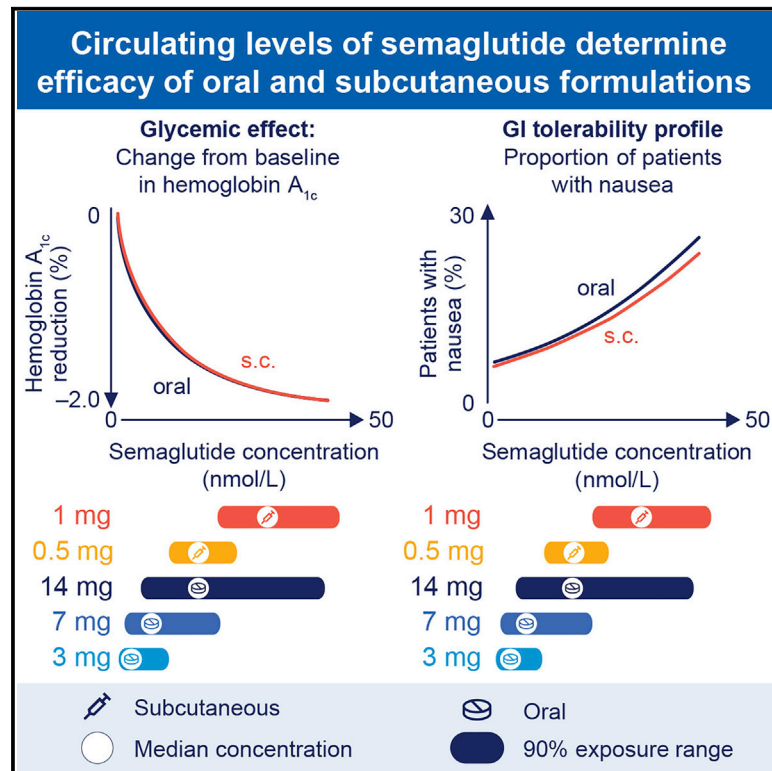


# Levels of circulating semaglutide determine reductions in HbA<sub>1c</sub> and body weight in people with type 2 diabetes

## Graphical abstract



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## In brief

Determinants of GLP-1 action include the gut-brain axis, interindividual differences in GLP-1 receptor expression, differential drug access to key tissues, and circulating levels of bioactive peptide. Overgaard et al. analyze oral versus parenteral routes of administration, revealing that circulating levels of semaglutide predict outcomes in people with type 2 diabetes.

## Highlights

- Circulating levels of semaglutide overlap with oral versus subcutaneous administration
- Pharmacokinetics of semaglutide predict reductions in HbA<sub>1c</sub> and body weight
- Route of semaglutide administration does not influence pharmacodynamic outcomes
- Body weight influences circulating levels of semaglutide in people with diabetes



## Article

# Levels of circulating semaglutide determine reductions in HbA<sub>1c</sub> and body weight in people with type 2 diabetes

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## SUMMARY

Glucagon-like peptide-1 receptor agonists (GLP-1RA) are used for the treatment of type 2 diabetes. Whether clinically important responses and adverse events (AEs) are dependent on the route of administration has not been determined. We demonstrate that nearly identical exposure-response pharmacodynamic relationships are determined by plasma semaglutide levels achieved through oral versus injectable administration for changes in HbA<sub>1c</sub>, body weight, biomarkers of cardiovascular risk, and AEs such as nausea and vomiting. At typical exposure levels for oral semaglutide, the estimated response is 1.58% (oral) versus –1.62% (subcutaneous) for HbA<sub>1c</sub> and 3.77% (oral) versus 3.48% (subcutaneous) reduction in body weight relative to baseline after 6 months. Increased body weight is the most important variable associated with reduced semaglutide exposure for both formulations. Hence, interindividual variation in GLP-1R responsivity or route of administration are not major determinants of GLP-1RA effectiveness in the clinic.

## INTRODUCTION

The treatment of type 2 diabetes (T2D) is centered around glycaemic control, based on strong relationships between blood glucose levels and the development of micro- and macrovascular complications.<sup>1</sup> The results of cardiovascular outcome studies in people with T2D have refined the approach to the selection of therapeutic agents, demonstrating the safety of three classes of glucose-lowering agents: the DPP-4 inhibitors, SGLT-2 inhibitors, and the glucagon-like peptide-1 receptor agonists (GLP-1RAs).<sup>2</sup> Moreover, the last two classes of drugs reduced the development of several complications of T2D, enabling strategies directed at the reduction of cardiovascular disease and progression of chronic kidney disease.<sup>3</sup>

GLP-1RAs exhibit highly different molecular structures and unique pharmacokinetics. These molecules encompass small non-modified naturally occurring peptide agonists such as exenatide administered twice daily, and lixisenatide, an exendin-4 derivative modified at the carboxyl terminus and developed as a once-daily injection.<sup>4</sup> Exenatide has also been developed as a long-acting microsphere preparation suitable for once-weekly administration.<sup>5</sup> Alternatively, acylated human GLP-1RAs such as liraglutide and semaglutide exhibit a longer circulating  $t_{1/2}$  via non-covalent binding to serum albumin.<sup>6</sup> Moreover, much larger molecules exemplified by dulaglutide, a once-weekly GLP-1RA containing two GLP-1 peptides coupled to an immunoglobulin (IgG4) Fc fragment, have been introduced for clinical use.<sup>4</sup> These substantial differences in structure and pharmaco-

netics necessitate scrutiny of the individual properties of and outcomes achieved with GLP-1RAs in the clinical setting.

An oral formulation of semaglutide was recently developed for clinical use, based on co-formulation with SNAC (sodium *N*-[8-(2-hydroxybenzoyl)amino] caprylate).<sup>7</sup> Historically, peptide-based drugs exhibited limited oral bioavailability because of their chemical nature and multiple physicochemical challenges in absorption via the gastrointestinal (GI) tract.<sup>8</sup> Oral semaglutide, administered once daily, in the morning on an empty stomach, was studied at 3 different doses, 3, 7, and 14 mg in the PIONEER (peptide innovation for early diabetes treatment) clinical trial program. Remarkably, the oral formulation of semaglutide achieved clinically relevant reductions of hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) and body weight, in people with T2D, either as monotherapy in combination with other oral glucose-lowering agents or when added to insulin regimens. The results of the PIONEER program enabled the approval of the first oral GLP-1-based therapy for the treatment of T2D.<sup>9–13</sup>

The development of the field of GLP-1 therapeutics has prompted a number of unresolved mechanistic questions. For example, is genetic variation in the GLP-1 receptor (GLP-1R) or GLP-1 resistance, as described in preclinical and clinical studies,<sup>14,15</sup> an important determinant of interindividual drug responses? Does the activity of local GI or neural GLP-1Rs preferentially activated by GLP-1RAs absorbed from the stomach contribute to differential efficacy of these oral agents?<sup>14</sup> Is the reporting of nausea and vomiting across trials reflective of interindividual sensitivity, modified by local GI concentrations of drug, or largely attributable to actual circulating levels of GLP-1R



**Table 1. Demographic characteristics of data from matched and unmatched subjects from SUSTAIN and PIONEER phase 3a trials**

Category	PIONEER		SUSTAIN (N = 1,551)
	Unmatched (N = 3,003)	Matched (N = 1,551)	
Age, y	59.4 ± 10.9	57.3 ± 10.5	56.0 ± 10.6
Female	1,345 (44.8)	653 (42.1)	658 (42.4)
HbA <sub>1c</sub> , %	8.1 ± 0.8	8.1 ± 0.9	8.1 ± 0.9
Body weight, kg	88.2 ± 21.9	87.2 ± 22.4	86.3 ± 22.7
Diabetes duration, y	9.5 ± 7.9	7.3 ± 6.3	7.2 ± 6.0
<b>Race</b>			
White, other	2,034 (67.7)	971 (62.6)	834 (53.8)
Asian	779 (25.9)	487 (31.4)	647 (41.7)
Black or African American	190 (6.3)	93 (6.0)	70 (4.5)
<b>Renal function</b>			
Normal	1,809 (60.2)	1,099 (70.9)	1,024 (66.0)
Mild impairment	865 (28.8)	415 (26.8)	502 (32.4)
Moderate impairment	329 (11.0)	37 (2.4)	25 (1.6)
<b>Background therapy</b>			
1–2 OADs	1,335 (44.5)	994 (64.1)	1,077 (69.4)
Monotherapy	703 (23.4)	431 (27.8)	345 (22.2)
Insulin	845 (28.1)	12 (0.8)	–
Diet and exercise	120 (4.0)	114 (7.4)	129 (8.3)
<b>Maintenance dose</b>			
Placebo	572 (19.0)	191 (12.3)	129 (8.3)
0.5 mg s.c.	–	–	556 (35.8)
1.0 mg s.c.	–	–	866 (55.8)
3 mg oral	629 (20.9)	345 (22.2)	–
7 mg oral	620 (20.6)	331 (21.3)	–
14 mg oral	1,182 (39.4)	684 (44.1)	–

Data are n (%) or means ± SDs. OAD, oral antidiabetic drug; s.c., subcutaneous; SD, standard deviation.

agonists, whether achieved through enteral or parenteral administration?

The availability of pharmacokinetic data from people treated with oral versus injectable semaglutide provided the opportunity to compare clinically meaningful outcomes achieved with different routes of drug administration. Here, we compared the extent of HbA<sub>1c</sub> and body weight reduction, changes in cardiovascular biomarkers, and commonly reported AEs such as nausea and vomiting, in people with T2D treated with oral or subcutaneous (s.c.) semaglutide. The data reveal that systemic exposure to circulating semaglutide, irrespective of the mode of administration, is the major predictor of semaglutide outcomes in people with T2D. These findings indirectly suggest that preferential activation of portal or enteric neuronal GLP-1Rs, or interindividual differences in GLP-1R signaling, are not likely to be substantial determinants of common clinical outcomes in people with T2D treated with oral GLP-1R agonists.

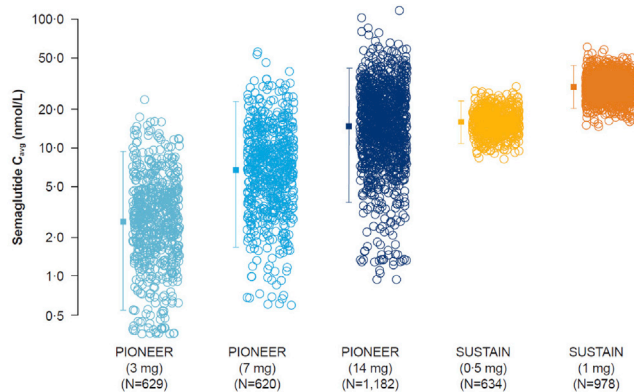
## RESULTS

Baseline parameters for the study populations enrolled in the PIONEER and SUSTAIN clinical trial programs are shown in

**Table 1.** To compare results with oral versus injectable semaglutide, we examined whether oral dosing of semaglutide in the PIONEER program was affected by variability in common clinical parameters. Based on population pharmacokinetic (PK) modeling described in [STAR Methods](#) and [Methods S1](#) and [S2](#), body weight was the covariate with the greatest impact on semaglutide exposure as illustrated for the model including all covariate factors ([Figure S1](#)) and via the parameter estimates for the final model ([Table S1](#)). Average semaglutide concentration levels at steady state were obtained for each subject planned for PK assessment in the PIONEER program. These exposure levels were subsequently used to derive exposure-response relationships as described below. Exposure-response relationships were evaluated after 26 weeks of treatment with once-daily oral semaglutide, which represents the primary evaluation time point in the 6 PIONEER trials.

### Oral semaglutide exposure-response relationships determine HbA<sub>1c</sub> reduction

The change in HbA<sub>1c</sub> from baseline until week 26 with once-daily oral semaglutide was exposure dependent and started to level off at higher exposures ([Figure S2A](#)). A reduction in HbA<sub>1c</sub> was observed across the concentration range evident with oral



**Figure 1. Semaglutide exposure is dose proportionate in the PIONEER and SUSTAIN trials**

Data are individual  $C_{avg}$  values (open symbols) and geometric means with 90% ranges (closed symbols with error bars). Data from PIONEER 1, 2, 3, 5, 8, and 9; SUSTAIN 1, 2, 3, and 6; and SUSTAIN Japan OAD.  $C_{avg}$ , average concentration; OAD, oral antidiabetic drugs.

semaglutide doses of 7 and 14 mg, with a 90% exposure range from 2 to 41 nmol/L. We next explored the exposure-response relationship for  $HbA_{1c}$  for various covariate effects in the PIONEER trials. The exposure-response relationship was primarily dependent on baseline  $HbA_{1c}$  (Figure S2B). Subjects with higher baseline  $HbA_{1c}$  had greater  $HbA_{1c}$  reductions compared to subjects with lower  $HbA_{1c}$  and did not reach the same absolute  $HbA_{1c}$  level compared to subjects with lower  $HbA_{1c}$  (Figure S2C). At high exposure levels of semaglutide, subjects with the highest baseline  $HbA_{1c}$  ( $\geq 9.1\%$ ) had a  $\sim 3\%$  decrease in  $HbA_{1c}$  compared to a  $\sim 1\%$  reduction in subjects with a lower baseline  $HbA_{1c}$  ( $< 7.5\%$ ). Other covariates such as baseline body weight, BMI, diabetes duration, gender, age group, race, ethnicity, and presence of upper GI disease were of no or only minor importance and did not affect the exposure response relationships for oral semaglutide (Figure S1).

Analysis of the proportion of subjects in the PIONEER trials achieving  $HbA_{1c}$  levels  $< 7.0\%$  or  $\leq 6.5\%$  is shown in Figure S3. Subjects with moderate renal impairment and those receiving insulin treatment had moderately lower  $HbA_{1c}$  responses compared to subjects with normal renal function or those treated with multiple oral antidiabetic drugs (OADs) (Figures S2D and S3E). Notably, a consistent relationship was observed between plasma levels of semaglutide achieved with oral semaglutide administration and  $HbA_{1c}$  targets, when analyzed across a range of PIONEER trials and starting levels of  $HbA_{1c}$  (Figures S3A, S3C, S3D, and S3F). As expected, the proportion of individuals achieving glycemic targets was inversely proportional to the starting  $HbA_{1c}$ . Some differences in exposure response across age groups were seen, but these differences appeared to be well described by other covariate factors, and age was not identified as a covariate in the model-based analysis (Figures S1 and S4).

#### Oral semaglutide exposure-response relationships for body weight are modulated by gender and baseline $HbA_{1c}$

Body weight loss after 26 weeks of treatment was observed across the entire concentration range investigated with oral

semaglutide 7 and 14 mg, and the slope of the weight loss effect did not appear to level off at the highest exposures obtained (Figure S5A). Greater weight loss was seen in females compared to males (Figure S5B); subjects with higher baseline  $HbA_{1c}$  appeared to have less weight loss (Figure S5C). Background medication did not affect weight loss responses by exposure (Figure S5D). Other demographic covariates had little or no effect on weight loss (Figures S5D and S5E).

#### Oral semaglutide exposure-response relationships predict rates of GI AEs

Exposure-response relationships were investigated for nausea and vomiting events reported during the first 26 weeks of treatment with oral semaglutide. The proportions of subjects reporting nausea or vomiting increased with greater semaglutide exposure (Figures S4C and S4D).

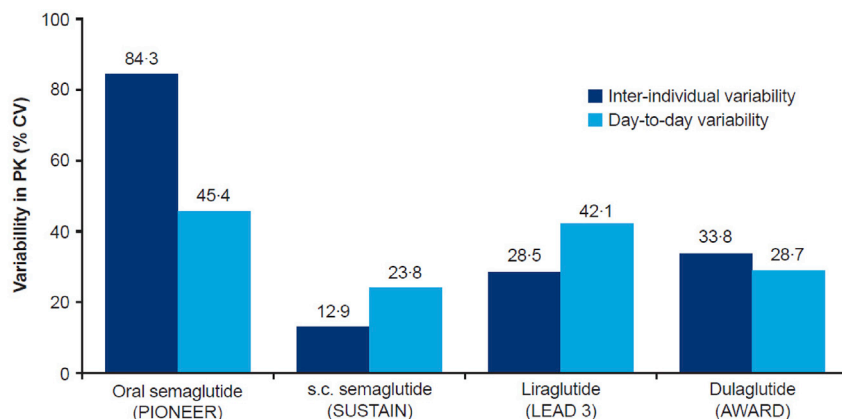
Larger proportions of female subjects reported GI side effects across the exposure range for both nausea and vomiting. The remaining covariate factors, including moderate renal impairment, background medication for T2D, ethnicity, upper GI disease, and race had no or little influence on reports of nausea or vomiting that remained proportional to circulating semaglutide concentrations (Figures S5F and S5G).

#### Overlapping plasma levels of semaglutide with oral versus injectable administration

Based on the PK data from the PIONEER trials (Figures 1 and S2A), the variability of oral semaglutide was assessed both between and within subjects and compared to different GLP-1RAs (Figure 2). Across subjects with similar demographic profiles (Table 1), the coefficient of variation (CV) between subjects was estimated to be 84%, with lower variability in plasma concentrations observed within subjects (45% CV) (Figure 2). PK variability was greater for oral semaglutide versus injectable semaglutide, liraglutide, or dulaglutide (Figure 2).

To compare circulating peptide levels obtained with oral versus s.c. semaglutide, the individual model derived steady-state exposure levels for subjects on oral semaglutide in the PIONEER program were compared to the individual estimates for subjects taking s.c. semaglutide in the SUSTAIN program.<sup>16</sup> The range of exposure levels for the two routes of administration overlapped considerably (Figure 1), but variability was smaller following s.c. versus oral semaglutide. The geometric mean exposure for oral semaglutide 14 mg was similar to that of 0.5 mg s.c. semaglutide, whereas the upper exposure ranges of 14 mg oral and 1.0 mg s.c. semaglutide doses overlapped, but the mean exposure levels were nominally higher with 1 mg s.c. semaglutide (Figure 1). Likewise, the upper exposure ranges for 7 mg oral and 0.5 mg s.c. doses were similar, although the geometric mean exposure for the 7 mg oral dose was lower than for 0.5 mg s.c. (Figure 1).

Previous semaglutide population PK analysis show that individual semaglutide exposure levels following s.c. administration in a Phase III diabetes program can be reasonably simulated based on baseline demographic data.<sup>16</sup> Simulated data for s.c. semaglutide was compared to the observed exposure levels following oral administration to estimate the relative differences in plasma semaglutide levels upon switching from



oral to s.c. semaglutide, or vice versa. This would mean, for subjects switching from 14 mg oral semaglutide to 0.5 mg s.c. semaglutide, 88% would have a <2-fold increase in exposure, which corresponds to the exposure increase seen during normal dose escalation (Figure 3). These findings may provide guidance for anticipating changes in semaglutide levels in individuals switching between oral and s.c. semaglutide formulations.

#### Plasma exposure-response relationships are identical with oral versus s.c. semaglutide

We next compared a range of clinically meaningful GLP-1-responsive therapeutic and safety endpoints across a range of semaglutide exposure levels obtained with oral versus s.c. semaglutide administration. Remarkably, exposure-response analyses showed highly similar relationships reflecting plasma levels of semaglutide obtained with oral versus s.c. semaglutide (Figure 1) and subsequent reductions in HbA<sub>1c</sub> and body weight (Figures 4A and 4B). Consistent with these findings, the proportions of subjects reporting nausea or vomiting were similar for oral and s.c. semaglutide administration over a range of plasma concentrations (Figures 4C and 4D). Differential effects for oral versus s.c. administration at 14.6 nM, corresponding to 14 mg, were computed based on a sensitivity analysis using the propensity-matched dataset. The predicted change in HbA<sub>1c</sub> was -1.58% (oral) versus -1.62% (s.c.) and the body weight change was 3.77% (oral) versus 3.48% (s.c.). Nausea was predicted to occur in 11.1% (oral) versus 11.8% (s.c.) of subjects and vomiting in 4.2% (oral) versus 4.2% (s.c.). Hence, the data illustrate the importance of plasma levels, irrespective of the route of dosing, or theoretical differences in GLP-1R sensitivity, for determining the clinical pharmacodynamic consequences of semaglutide administration.

The exposure-response relationships for changes in blood pressure (BP), C-reactive protein (CRP), and fasting levels of triglycerides, indirect biomarkers of cardiovascular risk, are shown in Figure 5. Progressive reductions in CRP and triglycerides (Figures 5A and 5B) were associated with increasing levels of semaglutide exposure, whether obtained with oral or s.c. administration. Changes in diastolic BP were modest over a range of semaglutide concentrations; however, a clear exposure-

**Figure 2. Variability estimates in exposure when adjusted for time since dose and covariate factors, assessed based on population PK analysis**

PIONEER estimates are based on the model presented in the text. Estimates from SUSTAIN, SCALE, and AWARD were based on similar population PK methodology and previously published.<sup>16,17,18</sup> PK, pharmacokinetic; CV, coefficient of variation; s.c., subcutaneous.

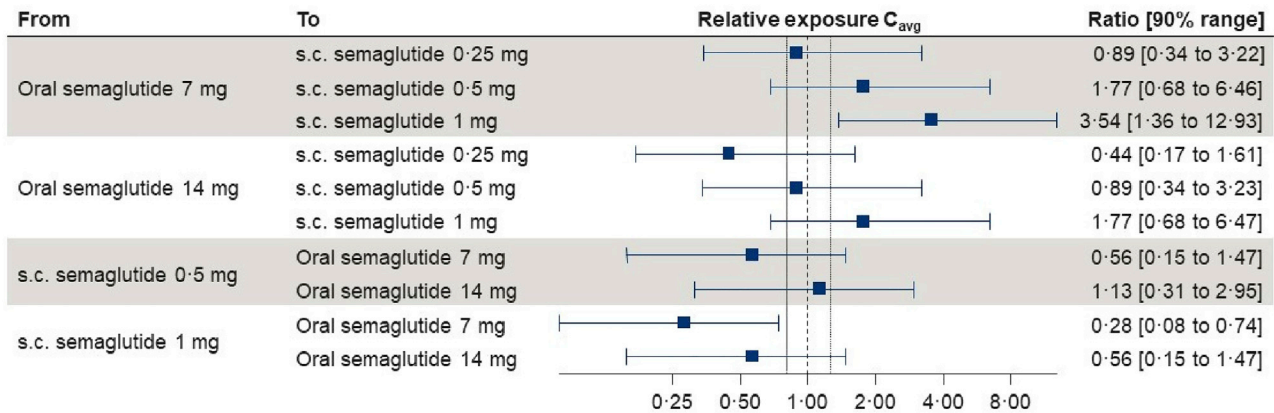
response relationship was observed for systolic BP (Figures 5C and 5D). Collectively, these findings highlight the dominant importance of circulating levels of semaglutide, irrespective of the route of administration, for the determination of a broad range of clinically meaningful endpoints in people with T2D.

#### DISCUSSION

Although GLP-1Ras have been used for the treatment of T2D for ~16 years,<sup>19</sup> the key determinants of clinical outcomes and their relationship to circulating drug levels in people with T2D have not been systematically compared using different routes of peptide administration. Here, we demonstrate that the plasma levels of semaglutide, independent of a wide range of co-variables, or theoretical interindividual differences in GLP-1R expression and sensitivity, are the major determinants of efficacy, as judged by reductions in HbA<sub>1c</sub> and body weight in people treated with oral or s.c. semaglutide. Not surprisingly, semaglutide plasma exposure also correlated with reports of nausea and vomiting in the same individuals, and the dose-response relationships for these endpoints were not different in the PIONEER versus SUSTAIN clinical trial programs.

Previous studies have identified neural circuits triggered by endogenous GLP-1 released from the gut that communicate with the autonomic and central nervous systems to mediate multiple actions of GLP-1, including control of satiety, glycemia, and gastric emptying.<sup>20,21</sup> Notably, oral semaglutide is administered before food ingestion in the morning, whereas s.c. semaglutide may be administered at any time throughout the day. Despite an extensive preclinical literature highlighting the existence of a GLP-1R-dependent gut-brain axis triggered by oral nutrient ingestion and linked to the control of glucose homeostasis,<sup>22–24</sup> the importance of this axis has been questioned,<sup>25</sup> and its relevance to human physiology or the treatment of T2D remains uncertain. Critically, we did not detect evidence for preferential or detrimental actions of oral (relative to s.c. administered) semaglutide across a broad range of semaglutide plasma concentrations.

Notably, the venous drainage of the stomach via the splenic vein allows rapid access of semaglutide, administered orally, to the GLP-1Rs within the portal vein.<sup>24</sup> Nevertheless, in most species, the majority of L cells are located in the distal gut, and the quantitative contributions of proximal gut L cells to the control of glucose homeostasis and circulating levels of GLP-1



**Figure 3. Comparative exposures with oral versus injectable semaglutide**

Data are mean relative exposures with 90% ranges obtained using the individual exposure estimates from the final PK model for subjects in PIONEER 1, 2, and 8 populations. For each individual, s.c. exposure estimates were predicted based on a dose and demographic factors using a reduced population PK model developed based on s.c. semaglutide data.<sup>16</sup>

throughout the day remains questionable.<sup>26</sup> Our current observations with oral semaglutide absorbed through the stomach do not rule out the potential existence and importance of an endogenous GLP-1R-dependent gut-brain axis in humans, with physiological circuits potentially activated transiently by food ingestion triggering GLP-1 secretion from L cells within the distal gut enteroendocrine system.

Examining the clinical importance of PK variability reveals insights for oral peptide delivery. The variability estimates for oral semaglutide were compared to results obtained with similar methodology for s.c. semaglutide<sup>16</sup> and dulaglutide.<sup>17</sup> Interindividual variability of oral semaglutide was higher than for other GLP-1RAs dosed s.c., whereas intra-individual variability was just marginally higher, confirming previous findings from clinical pharmacology trials.<sup>16</sup> Importantly, the higher day-to-day variability was mitigated by frequent dosing and a long half-life of ~1 week for semaglutide, thereby leading to similar effects on metabolic outcomes and body weight.

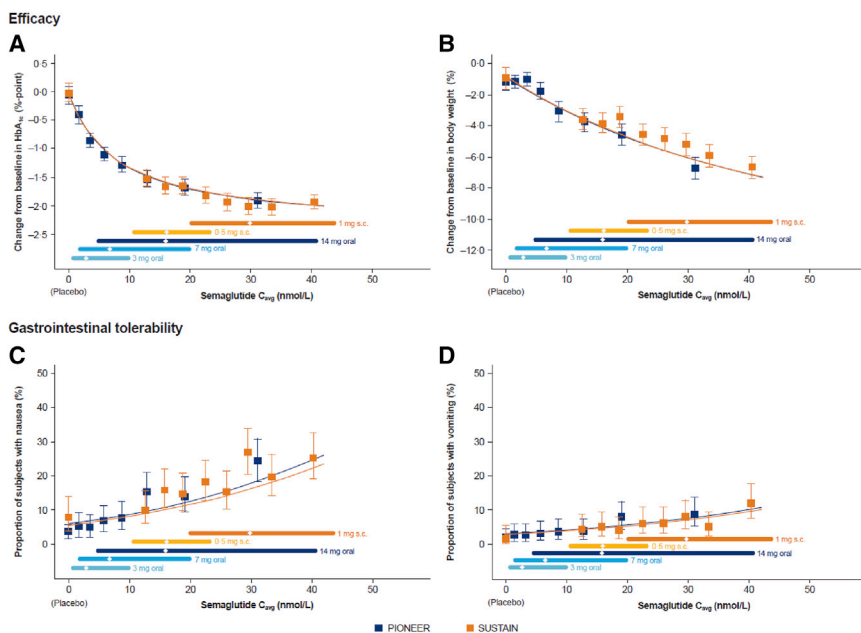
Similar exposure-response relationships with s.c. versus oral semaglutide for GI side effects further support the limited clinical impact of intra-individual variability in plasma concentrations for oral semaglutide. The starting dose of 3 mg semaglutide administered orally generally provides lower exposure than 0.25 mg s.c., which presumably helps to ensure tolerability across the exposure range and comparable tolerability for oral and s.c. semaglutide. Nevertheless, compared to s.c. semaglutide, more subjects achieved lower plasma exposures, leading to suboptimal pharmacodynamics effects. At 14 mg, a substantial proportion of the subjects achieved exposure and reductions in HbA<sub>1c</sub> and body weight similar to that achieved with s.c. semaglutide 1 mg.

Examining the subset of individuals on oral semaglutide 14 mg (19%) that achieved exposure levels below those obtained with 0.5–1.0 mg s.c. semaglutide, revealed that these subjects still achieved an ~1% reduction in HbA<sub>1c</sub>, which is considered a clinically relevant treatment outcome. Only a small fraction (3%) of subjects obtained exposure levels considered below the therapeutic range, with an expected HbA<sub>1c</sub> reduction <0.5%. Whether

these observations reflect real problems with drug absorption and/or compliance remains uncertain. These findings demonstrate that the variability associated with the oral administration of semaglutide has limited consequences for clinical responses in the majority of treated subjects.

It is widely appreciated that the extent of insulin resistance, and not just circulating levels of insulin, influences the biological response to insulin therapy. In contrast, despite the growing clinical use of GLP-1RAs, the potential importance of various factors modifying therapeutic responses to GLP-1RAs remains poorly defined. Notably, relative GLP-1 resistance and impairment of the gut-brain GLP-1 axis has been described in preclinical studies.<sup>14</sup> Clinically, genetic variation in the *GLP1R* has been associated with changes in receptor signaling and glycemia<sup>27</sup> and differential responses to GLP-1RA in some human studies.<sup>28,29</sup> Variables such as local tissue expression of the GLP-1R, interindividual differences in GLP-1 receptor binding, receptor structure, or downstream signaling activity are difficult to measure in the context of large clinical trials. Here, we determined that when corrected for plasma level of semaglutide achieved, irrespective of the mode of administration, factors such as body weight or BMI, race, and ethnicity did not meaningfully influence the exposure-response relationships for key determinants such as HbA<sub>1c</sub> and weight loss. In contrast, individuals on insulin, those with modest degrees of renal impairment, and subjects with lower starting baseline HbA<sub>1c</sub> exhibited diminution in exposure-response relationships, relative to other analyzed groups. The characteristics of the first two subgroups are related in part to a more prolonged duration of disease and so their diminished responses are in line with general experience surrounding the relative efficacy of glucose-lowering agents and the extent and duration of preexisting T2D.

The observations that multiple long-acting GLP-1RAs reduced the rates of CV events and all-cause mortality across a broad range of cardiovascular safety trials<sup>30</sup> have sparked tremendous interest in understanding mechanisms linking GLP-1R activation to cardioprotection. Notably, our analyses demonstrate a consistent



**Figure 4. Efficacy and tolerability by semaglutide exposure in the PIONEER and SUSTAIN trials**

Data are means and 95% CIs at week 26 for PIONEER and week 30 for SUSTAIN. Exposure is presented as quantiles of  $C_{avg}$  for semaglutide and 1 quantile for placebo (at  $C_{avg}$  of 0 nmol/L). The fitted solid line represents model-derived relations for each clinical program. The horizontal lines along the x axes represent medians and 90% exposure ranges, with the median exposure represented by a diamond. Data from SUSTAIN 1, 2, and 3; SUSTAIN-Japan; and PIONEER 1, 2, 3, 5, 8, and 9. The PIONEER and SUSTAIN populations differed somewhat with respect to demographic composition, and datasets were propensity matched. CI, confidence interval.

exposure-response relationship for biomarkers and physiological variables indirectly related to CV risk, such as CRP, BP, and plasma triglycerides, with both oral and s.c. semaglutide. These findings align with the results of the SUSTAIN-6 and PIONEER-6 trials, showing a 26% and non-significant 21% reduction, respectively, in the hazard ratio of major adverse cardiovascular events (MACE) in T2D populations with high CV risk.<sup>31,32</sup> Hence, the effects of semaglutide in modifying cardiovascular risk factors are not dissociated from plasma levels of semaglutide achieved using different modes of drug administration.

The finding that increasing semaglutide exposure is linked to the extent of  $HbA_{1c}$  reduction and weight loss provides further guidance for the ongoing development of GLP-1RAs for the treatment of obesity. These observations are consistent with more limited analysis of the pharmacodynamic differences obtained for 0.5 versus 1.0 mg of injectable semaglutide,<sup>16,33</sup> as well as the greater weight loss achieved with 3.0 versus 1.8 mg liraglutide<sup>34,35</sup> and collectively support the evaluation of higher doses of oral semaglutide, and the higher dose of 2.4 mg semaglutide once weekly investigated in the STEP program. These findings may also inform the dose selection of semaglutide in some subjects with monogenic obesity, an important population that may benefit from therapy with GLP-1RAs.<sup>36</sup> Interestingly, an upper dose and plasma concentration range for semaglutide, or for that matter, any other GLP-1RA, that is not associated with additional incremental weight loss, has not yet been defined. These findings highlight the importance of progressive dose escalation, while balancing tolerability, for the development of new GLP-1RAs with improved weight loss profiles.

### Translational relevance

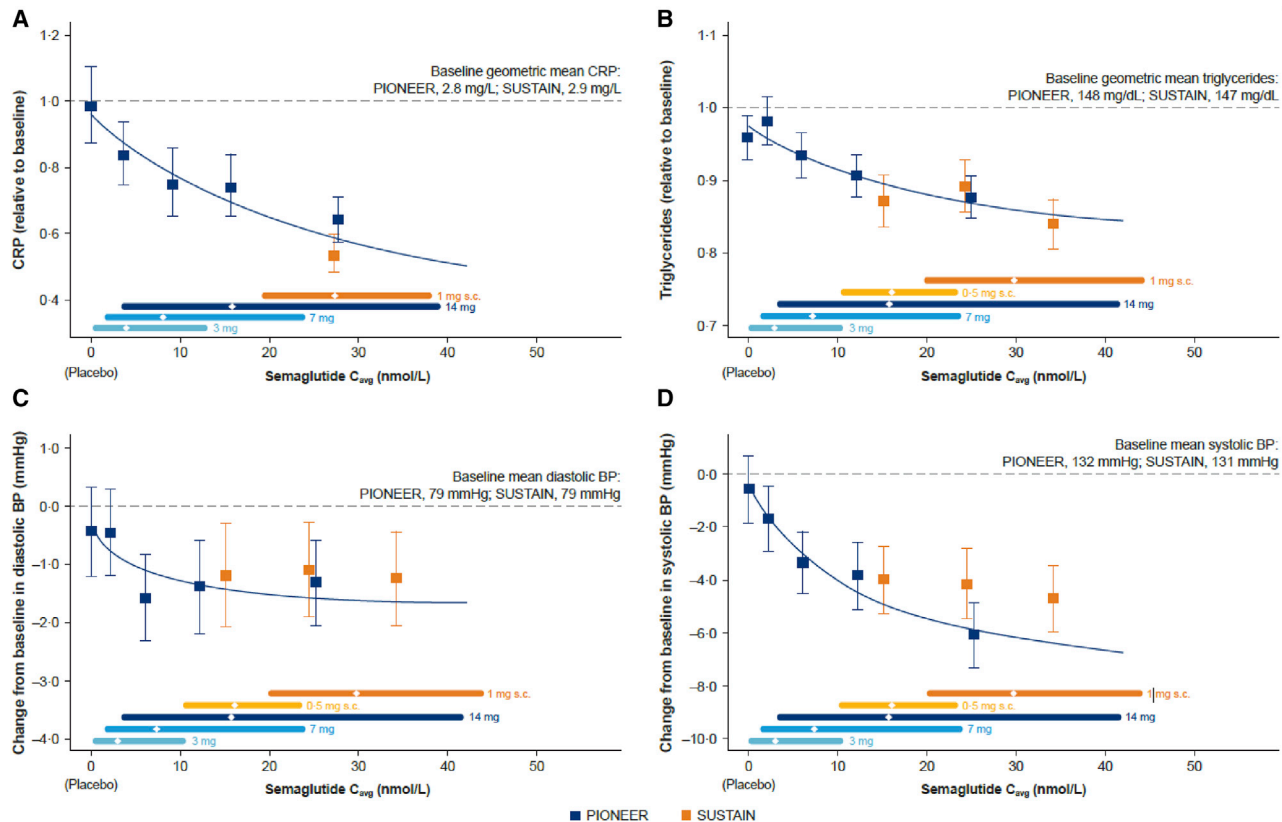
Importantly, body weight was a relevant modifier of the plasma levels of semaglutide achieved across a range of semaglutide dosing regimens, although its impact on semaglutide plasma

levels does not substantially modify clinical outcomes and does not imply dose adjustments based on baseline body weight. Nevertheless, the body weight-exposure relationship may influence the option of increasing or decreasing dose

levels on an individual basis. Individuals with greater degrees of obesity not achieving glycemic and weight goals on oral semaglutide may consider switching to injectable semaglutide. These relationships may also explain in part the frequency of AEs in people across a range of BMIs. The presence of upper GI disorders may modify the absorption of oral semaglutide<sup>37</sup> in some individuals; however, 85% of subjects with GI disorders studied at 14 mg (81% overall) achieved circulating levels of semaglutide comparable to that achieved with s.c. administration. Higher exposure with GI disease was driven by gastroesophageal reflux disease, which constituted the majority of reported GI diseases (412 subjects). Gastric ulcer was less common in 34 patients; a non-significant 11% lower exposure was seen in these subjects compared to subjects not reporting GI disease. No effect differences were seen in 174 subjects with chronic gastritis. For subjects in whom switching between oral versus s.c. semaglutide is relevant, our data provide guidance on how to avoid exposure increases leading to tolerability issues. The interindividual and, to a lesser extent, day-to-day intra-individual variability, in plasma semaglutide levels was greater with oral versus s.c. semaglutide administration, likely reflecting the greater reproducibility of semaglutide delivery with parenteral administration.

Our comparative findings simplify the interpretation of outcomes in thousands of people studied in the PIONEER and SUSTAIN clinical trials. The predictability of the dose-response relationships for oral semaglutide support ongoing trials that include higher dose levels of oral semaglutide in people with T2D and obesity, to maximize efficacy by achieving levels of circulating semaglutide with oral dosing that more approximates levels achieved with higher dose of s.c. semaglutide.

The complexity of interpreting dose-response relationships for clinical insulin action requires consideration that insulin bioactivity is greatly influenced by interindividual differences in



**Figure 5. Change in CRP, triglycerides, and blood pressure reflect semaglutide exposure in the PIONEER and SUSTAIN trials**

Data are means and 95% CIs. Exposure is presented as quantiles of  $C_{avg}$  for semaglutide and 1 quantile for placebo (at  $C_{avg}$  of 0 nmol/L). The fitted solid line represents model-derived relations not adjusted for covariate factors, based on the PIONEER program. Triglycerides and systolic and diastolic BP: data from PIONEER 1, 2, 3, 5, 8, and 9 at week 26 and SUSTAIN 1, 2, and 3 and SUSTAIN Japan OAD at week 30. CRP: PIONEER 1, 2, and 5 data at week 26 and SUSTAIN 3 data at week 56. BP, blood pressure; CRP, C-reactive protein.

insulin resistance, and potentially through differential routes of administration such as selective preferential targeting of hepatic versus adipose tissue insulin receptors.<sup>38</sup> In contrast, the present data highlight the predominant importance of plasma semaglutide levels, generally independent of a range of potential covariables, as critical for determining the efficacy of GLP-1RA therapy for the management of T2D.

### Conclusions

The present data advance our understanding of key variables potentially modifying the efficacy of oral and injectable semaglutide for the therapy of T2D. Neither gender nor age was found to be an important predictor of semaglutide action; however, plasma levels trended higher, and weight loss achieved was somewhat greater in female subjects at a given exposure level. Intra-individual variability did not appear to have a clinical impact, and plasma levels of semaglutide achieved with oral semaglutide overlapped substantially with those detected after s.c. semaglutide administration. Neither renal impairment nor hemodialysis meaningfully affected dose-response relationships. Moreover, plasma semaglutide levels increased in a dose-proportional manner with both modes of administration and represented the major determinant of the magnitude of clinical benefit.

### Limitations of the study

Exposure-response data following oral and s.c. administration were obtained in separate large clinical programs with potential differences in trial population and conduct; differences were corrected for in part by propensity matching. Additional limitations of the present analysis include the lack of measurements of insulin, glucagon, and gastric emptying or the assessment of *GLP1R* expression in tissues from study subjects, and the absence of genotyping for individual variation within the *GLP1R*. We were unable to measure free semaglutide; hence, the measurement of total semaglutide concentrations is an indirect surrogate of true semaglutide bioavailability *in vivo*. Nevertheless, the present data solidify the importance of plasma exposure, independent of the route of administration, as a key determinant of semaglutide action in the therapy of T2D.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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#### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xcrm.2021.100387>.

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#### AUTHOR CONTRIBUTIONS

R.V.O., C.L.H., S.H.I., and A.N. carried out the initial primary analyses and wrote and edited the paper. D.J.D. analyzed the data and wrote the paper.

#### DECLARATION OF INTERESTS

R.V.O., C.L.H., S.H.I., and A.N. are full-time employees of and own stock in Novo Nordisk A/S. D.J.D. has served as an advisor or consultant or speaker within the past 12 months to Eli Lilly, Forkhead Biotherapeutics, Intarcia Therapeutics, Kallyope, Merck Research Laboratories, and Novo Nordisk, and holds non-exercised options in Kallyope.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
NONMEM version 7.3	ICON Development Solutions	<a href="https://www.iconplc.com/innovation/nonmem/">https://www.iconplc.com/innovation/nonmem/</a>
R version 3.2.3	R-Project	<a href="https://www.r-project.org/">https://www.r-project.org/</a>
<a href="#">STAR Methods</a> . Oral semaglutide population PK model: Parameterization and NONMEM settings for parameter estimation	This paper Supplemental <a href="#">Methods S1</a>	N/A
<a href="#">STAR Methods</a> . Subcutaneous semaglutide population PK model: Parameterization and NONMEM settings for parameter estimation	This paper Supplemental <a href="#">Methods S2</a>	N/A
Other clinical trial pharmacokinetic data		
SUSTAIN clinical trial program	Published analyses	PMID 29907893
PIONEER clinical trial program	Blood samples analyzed from PIONEER trials	PMID: 31186300
PIONEER 1,2,3,5,8, and 9 trials		PMID: 31530666 PMID: 30903796 PMID: 31189517 PMID: 31530667 PMID: 32333875

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Daniel Drucker ([danieljdrucker@gmail.com](mailto:danieljdrucker@gmail.com) or [drucker@lunenfeld.ca](mailto:drucker@lunenfeld.ca)).

#### Materials availability

No new reagents or research materials were generated in this study.

#### Data and code availability

- Original data may be reviewed consistent with conditions within the informed consent signed by participants in the PIONEER and SUSTAIN clinical trial programs, upon contacting the authors, upon request to the lead contact, Daniel Drucker.
- No new code was generated in this study. For the oral semaglutide pharmacokinetics model, parameterization and NONMEM computer program settings for parameter estimation are provided in the [Methods S1](#) and [S2](#).
- The patient level data reported in this study cannot be deposited in a public repository because of limitations associated with the informed consent for the SUSTAIN and PIONEER trials.
- To request access, contact the Lead contact [DanielJDrucker@gmail.com](mailto:DanielJDrucker@gmail.com).
- All of the data reported in this manuscript will be shared upon contact of the lead author [Danieljdrucker@gmail.com](mailto:Danieljdrucker@gmail.com).
- Any additional information required to re-analyze the data reported in this work paper is available from the Lead Contact upon request.

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

This study presents exposure data and exposure-response analyses for oral semaglutide in male and female subjects with type 2 diabetes and furthermore, compares with previously published data on s.c. semaglutide for exposure<sup>16</sup> and exposure-response.<sup>33</sup> The pharmacokinetic analyses presented here are consistent with the informed consent forms approved by participating institutions and signed by participants enrolling in the PIONEER and SUSTAIN clinical trial programs.

## Clinical data

Data from six phase 3 trials of oral semaglutide was used for assessment of semaglutide PK and exposure-response analysis (global trials PIONEER 1, 2, 3, 5, 8 and Japanese trial PIONEER 9).<sup>9–13,32,39,40</sup> Each trial was powered for efficacy versus comparator or placebo, and not for population PK or exposure-response analyses. Randomization across the dose levels were 1:1:1 for PIONEER 1, 3, 8, and 9, whereas PIONEER 2 and 5 included only the 14 mg dose. The current exposure-pharmacodynamic relationships for oral semaglutide in the PIONEER trials were compared to those obtained from the phase 3a program of injectable semaglutide in subjects with T2D (SUSTAIN trials). Overall, the PIONEER and SUSTAIN exposure-response populations were similar, with the main difference being the inclusion of a dedicated study of patients with moderate renal impairment and a trial with concomitant insulin treatment in the PIONEER program. Propensity score matching was used to analyze the effect of balancing the differences between SUSTAIN and PIONEER populations. Patient characteristics are summarized in [Table S1](#).

Oral semaglutide dosing was administered with up to 120 mL of water, at least 30 min before the first meal of the day and any other oral medication. The PK population was defined as all subjects from the full analysis sets who were randomized to oral semaglutide, except for PIONEER 3 which had PK sampling scheduled for subjects recruited in USA, Germany and Japan, constituting approximately 50% of the subjects in PIONEER 3. Only PK data obtained during treatment was included, thus excluding data obtained at baseline and following premature discontinuation of treatment. Blood samples for estimation of semaglutide concentrations in plasma were obtained at week 4, 8, 14 and 26 (PIONEER 1), week 4, 26 and 52 (PIONEER 2), week 4, 8, 14, 26, 38, 52 and 78 (PIONEER 3), week 4, 8, 14, 26 (PIONEER 5), week 4, 14, 26, 38, 52 (PIONEER 8) and week 4, 14, 26, 38 and 52 (PIONEER 9). A single blood sample was obtained at each occasion, except for PIONEER 9 which had two samples obtained at week 26 (one pre-dose and one 60–90 min post-dose). Timing of blood sampling was at the investigator's discretion except in PIONEER 2 which aimed at sampling 25 min post-dose to approximately capture  $C_{max}$  of SNAC. Actual sampling times as well as dosing times for the last dose prior to sampling were recorded. Total protein bound semaglutide concentrations were estimated in plasma by a validated liquid chromatography-mass spectrometry assay with a lower limit of quantification (LLOQ) of 0.729 nmol/L.<sup>41</sup>

## METHOD DETAILS

### Population PK assessment of oral semaglutide

In total, 10,527 PK observations from 2,345 subjects during treatment with oral semaglutide from the PIONEER 1,2,3,5,8, and 9 trials were included in the PK dataset. Of these, 914 observations were below the LLOQ and were assigned the value LLOQ/2. 154 PK observations (1.5%) were excluded due to inadequate dosing history.

The population PK model for oral semaglutide was pre-specified based on the model obtained from data across clinical pharmacology trials of oral, s.c. and intravenous administration of semaglutide.<sup>42</sup> This was a two compartment model with separate first order absorption processes for oral and s.c. administration, and with the same distribution and elimination processes across all routes of administration. All parameter values in the oral PK model were based on these previously established results, except for bioavailability, which was estimated on PIONEER PK data. The PK model was used to characterize variability in exposure (within and between subjects), demographic factors influencing exposure, as well as individual average steady state exposure levels ( $C_{avg}$ ) used for exposure-response. A single  $C_{avg}$  value was computed for each subject based on the individual parameter estimates, as the average concentration in a dosing interval at steady state at the target dose level. Several sensitivity analyses with different structural PK parameters and different procedures for LLOQ handling were conducted, all demonstrating robust  $C_{avg}$  values, with above 0.99 correlation to the final estimates.

A confirmatory approach with estimation of a base model without covariates, a full model including all investigated covariates and a final model including only significant covariates, was applied.<sup>43</sup> The full model used for covariate analysis included the following covariates on exposure: gender (male, female), age group (18–64, 65–74, 75–92 years), race (White, Black or African American, Asian), ethnicity (Non-Hispanic or Latino, Hispanic or Latino), body weight as continuous covariate, renal function (normal, mild and moderate impairment), semaglutide dose (3, 7, 14 mg), subjects with/without upper GI disease and trial effects for PIONEER 2, 3, 5 and 8. Presence of upper GI disease was defined as having ticked 'yes' to an ongoing diagnosis of gastroesophageal reflux disease, chronic gastritis, gastric ulcer and/or gastroparesis on the GI disease history form filled in by the investigator at the baseline visit. In the final model, the following covariates were retained with minor impact: gender, race (Asian and Whites), body weight, upper GI disease and trial effects for PIONEER 3 and 5. The latter corrected for overall lower exposures in PIONEER 3 and 5 compared to PIONEER 1, 2, 8 and 9. The final model included log-normal inter-individual variability model for bioavailability (F), and a proportional residual error model. Details on the parameterization and software settings for parameter estimation is given in the [Supplemental information](#) section.

### Population PK assessment of s.c. semaglutide

Assessment of s.c. semaglutide exposure levels in type 2 diabetes from SUSTAIN trials has previously been reported.<sup>16</sup> Due to the relatively slow absorption of s.c. semaglutide, a one-compartment model was applied as this was the simplest model to adequately describe steady PK for s.c. semaglutide.<sup>16</sup> The one-compartment model and the two-compartment model used for oral semaglutide were assessed to provide almost identical  $C_{avg}$  values for s.c. semaglutide across individual subjects,<sup>42</sup> thus reassuring that  $C_{avg}$  levels can be compared across SUSTAIN and PIONEER. The final model included the same demographic covariates as for oral

semaglutide described above, with the exception of GI disease and the addition of injection site. log-normal inter-individual variability model for the apparent clearance (CL/F) and the apparent volume of distribution (V/F), and a proportional residual error model.

## QUANTIFICATION AND STATISTICAL ANALYSIS

Exposure-response analyses aim to reflect the efficacy observed for subjects adhering to treatment and not taking anti-diabetic rescue medication.

Subjects scheduled for PK assessment, but without valid concentration data were assigned the population PK parameter value based on the subject's covariate profile. Missing efficacy data for efficacy-response analyses were predicted using a mixed model repeated-measures (MMRM) model.

Propensity score matching based on baseline HbA<sub>1c</sub>, diabetes duration, race/ethnicity, and gender was used to obtain a dataset of subjects with matched baseline characteristics from SUSTAIN and PIONEER.

All statistical tests, e.g., used during model development for the population PK models and exposure-response models, were performed using likelihood ratio tests. Details regarding summary statistics presented is given in Figure Legends.

### Software

NONMEM (ICON Development Solutions, Ellicott City, MD, USA) version 7.3 was used for the population PK analysis. Parameter uncertainty was obtained via bootstrapping via PsN version 4.60.

R version 3.2.3 was used for data file processing, exposure-response modeling and plotting. *Nls* was used for the non-linear regression models of HbA<sub>1c</sub> and body weight change from baseline. *Gnls* was used for non-linear regression models of HbA<sub>1c</sub> responder endpoints on a logit scale. *Gnls* was used to implement a weighted regression consistent with modeling of the probability of response. *Glm* was used for the more standard linear logistic regression models for proportion of subjects reporting nausea or vomiting.

Propensity matching was performed based on baseline HbA<sub>1c</sub>, trial population, diabetes duration, race, ethnicity and gender, using the MatchIt library with default settings for a logit distance and a nearest neighbor method.

### Exposure-response analysis

The exposure-response population was comprised of the PK population with addition of placebo-treated subjects from PIONEER 1, 5, 8 and 9, adding up to a total of 3,003 subjects. Pre-specified exposure-response analysis for efficacy was conducted for change from baseline of HbA<sub>1c</sub> and body weight as well as for the proportions of subjects reaching the HbA<sub>1c</sub> target defined by ADA (HbA<sub>1c</sub> < 7.0%) and AACE (HbA<sub>1c</sub> ≤ 6.5%), respectively, following 26 weeks of treatment. Pre-specified exposure-response analysis for tolerability was conducted for proportions of subjects reporting nausea and vomiting, respectively, at any time and severity during 26 weeks of treatment. Stepwise model development was executed for each endpoint, ensuring that each covariate factor in the final model demonstrated an impact on the exposure-response that was statistically significant and reasonably identifiable from exposure-response figures.

HbA<sub>1c</sub> change from baseline data was described by a maximum response ( $E_{max}$ ) model with baseline HbA<sub>1c</sub> and trial population influencing the treatment effect, and additional placebo effects of diabetes duration and baseline HbA<sub>1c</sub>. The same model structure was used to model the logit of the probability for reaching HbA<sub>1c</sub> targets.

Body weight change from baseline (%) was described by an  $E_{max}$  model with gender and baseline HbA<sub>1c</sub> influencing the treatment effect and additional placebo effects of diabetes duration, race and ethnicity.

For binary safety endpoints (patients reporting nausea and vomiting), linear models on the logit scale were used. For nausea, covariate factors were identified as trial population, gender, race, ethnicity and upper GI disease. For vomiting, covariate factors were identified for trial population and gender.

Exposure-response was illustrated in quantiles of subjects based on individual steady-state exposure levels ( $C_{avg}$ ) or placebo with up to 200 subjects per quantile (100 subjects when further subgroup stratification is used). To correct for confounding factors due to differences in demographic profiles across quantiles, mean exposure-response model predictions are provided to show estimated outcomes of changing the exposure without a simultaneous change in the distribution of subjects.<sup>44</sup> The model lines were generated as the mean model prediction across subjects, for each exposure level.

Comparison of exposure-response for oral and s.c. data was conducted by adding previously published exposure-response data from SUSTAIN trials. Propensity matching was conducted to identify the subjects treated with oral semaglutide that best match subjects investigated with s.c. semaglutide. Previous exposure-response models for s.c. semaglutide support the relationship observed across exposure quantiles for s.c. semaglutide,<sup>33,42</sup> and no updated exposure-response modeling of s.c. semaglutide are provided in the present report. The exposure-response model obtained for oral semaglutide was used to provide model derived exposure-response lines both for a population with baseline characteristics as in the matched PIONEER population and for a population with baseline characteristics as in the SUSTAIN population.

The covariate factors tested for inclusion in the model were: "baseline HbA<sub>1c</sub>, body weight, BMI, diabetes duration, gender, age, Hispanic ethnicity, Asian race, upper GI disease, renal impairment, Trial effects, and Trial population, i.e. "Monotherapy"

corresponding to PIONEER 1 and 9, “1-2 OAD” corresponding to PIONEER 2 and 3, “Moderate renal impairment” corresponding to PIONEER 5, and “Insulin treatment” corresponding to PIONEER 8.

#### Model qualification

The population PK model was qualified to meet the assumptions in the prespecified model. Model qualification included 1) Model fit to median concentration profiles, overall and across covariate subgroups 2) Standard GOF plots, including investigation of observed versus predicted PK observations, conditional weighted residuals versus predictions and time, and the distribution of the conditional weighted residuals. 3) Visual predictive checks, investigating if simulations from the model could reproduce the median and 90% range in PK observations over time. 4) Sensitivity analyses were conducted, investigating sensitivity of estimated covariate effects and individual  $C_{avg}$  values with different fixed structural PK parameters (changing to 50% higher value) and different procedures for LLOQ handling (either excluding values below LLOQ, or handling these using a censoring technique).

The exposure-response models were qualified by model fit to mean response data overall and across covariate subgroups as included in [Figures S4](#) and [S5E–S5G](#).

#### ADDITIONAL RESOURCES

The PIONEER and SUSTAIN clinical trials used to generate the pharmacokinetic data are registered and described within the following clinical trial online registration sites.

PIONEER 9: NCT03018028  
PIONEER 1: NCT02906930  
PIONEER 3: NCT02607865  
PIONEER 5: NCT02827708  
PIONEER 2: NCT02863328  
PIONEER 8: NCT03021187  
SUSTAIN 1: NCT02054897  
SUSTAIN 2: NCT01930188  
SUSTAIN 3: NCT01930188  
SUSTAIN 6: NCT01720446  
SUSTAIN Japan OAD: NCT02207374