Original Article

MOLECULAR METABOLISM

A once-daily GLP-1/GIP/glucagon receptor tri-agonist (NN1706) lowers body weight in rodents, monkeys and humans

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ABSTRACT

Single molecules that combine complementary modes of action with glucagon-like peptide-1 receptor (GLP-1R) agonism are best-in-class therapeutics for obesity treatment. NN1706 (MAR423, RO6883746) is a fatty-acylated tri-agonist designed for balanced activity at GLP-1R and glucose-dependent insulinotropic peptide receptor (GIPR) with lower relative potency at the glucagon receptor (GcgR). Obese mice, rats and non-human primates dosed with NN1706 showed significant body weight reductions and improved glycemic control. In human participants with overweight or obesity, daily subcutaneous NN1706 treatment resulted in substantial body weight loss in a dose-dependent manner without impairing glycemic control (NCT03095807, NCT03661879). However, increased heart rate was observed across NN1706 treatment cohorts, which challenges further clinical development of NN1706.

Keywords Obesity; Incretins; Glucagon; Clinical

1. INTRODUCTION

Obesity is a chronic disease associated with a large number of metabolic risk factors and comorbidities resulting in health complications and premature mortality [1]. Although intensive lifestyle modification through diet and exercise is an important early intervention, it is usually insufficient to reverse disease progression [2]. Glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) mono-agonists are effective for weight management, with once-daily liraglutide and once-weekly semaglutide both approved as an adjunct to diet and exercise in individuals living with obesity or overweight with co-morbidities [3,4]. Liraglutide reduces body weight with meaningful impact on health, but relatively modest efficacy (5—10% in people without type 2 diabetes [T2D]) [5]. Semaglutide offers greater mean weight reduction in this population (~15%) [6], demonstrating that chemical optimization provides additional weight loss.

Despite initial successes with GLP-1R selective mono-agonists, pharmacotherapies that further lower body weight and alleviate obesity-related comorbidities are needed. Single molecules that integrate complementary action to GLP-1R agonism have been shown to offer superior efficacy for the treatment of obesity. Tirzepatide, a single-molecule GLP-1R/glucose-dependent insulinotropic peptide receptor (GIPR) co-agonist with receptor activity favoring GIPR relative to GLP-1R, demonstrated greater body weight reduction and

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glycemic control in individuals with T2D than semaglutide [7]. In individuals with obesity without T2D, mean body weight reduction of up to 22.5% from baseline was achieved with tirzepatide [8]. The exact magnitude of differential efficacy of tirzepatide relative to semaglutide in obesity requires further head-to-head clinical assessment.

GLP-1R/glucagon receptor (GcgR) co-agonists are proven to lower body weight and improve metabolic outcomes in obese animal models [9–11], and several clinical translation studies have been reported [10,12–14]. Similar drug candidates with sustained pharmacokinetics and different ratios of potency have reported better clinical results [15–21]. More extensive, longer comparative clinical studies are required to fully interrogate the risk-benefit of such co-agonism relative to GLP-1R agonists and GLP-1R/GIPR co-agonists.

Unimolecular integration of GcgR agonism with GLP-1R/GIPR co-agonism displays even greater efficacy in preclinical studies relative to matched dual co-agonists [22–24]. Coordinated activation of these receptors synergistically reduces body weight, while the incretin properties maintain glycemic control by opposing the hyperglycemic liability of GcgR agonism. Results from a single ascending dose (SAD) study of the once-daily tri-agonist SAR441255 in healthy participants show a beneficial effect on glycemic control [24]. More recently, once-weekly tri-agonist retatrutide (LY3437943), which like tirzepatide favors GIPR over GLP-1R and GcgR activity, has been investigated in clinical studies. An acceptable safety profile was observed in the SAD and multiple ascending dose (MAD) studies in healthy participants and individuals with T2D, respectively [23,25]. Furthermore, the 12-week MAD study for retatrutide showed significant reductions in mean daily plasma glucose, glycated hemoglobin (HbA_{1c}) and body weight, supporting tri-agonism for the management of obesity and associated metabolic conditions [25,26]. In phase 2 studies, retatrutide reduced body weight up to 24.2% from baseline at 48 weeks in individuals with obesity [26] and up to 16.9% from baseline at 36 weeks in individuals with T2D [27]. Notably, HbA_{1c} was reduced by 2.16%-points in the T2D study. However, increased heart rate was observed but the effect diminished with continued treatment. Longer studies utilizing methods that provide more resolution will allow to determine with certainty if the hemodynamic effects present a safety risk. Collectively, these data support continued drug discovery and development of gut hormone tri-agonists.

NN1706 is a fatty acid-acylated unimolecular peptide with tri-agonist activities at GLP-1R, GIPR, and GcgR. Here, we report preclinical characterization of NN1706 (MAR423, RO6883746) and first-in-human clinical results in individuals with overweight or obesity.

2. RESULTS

2.1. Chemical structure of NN1706 and *in vitro* profile

NN1706 is based on IUB447, a precursor GLP-1R/GIPR/GcgR tri-agonist with a balanced pharmacological profile at all three receptors relative to the respective native ligand as measured by cAMP accumulation [22]. NN1706 was designed to be equally potent at GLP-1R and GIPR but with a purposefully engineered reduction in relative potency at human GcgR estimated to 3-10-fold depending on assay conditions. The primary rationale for this targeted reduction in relative GcgR potency arose from concerns at the time of molecule design and iteration about the narrow therapeutic window relating to hyperglycemia. NN1706 is a 39 amino acid linear peptide conjugated to a C16 fatty monoacid at the lysine residue in position 10 via a gamma-glutamic acid linker (Figure 1A). The peptide backbone contains an Nα-acetylated D-stereoisomer of tyrosine (Ac-[D]Tyr) at the N-terminus, which permits substitution of the alpha-amino isobutyric acid (Aib) at position 2 of IUB447 with alanine while still protecting against dipeptidyl peptidase-4 degradation. These modifications reduce the GcgR potency with little impact on the potency for stimulation of cAMP production from GLP-1R and GIPR activation. NN1706 also contains a glutamic acid at position 28, which prevents isoaspartimide formation that is seen in the same position of IUB447.

We evaluated the potency for receptor activation in mammalian CRE-Luc reporter cell lines overexpressing either human GLP-1R, GIPR or GcgR. NN1706 possessed high-potency tri-agonism at all three receptors (Figure 1B). In these assay conditions, NN1706 has comparable *in vitro* potency at human GLP-1R relative to liraglutide, the GLP-1R/GIPR co-agonist NN0090-2746 (RG7697, MAR709) [28], the GLP-1R/GcgR co-agonist cotadutide [29], and the GLP-1R/GIPR/GcgR tri-agonist SAR441255 (Figure 1B). NN1706 has comparable *in vitro* potency at human GIPR to NN0090-2746, resulting in balanced activities at human GLP-1R and GIPR. NN1706 is less potent at GcgR than its precursor (IUB447), but more potent than cotadutide (Figure 1B). These results justified testing NN1706 on weight loss in animal models of obesity.

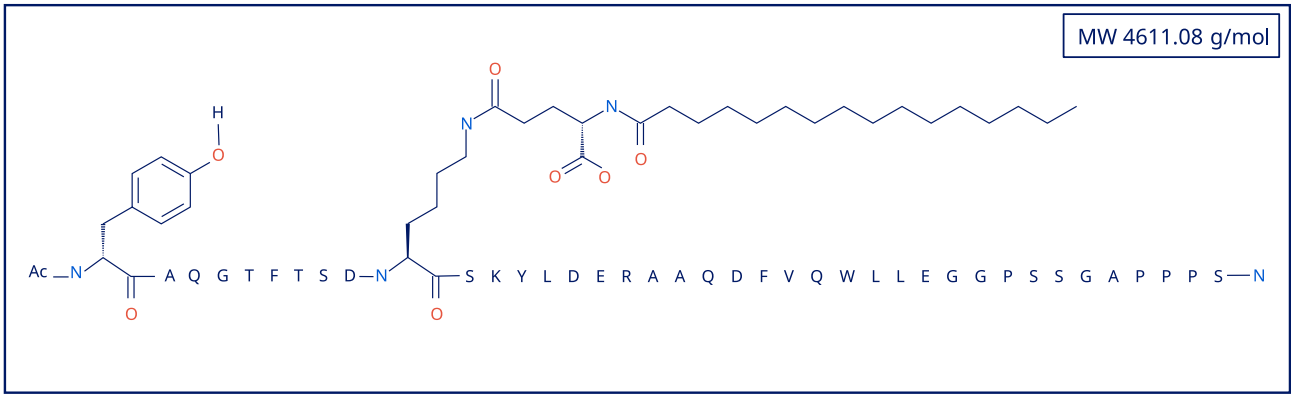
2.2. NN1706 lowers body weight in diet-induced obese rodent models and increases heart rate in Wistar rats

Pharmacokinetic (PK) studies in lean Wistar rats demonstrated sustained exposure of NN1706, with elimination following subcutaneous (s.c.) injection being absorption-limited. NN1706 $t_{½}$ was 1.2 h following intravenous (i.v.) administration, 6.0 h following s.c. administration, and bioavailability after s.c. dosing was 25.0% (Supplementary Information Table 1).

The effects of NN1706 on body weight and food intake were examined in diet-induced obese (DIO) mouse and rat models. DIO mice were injected once-daily with NN1706, IUB447 or SAR441255 (0.5, 1.0 or 2.0 nmol/kg, s.c.) for 28 days. NN1706 treatment resulted in dose-dependent body weight loss, which was more pronounced than with IUB447 or SAR441255 (Figure 2A). NN1706 treatment also caused a dose-dependent decrease in cumulative food intake over 28 days (Figure 2B). Glucose tolerance improved compared with vehicle, both when assessed at the NN1706 estimated time to maximal concentration (t_{max}) at steady-state on day 0 (Figure 2C) as well as at estimated time to minimal concentration (t_{min}) on day 27 (Figure 2D), albeit without apparent dose-correlation. Furthermore, NN1706 treatment resulted in greater body weight loss compared to pair-fed mouse controls (Figure 2E), suggesting non-food intake mechanisms contribute to the weight-lowering efficacy, consistent with functional activity at GcgR [22]. Indirect calorimetry analysis confirmed increased energy expenditure in DIO mice treated with NN1706 vs vehicle and pair-fed controls (Figure 2F). Overall, these findings are aligned with previous pharmacodynamic (PD) findings of tri-agonists in DIO mice [22–24].

DIO rats were injected (s.c.) for 14 days with either NN1706 (5 nmol/kg/day) or liraglutide (75 nmol/kg/day). NN1706 reduced body weight over 14 days with weight loss greater at 5 nmol/kg NN1706 than 75 nmol/kg liraglutide (Supplementary Information Fig. 1A). NN1706 reduced acute food intake similar to liraglutide by day 3, and the effect was sustained throughout the study while food intake increased close to baseline level in liraglutide treated animals (Supplementary Information Fig. 1B). NN1706 also reduced postprandial glucose and

A



B

Agonist potency in CRE-Luc reporter assay as geometric mean EC_{50} (pM; 0% HSA)			
	hGLP-1R	hGIPR	hGcgR
NATIVE LIGAND	2.5	3.2	5.0
NN1706	2.2	3.5	10.5
LIRAGLUTIDE	1.9	-	-
NN0090-2746	2.4	2.2	-
COTADUTIDE	1.7	-	18.6
SAR441255	1.2	10.1	7.8
IUB447	1.3	3.8	5.9

Figure 1: NN1706 is a potent and balanced tri-agonist of the GLP-1, GIP, and Gcg receptors. **A.** Structure schematic of NN1706 (MW 4611.08 g/mol). **B.** Agonist potencies (mean apparent 50% stimulatory concentration [EC_{50}] values) from *in vitro* CRE-Luc 0% HSA assays in BHK cells expressing human recombinant GLP-1R, GcgR, or GIPR (n≥3). *BHK, baby hamster kidney; cAMP, cyclic adenosine monophosphate; CRE-Luc, cAMP response element-luciferase; EC_{50}, half maximal effective concentration; Gcg, glucagon; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; hGcgR, human Gcg receptor; hGIPR, human GIP receptor; hGLP-1R, human GLP-1 receptor; HSA, human serum albumin; MW, molecular weight.*

insulin levels relative to vehicle and liraglutide (Supplementary Information Figs. 1C and 1D), consistent with PD results in DIO mice. The effects of single and repeated s.c. NN1706 administration on blood pressure and heart rate were investigated up to 7 days in conscious telemetered lean male Wistar rats at doses of 5, 20, or 45 μg/kg/day. On day 1 of dosing, sustained increases in heart rate were evident with all doses immediately post-injection (maximum effect ∼+120 beats per minute, bpm) (Figure 2G). Increases in mean blood pressure occurred 2–14 h post-injection at mid and top doses (maximum effect ∼+17 mmHg), but no effect was observed at 5 μg/kg (Figure 2H). No concurrent changes in other cardiovascular parameters were observed. Repeated once-daily NN1706 administration for 7 days had no impact on the magnitude of increased heart rate but response duration reduced (Figure 2I). Effects on blood pressure lessened over time with repeated dosing (no impact by day 7) (Figure 2J).

2.3. NN1706 reduces glucose excursions and lowers body weight in lean and DIO cynomolgus monkeys

The effects of NN1706 on glucose metabolism and food intake were examined in lean and DIO cynomolgus monkeys [29]. The half-life of NN1706 in monkeys following s.c. administration is ∼6 h (Supplementary Information Table 1), consistent with other peptides protracted with C16 fatty monoacids [29,30]. In lean monkeys, NN1706 (1.5 or 4.9 μg/kg s.c.) dose-dependently lowered glucose excursions during an oral glucose tolerance test (OGTT) without a prolonged increase in insulin secretion (Supplementary Information Figs. 2A–B), suggesting inhibition of gastric emptying is responsible for lower glucose excursions. NN1706 also decreased glucose excursions but led to increased insulin secretion during an intravenous glucose tolerance test (ivGTT) in DIO cynomolgus monkeys (Supplementary Information Figs. 2C–D), showing the insulin secretion activity of NN1706, predominantly due to GLP-1R/GIPR agonism. In DIO monkeys, treatment with NN1706 (5 μg/kg/day, s.c.) for 8 days significantly reduced body weight by 0.77 kg from baseline. This effect was greater than the 0.32 kg weight loss from baseline seen with liraglutide (20 μg/kg/day, s.c.), despite the lower NN1706 dose (Figure 3A).

In an ascending dose study, DIO cynomolgus monkeys were treated s.c. once-daily with escalating NN1706 or liraglutide doses over 39 days. Treatment with NN1706 titrated up to 2.0 μg/kg/day lowered body weight by 6.8% after 39 days of treatment, whereas liraglutide

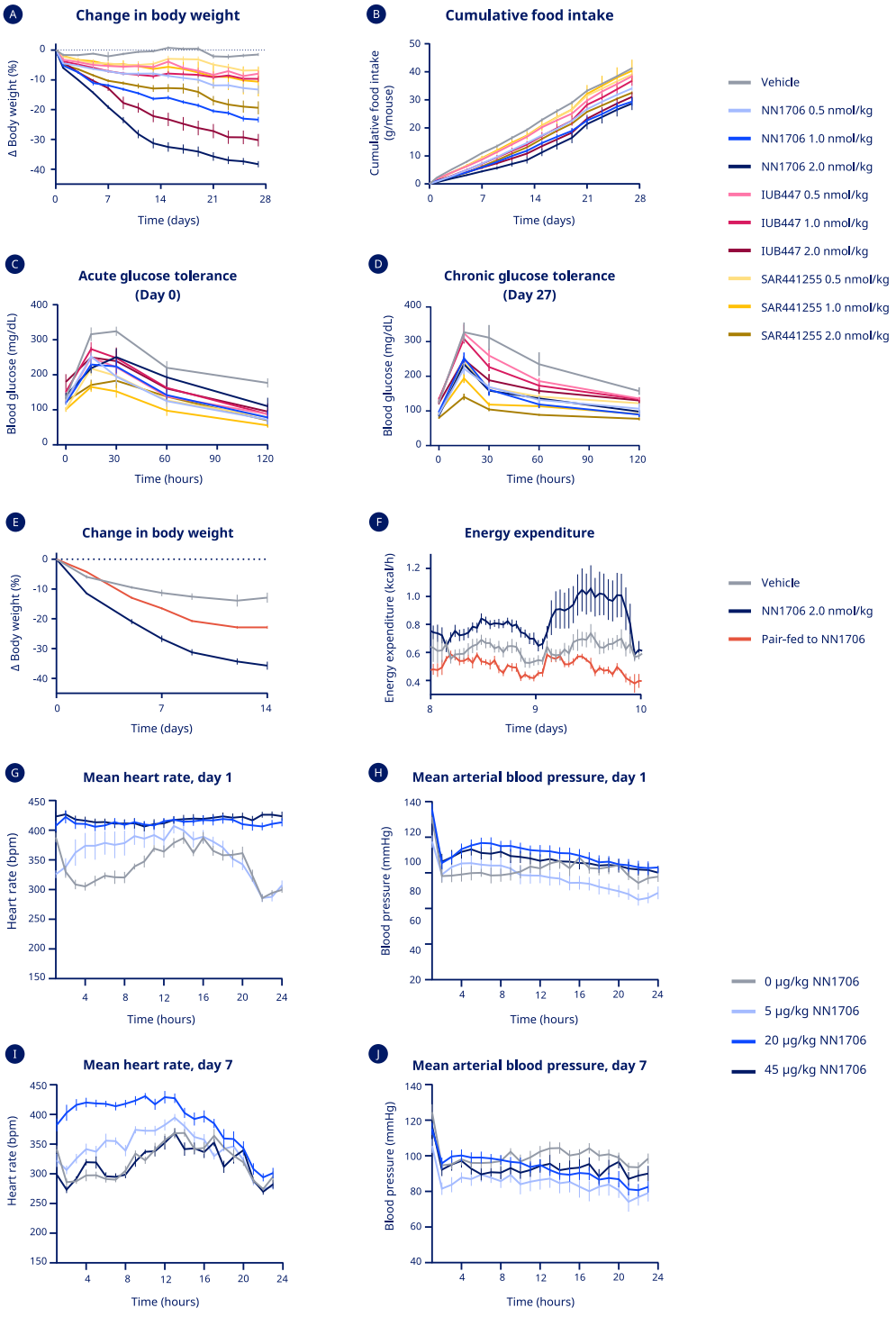


Figure 2: NN1706 lowers body weight and improves glycemic control in diet-induced obese mouse models and increases heart rate in telemetered Wistar Han rats. **A-B.** Relative change in body weight (mean baseline body weight 62.3 g; n = 8) (A) and cumulative food intake (B), in male DIO mice administered NN1706, IUB447 or SAR441255 over 28 days (n = 8). **C-D.** Acute glucose tolerance assessed at day 0 (C) and chronic glucose tolerance assessed at day 27 (chronic; D) of vehicle, NN1706, IUB447 or SAR441255 treatment in male DIO mice (n = 8). **E-F.** Body weight loss (E) and energy expenditure (F) in DIO mice treated with NN1706 over 10 days in comparison to vehicle and pair-fed controls (n = 8). **G-J.** Effect of NN1706 or vehicle on mean heart rate and mean blood pressure after a single dose (G—H), and after 7 days of dosing (I—J) in male telemetered Wistar Han rats (n = 8). One-minute mean data were collected every 4 min. Rats were injected at time = 0 h, and data are reported from 1 h post-dose. The 45 µg/kg dose was stopped after 2 days as heart rate and blood pressure increases were similar to those produced by the 20 µg/kg dose. Rats in the 45 µg/kg group received no treatment on days 3—7. Values represent mean ± SEM. *DIO, diet-induced obese.*

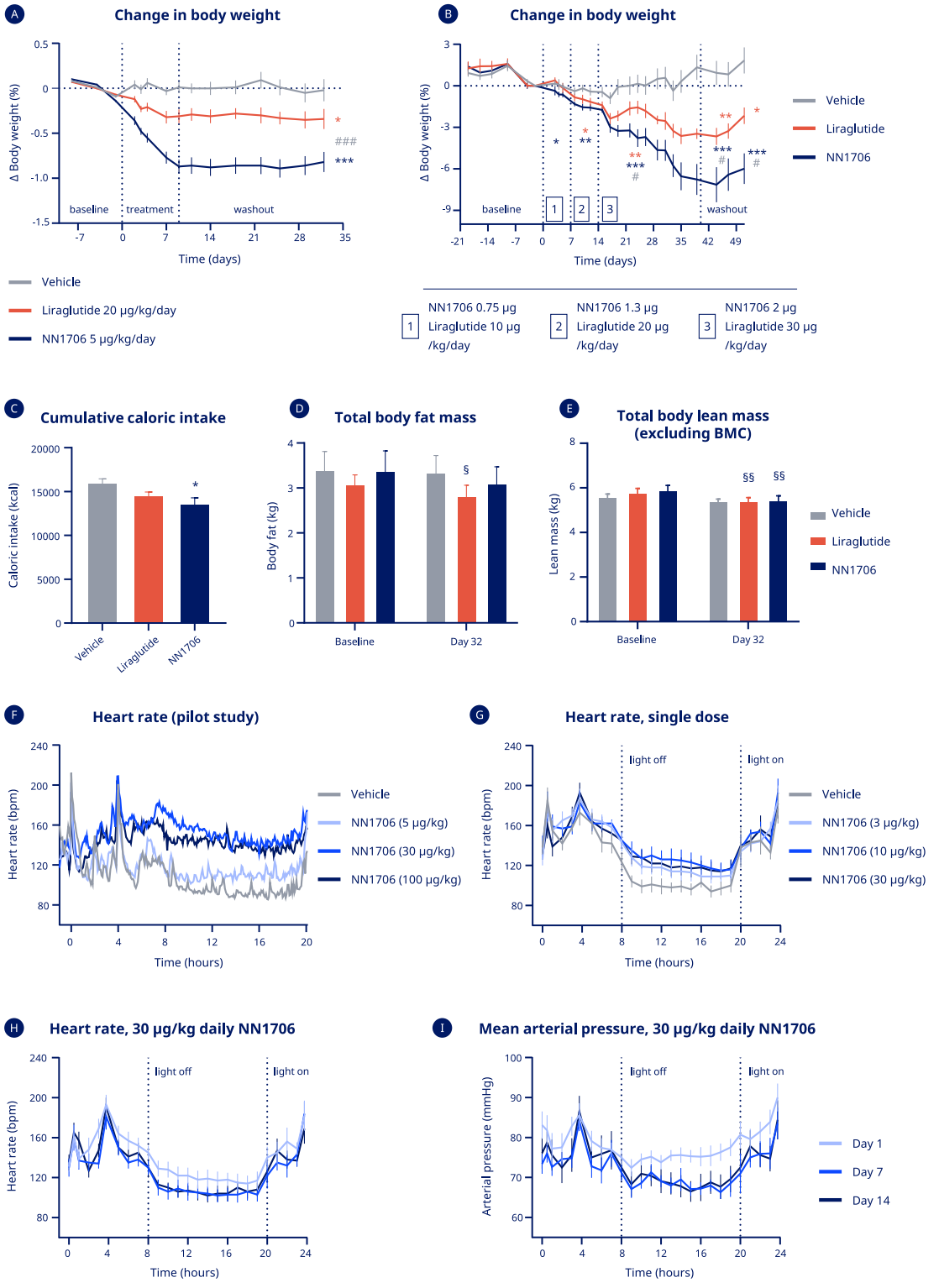


Figure 3: NN1706 reduces body weight and caloric intake in DIO cynomolgus monkeys and increases heart rate in lean cynomolgus monkeys. **A.** Relative change in body weight over 8 days s.c. NN1706 (5 µg/kg/day), liraglutide (20 µg/kg/day) or vehicle in DIO cynomolgus monkeys (n = 8; mean baseline body weight 10.4 kg). **B-E.** Ascending dose study of NN1706 in DIO cynomolgus monkeys treated with s.c. vehicle (n = 9) or escalating doses of NN1706 (n = 9) or liraglutide (n = 8) for 39 days (mean baseline body weight 9.6 kg). Relative change in body weight (B), cumulative caloric intake over days 0—38 (C) and total body fat (D) and lean mass (E) at day 32. **F—I.** Cardiovascular outcomes in conscious telemetered lean cynomolgus monkeys. Heart rate in a single dose pilot study with vehicle or 5, 30 or 100 µg/kg s.c. NN1706 (n = 3—4; F), and follow-up (n = 6; G) study of vehicle or 3, 10 or 30 µg/kg s.c. NN1706. Heart rate (H) and mean arterial blood pressure (I) at day 1, 7 and 14 after repeated doses of 30 µg/kg NN1706 (s.c.). A-E, G—I: Values represent mean ± SEM. F: Mean values without error are plotted. Data were analyzed using analysis of variance (ANOVA) for repeated measurements (A), ANOVA for repeated measures for the entire study and per study period (B), ANOVA followed by Dunnett's test (C), ANOVA followed by Dunnett's test and paired t-test (D—E), descriptive statistics (F), or ANOVA for repeated measurements (G—I). Statistical significance indicated for tests of treatment group vs vehicle (*p<0.05, **p<0.01, ***p<0.001), Liraglutide vs NN1706 (#p<0.05, ###p<0.001), baseline vs follow-up (§p<0.05, §§p<0.01). *ANOVA, analysis of variance; BMC, bone mineral content; DIO, diet-induced obese.*

titrated up to 30.0 μg/kg/day lowered body weight by 3.5% (Figure 3B). Despite superior body weight loss with NN1706, there was no difference in cumulative caloric intake between treatments (Figure 3C), suggesting non-food intake mechanisms contribute to greater NN1706 efficacy as seen in DIO mice. Weight loss was driven primarily by reduced total fat mass, although a loss in lean mass was evident with both treatments (Figure 3D–E).

2.4. Cardiovascular hemodynamics of NN1706 in lean telemetered cynomolgus monkeys

Two cardiovascular hemodynamic studies were conducted in conscious lean cynomolgus monkeys. In a pilot study, heart rate after a single s.c. injection of NN1706 at ascending doses of 5, 30 or 100 μg/kg was characterized. A sustained increase in heart rate up to 53 bpm 5–20 h post-dose was observed for 30 and 100 μg/kg NN1706 (Figure 3F). In the second study, effects of s.c. injections of NN1706 on heart rate and arterial pressure were investigated. A slight, non-significant increase in heart rate was observed after single injections with 3, 10 or 30 μg/kg NN1706 6–20 h post-dose (Figure 3G). After repeated dosing with 30 μg/kg for 14 days, heart rates returned to levels observed in vehicle controls (Figure 3H). A non-significant decrease in arterial pressure became apparent on days 7 and 14 (Figure 3I). Based on findings from the preclinical studies, NN1706 was advanced to clinical testing in individuals with overweight and obesity.

2.5. Single-dose phase 1 study of NN1706 in humans with overweight or obesity

A first-in-human, single ascending dose (SAD), randomized, double-blind, single-center, placebo-controlled study was conducted to investigate the safety, tolerability, PK, and PD of NN1706 (NCT03095807) in male participants aged 18–55 years with overweight or obesity (BMI 25.0–34.9 kg/m^2). Thirty-four participants were randomly assigned to 5 dose cohorts of NN1706: 5, 15, 50, 150, and 300 μg, or placebo (Figure 4A and Supplementary Information Fig. 3A). Seven cohorts (doses up to 2 mg) were planned, but after a single participant who received a dose of 300 μg experienced an unacceptable adverse event (AE; described below), it was determined not safe to dose at this level or above. Demographic and baseline characteristics are reported in Supplementary Information Table 2.

Noncompartmental PK parameters based on measured NN1706 plasma concentrations across the dose range are summarized in Supplementary Information Table 3 (participants dosed with 5 μg are excluded from the following as too few samples had detectable concentrations above the lower limit of quantification). The median t_{max} range was 9.1–11.7 h and elimination was monophasic with a geometric mean $t_{½}$ range of 13.8–17.6 h (Figure 4B). Exposure, as determined by C_{max}, showed dose-proportionality in the investigated dose range, but dose-proportionality for $AUC_{0\text{-inf}}$ was not clearly established (Supplementary Information Fig. 3B). Actual differences in the estimated treatment ratios between doses for C_{max} and $AUC_{0\text{-inf}}$ were small and therefore dose-proportionality evaluations should be interpreted with caution, which is also confounded by limited data. Collectively, NN1706 PK properties from the SAD study support once-daily dosing in humans.

Following a single dose of NN1706, a decrease in body weight was observed within the first 4 days after dosing with 50 or 150 μg (Figure 4C). The greatest reduction in mean body weight from baseline to day 4 was seen with 150 μg of NN1706 (−2.2%). A decrease in fasting plasma glucose was observed with all NN1706 doses within the first 2 days after administration (Figure 4D), and a decrease in non-fasting plasma glucose was observed at all tested NN1706 doses as well as placebo within 4 h after dosing (Figure 4E). Increases in fasting insulin and fasting C-peptide levels were observed at all NN1706 doses except 5 μg (Figure 4F,G).

Overall, treatment emergent adverse events (TEAEs) were reported in 13 participants (38.2%; 33 events); 6 (17.6%) participants had 21 TEAEs considered related to treatment and the majority of these (13 events in 4 participants [11.8%]) were of gastrointestinal (GI) origin. There were no deaths or serious TEAEs (Table 1), but there was an apparent dose-dependent increase in TEAE number and severity. The most frequent TEAEs were GI-related, all of which occurred at the two highest doses (150 and 300 μg NN1706) (Table 1). Majority of reported TEAEs were mild or moderate in intensity. Although NN1706 was well-tolerated up to 150 μg, 1 participant at the highest dose (300 μg) experienced a relatively large number of TEAEs (11; 6 of GI origin), of which 2 were severe (nausea, which occurred ∼3 h after dosing and lasted for 1 day; retching, which occurred ∼8.5 h after dosing and lasted for 5.5 h).

With the exception of increased heart rate, no relevant changes in vital sign measurements, laboratory parameters, or physical examination findings were reported. A dose-dependent transient increase in heart rate during the first 48 h after dosing was observed with NN1706 at 50 and 150 μg. The maximum increase in mean heart rate from baseline after NN1706 dosing was 19 and 25 bpm for 50 and 150 μg, respectively (placebo: 9 bpm) (Figure 4H).

2.6. Multiple ascending doses of NN1706 lowers body weight and increases heart rate in humans with overweight or obesity

A multiple ascending dose (MAD), randomized, double-blind within cohorts, single-center, placebo-controlled study (NCT03661879) was conducted to investigate safety, tolerability, PK, and PD of NN1706 over 10 weeks (with 2-week follow-up). Participants were 18–55 years old with overweight or obesity (BMI 25.0–39.9 kg/m^2) without T2D. Sixty-one participants (mean age: 37.3 years; mean body weight: 92.8 kg) were randomized to five NN1706 dose cohorts or placebo in a 9:3 ratio (Supplementary Information Figure 4A and Figure 5A). Planned ascending doses were 25 (C1), 60 (C2), 150 (C3), 300 (C4), and 600 μg (C5). However, three cases of second degree atrioventricular (AV) block were reported in three participants in the 150-μg cohort with temporal relationship between dose escalation to the target dose and onset of event. The study safety group and safety committee decided to stop dosing in the 150-μg cohort (C3) and amend dosing for the 300-μg cohort (C4) to 100 μg and repeat 150 μg dosing in the 600-μg cohort (C5). Demographic and baseline characteristics are reported in Supplementary Information Table 4.

Noncompartmental PK parameters based on measured plasma concentrations of NN1706 across the dose range are summarized in Supplementary Information Table 5. Steady-state plasma concentrations of NN1706 as well as $AUC_{0-24h,ss}$ and $C_{max,ss}$ increased proportionally with increasing dose (Figure 5B and Supplementary Information Fig. 4B). Median t_{max} of NN1706 at steady-state was similar across doses at 8–10 h, and the geometric mean $t_{½}$ range was 17.5–25.6 h at steady-state (Supplementary Information Table 5).

Greater body weight reductions were observed with increasing NN1706 dose (Figure 5C). After the 10-week treatment period, weight loss induced by 100 and 150 μg was statistically significant vs placebo (Figure 5D) and did not plateau. In OGTT, there was no deterioration induced by NN1706 vs placebo at day 36 or end of treatment, as assessed by glucose AUC_{0-2h} (Supplementary Information Table 6). Increased insulin secretion during the OGTT was observed with all NN1706 doses vs placebo (Supplementary Information Table 6).

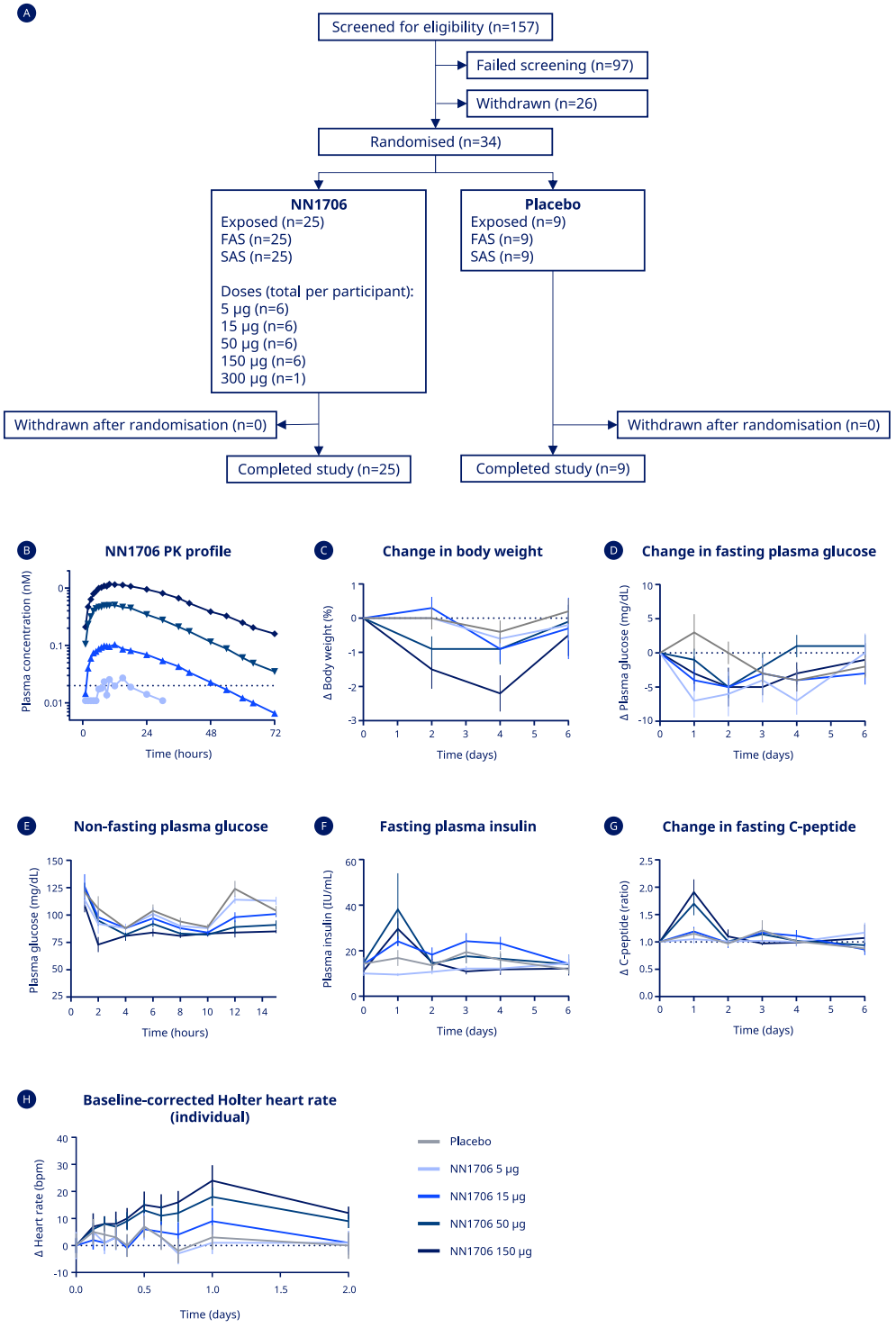


Figure 4: NN1706 in male participants in a phase 1 single ascending dose clinical study.
A. Participant disposition. **B.** NN1706 plasma concentrations after single dose of NN1706. Dotted line indicates the LLOQ. Values below LLOQ were imputed. **C.** Relative change in body weight after a single dose of NN1706. **D-H.** Changes in clinical laboratory parameters and heart rate after a single dose of NN1706. Change in fasting plasma glucose (D), non-fasting plasma glucose (E), fasting insulin (F), and fasting C-peptide (G) from baseline as well as baseline-corrected Holter heart rate (H). B: Values represent geometric mean. C–H: Values represent mean ± SEM. *bpm, beats per minute; FAS, full analysis set; LLOQ, lower limit of quantification; SAS, safety analysis set.*

Table 1 — Treatment emergent adverse events (TEAEs) in the single ascending dose (SAD) study.

Treatment emergent adverse events, n (%) [E]	Placebo n = 9	NN1706				
		5 μg n = 6	15 μg n = 6	50 μg n = 6	150 μg n = 6	300 μg n = 1
Events	2 (22.2) [2]	0	2 (33.3) [3]	3 (50.0) [5]	5 (83.3) [12]	1 (100.0) [11]
Serious	0	0	0	0	0	0
Leading to withdrawal	0	0	0	0	0	0
Severity						
Severe	0	0	0	0	0	1 (100.0) [2]
Moderate	2 (22.2) [2]	0	1 (16.7) [2]	1 (16.7) [1]	2 (33.3) [2]	1 (100.0) [3]
Mild	0	0	1 (16.7) [1]	3 (50.0) [4]	4 (66.7) [10]	1 (100.0) [6]
Related to study drug[a]						
Probable	0	0	0	0	1 (16.7) [3]	1 (100.0) [8]
Possible	0	0	0	1 (16.7) [1]	3 (50.0) [7]	1 (100.0) [2]
Unlikely	2 (22.2) [2]	0	2 (33.3) [3]	3 (50.0) [4]	2 (33.3) [2]	1 (100.0) [1]
Death	0	0	0	0	0	0
By system organ class and preferred term						
TEAE class						
Gastrointestinal disorders						
Nausea	0	0	0	0	3 (50.0) [3]	1 (100.0) [1]
Abdominal discomfort	0	0	0	0	1 (16.7) [1]	1 (100.0) [1]
Eructation	0	0	0	0	1 (16.7) [1]	1 (100.0) [1]
Vomiting	0	0	0	0	2 (33.3) [2]	0
Defecation urgency	0	0	0	0	0	1 (100.0) [1]
Dyspepsia	0	0	0	0	0	1 (100.0) [1]
Retching	0	0	0	0	0	1 (100.0) [1]
Cardiac disorders						
Rhythm idioventricular	0	0	0	1 (16.7) [1]	0	0
Supraventricular tachycardia	0	0	0	0	0	1 (100.0) [1]
Ventricular extrasystoles	0	0	0	0	1 (16.7) [1]	0
Infections and infestations						
Rhinitis	0	0	0	1 (16.7) [1]	0	0
Upper respiratory tract infection	1 (11.1) [1]	0	0	0	0	0
General disorders and administration site conditions						
Medical device site reaction	1 (11.1) [1]	0	0	2 (33.3) [2]	3 (50.0) [3]	0

Data are expressed as n (%) [E], where n is the number of participants reporting an event, % is the percentage of participants reporting an event and E is the number of events.
TEAE, treatment emergent adverse event.
[a] Relationship to study product was based on investigator assessment.

TEAEs were reported in 42 participants (93%; 311 events) receiving NN1706, with 38 participants (84%; 247 events) having TEAEs considered probably related to study product (Table 2). No deaths or other serious TEAEs occurred during the study. The most reported TEAEs were GI-related (70 events reported by 28 participants) that appeared to be dose-related. For example, nausea and vomiting events occurred predominantly during the dose escalation phase and were short in duration. Higher doses were better tolerated using an escalating dose titration scheme, which extensively increased exposure compared to the SAD study. A total of 23 participants reported 45 events of cardiac disorder, with the largest number reported at the highest NN1706 dose (150 μg) (Table 2). The most frequently reported cardiac event was sinus tachycardia (40 events in 23 participants). A total of 4 TEAEs led to withdrawal from the study. Two participants (1 in 60-μg cohort and 1 in 150-μg cohort) were withdrawn due to reaching the protocol-defined heart rate stopping rule following AEs reported as sinus tachycardia. One participant in the 60-μg cohort was withdrawn due to a TEAE reported as ventricular tachycardia; this event had a duration of <1 min. One participant in the 60-μg cohort was withdrawn due to vomiting. All withdrawal events were assessed as probably related to the study product and all were resolved.

There were no changes in vital signs with NN1706 except for pulse rate, which increased in all NN1706 cohorts without dose-dependency or evidence of tachyphylaxis throughout the 10-week treatment and returned to baseline upon treatment cessation (Figure 5E). The increase in pulse rate was comparable to increases in heart rate observed across doses using 12-lead electrocardiogram (ECG) (Figure 5F) and Holter monitoring. In the 12-lead ECG, the observed change from baseline to end of treatment in mean heart rate amounted to 7–16 bpm across NN1706 cohorts vs −5 bpm for placebo (Figure 5G). There was no apparent tachyphylaxis of the heart rate increase observed over the 10-week treatment period. Three participants developed second-degree AV blocks at the 150-μg NN1706 dose within 48 h of escalation to target dose. Analysis of these events determined that a causal relationship between NN1706 and second-degree AV blocks cannot be excluded as medical history and concomitant medication did not provide an alternative etiology. One additional second-degree AV block was reported in the 60-μg cohort but classified as related to concomitant disease. No AV blocks were reported at the 100-μg dose, nor were there any AV blocks reported when repeating the 150-μg dose. There was a small but non-significant increase in the individual corrected QT interval (QTcI) with increasing NN1706 concentrations (Figure 5H).

Of the 45 participants exposed to NN1706, 44 were assessed for NN1706 antibodies; 11 participants (25%) were antibody positive at some point post-baseline across all doses. Of those, no participants had cross-reactive antibodies against GIP or GLP-1, although one participant in the 150-μg cohort was positive for cross-reactivity to endogenous glucagon at follow-up (*in vitro* neutralizing effect was negative).

Beta-hydroxybutyrate, a ketone body produced during lipolysis, increased in all NN1706 cohorts (Supplementary Information Fig. 5A).

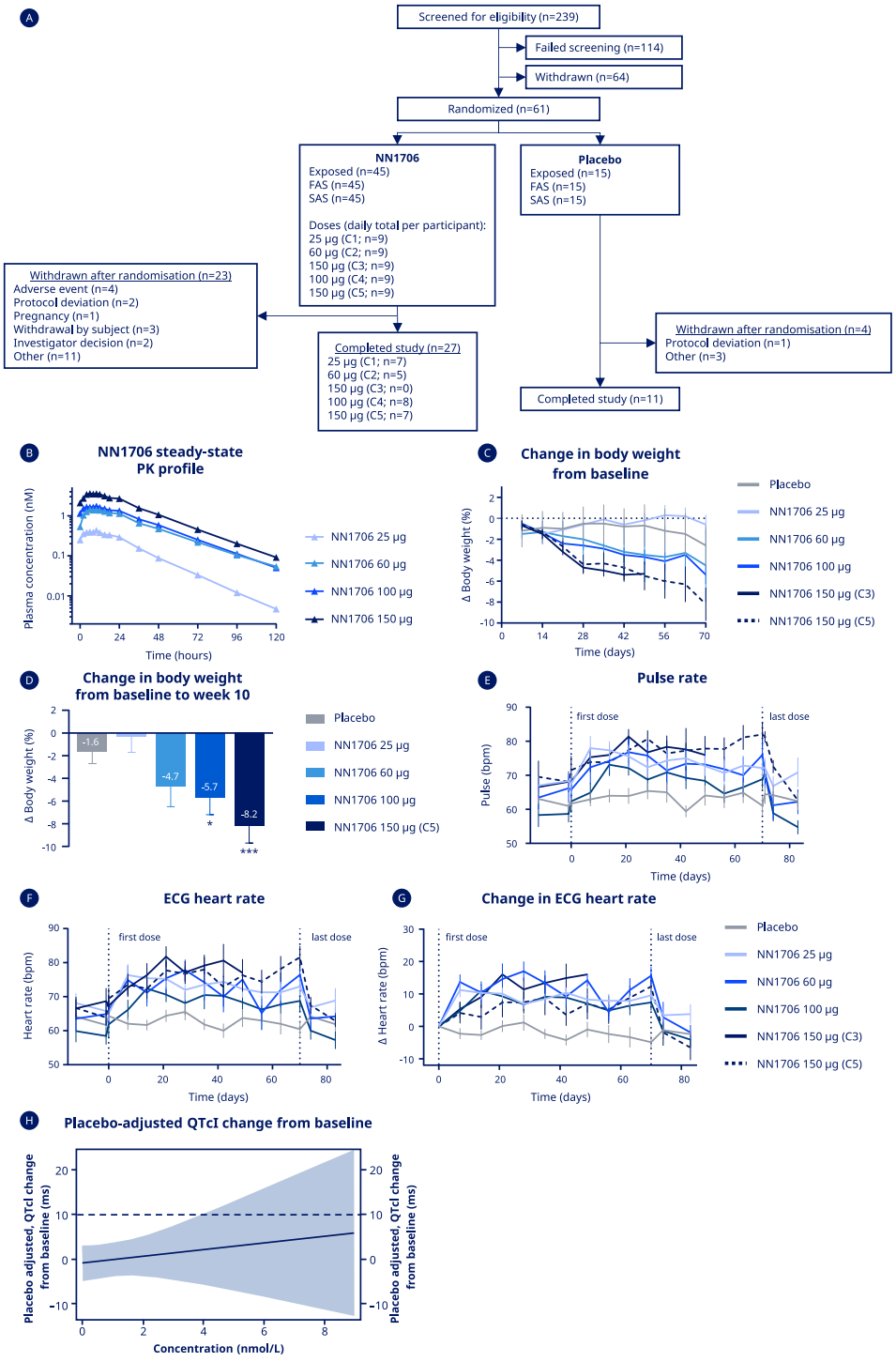


Figure 5: NN1706 in a phase 1 multiple ascending dose clinical study. **A.** Participant disposition. Three cases of second degree atrioventricular (AV) block were reported in three participants in the 150-µg cohort (C3); dosing was stopped in the 150-µg cohort (C3), amended to 100 µg in the 300-µg cohort (C4) and repeated with 150 µg in the 600-µg cohort (C5). **B.** NN1706 plasma concentration at steady state. **C-D.** Relative change in body weight after repeated NN1706 dosing. Change from baseline over 70 days (C) and relative body weight change from baseline after 10 weeks (D; ***$p < 0.001$, *$p < 0.05$).
E-H. Cardiovascular outcomes after repeated NN1706 dosing. Mean pulse rate (E), mean heart rate (F) as well as change in heart rate from baseline (G) by 12-lead ECG over the study as well as placebo adjusted QTcI change from baseline (H). B: Values represent geometric mean. C–G: Values represent mean ± SEM. E–G: Vertical dotted lines in graphs represent first and last dosing of NN1706. H: Solid line with shaded area denotes model-predicted mean placebo-adjusted delta QTcI with 90% CI. Reference line at 10 msec represents unacceptable prolongation of QTcI interval [57]. *ECG, electrocardiogram; FAS, full analysis set; QT, specific ECG interval corresponding to ventricular depolarization/repolarization; QTcI, individual corrected QT interval; SAS, safety analysis set.*

Table 2 — Treatment emergent adverse events (TEAEs) in the multiple ascending dose (MAD) study.

Treatment emergent adverse events, n (%) [E]	Placebo n = 15	NN1706				
		25 μg n = 9	60 μg n = 9	150 μg[a] n = 9	100 μg[a] n = 9	150 μg[a] n = 9
Events	12 (80.0) [52]	7 (77.8) [30]	9 (100.0) [46]	9 (100.0) [72]	8 (88.9) [59]	9 (100.0) [104]
Serious	0	0	0	0	0	0
Leading to withdrawal	0	0	3 (33.3) [3]	1 (11.1) [1]	0	0
Severity						
Severe	0	0	0	0	0	0
Moderate	4 (26.7) [6]	2 (22.2) [3]	2 (22.2) [2]	2 (22.2) [2]	4 (44.4) [6]	6 (66.7) [7]
Mild	12 (80.0) [46]	7 (77.8) [27]	9 (100.0) [44]	9 (100.0) [70]	8 (88.9) [53]	9 (100.0) [97]
Related to study product[b]						
Probable	9 (60.0) [22]	6 (66.7) [19]	8 (88.9) [31]	8 (88.9) [64]	7 (77.8) [41]	9 (100.0) [92]
Possible	8 (53.3) [16]	3 (33.3) [8]	4 (44.4) [7]	3 (33.3) [5]	5 (55.6) [13]	5 (55.6) [6]
Unlikely	7 (46.7) [14]	3 (33.3) [3]	2 (22.2) [8]	3 (33.3) [3]	4 (44.4) [5]	5 (55.6) [6]
Death	0	0	0	0	0	0
General disorders and administration site conditions[c]	6 (40.0) [10]	3 (33.3) [9]	4 (44.4) [14]	7 (77.8) [37]	6 (66.7) [22]	7 (77.8) [38]
Injection site reaction	5 (33.3) [9]	3 (33.3) [9]	1 (11.1) [9]	3 (33.3) [32]	2 (22.2) [17]	7 (77.8) [35]
Early satiety	1 (6.7) [1]	0	2 (22.2) [2]	4 (44.4) [4]	3 (33.3) [3]	2 (22.2) [2]
Gastrointestinal disorders	5 (33.3) [9]	1 (11.1) [3]	4 (44.4) [12]	7 (77.8) [14]	7 (77.8) [15]	9 (100) [26]
Nausea	2 (13.3) [3]	0	4 (44.4) [5]	3 (33.3) [3]	1 (11.1) [1]	4 (44.4) [4]
Dyspepsia	1 (6.7) [1]	0	0	5 (55.6) [5]	6 (66.7) [6]	1 (11.1) [1]
Diarrhea	0	1 (11.1) [1]	1 (11.1) [1]	1 (11.1) [1]	3 (33.3) [4]	2 (22.2) [4]
Abdominal pain	1 (6.7) [2]	1 (11.1) [2]	2 (22.2) [2]	1 (11.1) [1]	0	2 (22.2) [2]
Constipation	1 (6.7) [1]	0	0	1 (11.1) [1]	1 (11.1) [1]	4 (44.4) [4]
Vomiting	1 (6.7) [1]	0	2 (22.2) [3]	1 (11.1) [1]	0	3 (33.3) [8]
Cardiac disorders	6 (40.0) [10]	4 (44.4) [6]	5 (55.6) [7]	3 (33.3) [7]	4 (44.4) [5]	7 (77.8) [20]
Sinus tachycardia	6 (40.0) [9]	4 (44.4) [6]	5 (55.6) [6]	3 (33.3) [4]	4 (44.4) [5]	7 (77.8) [19]
Metabolism and nutrition disorders	3 (20.0) [3]	2 (22.2) [2]	3 (33.3) [3]	8 (88.9) [8]	6 (66.7) [6]	4 (44.4) [4]
Decreased appetite	2 (13.3) [2]	1 (11.1) [1]	3 (33.3) [3]	8 (88.9) [8]	6 (66.7) [6]	4 (44.4) [4]
Nervous system disorders	3 (20.0) [6]	2 (22.2) [3]	3 (33.3) [3]	2 (22.2) [2]	3 (33.3) [6]	6 (66.7) [9]
Headache	3 (20.0) [6]	2 (22.2) [3]	2 (22.2) [2]	1 (11.1) [1]	3 (33.3) [3]	5 (55.6) [6]
Investigations	3 (20.0) [5]	3 (33.3) [3]	2 (22.2) [2]	1 (11.1) [1]	0	1 (11.1) [1]
Infections and infestations	1 (6.7) [1]	1 (11.1) [2]	1 (11.1) [1]	2 (22.2) [2]	0	2 (22.2) [2]

Data are expressed as n (%) [E], where n is the number of participants reporting an event, % is the percentage of participants reporting an event and E is the number of events.
TEAE, treatment emergent adverse event.

[a] Three cases of second degree atrioventricular (AV) block were reported in three participants in the 150-μg cohort; dosing was stopped in the 150-μg cohort, amended to 100 μg in the 300-μg cohort and to 150 μg in the 600-μg cohort.

[b] Relationship to study product was based on investigator assessment.

[c] Included where >10% of the total population experienced events.

At the highest dose, fasting levels of beta-hydroxybutyrate progressively increased throughout treatment, but subsequently returned to baseline during the 2-week follow up period. Fasting total cholesterol (Supplementary Information Fig. 5B) and triglycerides (Supplementary Information Fig. 5C) were decreased from baseline during the initial phase of treatment at doses ≥60 μg, then rebounding during the later phase of the treatment regimen. The decrease in circulating lipids occurred in parallel with body weight loss but stabilized or trended upwards while weight loss continued. A decrease in fasting leptin from baseline to end of treatment was observed for the 60-, 100- and 150-μg doses (Supplementary Information Fig. 5D), and an increase in fasting soluble leptin receptor was observed for the 100- and 150-μg cohorts (Supplementary Information Fig. 5E). Mean fibroblast growth factor 21 (FGF-21) levels fluctuated with no specific pattern, with an increase observed in the 150-μg dose cohort, but a decrease observed in all other active treatment cohorts (Supplementary Information Fig. 5F). No other statistically significant changes in biomarkers were observed.

3. DISCUSSION

GLP-1R agonism is a highly effective treatment of T2D and obesity, including additional benefits beyond glycemic control and chronic weight management, such as reduced cardiovascular complications [31—33]. The concept of GLP-1R co-agonism with other incretin hormone receptors progressed to early clinical testing with a once-daily GLP-1R/GIPR co-agonist [28], which set the foundation for the ultimate discovery, development and approval for the once-weekly GLP-1R/GIPR co-agonist tirzepatide for the treatment of T2D and obesity [34,35]. The addition of GcgR activity to GLP-1R and GIPR agonism offers the potential for greater weight loss by additional mechanisms, including increased energy expenditure [22]. It also offers additional potential for obesity related comorbidities due to direct effects in the liver and kidneys [36,37] Additionally, GcgR agonism synergizes with the effects of incretin agonists on appetite [38], while dual incretin receptor agonism mitigates glycemic liability arising from chronic GcgR agonism [39]. Emerging phase 2 clinical data with the once-weekly GLP-1R/GIPR/GcgR tri-agonist retatrutide demonstrate substantial efficacy in individuals with obesity [26] and T2D [27], confirming preclinical observations. However, cardiovascular adverse effects remain evident with continued dosing, even though lessened by dose escalation and sustained treatment. It is unknown if the degree of these effects is unique to retatrutide, as there was no active comparator, and increases in heart rate have been reported for GLP-1-based molecules [40]. It is quite conceivable that PK, relative biodistribution and balance in receptor activities might present differing cardiovascular effects within the class of tri-agonists.

We report here that NN1706 is a highly potent tri-agonist targeting GLP-1R/GIPR/GcgR with balanced activity at GIPR and GLP-1R and modestly reduced relative potency at GcgR. Significant body weight

reductions were achieved with NN1706 in obese mice, rats, and non-human primates along with improvements in glycemic control, substantiating preclinical results with multiple other tri-agonists [22–24,41]. Substantial dose-dependent weight loss was also observed in human participants with overweight or obesity. In the 10-week MAD study, the weight loss was comparable to that of the most advanced treatments for obesity, in which dosage has been optimized [8,26]. However, in non-clinical studies in rats and non-human primates, along with human studies, we observed significant increases in heart rate with different dynamics across species. These findings question the risk-benefit profile of NN1706 for chronic weight management and may foretell the challenges of other drug candidates that include GcgR agonism.

GcgR agonism increases energy expenditure via discrete and integrated catabolic actions that include nutrient partitioning, thermogenesis [42], hepatic substrate utilization, adipocyte lipolysis, and glucose disposal [43]. When coupled with incretin receptor agonism, these multiple modes of action can drive a substantial negative energy balance and substrate cycling to elicit beneficial effects on body weight and glycemic control as demonstrated with single molecule GLP-1R/GIPR/GcgR tri-agonists in preclinical studies [22,24,41]. Importantly, mechanisms that simultaneously decrease energy intake coupled with increases in energy expenditure are expected to result in not just more body weight loss but also sustainable body weight loss with better subsequent maintenance due to improved regulation of metabolic adaptation.

Balancing receptor potency to an optimal ratio across the three receptors in a unimolecular tri-agonist creates many translational challenges including medicinal chemistry, *in vitro* characterization, preclinical *in vivo* pharmacology and safety evaluations. Although the addition of GcgR agonism enhances weight reduction, relative potency at the incretin receptors must be sufficiently high to mitigate the glycemic effect of GcgR agonism [44]. Therefore, most GLP-1R/GcgR co-agonists have purposefully and substantially reduced GcgR potency relative to GLP-1R potency [45], which can explain the relatively modest differential clinical efficacy to GLP-1R mono-agonists. High agonist potency at GLP-1R and GIPR offers glycemic benefits that permit higher relative GcgR potency to achieve greater weight loss when compared to dual GLP-1R/GIPR agonism alone [22]. These findings were corroborated in mice with multiple GLP-1R/GIPR/GcgR tri-agonists, including SAR441255, retatrutide, and next-generation tri-agonists [23,24,41]. However, an ideal potency ratio in humans remains to be elucidated.

Results with GLP-1R/GcgR co-agonists in phase 2 studies vary. JNJ-64565111 showed significant weight reduction but no improvement in glycemic parameters, while cotadutide demonstrated significant reductions in both body weight and HbA_{1c} vs placebo in individuals with T2D and overweight or obesity [14,16,19]. NN1177, a fatty diacid-acylated co-agonist with reduced relative GcgR potency, achieved clinically meaningful weight loss (up to 12.6% after 12 weeks), but several treatment-related safety liabilities were observed, notably a persistently increased heart rate [13]. Other fatty diacid-acylated co-agonists, mazdutide and survodutide, which each have reduced GcgR potency relative to GLP-1R potency, decreased body weight albeit with increased heart rate above baseline in clinical studies, but both the magnitude and transient nature of the effect differ from those of NN1177 [15,17]. Collectively, a commonality through these results with GLP-1R/GcgR co-agonists exemplify the uncertainties identifying and investigating the ideal fixed receptor ratio in a single-molecule co-agonist. These challenges are not exclusive to GLP-1R/GcgR co-agonists, as the ideal ratio and receptor signaling bias in GLP-1R/GIPR co-agonists also requires substantiating. The GLP-1R/GIPR co-agonist NN0090-2746 (RG7697, MAR709), which is equipotent at GLP-1R and GIPR according to cAMP potency assays, significantly reduced body weight and improved glycemic control in individuals with T2D and overweight or obesity [46,47]. However, it cannot be concluded that NN0090-2746 is different from GLP-1R mono-agonism despite post-hoc analyses suggesting it relative to an open-label liraglutide comparator [46]. The differential effects of agonism at each of the three constituent receptors also implies that the optimal relative potency at each receptor may differ depending on the desired endpoint (e.g., weight loss, glycemic control, heart rate) and therapeutic indication [45].

The rodent pharmacology studies presented here show that NN1706 causes greater body weight loss than its precursor tri-agonist IUB447 [22], as well as the once-daily tri-agonist SAR441255 [24]. The weight loss induced by NN1706 cannot be solely explained by food intake mechanisms, which is inferred from preclinical studies where greater weight loss was achieved with NN1706 treatment in DIO mice relative to pair-fed controls. Indirect calorimetry demonstrates that energy expenditure is enhanced in DIO mice administered NN1706 compared with pair-fed controls, even without factoring in the smaller body weight of the NN1706-treated mice [48]. It is noteworthy that energy expenditure was maintained in the context of substantial body weight loss with chronic NN1706 treatment relative to vehicle controls. The energy expenditure is driven predominantly by the GcgR activity in NN1706 as inferred by comparative studies of NN1706 to GLP-1R mono-agonists and GLP-1R/GIPR co-agonists, and supported by other preclinical studies with GcgR agonist-containing mixed agonists in which the GcgR component is attenuated or neutralized [10,24,41]. The NN1706 PK profile in humans supports once-daily dosing, consistent with the fatty monoacid based protraction comparable to liraglutide, cotadutide and SAR441255 [24,49,50]. The half-life of liraglutide in humans is 13 h [49] whereas that of NN1706 ranged from 17 to 25 h in the MAD study, which promotes greater drug exposure profile and potential for greater accumulation with the once-daily dosing regimen. Repeated daily treatment with NN1706, notably at 10-fold lower doses than liraglutide, resulted in profound weight loss in participants with overweight or obesity without T2D, with up to 8.2% body weight loss from baseline at 10 weeks at the highest dose tested. The magnitude of the weight loss over 10 weeks is competitive to that observed with semaglutide [6], tirzepatide [8], and the once-weekly amylin analog cagrilintide in combination with semaglutide [51] in comparable participants employing optimized dose titration. The results with NN1706 are even more striking since the other molecules employ fatty diacid acylation which has been shown to enhance efficacy [52]. The magnitude of weight loss exemplifies the multi-mechanism to reduce food intake and increase energy expenditure. It appears that tri-agonism therapy provides maximal efficacy, which has been further validated by the emerging results with retatrutide [26]. Importantly, there was no apparent deterioration of glycemic control with NN1706, much like retatrutide [26]. Signs of improved glycemic parameters were observed throughout the studies, although there was no dose-responsive effect. The observations with NN1706 on glycemic control are in contrast to what was observed with the GLP-1R/GcgR co-agonist NN1177, where impaired glycemic control was evident at high exposure and with chronic treatment [13]. This indirectly corroborates that addition of GIPR agonism to GcgR agonists provides greater glycemic buffering than GLP-1R agonism alone.

The dose-limiting adverse effects of NN1706 are predominantly cardiovascular. In the SAD and MAD studies, there were no unusual changes in systolic or diastolic blood pressure, but both studies revealed an increase in heart rate. It was dose-responsive at 50 and 150 μg in the SAD study, and in all NN1706 treatment cohorts without

apparent dose-dependency in the MAD study. There was no evidence of tachyphylaxis at any point in the 10-week MAD study, which is incongruent to what was observed after chronic dosing in monkeys, where the increased heart rate dissipated over time to levels similar to vehicle controls at study end. It is important to note that heart rate did return to baseline upon treatment cessation in the MAD study. It is to be determined whether persistent increase in heart rate might dissipate over time with continued treatment, more intermittent exposure, or with different dose levels. Increases in heart rate are well-characterized effects of GLP-1R agonism [53,54]. The observed increase in heart rate for NN1706 exceeds that expected for GLP-1R mono-agonism, however conclusions cannot be made on the differential magnitude of the effect with NN1706 due to the nature of the short-term study. A meta-analysis comparing the therapeutic index, particularly heart rate in relation to weight loss, for liraglutide, MAR709, NN1706, semaglutide, tirzepatide and retatrutide would be informative.

Supraphysiological levels of glucagon are also known to have a chronotropic effect [55,56], and Bossart et al. noted a trend toward increased heart rate in the 150-μg cohort of SAR441255 over the whole observation period [24]. Reports on retatrutide also observed increases in heart rate (up to 32 and 13 bpm from baseline in the SAD and MAD trials, respectively) [23,25]. In the phase 2 study of retatrutide in participants with obesity, dose-dependent increases in heart rate were observed, with a peak of 10 bpm at the study midpoint that declined thereafter to 6 bpm with the highest dose (12 mg) at 48 weeks [26]. This suggests that with optimized dose titration, maximal increases in heart rate lessened and that chronic treatment results in a partial desensitization or tachyphylaxis of the effect. In the same study, arrhythmias were reported in a small percentage of participants who were randomized to the faster dose escalation schedule (4 and 8 mg doses) or received the highest dose (12 mg). This effect was not apparent in participants employing a slower dose escalation schedule (4 and 8 mg doses) [26]. It remains to be determined how the molecular pharmacology and drug exposure of GLP-1R/GIPR/GcgR triagonists impact hemodynamic effects. The observations with NN1706 are noteworthy and suspected to be a function of its high potency and overall PK profile relative to retatrutide. The cardiovascular safety with NN1706 is the key area for further investigation.

3.1. Limitations of this study

The lack of long-term studies conducted in humans with long-acting GIPR or GcgR mono-agonists, alone or in combination with GLP-1R mono-agonists, makes the relative contributions of individual receptor agonism difficult to ascertain. The first-in-human SAD and MAD studies reported here had typical phase 1 study limitations, such as small sample size and short duration which limit the strength and generalizability of the results. Obesity being a chronic disease requires a steady, sustained approach for intervention, which is not captured in a 10-week study. This is exemplified when studying drug candidates with GcgR agonism, such as NN1706, where longer studies are needed to observe their complete action profile.

4. CONCLUSION

NN1706 was designed to possess balanced pharmacological activity at GIPR/GLP-1R with reduced but potent activity at GcgR. Substantial body weight reductions were achieved at low doses with NN1706 in obese mice, rats, and monkeys along with improved glycemic control. Dose titration in participants with overweight or obesity who received repeated daily doses of NN1706 in the MAD study demonstrated improved tolerability relative to the observations in the SAD study. Notably, body weight reduction in humans was substantial and achieved with dose-levels an order of magnitude lower than chemically equivalent selective GLP-1R agonists or GLP-1R/GIPR co-agonists. The efficacy in this relatively short 10-week study rivals that achieved with molecules such as semaglutide, tirzepatide, retatrutide and cagrilintide that are designed for once-weekly dosing. Cardiovascular safety emerged as a primary concern associated with NN1706. Heart rate increased across all NN1706 treatment cohorts without dose-dependency or tachyphylaxis. Collectively, these results point to the unique and powerful ability of GcgR agonism to further supplement weight loss induced by GLP-1R/GIPR co-agonism but also the potential for adverse cardiovascular effects. There is need for continued iteration in setting initial dose levels and adjustment with time. Whether more sustained-action tri-agonists, such as retatrutide, will ultimately prove safer and efficacious is to be determined in phase 3 clinical studies. It may yet prove that the addition of GcgR agonism might require closer clinical monitoring relative to other medications for obesity. If GcgR agonism proves to be indispensable in maximizing weight loss and improving comorbidities, then determining how best to use it constitutes a justifiable objective in moving forward.

5. METHODS

5.1. Functional *in vitro* assay

Cyclic AMP (cAMP) production was assessed using a stably transfected baby hamster kidney (BHK) cell line expressing one of the following human receptors: GLP-1R, GIPR or GcgR, and a firefly luciferase reporter gene linked to the cAMP response element (CRE). Conditions have been reported previously [22]. Cells were kept in continuous culture at 37°C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS) and receptor-specific selective antibiotics, were plated in a 96-well poly-D-lysine-coated plate at 5,000 cells per well in growth media and incubated overnight at 37°C with 5% CO_2. Following day, media was removed, and the plate was washed once in Dulbecco's phosphate-buffered saline (DPBS) before 50 μL of assay buffer (DMEM without phenol red, 10 mM HEPES, 1x Glutamax, 1% ovalbumin, 0.1% Pluronic F-68) was added per well. Compounds to be tested were serially diluted 3.5-fold to create a 12-point dilution curve in assay buffer. Aliquots of the dilution curves were added to the cell plate in a volume of 50 μL per well resulting in final assay concentrations ranging from 1×10^{-14} to 1×10^{-7} M. The assay plate was incubated for 3 h at 37°C and 5% CO_2. After the incubation, the assay plate was washed with DPBS. A 100 μL aliquot of DPBS was added to each well followed by 100 μL of steadylite plus™ reagent (PerkinElmer). The assay plate was covered to protect reagent from light, shaken at 250 rpm at room temperature for 30 min, and read in a luminometer. Potency (EC50) values were calculated using Prism software (GraphPad) with the nonlinear regression log (agonist) vs response. A minimum of three replicates were measured for each sample.

5.2. *In vivo* studies in obese mice

Studies were approved by and performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Cincinnati and in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male 6–8-week-old C57BL/6J mice (stock #00664, The Jackson Laboratory, ME, USA) were provided ad libitum access to water and a 58% fat, high-sugar diet (D12331, Research Diets, NJ, USA) for 9 months to promote diet-induced obesity (DIO). Mice were housed 4 per

cage, exposed to a controlled 12-h light–dark cycle at room temperature (22°C). Mice were approximately 11 months old when experiments were conducted.

To study body weight, food intake and glucose tolerance, mice (n = 8/group) were treated for 28 days with once-daily s.c. NN1706, IUB447 or SAR441255 at 0.5, 1.0 or 2.0 nmol/kg. Test compounds were dissolved in a vehicle (pH 7.4) containing 0.05% polysorbate-80, 50 mM sodium phosphate and 70 mM sodium chloride. Body weight and food intake were measured immediately prior to dosing each day, which occurred early in light cycle (1–4 h dosing window). Intraperitoneal (i.p.) glucose tolerance tests (GTT) were performed on day 0 at estimated time to maximal concentration (t_{max}; acute) and on day 27 at estimated time to minimal concentration (t_{min}; chronic). The animals were fasted for 6 h prior to the test but had access to water. Fasting blood glucose levels were measured before injecting the mice with 2 g/kg glucose (200 mg/mL aqueous solution). Tail blood glucose levels were measured 15, 30, 60, 90 and 120 min following the glucose load. A handheld glucometer (Freestyle, Abbot) was used for all glucose measurements.

For assessments of energy expenditure, mice were treated for 10 days with daily s.c. NN1706 or vehicle or were pair-fed to match the food intake of the NN1706 group for 14 days. Body weight and food intake were measured throughout the study, which occurred early in light cycle (1–4 h dosing window). On day 8–10, energy expenditure was assessed using an indirect calorimetry system (TSE Systems, Chesterfield, MO, USA). O_2 consumption and CO_2 production were measured every 20 min to determine energy expenditure using the Weir equation (EE=(3.94 x vO_2) + (1.1 x vCO2)).

5.3. PK evaluation of NN1706 in lean Wistar rats

The NN1706 PK profile was studied in male Wistar rats at the F. Hoffmann-La Roche, Pharma Research Basel testing facility, fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Study permission number is 244. The animals (n = 3/group) received either 0.00616, 0.02 or 0.10 mg/kg NN1706 s.c. or 0.02 mg/kg NN1706 i.v. dissolved in 20 mM sodium phosphate and 240 mM trehalose at pH 7.5. Plasma samples were collected at 0.25, 0.5, 1, 2, 4, 7 and 24 h post dose in all groups and additionally at 0.0833 h post dose in the i.v. group. NN1706 concentration in plasma was measured using liquid chromatography tandem-mass spectrometry (LC-MS/MS), and PK parameters were determined by non-compartmental analysis using ToxKin™.

5.4. *In vivo* studies in obese Sprague Dawley rats

These studies took place at the F. Hoffmann-La Roche, Pharma Research Basel testing facility under animal permission 2138. Nine-week old male Sprague Dawley (SD) rats received a high-fat, high-sucrose diet (Ssniff, Germany) ad libitum for 20 weeks to generate DIO rats. The animals were pair-housed in a controlled room on a 12-hour light–dark cycle with free access to food and water. The rats were single-caged at 27 weeks of age, and baseline values for body weight and food intake were recorded over 3 consecutive days. Baseline post-prandial glucose (4-hour fast) was measured a day prior to the start of treatment by tail-tip puncture using a hand-held glucose meter (AlphaTrak®, Abbott Laboratories, USA). The animals were distributed into 5 homogenous study groups (n = 7–8/group) based on body weight, food intake and post-prandial blood glucose. At 29 weeks of age, the rats were treated for 14 days with either vehicle, NN1706 (5 nmol/kg/day) or liraglutide (75 nmol/kg/day) by daily s.c. injections within an hour prior to the onset of the dark cycle. Due to an initial large decrease in body weight, treatment was administered every other day from day 10–14. Body weight and food intake were measured daily throughout the study and 4-hour post-prandial glucose was measured at day 8 by tail-tip puncture using a hand-held glucose meter (AlphaTrak®, Abbott Laboratories, USA). Blood was collected in EDTA-coated tubes containing aprotinin at the end of the study (day 15) after an overnight fast to assess fasting plasma glucose and insulin. Glucose was measured on the COBAS 6000 system (04404483 190, Roche) while insulin was measured by ELISA (10-1248-10, Mercodia).

5.5. Cardiovascular assessment in Wistar Han rats

The cardiovascular rat study was conducted at the F. Hoffmann-La Roche, Pharma Research Basel testing facility. Wistar Han rats were housed in a controlled room (temperature 21°C ± 2°C, relative humidity range of 55–65%) on a 12-hour light–dark cycle with free access to standard chow (Provimi Kliba, 3436) and water. The effects of single and repeat NN1706 administration for up to 7 days on blood pressure and heart rate were investigated in freely moving conscious telemetered rats. Sixteen male Wistar Han rats, weighing between 350 and 500 g and aged 3–12 months on the first dosing day, were included in the study. Eight rats served as the parallel control group that received s.c administration of vehicle (20 mM PBS + 240 mM trehalose, pH 7.5) while 8 rats received s.c administration of vehicle or 5, 20 or 45 μg/kg NN1706. Treatment was given daily for 7 days with a ≥7-day washout period between doses. The 45 μg/kg dose was discontinued after 2 days due to a large increase in heart rate.

Animals had been previously implanted with a telemetric transmitter system (Data Sciences International; TA11PA-C40) able to measure blood pressure, heart rate and activity. The animals were free to move about throughout the measurement period. Telemetric measurements were started at least 1 h prior to dosing and were continued for the duration of the study. Data were sampled for a 1-minute period every 4 min in each animal and compared to the parallel control group.

5.6. PK evaluation of NN1706 in cynomolgus monkeys

Two male cynomolgus monkeys at the F. Hoffmann-La Roche, Pharma Research Basel testing facility were used for the PK study. NN1706 was dosed at 0.0211 or 0.016 mg/kg s.c. (dissolved in PBS) or 0.0031 mg/kg i.v (dissolved in DPBS). The monkeys were fasted before dosing, and food was offered again 7 h after s.c. dosing and 4 h after i.v. dosing. Plasma samples were collected pre-dose and at 0.25, 0.5, 1, 2, 4, 7, 24 and 31 h post-dose, and additionally at 0.0083 h post-dose after i.v. dosing. NN1706 concentration in plasma was measured using LC-MS/MS, and PK parameters were determined by non-compartmental analysis using ToxKin™.

5.7. Glucose tolerance study in lean cynomolgus monkeys

An OGTT was conducted in adult male lean cynomolgus monkeys at the F. Hoffmann-La Roche, Pharma Research Basel testing facility. The monkeys (n = 3 animals/peptide or dose group) were fasted overnight, and each animal served as its own control and underwent a baseline OGTT with vehicle treatment prior to dosing. A single s.c. injection of vehicle or NN1706 (1.5 or 4.9 μg/kg) was administered 4 h prior to an oral gavage glucose challenge of 2 g/kg. Blood samples were taken, and blood glucose was measured at 0 (pre-dose) as well as 3, 4, 4.25, 4.5, 5, 6, 8 and 28 h post-dose. Blood was collected in K_2EDTA-coated tubes containing aprotinin, and plasma insulin was measured by ELISA according to the manufacturer's instructions (Mercodia® 10-1113-01).

5.8. Glucose tolerance study in obese cynomolgus monkeys

An ivGTT was conducted at the KBI facility, Yunnan, China. This was conducted in accordance with Safety and Quality Assurance guidelines set out in the Guideline for Experiments document of Kunming Biomed International (KBI-01-GE v2.0) with approval from F. Hoffmann-La Roche and Kunming Biomed International Institutional Animal Care and Use Committees. The test facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Male pre-diabetic, obese, HFD-fed cynomolgus monkeys were selected for the study according to homeostatic model assessment for insulin resistance (HOMA IR) and product of HOMA IR x body weight. These monkeys underwent a baseline ivGTT with vehicle treatment. Monkeys ($n = 6$) were enrolled according to glucose and insulin AUC for treatment with either 1.3 or 5 μg/kg NN1706.

Animals were fasted for 15 h prior to the ivGTT. and were sedated with ketamine (10 mg/kg; intramuscular). Baseline blood was collected and thereafter a 0.5 g/kg glucose bolus over 30 s was administered via an i.v. butterfly needle in the upper cephalic vein. Timepoint T0 began at the end of glucose administration, and serial blood samples were collected 1, 3, 5, 7, 10, 20, 40 and 60 min later. Blood was collected into K_2EDTA-coated tubes containing aprotinin. Plasma glucose concentrations were measured on a Roche C311 biochemical analyzer while plasma insulin concentrations were measured on a Roche C411 Immunoanalyzer.

5.9. Body weight, food intake and body composition assessments with multiple and ascending doses in obese cynomolgus monkeys

The multiple and ascending dose studies were conducted in adult male cynomolgus monkeys the KBI facilities in Yunnan, China. Animals were provided 3 meals per day consisting of 50 g of standard monkey formula feed, one apple, and 100 g of KBI proprietary high-fat diet feed. Forty obese pre-diabetic monkeys were selected for the studies based on the following criteria: age >10 years, body weight >10 kg, body mass index (BMI) >40 kg/m^2, fasting glucose >4.5 mM, fasting triglycerides >0.6 mM, body fat measurement by dual-energy X-ray absorptiometry (DEXA) and adaptation to training. Monkeys were individually housed in species- and size-appropriate metabolic caging for the duration of the study with *ad libitum* access to water. The study was originally designed to consist of a 4-week acclimatization period, a 1-week baseline run-in period, an 8-week dosing period with either vehicle, 5 μg/kg/day NN1706 or 20 μg/kg/day liraglutide (all dosed within 1 h during early part of the day), followed by a 2-week washout period. Upon treatment initiation, animals treated with NN1706 reduced their food intake almost completely; therefore, dosing was discontinued after 8 days of treatment (Day 15) and the animals underwent a washout period of 5.5 weeks before being enrolled in a subsequent ascending dose study. Caloric intake was recorded daily, and body weight was assessed regularly during the baseline, treatment, and washout phase.

Caloric intake reached baseline levels 7 days after stopping daily treatment; however, the animals did not recover completely from body weight loss. Obvious hypersensitive animals with regards to food intake were identified and excluded from the ascending dose study. Before beginning the study run-in period, the monkeys underwent further training for habituation to blood sampling and body weight measurement. The study tested the effects of ascending doses of NN1706 ($n = 9$) and liraglutide ($n = 8$) alongside vehicle control ($n = 9$), and included a 17-day run-in period, a 39-day dose escalation period and a 13-day washout period. Increasing doses of NN1706 were administered at the following intervals: 0.75 μg/kg/day (Days 1–7), 1.30 μg/kg/day (Days 8–14), and 2.00 μg/kg/day (Days 15–39). Liraglutide was administered at 10, 20 and 30 μg/kg/day at the same intervals. One animal was removed from the NN1706 treatment group on day 3 due to health concerns (loose feces and low food intake). Body weight was measured three times per week during the treatment phase and total caloric intake was quantified daily throughout the study. Baseline and post-treatment DEXA body composition analysis was performed using a Hologic Discovery QDR Series Densitometer (Bedford, MA) according to the manufacturer's instructions. The animals were sedated with ketamine (10 mg/kg intramuscular) and secured inside the scanning region on the DEXA bed. Scans were analyzed for total and regional body composition (fat and lean mass) using the Hologic Body Composition Software.

5.10. Cardiovascular assessment in lean cynomolgus monkeys

A cardiovascular assessment pilot study was conducted on adult male lean cynomolgus monkeys at the F. Hoffmann-La Roche, Pharma Research Basel testing facility. Animals were single housed during telemetry measurement, in a controlled room (temperature 23°C ± 1°C, relative humidity range of 40–60%) on a 12-hour light–dark cycle. They had free access to water and food pellets (Provimi Kliba, 3448), and their diets were supplemented with fresh vegetables. The effect of NN1706 on cardiovascular function was studied in freely moving conscious telemetered animals. The pilot study ($n = 4$) was conducted to characterize effects on heart rate after a single s.c. injection of NN1706 at ascending doses of 5, 30 or 100 μg/kg or vehicle control. Animals weighed between 5.6 and 10.2 kg on the first dosing day. Animals had been previously implanted with a telemetric transmitter system, with pressure transducers inserted in the descending aorta and the left ventricle of the heart. Telemetric measurements started at least 1 h prior to dosing and were continued for 21 h after dosing.

Further cardiovascular experiments on telemetered adult lean cynomolgus monkeys were conducted at the European Research Biology Center, Baugy, France. Animals were procured from Noveprim Ltd and Le Tamarinier LFee (Mauritius). Upon arrival at the study site, each animal was acclimated for at least 2 weeks. Animals were single-housed during the study, in a controlled room (temperature 20–24°C, relative humidity range of 45–65%) on a 12-h light–dark cycle, with *ad libitum* access to water. Food pellets (DIETEX Old World Monkey) were available *ad libitum* and were distributed in the morning and afternoon. Diet was supplemented with fresh vegetables, fruits, and other supplements after the approval of the attending veterinarian. Three males and 3 females weighing between 4.4 and 6.5 kg on the first dosing day were used for the study. Monkeys received single s.c. injections of vehicle or NN1706 at 3, 10 or 30 μg/kg or repeated injections of 30 μg/kg NN1706 for 14 days. Telemetry measurements started at least 2 h before each administration and continued for 24 h after dosing. Telemetry measurements were also recorded on days 7 and 14 during repeated administration of NN1706 at 30 μg/kg. On these days, food pellets were available ad libitum and animals received a supplement only before dosing. Telemetry data were collected using ART™ acquisition system (version 4.2, Data Sciences International) and analyzed using RS/1 software (version 6.3, APPLIED MATERIALS).

5.11. Phase 1 single ascending dose study of NN1706 in humans with overweight or obesity

5.11.1. Study design and participants

This was a first-in-human, placebo-controlled, double-blinded, randomized (within cohort), single ascending dose study to study safety, tolerability and PK of NN1706 in adult male participants with

overweight or obesity without T2D. The study was conducted at Novo Nordisk Investigational Site, Baltimore, Maryland, USA, between March and August 2017. The study (ClinicalTrials.gov: NCT03095807) was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practices Guideline. Local institutional review boards approved the protocol. All participants provided written informed consent before participating in the study.

Adult males (18–55 years of age) with overweight or obesity (BMI 25.0–34.9 kg/m^2) due to an excess of adipose tissue (as judged by the investigator) were eligible to participate. Exclusion criteria included: blood pressure outside the range 90–159 mmHg (systolic) or 50–99 mgHg (diastolic); resting heart rate $\geq$90 bpm; ECG abnormalities; neck circumference $\geq$50 cm; abnormal laboratory parameters; $\geq$5% estimated 10-year atherosclerotic CVD risk in participants $\geq$40 years old; history or presence of diabetes or any clinically relevant cardiovascular, respiratory, metabolic, renal, hepatic, gastrointestinal or endocrine conditions, or malignant neoplasm (in past 5 years; excluding basal or squamous cell skin cancer and in situ carcinoma); history of pancreatitis or major depressive disorder; family or personal history of multiple endocrine neoplasia type 2 (MEN 2) or medullary thyroid carcinoma. No concomitant medication was allowed during the study. Demographics and baseline characteristics are described in Supplementary Information Table 2.

In each of the cohorts receiving 5, 15, 50 or 150 μg NN1706, 6 participants were randomly assigned to receive NN1706 and 2 participants to receive placebo by using randomization numbers provided by Novo Nordisk A/S. Sentinel dosing was applied, and the first 2 participants in each cohort were randomized 1:1 to receive either active treatment or placebo, followed by a safety observation period of $\geq$24 h. If no safety concerns were raised, the remaining 6 participants in the cohort were dosed with active treatment or placebo (5:1). Based on safety data from these cohorts, the study safety group revised dosing in the cohort planned to receive 500 μg NN1706 to 300 μg. However, the stopping rule of unacceptable adverse events was met with the 300-μg dose, and it was decided to cease dosing after only one participant had received 300 μg NN1706 and one participant had received placebo in this cohort. Following the revised plan, 157 participants were screened and 34 were randomized to one of 5 dose cohorts receiving 5, 15, 50, 150 or 300 μg NN1706 or placebo. Participants remained at the clinic from screening until at least 4 days post-dosing. Participant disposition can be found in Figure 4A.

5.11.2. Study medication and endpoints

NN1706 or placebo were administered by a s.c. abdominal injection at t = 0 after a $\geq$8-hour fast at approximately 8:00 AM (−60/+120 min). The primary endpoint was the number of treatment emergent adverse events (TEAE) recorded from the time of dosing (day 1) and until completion of the post-treatment follow-up visit (days 10–13) in the safety analysis set, which consists of all subjects exposed to at least one dose. A TEAE was defined as an event that either had onset after administration of the study product and no later than the follow-up visit; or was present before the study product administration and increased in severity during the treatment period and no later than the follow-up visit.

Secondary safety endpoints included change from baseline (pre-dose on day 1) to the follow-up visit (days 10–13) in clinical laboratory parameters, 12-lead ECG, physical examinations, vital signs, injection site reactions and episodes of hypoglycemia, as well as change from baseline to all post-treatment assessments up to 48 h post-dose in QT interval corrected using Fridericia's formula (QTcF) in the safety analysis set.

Secondary PK endpoints derived from the concentration–time curves of samples collected from baseline to day 7 were assessed, including the area under the NN1706 plasma concentration–time curve from time 0 to infinity after administration of a single s.c. dose ($AUC_{0-\infty,SD}$), area under the NN1706 plasma concentration–time curve from time 0–24 h after administration of a single s.c. dose ($AUC_{0-24h,SD}$), area under the NN1706 plasma concentration–time curve from time 0 to the time point for the last quantifiable sample after administration of a single s.c. dose ($AUC_{0-tz,SD}$), maximum plasma concentration of NN1706 after administration of a single s.c. dose ($C_{max,SD}$), time to maximum plasma concentration of NN1706 after administration of a single s.c. dose ($t_{max,SD}$), terminal half-life of NN1706 after administration of a single s.c. dose ($t_{½,SD}$), mean residence time after administration of a single s.c. dose (MRT_{SD}), apparent total plasma clearance of NN1706 after administration of a single s.c. dos (CL/F_{SD}), and apparent volume of distribution of NN1706 (V_z/F_{SD}) after administration of a single s.c. dose, all in the full analysis set, which consists of all subjects who received at least one dose.

Secondary PD endpoints, including change from baseline to follow-up visit in body weight, plasma glucose, and levels of fasting insulin and C-peptide and glucagon, were assessed as secondary endpoints, all in the full analysis set.

5.11.3. Safety evaluation

Cardiovascular abnormalities were monitored by continuous ECG monitoring (telemetry), 12-lead ECG and Holter monitoring. The following conditions were used to inform decisions on ascending the dose in the next cohort (measured after $\geq$10 min rest in supine position): increase in QTcF >60 ms compared to baseline (pre-dose on day 1), absolute heart rate >115 bpm, or sustained absolute heart rate >100 bpm over a period of $\geq$24 h during the in-house stay.

5.11.4. Blood samples and analyses

Blood samples for PK assessment were taken on day 1 within 30 min prior to dosing, then every hour ± 10 min from 1 to 10 h(s) post-dose, 12 and 15 h ± 10 min post-dose, on day 2 every 6 h ± 10 min from 18 to 40 h post-dose, on day 3 at 48 h ± 10 min post-dose, then every 6 h ± 30 min from 54 to 64 h post-dose, day 4 at 72 h ± 30 min post-dose, day 5 at 96 h ± 30 min post-dose (end of in-house stay), and day 7 at 144 h ± 2 h post-dose.

Blood samples for plasma glucose were taken on day 1 from 120 to 30 min pre-dosing, then 1 h post-dose and every 2 h from 2 to 12 h post-dose, 15 h post-dose (all 0–15 min after PK sampling), then at 24, 48, 72 and 96 h ± 60 min post-dose, 114 ± 2 h post-dose, and at the follow-up visit 216–288 h post-dose. All blood samples, other than those post-dose on day 1, were taken in the fasted state. Blood samples for fasting insulin and C-peptide analysis were taken on day 1 at 120–30 min pre-dosing, and then at 24, 48, 72, 96 h ± 60 min post-dose. Blood sample for glucagon was taken on day 1 at 120–30 min pre-dosing.

The samples were collected and handled according to the laboratory manual. Blood samples of up to 500 mL were drawn into K_3EDTA treated sample tubes and plasma separated. Half the plasma was stored as a back-up sample and the other half sent to a special laboratory for analysis. NN1706 concentration in plasma was determined using a validated NN1706-specific LC-MS/MS assay.

5.12. Phase 1 multiple ascending dose study of NN1706 in humans with overweight or obesity

5.12.1. Study design and participants

A phase 1 single site, investigator and participant blinded, randomized, placebo-controlled, multiple dose study was conducted to evaluate the safety, tolerability and PK of NN1706 in adult males and females with overweight or obesity without T2D. The study was conducted at Novo Nordisk Investigational Site, Overland Park, Kansas, USA, between September 2018 and October 2019. The clinical study (ClinicalTrials.gov: NCT03661879) was conducted in accordance with the Declaration of Helsinki, and the International Conference on Harmonization Good Clinical Practices Guideline. Local institutional review boards approved the protocol. All participants provided written informed consent before participating in the study.

Adults (18–55 years of age) with body mass index (BMI) 25.0–39.9 kg/m^2 (with overweight or obesity due to an excess of adipose tissue, as judged by the investigator) were eligible to participate in the study. Exclusion criteria included blood pressure outside the range 90–160 mmHg (systolic) or 50–100 mmHg (diastolic); resting heart rate ≥90 bpm; ECG abnormalities; neck circumference ≥50 cm; abnormal laboratory parameters; ≥5% estimated 10-year atherosclerotic CVD risk in participants ≥40 years old; history or presence of diabetes or any clinically relevant cardiovascular, respiratory, metabolic, renal, hepatic, gastrointestinal or endocrine conditions, or malignant neoplasm (in past 5 years; excluding basal or squamous cell skin cancer and in situ carcinoma); history of pancreatitis or major depressive disorder; family or personal history of multiple endocrine neoplasia type 2 (MEN 2) or medullary thyroid carcinoma. No concomitant medication was allowed during the study. Demographic and baseline characteristics are described in Supplementary Information Table 4.

The initial plan was to screen 275 participants and randomize 60 to one of 5 dose cohorts (n = 12/cohort). Planned doses were 25, 60, 150, 300 and 600 μg with participants in each cohort randomized 9:3 to NN1706 or placebo by using randomization numbers provided by Novo Nordisk A/S. Sentinel dosing was applied, and the first 4 participants in each cohort were randomized 3:1 to receive either active treatment or placebo, followed by a safety observation period. A sequential dose scheme was applied with the remaining participants in the cohort randomized in a 6:2 manner to NN1706 or placebo. Safety data from the 60-μg and 150-μg cohorts showed 4 events of second-degree atrioventricular (AV) block, and the study safety group decided to cease dosing in the 150-μg cohort. Furthermore, dosing was adjusted from the planned 300 μg to 100 μg and from 600 μg to 150 μg. Thus, the final cohorts were 25 μg (cohort 1 or C1), 60 μg (C2), 150 μg (C3; dosing ceased), 100 μg (C4) and 150 μg (C5). There was no dose escalation in the 25 and 60 μg cohorts while doses were escalated weekly from a starting dose of 25 μg in the 100- and 150-μg cohorts. In the revised study, 239 participants were screened, 61 randomized and 60 exposed to treatment. The 25-, 60- and 100-μg cohorts completed an in-house safety visit during the first 72 h at the planned final dose, while the 150-μg cohort completed in-house safety visits during the first 96 h at the planned final dose and the first 96 h at the dose escalation step prior to the final dose. All safety data assessed during the in-house safety visits were reviewed by the investigator prior to participants leaving the study site.

5.12.2. Study medication and endpoints

NN1706 or placebo was self-administered daily by s.c. abdominal injection, at 25, 60, 100 or 150 μg. Each subject was planned to receive 70 doses, i.e. 10 weeks of treatment. One subject receiving 150 μg NN1706 and 1 subject treated with placebo received reduced doses of study product between days 9 and 14.

The primary safety endpoint was the number of TEAEs recorded from baseline (day 1 pre-dose) until follow-up visit (day 84) in the safety analysis set. A TEAE was defined as an event that either had onset after administration of the study product and no later than the follow-up visit; or was present before the study product administration and increased in severity during the treatment period and no later than the follow-up visit.

A secondary safety endpoint assessed was change from baseline (pre-dose on day 1) to follow-up visit (day 84) in the time-profile of individual corrected QT interval (ΔQTcI) in the safety analysis set.

Secondary PK endpoints included the area under the NN1706 plasma concentration–time curve from time 0–24 h at steady state ($AUC_{0-24h,SS}$), and the maximum plasma concentration of NN1706 at steady state ($C_{max,SS}$) in the full analysis set.

Exploratory safety endpoints included change from baseline (day −1) to end of treatment (day 71) in alanine aminotransferase and aspartate aminotransferase, change from baseline (day 1) to end of treatment in heart rate, number of injection site reactions and occurrence of anti-NN1706 antibodies from baseline to follow-up visit in the safety analysis set.

Exploratory PK endpoints derived from the concentration–time curves of samples collected from baseline (day 1) to follow-up visit, included time to maximum plasma concentration of NN1706 at steady state ($t_{max,SS}$), terminal half-life of NN1706 at steady state ($t_{½,SS}$), apparent total plasma clearance of NN1706 at steady state (CL/F_{SS}), apparent volume of distribution during elimination (Vz/F_{SS}), apparent volume of distribution at steady state (Vss/F_{SS}), and mean residence time at steady state (MRT_{SS}) all in the full analysis set.

Exploratory PD endpoints included change from baseline (day 1) to end of treatment in body weight, fasting lipids and hormones (fasting leptin, soluble leptin receptor and FGF-21) and change from baseline (day −1) to end of treatment in HbA_{1c}, fasting glucose, insulin, C-peptide and glucagon and OGTT parameters in the full analysis set.

5.12.3. Safety evaluation

Safety and tolerability of NN1706 were assessed by physical examination, vital signs, continuous ECG monitoring, 12-lead ECG recording, clinical laboratory tests, anti-NN1706 antibody testing, assessment of hypersensitivity to study product, mental health assessments, injection site reaction assessment and adverse event reporting. All protocol-required laboratory assessments were conducted in accordance with the laboratory manual and the flowchart.

Continuous electrocardiograms (ECG) were obtained at screening, approximately 24 h prior to first dosing and at multiple post-dose timepoints to determine if any abnormal findings were present. Clinically significant abnormalities were recorded as adverse events.

Twelve-lead ECGs were obtained at screening, prior to dosing and at multiple post-dose timepoints, and measured heart rate, ECG interval from atrial depolarization to ventricular depolarization (PR), ECG interval corresponding to ventricular depolarization (QRS), ECG interval corresponding to ventricular depolarization/repolarization (QT) and corrected QT (QTc) intervals. Participants rested in a supine position for ≥5 min prior to screening ECG and ≥10 min prior to subsequent ECGs and remained at rest during the recording. The investigator determined whether any abnormal findings were present, and the presence of clinically significant findings on a recording made after the screening visit was recorded as an AE or SAE.

Holter monitoring was performed at 24 h prior to dosing and at multiple post-dose timepoints.

5.12.4. Blood samples and analyses

The procedures for blood sampling were carried out in accordance with the protocol and laboratory manual. Blood samples for PK assessment were taken at 8:00 AM (−60 to 0 min pre-dose) on days 1−4, 8−11, 15−18, 22−25, 29−32, 36−39, 43−46, 50, 57, 64, and 70, at 12:00, 16:00 and 22:00 ± 60 min on days 3, 10, 17, 24, 31, 38 and 45, at 10:00, 12:00, 14:00, 16:00, 18:00, 20:00 and 23:00 ± 30 min on day 70, at 02:00, 08:00, 20:00 ± 30 min on day 71, at 08:00 ± 60 min on days 72 and 73, and at 08:00 ± 120 min on days 74, 75 and 84. NN1706 concentration in plasma was determined using a validated LC-MS/MS assay.

Blood samples for serum antibodies against NN1706 were taken on days 1, 29, 57, 71 (end of treatment) and 84 (follow-up) and analyzed by Novo Nordisk. Determination of antibodies against NN1706 was performed using a validated antibody binding assay developed by Novo Nordisk to specifically determine antibody levels against NN1706. Antibody positive samples were tested for cross-reactivity to endogenous glucagon, endogenous GIP and endogenous GLP-1. Samples taken at the follow-up visit and confirmed positive and cross-reactive to endogenous glucagon, GLP-1 and/or GIP were tested for neutralizing effect towards the relevant endogenous counterpart to which the antibodies are cross-reactive (e.g. endogenous glucagon, GLP-1 and/or GIP).

Blood samples for PD laboratory assessments encompassed glucose metabolism (fasting glucose, insulin, C-peptide and glucagon as well as HbA_{1c}), hormones (fasting leptin, soluble leptin receptor and fibroblast growth factor-21 [FGF-21]) as well as lipids (fasting beta-hydroxybutyrate, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, very low density lipoprotein (VLDL) cholesterol and free fatty acids). Glucose metabolism samples were collected on day −1, 8 (no HbA_{1c} or glucagon), 15 (no HbA_{1c}), 22 (no HbA_{1c} or glucagon), 29 (no HbA_{1c}), 36, 43 (no HbA_{1c}), 43 (no HbA_{1c}), 50 (no HbA_{1c} or glucagon), 57 (no HbA_{1c}), 64 (no HbA_{1c} or glucagon) and 71 (end of treatment). Samples for hormones were only collected on day 1 and 71. Lipid samples were collected on day 1, 15, 29, 43 57 and 71.

5.12.5. Oral glucose tolerance test

The OGTT was initiated on study days 1 (baseline), 36 and 71 (end of treatment) between approximately 7:00 and 9:00 AM in participants fasted overnight or for ≥8 h. Participants were asked to drink a fluid containing a 75-g glucose load over a maximum of 5 min, and blood samples were collected via venipuncture, or IV catheter if deemed necessary, at 0, 10, 20, 30, 60, 90 and 120 min after starting the glucose intake. Samples were assessed for plasma glucose and insulin.

5.13. Statistical analysis

Details about data and statistical analysis of *in vitro* and animal experiments are included in the above method description where applicable.

5.13.1. Phase 1 single ascending dose study of NN1706 in humans with overweight or obesity

As this was an exploratory study, sample size was not based on a formal statistical assessment, however, it was considered sufficient to evaluate the primary endpoint. No formal statistical analysis was performed for the primary endpoint (TEAEs) that are summarized in Table 1.

Clinical laboratory safety parameters and vital signs were summarized by dose cohort and visit using descriptive statistics. Change from baseline in these parameters were summarized in the same way.

Participants receiving placebo treatment were pooled across cohorts for analysis of PD endpoints. Change from baseline in body weight and glucose metabolism parameters were summarized by dose cohort and day using descriptive statistics.

Regarding PK data, mean plasma concentration over time after single dose of NN1706 are presented. Each of the PK endpoints for NN1706 ($AUC_{0-\infty,SD}$, and $C_{max,SD}$) was logarithmically transformed and analyzed separately by an analysis of variance model with dose as factor, allowing for different variation on the different dose levels. The estimated least square means were back-transformed to original scale and presented together with 95% confidence intervals. Also, appropriate treatment ratios are presented with corresponding 95% confidence intervals. The dose-proportionality of PK endpoints after administration of a single dose of NN1706 was explored. This was done by estimating the slope β in the linear regression model of the logarithm of the relevant endpoint versus log (dose). Here $\beta = 1$ would mean that the PK endpoint increased in a dose-proportional manner with increasing dose. The estimated quantity 2^{β} with 95% confidence interval was reported. The model was applied to $AUC_{0-\infty,SD}$, and $C_{max,SD}$.

AUCs were calculated using the linear trapezoidal method based on observed values and actual measurement times. The actual time for the pre-dose sample (nominal time equal to 0) was substituted with 0. If the 24-hour observation was missing and there was a quantifiable observation after 24 h, linear interpolation was used to impute a value at 24 h. This value was used in the calculation of the AUC. If the last quantifiable sample, denoted t_z, occurred before 24 h, and it was determined that the terminal phase had commenced with an estimable $t_{½}$, the area under the time−concentration curve from t_z to 24 h was calculated based on the estimated $t_{½}$ and the predicted concentration at t_z. If $t_{½}$ was not estimable, the first value after the last quantifiable sample was set to lower limit of quantification (LLOQ)/2 and the following values to 0. Any values below LLOQ before t_z were set to LLOQ/2. $C_{max,SD}$ was derived as the maximum of all valid observed concentrations of NN1706, and $t_{max,SD}$ was determined as the corresponding time point.

5.13.2. Phase 1 multiple ascending dose study of NN1706 in humans with overweight or obesity

This was an exploratory study to assess safety and tolerability of NN1706. No formal statistical sample size calculation was performed, however the selected sample size was considered sufficient to evaluate the primary objective. In each of the 5 cohorts, 12 participants (9 on active and 3 on placebo) were planned, allowing for an estimated drop-out of 15−20% and 9 completers in each cohort. Participants were replaced as needed to have at least 8 with sufficient evaluable safety data to complete the cohort safety evaluation.

Analyses of PK and PD endpoints were based on the full analysis set, and no statistical adjustments were performed to factor in subject withdrawal. Analyses of safety endpoints were based on the safety analysis set and were descriptive. Participants receiving placebo treatment were pooled across cohorts.

Supportive secondary PK endpoints were logarithmically transformed and analyzed separately by an analysis of variance model with

treatment as factor and allowing residual variance to depend on dose cohort. The estimated least square means was back-transformed to original scale and presented together with 95% confidence intervals and p-values. Dose-proportionality of the $AUC_{0-24h,SS}$ endpoint at steady state was explored by estimating the slope β in the linear regression model of the logarithm of the relevant endpoint versus log (dose), $\beta = 1$ meaning that the PK endpoint increases in a dose-proportional manner with increasing dose. Descriptive analyses were used for the remaining PK endpoints.

Change in body weight was analyzed using a mixed model for repeated measurements, where all post-baseline changes in body weight measurements were entered as the dependent variable, treatment (NN1706 dose and placebo) and visit entered as factors, and baseline body weight as covariate, all as fixed effects. Treatment and baseline body weight were nested within visit, which technically corresponds to interactions with visit. Within subject, repeated measurements were incorporated with a Toeplitz covariance matrix. Least square means of the PD endpoint by treatment were presented, along with estimated treatment differences with corresponding 95% CI.

Clinical laboratory safety parameters and vital signs were summarized by dose cohort and visit using descriptive statistics. Change from baseline in these parameters was summarized in the same way.

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DECLARATION OF COMPETING INTEREST

Brian Finan: Shareholder and employee of Eli Lilly; shareholder and former employee of Novo Nordisk A/S Jonathan D. Douros: Shareholder and former employee of Novo Nordisk A/S Ronald Goldwater: Employee of Parexel International Ann Maria Kruse Hansen: Shareholder and employee of Novo Nordisk A/S Julie B. Hjerpsted: Shareholder and employee of Novo Nordisk A/S Karina Rahr Hjøllund: Shareholder and employee of Novo Nordisk A/S Martin K. Kankam: Employee at Altasciences Clinical Kansas, Inc., which has received research funding from Novo Nordisk A/S, Merck, Vertex, Camino, Ionis, EncuraGen Inc., Eliem Therapeutics, Arthrosi Therapeutic, Staidson Biopharma, NIAID/NIH, Amgen and Biogen Patrick J. Knerr: Shareholder and former employee of Novo Nordisk A/S Anish Konkar: Shareholder and employee of AstraZeneca Stephanie A. Mowery: Shareholder and former employee of Novo Nordisk A/S Timo D. Müller: receives research funding from Novo Nordisk and has received speaking fees from Eli Lilly, AstraZeneca, Novo Nordisk and Merck John Rømer Nielsen: Shareholder and employee Novo Nordisk A/S Sune Boris Nygård: Shareholder and employee Novo Nordisk A/S Diego Perez-Tilve: Received research funds from Novo Nordisk A/S Kirsten Raun: Shareholder and employee of Novo Nordisk A/S Bin Yang: Employee of Dexatide LLC; shareholder and former employee of Novo Nordisk A/S Matthias H. Tschöp: Advisory board of ERX Pharmaceuticals, Inc., Cambridge, MA (2019), Research Cluster Advisory Panel (ReCAP) of the Novo Nordisk Foundation (2017—2019), research funding from Novo Nordisk (2016—2020) and Sanofi-Aventis (2012—2019), consultations for Böhringer Ingelheim Pharma GmbH & Co. KG (2020 & 2021), scientific lectures for Sanofi-Aventis Deutschland GmbH (2020) and Astra-Zeneca GmbH (2024); As CEO and CSO of Helmholtz Munich, co-responsible for collaborations of the employees with a multitude of companies and institutions worldwide, including but not limited to Boehringer Ingelheim, Novo Nordisk A/S, Roche Diagnostics, Arbormed, Eli Lilly, SCG Cell Therapy and others, and overall responsible for commercial technology transfer activities. Richard D. DiMarchi: Shareholder and former employee of Novo Nordisk A/S; Co-inventor of intellectual property at Indiana University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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DATA AVAILABILITY

Data will be made available on request.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2025.102129.

REFERENCES

[1] Prospective Studies C, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet 2009;373(9669):1083—96.

[2] Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med 2011;365(17):1597—604.
[3] Novo Nordisk. WEGOVY (semaglutide) injection, for subcutaneous use. 2021. https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/215256s000lbl.pdf.
[4] Novo Nordisk. SAXENDA (liraglutide) injection, for subcutaneous use. 2018. https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/206321s007lbl.pdf.
[5] Pi-Sunyer X, Astrup A, Fujioka K, Greenway F, Halpern A, Krempf M, et al. A randomized, controlled trial of 3.0 mg of liraglutide in weight management. N Engl J Med 2015;373(1):11—22.
[6] Wilding JPH, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, et al. Once-weekly semaglutide in adults with overweight or obesity. N Engl J Med 2021;384(11):989.
[7] Frias JP, Davies MJ, Rosenstock J, Perez Manghi FC, Fernandez Lando L, Bergman BK, et al. Tirzepatide versus semaglutide once weekly in patients with type 2 diabetes. N Engl J Med 2021;385(6):503—15.
[8] Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, et al. Tirzepatide once weekly for the treatment of obesity. N Engl J Med 2022;387(3):205—16.
[9] Day JW, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J, et al. A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. Nat Chem Biol 2009;5(10):749—57.
[10] Simonsen L, Lau J, Kruse T, Guo T, McGuire J, Jeppesen JF, et al. Preclinical evaluation of a protracted GLP-1/glucagon receptor co-agonist: translational difficulties and pitfalls. PLoS One 2022;17(3):e0264974.
[11] Pocai A, Carrington PE, Adams JR, Wright M, Eiermann G, Zhu L, et al. Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. Diabetes 2009;58(10):2258—66.
[12] Säll C, Alifrangis L, Dahl K, Friedrichsen MH, Nygård SB, Kristensen K. *In vitro* CYP450 enzyme down-regulation by GLP-1/glucagon co-agonist does not translate to observed drug-drug interactions in the clinic. Drug Metab Dispos 2022;50(8). DMD-AR-2022-000865.
[13] Friedrichsen MH, Endahl L, Kreiner FF, Goldwater R, Kankam M, Toubro S, et al. Results from three phase 1 trials of NNC9204-1177, a glucagon/GLP-1 receptor co-agonist: effects on weight loss and safety in adults with overweight or obesity. Mol Metabol 2023:101801.
[14] Nahra R, Wang T, Gadde KM, Oscarsson J, Stumvoll M, Jermutus L, et al. Effects of cotadutide on metabolic and hepatic parameters in adults with overweight or obesity and type 2 diabetes: a 54-week randomized phase 2b study. Diabetes Care 2021;44(6):1433—42.
[15] Zhang B, Cheng Z, Chen J, Zhang X, Liu D, Jiang H, et al. Efficacy and safety of mazdutide in Chinese patients with type 2 diabetes: a randomized, double-blind, placebo-controlled phase 2 trial. Diabetes Care 2024;47(1):160—8.
[16] Ji L, Jiang H, Cheng Z, Qiu W, Liao L, Zhang Y, et al. A phase 2 randomised controlled trial of mazdutide in Chinese overweight adults or adults with obesity. Nat Commun 2023;14(1):8289.
[17] le Roux CW, Steen O, Lucas KJ, Startseva E, Unseld A, Hennige AM. Glucagon and GLP-1 receptor dual agonist survodutide for obesity: a randomised, double-blind, placebo-controlled, dose-finding phase 2 trial. Lancet Diabetes Endocrinol 2024;12(3):162—73.
[18] Alba M, Yee J, Frustaci ME, Samtani MN, Fleck P. Efficacy and safety of glucagon-like peptide-1/glucagon receptor co-agonist JNJ-64565111 in individuals with obesity without type 2 diabetes mellitus: a randomized dose-ranging study. Clin Obes 2021;11(2):e12432.
[19] Di Prospero NA, Yee J, Frustaci ME, Samtani MN, Alba M, Fleck P. Efficacy and safety of glucagon-like peptide-1/glucagon receptor co-agonist JNJ-64565111 in individuals with type 2 diabetes mellitus and obesity: a randomized dose-ranging study. Clin Obes 2021;11(2):e12433.
[20] Jungnik A, Arrubla J, Plum-Mörschel L, Kapitza C, Lamers D, Thamer C, et al. Phase I studies of the safety, tolerability, pharmacokinetics and pharmacodynamics of the dual glucagon receptor/glucagon-like peptide-1 receptor (GCGR/GLP-1R) agonist BI 456906. Diabetes Obes Metabol 2022;25(4):1011—23.
[21] Klein S, Nestor JR JJ, Harris MS, Suyundikov A, Casper R, Steele SM, et al. 334-OR: pemvidutide (ALT-801) , a balanced (1:1) GLP-1/glucagon dual receptor agonist, induces rapid and marked weight loss without the need for dose titration in people with overweight/obesity. Diabetes 2022;71(Supplement_1).
[22] Finan B, Yang B, Ottaway N, Smiley DL, Ma T, Clemmensen C, et al. A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. Nat Med 2015;21(1):27—36.
[23] Coskun T, Urva S, Roell WC, Qu H, Loghin C, Moyers JS, et al. LY3437943, a novel triple glucagon, GIP, and GLP-1 receptor agonist for glycemic control and weight loss: from discovery to clinical proof of concept. Cell Metab 2022;34(9):1234—1247 e1239.
[24] Bossart M, Wagner M, Elvert R, Evers A, Hubschle T, Kloeckener T, et al. Effects on weight loss and glycemic control with SAR441255, a potent unimolecular peptide GLP-1/GIP/GCG receptor triagonist. Cell Metab 2022;34(1):59—74 e10.
[25] Urva S, Coskun T, Loh MT, Du Y, Thomas MK, Gurbuz S, et al. LY3437943, a novel triple GIP, GLP-1, and glucagon receptor agonist in people with type 2 diabetes: a phase 1b, multicentre, double-blind, placebo-controlled, randomised, multiple-ascending dose trial. Lancet 2022;400(10366):1869—81.
[26] Jastreboff AM, Kaplan LM, Frías JP, Wu Q, Du Y, Gurbuz S, et al. Triple-hormone-receptor agonist retatrutide for obesity - a phase 2 trial. N Engl J Med 2023;389(6):514—26.
[27] Rosenstock J, Frias J, Jastreboff AM, Du Y, Lou J, Gurbuz S, et al. Retatrutide, a GIP, GLP-1 and glucagon receptor agonist, for people with type 2 diabetes: a randomised, double-blind, placebo and active-controlled, parallel-group, phase 2 trial conducted in the USA. Lancet 2023;402(10401):529—44.
[28] Finan B, Ma T, Ottaway N, Muller TD, Habegger KM, Heppner KM, et al. Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. Sci Transl Med 2013;5(209):209ra151.
[29] Henderson SJ, Konkar A, Hornigold DC, Trevaskis JL, Jackson R, Fritsch Fredin M, et al. Robust anti-obesity and metabolic effects of a dual GLP-1/glucagon receptor peptide agonist in rodents and non-human primates. Diabetes Obes Metabol 2016;18(12):1176—90.
[30] King-Ahmad AJ, Kalgutkar AS, Niosi M, Eng H, Holliman C. A sensitive method for the quantitation of the peptide-based glucagon-like peptide-1 receptor agonist liraglutide in plasma using microfluidics chromatography tandem MS. Bioanalysis 2018;10(5):357—68.
[31] Lincoff AM, Brown-Frandsen K, Colhoun HM, Deanfield J, Emerson SS, Esbjerg S, et al. Semaglutide and cardiovascular outcomes in obesity without diabetes. N Engl J Med 2023;389(24):2221—32.
[32] Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. N Engl J Med 2016;375(19):1834—44.
[33] Kosiborod MN, Abildstrøm SZ, Borlaug BA, Butler J, Rasmussen S, Davies M, et al. Semaglutide in patients with heart failure with preserved ejection fraction and obesity. N Engl J Med 2023;389:1069—84.
[34] Eli Lilly and Company. FDA approves Lilly's Mounjaro™ (tirzepatide) injection, the first and only GIP and GLP-1 receptor agonist for the treatment of adults with type 2 diabetes. 2022. https://investor.lilly.com/news-releases/news-release-details/fda-approves-lillys-mounjarotm-tirzepatide-injection-first-and.
[35] Eli Lilly and Company. FDA approves lilly's Zepbound™ (tirzepatide) for chronic weight management, a powerful new option for the treatment of obesity or overweight with weight-related medical problems. 2023. https://investor.lilly.com/news-releases/news-release-details/fda-approves-lillys-zepboundtm-tirzepatide-chronic-weight.

[36] Kim T, Nason S, Holleman C, Pepin M, Wilson L, Berryhill TF, et al. Glucagon receptor signaling regulates energy metabolism via hepatic farnesoid X receptor and fibroblast growth factor 21. Diabetes 2018;67(9):1773–82.
[37] Wang MY, Zhang Z, Zhao S, Onodera T, Sun XN, Zhu Q, et al. Downregulation of the kidney glucagon receptor, essential for renal function and systemic homeostasis, contributes to chronic kidney disease. Cell Metab 2024;36(3):575–597.e577.
[38] Hope DCD, Vincent ML, Tan TMM. Striking the balance: GLP-1/Glucagon Co-agonism as a treatment strategy for obesity. Front Endocrinol 2021;12:735019.
[39] Müller TD, Finan B, Clemmensen C, DiMarchi RD, Tschöp MH. The new Biology and pharmacology of glucagon. Physiol Rev 2017;97(2):721–66.
[40] Robinson LE, Holt TA, Rees K, Randeva HS, O'Hare JP. Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. BMJ Open 2013;3(1):e001986-e001986.
[41] Knerr PJ, Mowery SA, Douros JD, Premdjee B, Hjollund KR, He Y, et al. Next generation GLP-1/GIP/glucagon triple agonists normalize body weight in obese mice. Mol Metabol 2022:101533.
[42] Beaudry JL, Kaur KD, Varin EM, Baggio LL, Cao X, Mulvihill EE, et al. The brown adipose tissue glucagon receptor is functional but not essential for control of energy homeostasis in mice. Mol Metabol 2019;22:37–48.
[43] Kim T, Holleman CL, Nason S, Arble DM, Ottaway N, Chabenne J, et al. Hepatic glucagon receptor signaling enhances insulin-stimulated glucose disposal in rodents. Diabetes 2018;67(11):2157–66.
[44] Day JW, Gelfanov V, Smiley D, Carrington PE, Eiermann G, Chicchi G, et al. Optimization of co-agonism at GLP-1 and glucagon receptors to safely maximize weight reduction in DIO-rodents. Biopolymers 2012;98(5):443–50.
[45] Capozzi ME, DiMarchi RD, Tschöp MH, Finan B, Campbell JE. Targeting the incretin/glucagon system with triagonists to treat diabetes. Endocr Rev 2018;39(5):719–38.
[46] Frias JP, Bastyr EJ, Vignati L, Tschöp MH, Schmitt C, Owen K, et al. The sustained effects of a dual GIP/GLP-1 receptor agonist, NNC0090-2746, in patients with type 2 diabetes. Cell Metab 2017;26(2):343–352.e342.
[47] Schmitt C, Portron A, Jadidi S, Sarkar N, DiMarchi R. Pharmacodynamics, pharmacokinetics and safety of multiple ascending doses of the novel dual glucose-dependent insulinotropic polypeptide/glucagon-like peptide-1 agonist RG7697 in people with type 2 diabetes mellitus. Diabetes Obes Metabol 2017;19(10):1436–45.
[48] Tschop MH, Speakman JR, Arch JR, Auwerx J, Bruning JC, Chan L, et al. A guide to analysis of mouse energy metabolism. Nat Methods 2011;9(1):57–63.
[49] Jacobsen LV, Flint A, Olsen AK, Ingwersen SH. Liraglutide in type 2 diabetes mellitus: clinical pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 2016;55(6):657–72.
[50] Ambery PD, Klammt S, Posch MG, Petrone M, Pu W, Rondinone C, et al. MEDI0382, a GLP-1/glucagon receptor dual agonist, meets safety and tolerability endpoints in a single-dose, healthy-subject, randomized, Phase 1 study. Br J Clin Pharmacol 2018;84(10):2325–35.
[51] Enebo LB, Berthelsen KK, Kankam M, Lund MT, Rubino DM, Satylganova A, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of concomitant administration of multiple doses of cagrilintide with semaglutide 2.4 mg for weight management: a randomised, controlled, phase 1b trial. Lancet 2021;397(10286):1736–48.
[52] Knudsen LB, Lau J. The discovery and development of liraglutide and semaglutide. Front Endocrinol 2019;10:155.
[53] Heuvelman VD, Van Raalte DH, Smits MM. Cardiovascular effects of glucagon-like peptide 1 receptor agonists: from mechanistic studies in humans to clinical outcomes. Cardiovasc Res 2020;116(5):916–30.
[54] Lorenz M, Lawson F, Owens D, Raccah D, Roy-Duval C, Lehmann A, et al. Differential effects of glucagon-like peptide-1 receptor agonists on heart rate. Cardiovasc Diabetol 2017;16(1):6.
[55] Neumann J, Hofmann B, Dhein S, Gergs U. Glucagon and its receptors in the mammalian heart. Int J Mol Sci 2023;24(16).
[56] Hernández-Cascales J. Does glucagon have a positive inotropic effect in the human heart? Cardiovasc Diabetol 2018;17(1):148.
[57] International Conference on Harmonization (ICH). The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs E14. 2005.

Glossary

Ac-[D]Tyr: Nα-acetylated D-stereoisomer of tyrosine
AE: adverse event
Aib: alpha-amino isobutyric acid
ANOVA: analysis of variance
AUC: area under curve
AV: atrioventricular
BHK: baby hamster kidney
BMC: bone mineral content
BMI: body mass index
bpm: beats per minute
cAMP: cyclic adenosine monophosphate
Cmax: maximum concentration
CRE-Luc: cAMP response element-luciferase
DIO: diet-induced obesity/obese
EC50: half maximal effective concentration
ECG: electrocardiogram
FAS: full analysis set
Gcg: glucagon
GIP: glucose-dependent insulinotropic peptide
GIPR: glucose-dependent insulinotropic peptide receptor
GLP-1: glucagon-like peptide-1
GLP-1R: glucagon-like peptide-1 receptor
Gcg: glucagon
GcgR: Gcg receptor
GI: gastrointestinal
GTT: glucose tolerance test
h: human
HbA_{1c}: glycated hemoglobin
HSA: human serum albumin
i.p.: intraperitoneal
ivGTT: intravenous glucose tolerance test
LLOQ: lower limit of quantification
MAD: multiple ascending dose
OGTT: oral glucose tolerance test
PD: pharmacodynamic
PK: pharmacokinetic
QD: quaque die (once daily)
QT: specific ECG interval corresponding to ventricular depolarization/repolarization
QTcI: individual corrected QT interval
SEM: standard error of mean
SAD: single ascending dose
SAS: safety analysis set
s.c.: subcutaneous
T2D: type 2 diabetes
TEAE: treatment emergent adverse event
Tmax: time to maximal concentration
Tmin: time to minimal concentration

