Males were dosed for 4 weeks before mating, and females were dosed for 2 weeks prior to mating and throughout organogenesis until Gestation Day 17. No effects were observed on male fertility. In females, an increase in estrus cycle length was observed at all dose levels, together with a small reduction in numbers of corpora lutea at ≥0.03 mg/kg/day. These effects were likely an adaptive response secondary to the pharmacological effect of semaglutide on food consumption and body weight (http://www.novonordisk-us.com/products/product-patents.html.).

3.2.1 Animal Toxicology and Pharmacology

An increase in lactate levels and decreased glucose levels in the plasma and cerebrospinal fluid (CSF) were observed in mechanistic studies with SNAC in rats. Small but statistically significant increases in lactate levels were observed in a few animals at approximately the clinical exposure. These findings were associated with moderate to marked adverse clinical signs (lethargy, abnormal respiration, ataxia,

reduced activity, body tone, and reflexes) and marked decreases in plasma and CSF glucose levels at higher exposures. These findings are consistent with inhibition of cellular respiration and lead to mortality at SNAC concentrations >100-times the clinical Cmax (http://www.novonordisk-us.com/products/product-patents.html.).

4 CLINICAL TRAILS

4.1 Overview of Clinical Development of Semaglutide

The clinical trial is a carefully designed study that tests the benefits and risks of a specific medical treatment or intervention in humans. It is to determine the efficacy and safety of the trial drug. It consists of four phases, phase I, phase II, phase III, and phase IV (after the drug marketed).

As shown in Figure 4, a comprehensive global clinical development program was conducted for semaglutide. At the time of cut-off for the NDA, 25 trials with semaglutide s.c. once-weekly had been completed: 16 phases 1 clinical pharmacology trials, one phase 2 dose-finding trial, and 8 phase 3a trials (including a 2-year cardiovascular outcomes trial [CVOT]). A total of 9,384 individuals were included in the clinical development program, of whom 5,710 were exposed to semaglutide and 3,674 to comparators, including placebo. Approximately 1/3 of the total population was recruited from sites in the US. (https://www.fda.gov/media/108291/download).

Completed Trials		
Phase 1	**Phase 2 trial**	**Phase 3a trials**
Clinical pharmacology trials	1821-Dose finding	SUSTAIN 1(3623) sema vsplacebo (Mono)
Healthy subjects		SUSTAIN 2(3626) sema vs sita (OADs)
1820-First human dose		SUSTAIN 3(3624) sema vs exe ER (OADs)
3697-Equivalence-product strength		SUSTAIN 4(3625) sema vs IGlar (OADs)
3687-Equivalence/bioavailability		SUSTAIN 5(3627) sema vs placebo (Insulin)
4010-Bioequivalence-manufacturing process		SUSTAIN JP Mono (4092) sema vs sita (Mono), JP
3633-Multiple dose-Caucasian/JP		SUSTAIN JP OADs (4091) sema vs OAD (OAD), JP
3634-Pk/Pd-Caucasian/JP		
3789-Metabolism		
3652-QTc		
Special populations		**Phase 3b trial**
3616-Renal impairment		***Long-term outcomes trial***
3651-Hepatic impairment		SUSTAIN 6 COVT (3744) sema vs placebo (SoC)
Drug-drug interaction		
3817-DDI-metformin and warfarin		
3818-DDI atorvastatin and digoxin		
3819-DDI-oral contraceptives		
Pharmacodynamics		
3635-Beta-cell function		
3684-Hypoglycaemia		
3685-Energy intake		

Figure 4: Semaglutide development program: Overview of completed clinical trials.

4.2 Phase 1

4.2.1 Trail Design

The purpose of phase 1 clinical trial is to investigate the first safety, pharmacokinetic and pharmacodynamic data for oral semaglutide in healthy subjects and subjects with T2D.

In the first-in-human experiment, there are two kinds of trials, single-dose trial, and multiple-dose trial. The single-dose trial is tested in healthy subjects, and the multiple-dose trial is tested between the healthy subjects and the subjects with T2D. Both trials were randomized, placebo-controlled, double-blind trials, each conducted at single sites (Parexel International, Harrow, UK, and Parexel International GmbH, Berlin, Germany, respectively). (Granhall 2019)

4.2.2 Selection Condition of Subjects

Table 6 shows the selection conditions of subjects. In the single-dose trial, eligible subjects are healthy men aged 18-50, weighing 65.0-95.0 kg, and having a

body mass index (BMI) of 18.5-27.5 kg/m^2. In the multi-dose trial, eligible subjects are healthy men aged 18-64 with a BMI of 20.0-29.9kg/m^2 and men who have been diagnosed with T2D after diet, exercise, and/or metformin treatment within the last 10 years, aged 18-64 years old, with a BMI of 20.0-37.0 kg/m^2, glycosylated hemoglobin (HbA1c) of 6.5-9.0%.

If the subject has a clinically significant concomitant disease or disorder, clinically significant outliers in laboratory screening tests, any history of gastrointestinal surgery (except for simple surgical procedures such as appendectomy and hernia surgery), or if they smoked more than five cigarettes or the equivalent per day. (Granhall 2019).

Table 6: The selection conditions of subjects.

Single-dose trial	Multiple-dose trial	
Healthy males	Healthy males	Males with T2D (Within the last 10 years, treated with diet and exercise and/or metformin)
● 18~50 years old ● 65.0~95.0 kg ● BMI 18.5~27.5 kg/m^2	● 18~64 years old ● BMI 20.0~29.9 kg/m^2	● 18~64 years old ● BMI 20.0~37.0 kg/m^2 ● HbA1c 6.5~9.0%

4.2.3 Trail Methods

Figure 5 shows the details of the trail design. The single-dose trial is to investigate the best dose of oral semaglutide. It is divided into 3 parts, part 1a, part 1b, and part 2.

In part 1a, four ascending dose groups are tested in a sequential design. When the first dose level is proved safe, test the next dose level until it reached the specified maximum dose. In part 1b, there are three additional dose groups in a parallel design. This process is to confirm which dose level of the semaglutide with SNAC has a better curative effect. In part 2, three of the doses from part 1 were selected to be repeated in a parallel design in another three groups. At the same time, intravenous and subcutaneous semaglutide are also tested to investigate absolute and relative biotechnology.

The multiple dose trial is to reveal the therapeutic potential in the treatment of T2D. As the subjects are divided into healthy males and males with T2D, their tests are also different. This trial is to identify if the semaglutide gets the therapeutic potential in the treatment of T2D. Healthy males receive oral semaglutide maintenance doses of 20 and 40 mg, and also compared them with receiving placebo and placebo with SNAC. But the subjects with T2D only receive an oral semaglutide dose of 40 mg. In the multiple-dose trial, subjects are randomized to once-daily treatment for 10 weeks with different tablets. (Granhall 2019)

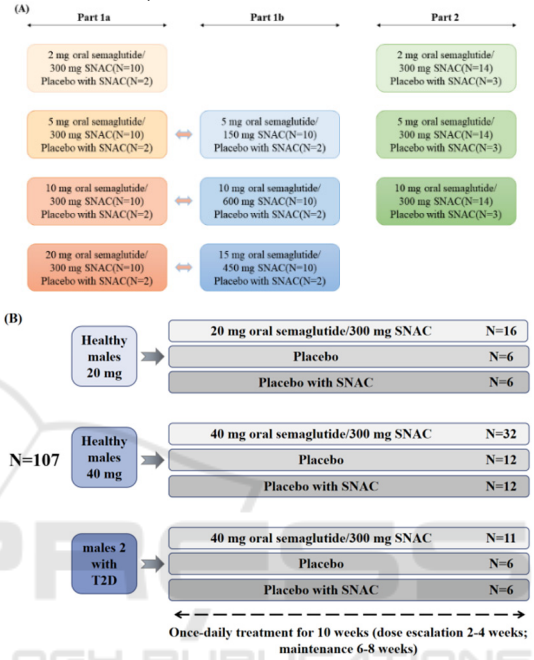

Figure 5: Trail design.

4.2.4 Results

Overview two trials, it confirmed that there are no safety concerns identified and the pharmacokinetic properties of oral semaglutide are comparable in healthy subjects and subjects with T2D.

About the single-dose trial, it concluded that semaglutide with 300mg SNAC gets the best pharmacokinetics. Through comparing different dose levels, 300mg compared with 150 or 600mg is the optimal amount of SNAC to enhance absorption of oral semaglutide. Next, at a fixed amount of 300 mg SNAC, both the proportion of subjects with measurable semaglutide in plasma and the semaglutide exposure appeared to increase with increasing dose of oral semaglutide from 2 to 10 mg, as shown in Figure 6. And in healthy subjects of the multiple-dose trial, semaglutide plasma exposure was about twofold higher with oral semaglutide 40 mg compared with 20 mg, as shown in Figure 7. Furthermore, semaglutide plasma exposure did not differ between healthy subjects receiving 40 mg and

subjects with T2D receiving 40 mg, as shown in Figure 8 and 9. (Granhall 2019)

Comparison of a $AUC_{0–24\ h,\ semaglutide,\ SS(steady\ state)}$ and b $C_{max,\ semaglutide,\ SS}$ between 20 and 40 mg doses of oral semaglutide in healthy males and between healthy males and males with T2D receiving 40 mg oral semaglutide (multiple-dose trial)

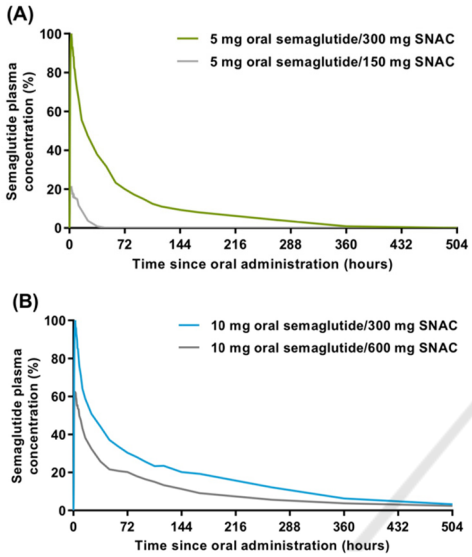

Figure 6: Arithmetic mean semaglutide plasma concentration-time pro-files after a single dose of oral semaglutide with varying amounts of SNAC in healthy male subjects in single-dose trial. (Granhall 2019)

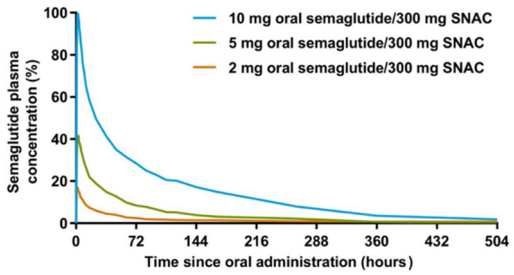

Figure 7: Arithmetic means semaglutide plasma concentration-time profiles after ascending single doses of oral semaglutide with 300 mg SNAC in healthy male subjects in single-dose trial. (Granhall 2019)

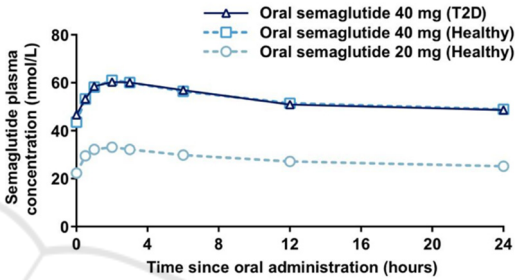

Figure 8: Geometric mean semaglutide plasma concentration-time pro-files at steady state in multiple-dose trial. Profiles represent geometric means of the last 3 days of once-daily oral semaglutide treatment for 10 weeks. T2D type 2 diabetes. (Granhall 2019).

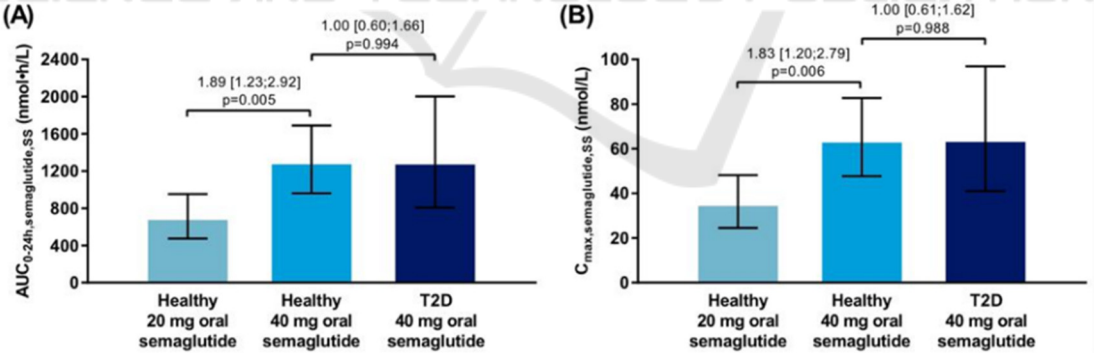

Figure 9. Comparison of a AUC0–24 h, semaglutide, SS and b Cmax, semaglutide, SS between 20 and 40 mg doses of oral semaglutide in healthy males and between healthy males and males with T2D receiving 40 mg oral semaglutide in multiple-dose trial. (Granhall 2019).

4.3 Phase 2

4.3.1 Trail Design

Phase 2 compares the effects of oral semaglutide with placebo (primary) and open-label subcutaneous semaglutide (secondary) on glycemic control in patients with type 2 diabetes.

From December 2013 to December 2014, a 2-week randomized, parallel grouping, the dose-determined 26-week trial was conducted in 100 locations (hospital clinics, general practice, and clinical research centers) in 14 countries/regions. 5-week follow-up. Among the 1106 participants evaluated, 632 patients with type 2 diabetes and insufficient blood glucose control only through diet

and exercise or a stable dose of metformin were randomly assigned. Randomization was stratified by metformin use. (Davies 2017)

4.3.2 Trail Methods

Subjects took orally semaglutide 2.5 mg (n=70), 5 mg (n=70), 10 mg (n=70), 20 mg (n=70), 40 mg orally once a day in 4 weekly dose escalations (standard Escalation; n=71), 40 mg 8-week dose escalation (slow escalation; n=70), 40 mg 2-week dose escalation (rapid escalation, n=70), oral placebo (n=71; double-blind) or every Semaglutide 1.0 mg (n=70) was injected subcutaneously once a week for 26 weeks. (Davies 2017)

4.3.3 Results

The baseline characteristics of each treatment group are comparable. Among 632 randomized patients, 583 (92%) completed the test. From baseline to week 26, the average change in HbA1c levels decreased with oral semaglutide (dose-dependent range, -0.7% to -1.9%) and subcutaneous semaglutide (-1.9%) and placebo (-0.3%); Compared with placebo, the reduction in oral semaglutide was significant compared to the dose (the dose-dependent) estimated treatment difference between oral semaglutide and placebo [ETD] ranged from –0.4% to –1.6%; for 2.5 mg, P = 0.01, for all other doses, <0.01). Oral simaglutide (dose-dependent range, -2.1 kg to -6.9 kg) and subcutaneous simaglutide (-6.4 kg) have greater weight loss than placebo (-1.2 kg), and oral simaglutide doses Significantly compared to placebo at 10 mg or higher (dose-dependent ETD range –0.9 to –5.7 kg; P<0.01). The reported incidence of adverse events was 63% to 86% in the oral semaglutide group (371 of 490 patients), 81% of the subcutaneous semaglutide group (56 of 69 patients), and placebo group 68% (48 of 71 patients); mild to moderate gastrointestinal events are the most common. Within 26 weeks, oral semaglutide can control blood sugar better than a placebo and is effective for patients with type 2 diabetes. (Davies 2017)

4.4 Phase 3

4.4.1 Trail Design

Phase 3 is a series of PIONEER programs. The PIONEER program includes 10 trials, including a pre-approval cardiovascular outcome trial, which aims to evaluate the efficacy and safety of oral semagluide in a wide range of patients with type 2 diabetes. The program includes eight global trials, including Japanese patients, and two trials conducted only in Japan. All trials started in 2016, and the main treatment period ended in 2018. (Rasmussen 2020)

4.4.2 Results

The PIONEER clinical trial plan includes several studies that recruit Japanese patients.

Throughout the plan, oral semaglutide 14 mg reduced HbA1c significantly more than placebo, empagliflozin, and sitagliptin, and was not inferior to liraglutide. In the PIONEER trial in Japan, the reduction in HbA1c of 14 mg of oral semaglutide was significantly higher than that of liraglutide 0.9 mg or dulaglutide 0.75 mg, and the reduction of HbA1c at a 7 mg dose was similar to that of dulaglutide 0.75 mg.

Compared with oral placebo, sitagliptin, and liraglutide, oral sitagutide 14 mg can also significantly reduce body weight. And the weight loss was similar to ipaglifozin. Oral semaglutide also has more beneficial effects in achieving blood sugar control and weight loss than sitagliptin, even when flexible dosage adjustments are made, which reflect the actual dosage setting.

Whether it is based on the estimated value of the treatment strategy (regardless of whether the trial product is discontinued or the use of emergency drugs) or the estimated value of the trial product (the patient continues to use the experimental drug and does not use emergency drugs), the results are usually consistent.

In all PIONEER trials, oral semaglutide was well tolerated, and its adverse events were consistent with other GLP-1RA administered subcutaneously. There were no unexpected safety risks in individual trials. For patients with moderate renal insufficiency, the safety of oral semaglutide seems to be acceptable. In Japanese patients, oral semaglutide is also well tolerated. The incidence of adverse events of oral semaglutide is similar to that of dulaglutide, and its safety is consistent with that of injectable GLP-1RA. In the PIONEER 6 trial, oral glucosamine showed good cardiovascular safety compared with conventional care and compared with placebo, cardiovascular death and all-cause mortality were significantly reduced. (Rasmussen 2020)

5 CONCLUSIONS

Oral semaglutide is a novel tablet containing the human glucagon-like peptide-1 (GLP-1) analogue semaglutide, co-formulated with the absorption enhancer SNAC. It has three major effects of

lowering blood glucose, weight loss, and reducing cardiovascular risk. For non-clinical species, intact semaglutide is a major component circulating in plasma and is metabolized prior to excretion. Additionally, it has been found to affect the thyroid's fertility and C cell adenomas in preclinical studies. In the single-dose experiment of clinical trials, it was found that semaglutide with 300mg SNAC will exert the maximum effect. Multi-dose experiments have proved that semaglutide has the therapeutic properties of T2D. In phase 2 trials, semaglutide has better potency and effects than other therapeutic drugs already on the market. Phase 3 trial further proved that semaglutide has the function of reducing HbA1c and body weight. In conclusion, the successful design of the oral formulation of Semaglutide paves the way for the subsequent development of oral forms of GLP-1RAs.

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