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Supplemental information

Levels of circulating semaglutide determine reductions in HbA1c and body weight in people with type 2 diabetes

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Supplemental Material

Levels of circulating semaglutide determine pharmacodynamic outcomes in people with type 2 diabetes

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Parameter	Estimate	$CI_{95\%}$ Lower bound	$CI_{95\%}$ Upper bound	RSE (%)	IIV (%CV)
Absorption rate, 1/h constant (k_a)	2.09	Fixed	Fixed	Fixed	NA
Clearance (CL), L/h	0.039	Fixed	Fixed	Fixed	NA
Central volume (V_c), L	3.59	Fixed	Fixed	Fixed	NA
Inter-compartmental clearance (Q), L/h	0.304	Fixed	Fixed	Fixed	NA
Peripheral volume (V_p), L	4.1	Fixed	Fixed	Fixed	NA
Body weight exponent on clearance	1.01	Fixed	Fixed	Fixed	NA
Body weight exponent on volume	0.923	Fixed	Fixed	Fixed	NA
Bioavailability (F)	0.00471	0.0044	0.00498	2.99	84.3
Sex factor on F	0.814	0.76	0.868	3.38	NA
Asian factor on F	1.1	1.02	1.19	4.02	NA
Body weight factor on F	0.374	0.229	0.519	19.8	NA
Upper GI disease factor on F	1.13	1.04	1.22	3.93	NA
Trial effect of PIONEER 2	0.6	0.559	0.642	3.57	NA
Trial effect of PIONEER 3	0.758	0.651	0.866	7.22	NA
Residual error (%CV)	45.4	44.4	46.5	1.17	NA

Supplementary Table 1: Parameter estimates for the final PK model for oral semaglutide, related to Table 1. A number of parameters were not estimated based on PIONEER, but fixed based on a PK model developed based on clinical pharmacology trials from oral semaglutide. CI: confidence interval, RSE: relative standard deviation, IIV: inter-individual (between-subject) variation, GI: gastrointestinal

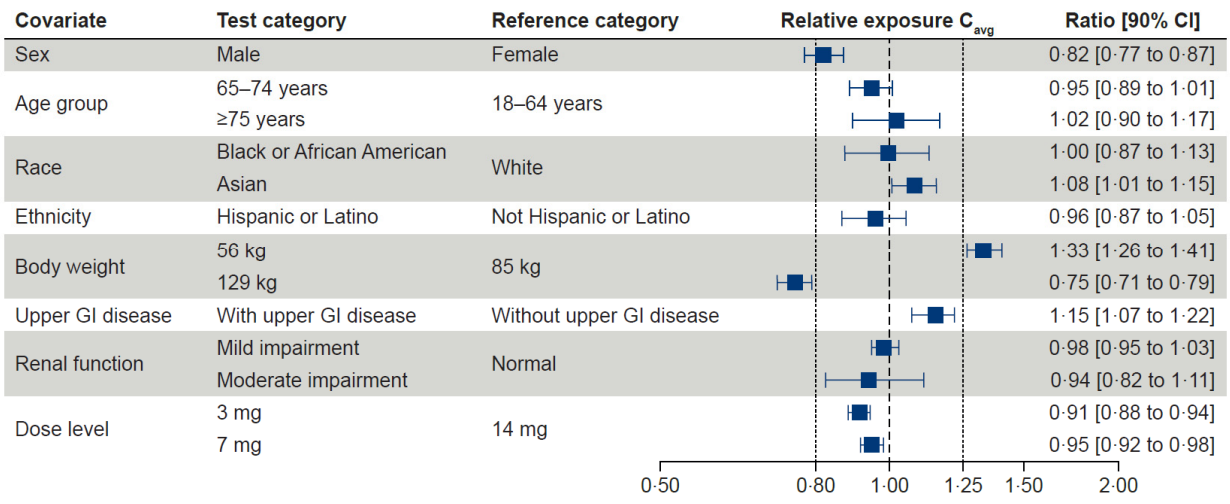

Figure S1 Relative exposure of oral semaglutide by multiple covariates, related to Table 1 Relative exposure of semaglutide by multiple covariates. Data are average dose-normalised semaglutide concentrations relative to a reference subject profile (White, non-Hispanic or Latino female aged 18-64 years, with body weight of 85 kg, without upper GI disease or renal impairment, dosed 14 mg). The plot and the right column show means and 90% CIs. Body weight categories (56 and 129 kg) represent the 5% and 95% percentiles in the data. Vertical dotted lines indicate the acceptance interval for bioequivalence [0.80–1.25]. Data from PIONEER 1, 2, 3, 5, 8 and 9. Sex, Asian race, body weight, and presence of upper GI disease were associated with statistically significant different exposure levels, whereas the remaining demographic covariates were not. C_{avg}, average concentration; CI, confidence interval; GI, gastrointestinal.

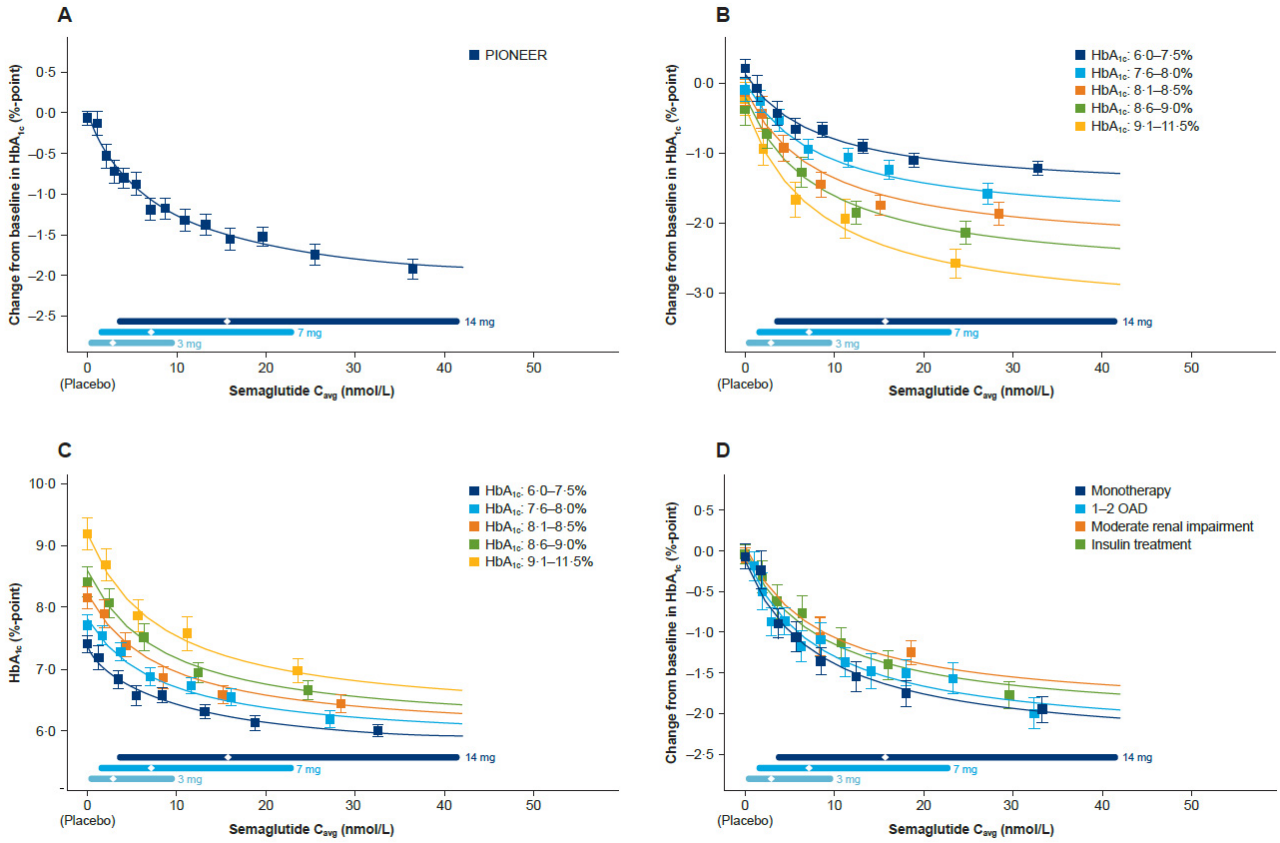

Figure S2A-D: HbA_{1c} change from baseline versus drug exposure after oral semaglutide administration in the PIONEER trials, related to Figures 1 and 3 HbA_{1c} change from baseline versus semaglutide exposure; overall (A), stratified by baseline HbA_{1c} (B), and by trial population (D). Panel C shows absolute values of HbA_{1c} versus exposure by baseline HbA_{1c}. Data are mean HbA_{1c} response values with 95% CI obtained after 26 weeks of treatment versus exposure expressed as quantiles of C_{avg} (plus placebo at C_{avg} of 0 mmol/L). The lines through data represent model-derived exposure-response relations for each population. Horizontal lines with diamonds along the x-axes represent medians and 90% exposure ranges. Data from PIONEER 1,2,3,5,8,9. Baseline HbA_{1c} and trial population (subgroups described in S2D) were associated with a statistically significant impact on the exposure-response of semaglutide in the final model for HbA_{1c}, whereas the remaining covariate factors were not. C_{avg}, average concentration; CI, confidence interval; OAD, oral antidiabetic drug

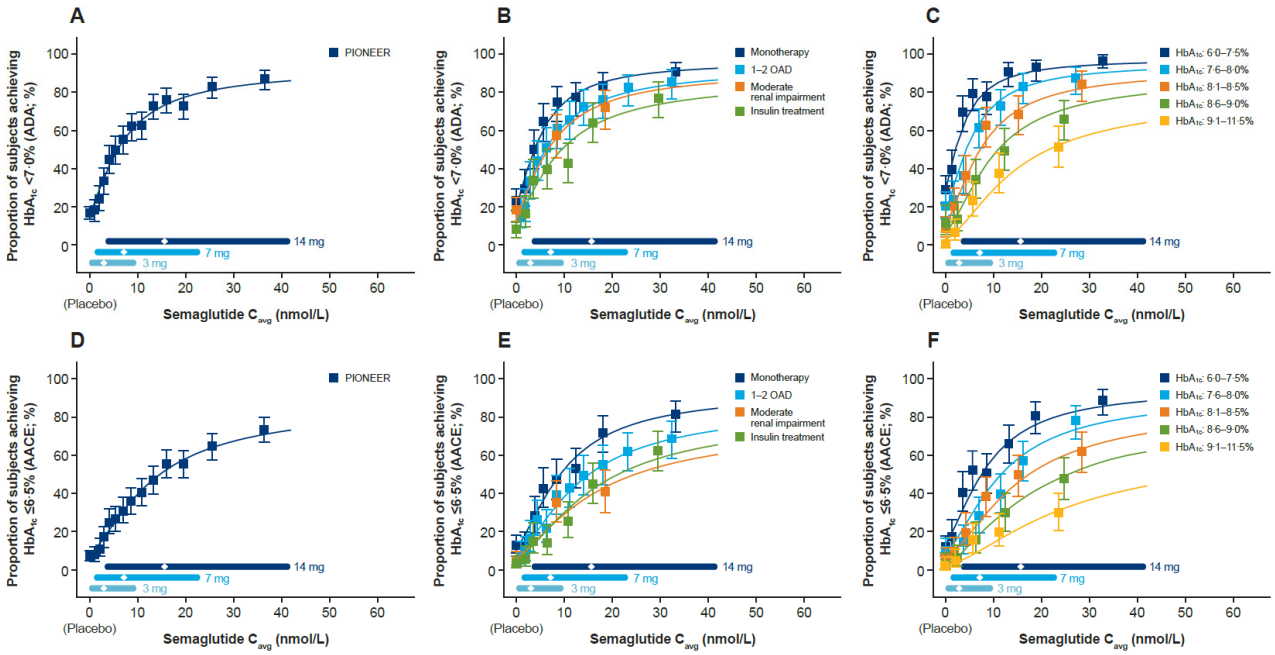

Figure S3A-F: Proportion of subjects reaching HbA_{1c} <7% and ≤6.5% reflects semaglutide exposure in the PIONEER trials, related to Figure 4 Proportion of subjects reaching HbA_{1c} <7% versus semaglutide exposure; overall (A), stratified by trial population (B), and by baseline HbA_{1c} (C). Proportion of subjects reaching HbA_{1c} ≤6.5% versus semaglutide exposure; overall (D), stratified by trial population (E), and by baseline HbA_{1c} (F). Data are proportion of subjects with 95% CI following 26 weeks of treatment versus exposure expressed as quantiles of C_{avg} (plus placebo at C_{avg} of 0 nmol/L). The lines through data represent model-derived exposure-response relations for each trial population. Horizontal lines with diamonds along the x-axes represent medians and 90% exposure ranges. Baseline HbA_{1c} and trial population (subgroups described in S3E) were associated with a statistically significant impact on the exposure-response of semaglutide in the final model for HbA_{1c}, whereas remaining covariate factors investigated were not. ADA, American Diabetes Association; AACE, American Association of Clinical Endocrinologists; C_{avg}, average concentration; CI, confidence interval; OAD, oral antidiabetic drug

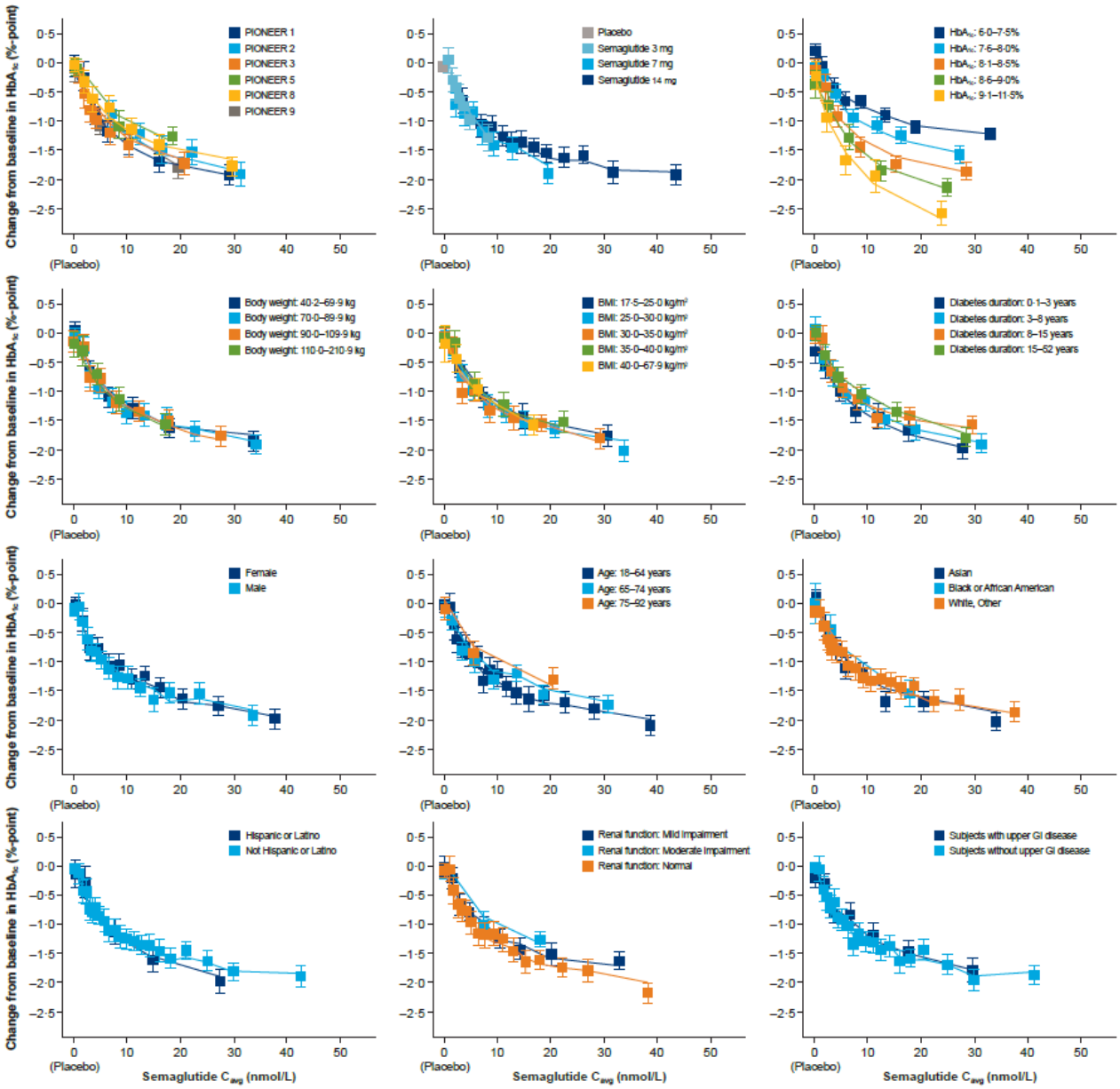

Figure S4: Model fit to observed data for HbA_{1c} change from baseline by trial, dose, HbA_{1c} baseline, body weight baseline, BMI, diabetes duration, sex, age group, race, ethnicity, renal dysfunction and presence of upper GI disease in the PIONEER trials, related to Figure 4 Data are mean HbA_{1c} response values with 95% CI obtained after 26 weeks of treatment versus exposure expressed as quantiles of C_{avg} (plus placebo at C_{avg} of 0 mmol/L). The lines through data represents mean model predictions for each exposure quantile. Data from PIONEER 1, 2, 3, 5, 8, 9. Baseline HbA_{1c} and trial population were associated with a statistically significant impact on the exposure-response of semaglutide in the final model for HbA_{1c}, whereas the remaining covariate factors investigated were not. BMI, body mass index; C_{avg}, average concentration; CI, confidence interval; GI, gastrointestinal

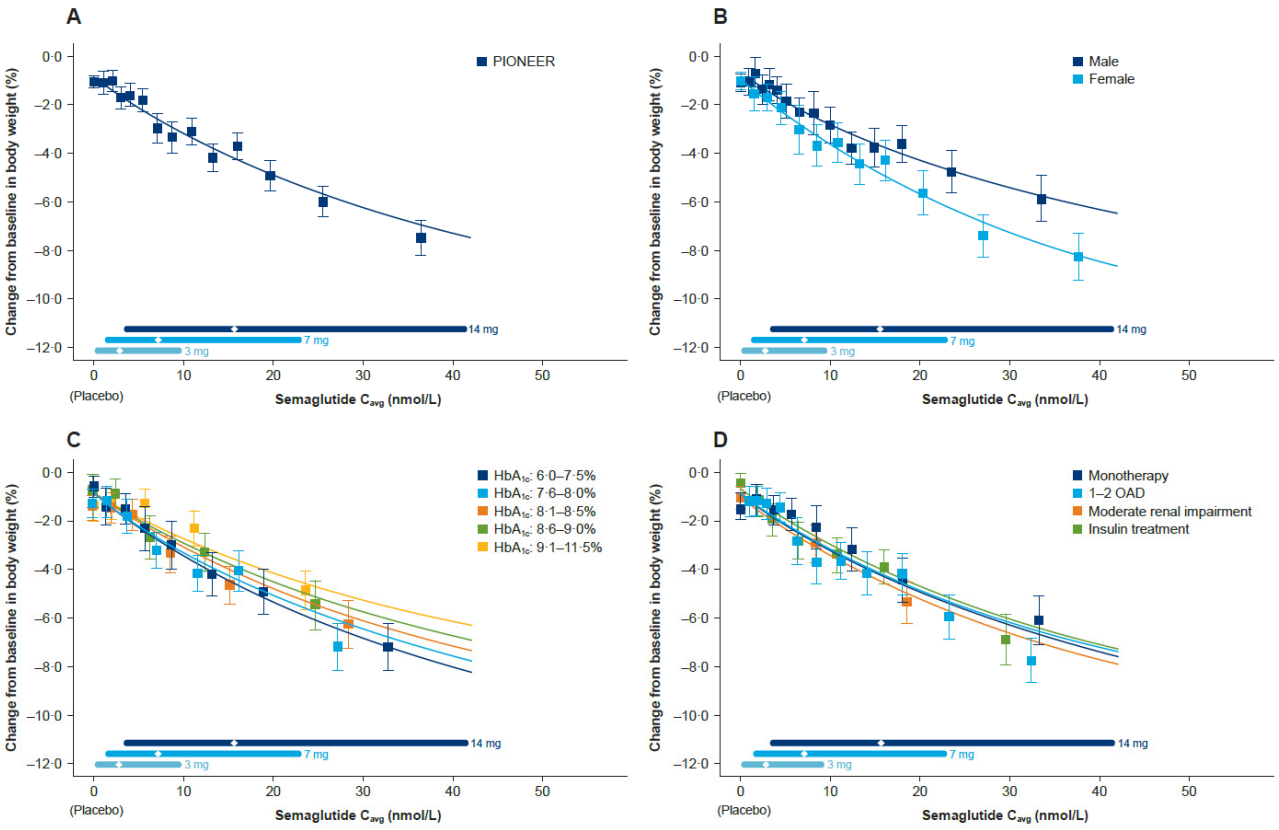

Figure S5A-D: Body weight change from baseline is determined by semaglutide exposure in the PIONEER trials, related to Figures 1 and 4 Body weight change (%) from baseline versus semaglutide exposure; overall (A), stratified by sex (B), by baseline HbA_{1c} (C) and by trial population (D). Data are mean %-body weight loss values with 95% CI following 26 weeks of treatment versus exposure expressed as quantiles of C_{avg} (plus placebo at C_{avg} of 0 nmol/L). The lines through data represent model-derived exposure-response relations for each population. Horizontal lines with diamonds along the x-axes represent medians and 90% exposure ranges. C_{avg}, average concentration; Baseline HbA_{1c} and sex were associated with statistically significant impact on the exposure-response of semaglutide in the final model for weight loss, whereas the remaining covariate factors investigated were not. CI, confidence interval; OAD, oral antidiabetic drug

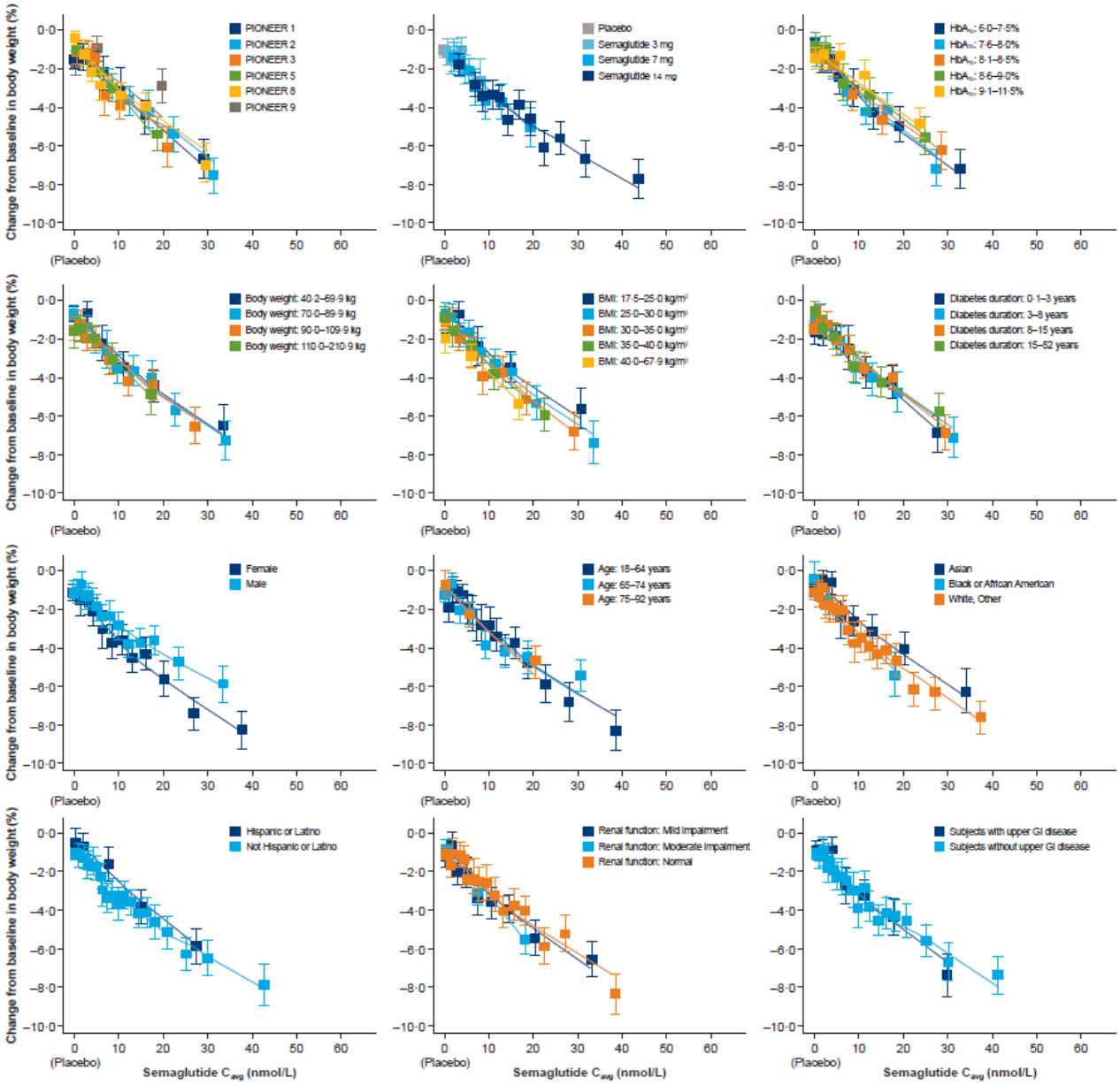

Figure S5E: Model fit to observed data for body weight change from baseline with oral semaglutide by trial, dose, HbA_{1c} baseline, body weight baseline, BMI, diabetes duration, sex, age group, race, ethnicity, renal dysfunction and presence of upper GI disease, related to Figures 1 and 4 Data are mean body weight response values with 95% CI obtained after 26 weeks of treatment versus exposure expressed as quantiles of C_{avg} (plus placebo at C_{avg} of 0 mmol/L). The lines through data represents mean model predictions for each exposure quantile. Data from PIONEER 1, 2, 3, 5, 8, 9. Baseline HbA_{1c} and sex were associated with a statistically significant impact on the exposure-response of semaglutide in the final model for weight loss, whereas the remaining covariate factors investigated were not. BMI, body mass index; C_{avg}, average concentration; CI, confidence interval; GI, gastrointestinal

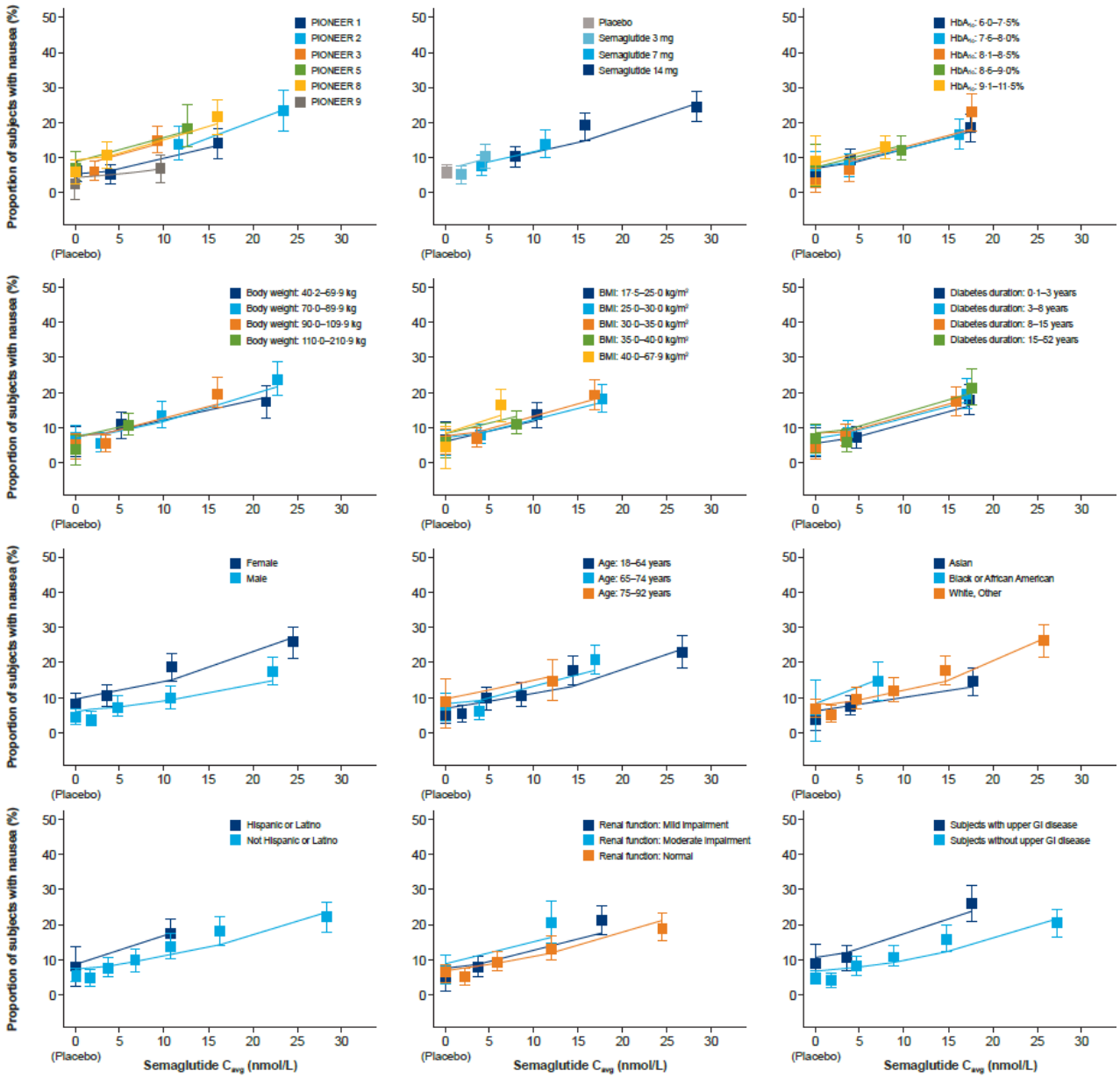

Figure S5F: Model fit to observed data for nausea by trial, dose, HbA_{1c} baseline, body weight baseline, BMI, diabetes duration, sex, age group, race, ethnicity, renal dysfunction and presence of upper GI disease with oral semaglutide administration, related to Figures 1 and 4 and Table 1 Data are proportion of subjects reporting nausea with 95% CI obtained after 26 weeks of treatment versus exposure expressed as quantiles of C_{avg} (plus placebo at C_{avg} of 0 mmol/L). The lines through data represents mean model predictions for each exposure quantile. Data from PIONEER 1, 2, 3, 5, 8, 9. Trial population, sex, Hispanic ethnicity, presence of upper GI, and Asian race were associated with a statistically significant impact on the exposure-response of semaglutide in the final model for nausea, whereas remaining covariate factors investigated were not. BMI, body mass index; C_{avg}, average concentration; CI, confidence interval; GI, gastrointestinal

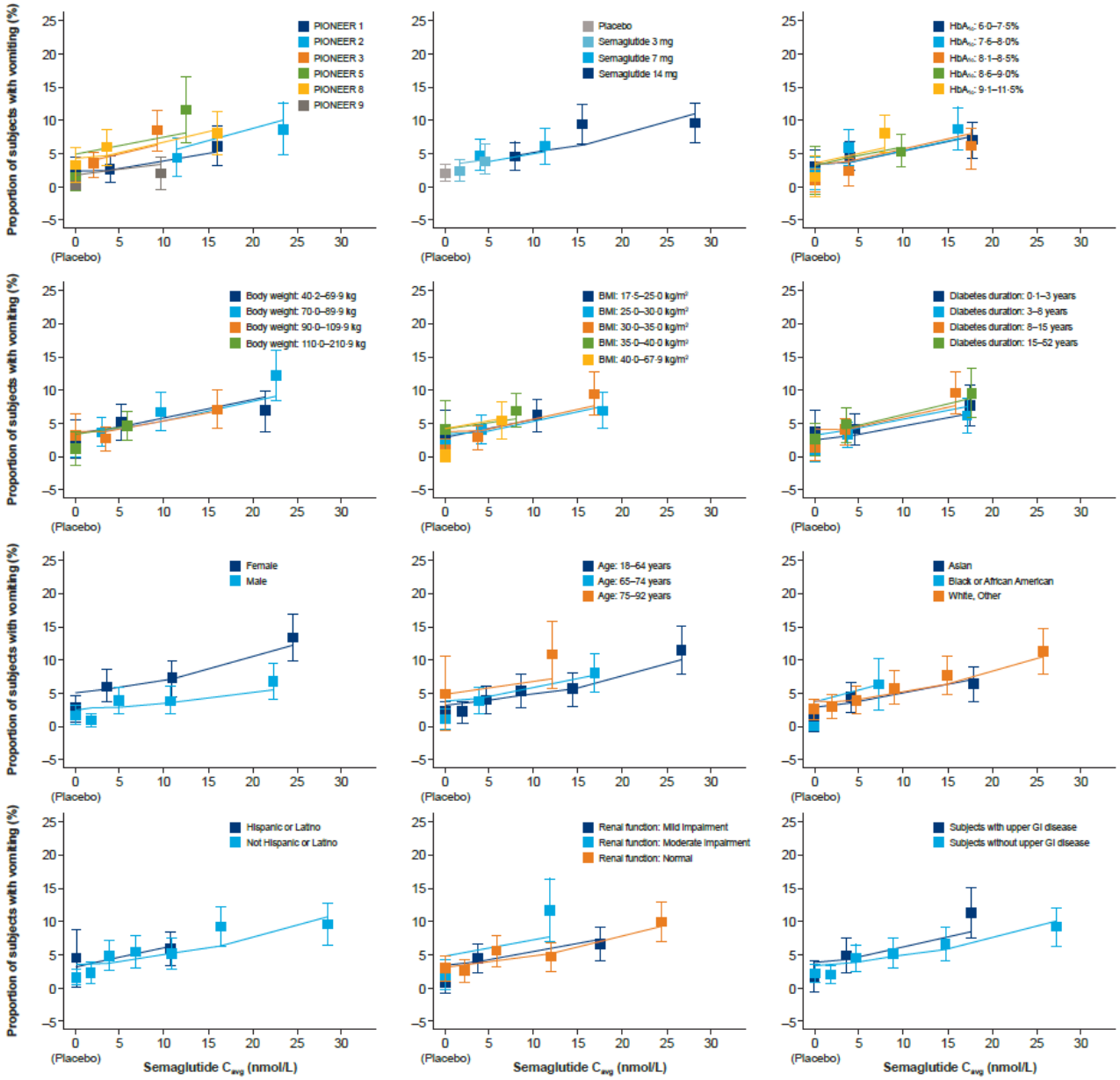

Figure S5G: Model fit to observed data for vomiting by trial, dose, HbA_{1c} baseline, body weight baseline, BMI, diabetes duration, sex, age group, race, ethnicity, renal dysfunction and presence of upper GI disease, related to Figures 1 and 4 and Table 1

Data are proportion of subjects reporting vomiting with 95% CI obtained after 26 weeks of treatment versus exposure expressed as quantiles of C_{avg} (plus placebo at C_{avg} of 0 mmol/L). The lines through data represents mean model predictions for each exposure quantile. Data from PIONEER 1, 2, 3, 5, 8, 9. Trial population and sex were associated with a statistically significant impact on the exposure-response of semaglutide in the final model for vomiting, whereas the remaining covariate factors investigated were not. BMI, body mass index; C_{avg}, average concentration; CI, confidence interval; GI, gastrointestinal

Methods S1. Oral semaglutide population PK model: Parameterization and NONMEM settings for parameter estimation related to STAR Methods

```
$SUBROUTINE ADVAN4 TRANS4
$THETA (0, 2.09, 10)  FIX    ; 1 ; KA [1/h]  ;Absorption rate constant
$THETA (0, 0.0390, 1) FIX    ; 2 ; CL [L/h]  ;Apparent Clearance
$THETA (0, 3.59, 20)  FIX    ; 3 ; V2  [L]    ;Apparent central volume of distribution
$THETA (0, 0.304, 1)  FIX    ; 4 ; Q [L/h]  ;Apparent Clearance
$THETA (0, 4.1, 20)   FIX    ; 5 ; V3  [L]    ;Apparent peripheral volume of distribution
$THETA (0, 1.01, 1.5) FIX    ; 6 ; BW CL []   ;BW exponent Clearance
$THETA (0, 0.923, 1.5)FIX    ; 7 ; BW V[]   ;BW exponent Volume
$THETA (0, 0.006, 1)         ; 8 ; BA []  ;Mean Bioavaiabillity
$THETA (0, 0.578, 5)           ; 9 ; SIGMA []
$THETA (0, 0.8, 4)             ; 10 ; MALE_BA []
$THETA (0, 1, 4)   FIX          ; 11 ; AGE_BA []
$THETA (0, 1., 4) FIX            ; 12 ; AGE_BA 75 []
$THETA (0, 1, 4)   FIX           ; 13 ; RACE_BLACK_BA []
$THETA (0, 1., 4)             ; 14 ; RACE_ASIAN_BA []
$THETA (0, 1, 4)   FIX          ; 15 ; HISPANIC_BA []
$THETA (-4, 0.39, 4)          ; 16 ; BW_BA []
$THETA (0, 1.15, 4)             ; 17 ; GI_BA []
$THETA (-4, 0.0, 4)  FIX        ; 18 ; EGFR_BA []
$THETA (0, 1, 4)  FIX           ; 19 ; DOSE_3_BA []
$THETA (0, 1, 4)  FIX           ; 20 ; DOSE_7_BA []
$THETA (0, 1, 4) FIX            ; 21 ; PIONEER2_BA []
$THETA (0, 0.6, 4)              ; 22 ; PIONEER3_BA []
$THETA (0, 0.7, 4)            ; 23 ; PIONEER5_BA []
$THETA (0, 1, 4)  FIX           ; 24 ; PIONEER8_BA []
$THETA (0, 1., 4) FIX            ; 25 ; PIONEER9_BA []

$OMEGA 0. FIX     ; 1 ; KA.IIV
$OMEGA 0.4        ; 2 ; BA.IIV
$OMEGA 0.0 FIX    ; 3 ; V2.IIV
$OMEGA 0.0 FIX    ; 4 ; CL.IIV

$SIGMA 1 FIX              ; Prop. Error

$PK
MW=4113.6/1000000 ; mg/nmol
SIG=THETA(9)

; Fixed parameters
TVKA=THETA(1)                       ; Absorption rate constant
TVCL=THETA(2)*(BW/85)**THETA(6)     ; Clearance
COVCL=(BW/85)**THETA(6)
TVV2=THETA(3)*(BW/85)**THETA(7)     ; Central volume
TVQ= THETA(4)*(BW/85)**THETA(6)     ; Intercompartmental clearance
TVV3=THETA(5)*(BW/85)**THETA(7)     ; Peripheral volume

KA=TVKA*EXP(ETA(1))
CL=TVCL*EXP(ETA(4))
V2=TVV2*EXP(ETA(3))
V3=TVV3*EXP(ETA(3))
Q=TVQ*EXP(ETA(4))


COV=(THETA(10)**MALE)*(THETA(11)**AGE65)*(THETA(12)**AGE75)*(THETA(13)**BLACK)*(THETA(14)**ASIAN)*
(THETA(15)**HISP)
COV=COV*((BW/85)**THETA(16))*(THETA(17)**GI)*((EGFR/100)**THETA(18))*(THETA(19)**DL3)*(THETA(20)**
DL7)
COV=COV*(THETA(21)**P2)*(THETA(22)**P3)*(THETA(23)**P5)*(THETA(24)**P8)*(THETA(25)**P9)

; Bioavailability
TVBA=THETA(8)*COV
BA=TVBA*EXP(ETA(2))       ; BA is the mean bioavailability
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
```

```
F1=BA

S2=V2*MW                  ; AMT_unit:mg, A2_unit:  mg, DV_unit: nM
AUC   = (BA*DLVL/CL) * (1/MW)
CAVG   = AUC/24
TVAUC   = (TVBA*DLVL/ TVCL) * (1/MW)
TVCAVG   = TVAUC/24


$ERROR
DEL=0
IF(F.LE.0) DEL=0.001
IPRE = F
Y    = F+ (F*SIG)*EPS(1)

$ESTIMATION MAXEVALS=9999 METHOD=1 INTER NSIG=3 SIGL=9 NOABORT PRINT=5 MSFO=msfo.msf
```

Methods S2. Subcutaneous semaglutide population PK model: Parameterization and NONMEM settings for parameter estimation related to STAR Methods

```
$SUBROUTINES ADVAN2 TRANS2

$THETA 0.0286 FIX       ; 1 ; KA [1/h]     ;Absorption rate constant
$THETA (0, 0.0479)      ; 2 ; CL/F [L/h]   ;Apparent Clearance
$THETA (0, 5.7)         ; 3 ; V/F [L]      ;Apparent Volume of Distribution
$THETA  (0, 0.774, 1.5)            ; 4  ;Body weight exponent on CL/F
$THETA  (0, 1.04, 2)               ; 5  ;Gender contrast (MALE/FEMALE) on CL/F
$THETA  (0, 0.988, 2)              ; 6  ;Age contrast 65-74 y / <65years on CL/F
$THETA  (0, 0.960, 2)              ; 7  ;Age contrast >74 / <65years on CL/F
$THETA  (0, 1.0, 2)                ; 8  ;Dose level 0.5 mg cohort /1.0 mg cohort on CL/F
$THETA  (0, 0.974, 2)              ; 9  ;Race contrast (BLACK A.A./WHITE) on CL/F
$THETA  (0, 0.988, 2)              ; 10 ;Race contrast (ASIAN/WHITE) on CL/F
$THETA  1 FIX                      ; 11 ;Race contrast (OTHER/WHITE) on CL/F
$THETA  (0, 1.07, 2)               ; 12 ;Ethnicity contrast (H.L/Non H.L.) on CL/F
$THETA  (0, 1.04, 2)               ; 13 ;Injection site (THIGH/ABDOMINAL SKIN) on CL/F
$THETA  (0, 1.08, 2)               ; 14 ;Injection site (UPPER ARM/ABDOMINAL SKIN) on CL/F
$THETA  (0, 0.95, 2)               ; 15 ;Renal impairment (MILD/NORMAL) on CL/F
$THETA  (0, 0.95, 2)               ; 16 ;Renal impairment (MODERATE/NORMAL) on CL/F
$THETA  (0, 0.95, 2)               ; 17 ;Renal impairment (SEVERE/NORMAL) on CL/F

$OMEGA 0 FIX            ; 1 ; KA.IIV
$OMEGA 0.0165           ; 2 ; CL.IIV
$OMEGA 0.162            ; 3 ; V.IIV
$SIGMA 0.05                   ; Prop. Error

$PK
MW = 4113.6/1000000  ; mg/nmol

NBW = 85 ;;; Chosen body weight (kg) for normalisation

;;; Absorption rate constant
TVKA = THETA(1)
KA = TVKA*EXP(ETA(1))  ; KA fixed to 0.0286, as in ClinPharm Ph1 single comp MD PK model. ETA(1)
to be set to 0 according to MAP.

TVCL = THETA(2)
TVV = THETA(3)
V = TVV*EXP(ETA(3))

;;; Covariates
; Body weight
```

```
CLBW=(BW/NBW)**THETA(4)

; Gender (reference: FEMALE)
CLGENDER=1
IF (MALE.EQ.1) CLGENDER = THETA(5)   ; MALE/FEMALE

; Age (reference: < 65 years)
CLAGE=1
IF (AGEFL.EQ.1) CLAGE = THETA(6)     ; 65-74 y / <65years
IF (AGEFL.EQ.2) CLAGE = THETA(7)     ; >74 / <65years

; Dose level (reference: 1.0 mg)
CLDLVL=1
IF (DLVL.EQ.0.5) CLDLVL = THETA(8)   ; 0.5 mg cohort /1.0 mg cohort

; RACE (reference: WHITE)
CLRAC=1
IF (RACE.EQ.2)  CLRAC = THETA(9)      ; BLACK A.A./WHITE
IF (RACE.EQ.3)  CLRAC = THETA(10)     ; ASIAN/WHITE
IF (RACE.EQ.0)  CLRAC = THETA(11)     ; OTHER/WHITE
IF (RACE.EQ.6)  CLRAC = THETA(11)     ; OTHER/WHITE
IF (RACE.EQ.-1) CLRAC = THETA(11)     ; OTHER/WHITE

; Ethnicity (reference: NOT HISPANIC OR LATINO)
CLETHN=1
IF (ETHN.EQ.2) CLETHN = THETA(12)    ; H.L./NOT H.L.

; Injection site (reference: ABDOMINAL SKIN)
CLINJ=1
IF (INJSIT.EQ.3) CLINJ = THETA(13)       ; THIGH/ABDOMINAL SKIN
IF (INJSIT.EQ.2) CLINJ = THETA(14)       ; UPPER ARM/ABDOMINAL SKIN

; Renal impairment (reference: Normal, also including one NA)
CLREN=1
IF (RENAL.EQ.2) CLREN = THETA(15)       ; MILD/NORMAL
IF (RENAL.EQ.3) CLREN = THETA(16)       ; MODERATE/NORMAL
IF (RENAL.EQ.4) CLREN = THETA(17)       ; SEVERE/NORMAL

;;; Combine all covariate effects on clearance
TVCL = TVCL * CLBW * CLGENDER * CLAGE * CLDLVL * CLRAC * CLETHN * CLINJ * CLREN

;;; Add inter-individual variation
CL = TVCL * EXP(ETA(2))

;;; Output scaling
S2 = V*MW   ; scale ug dose to nmol/L concentrations
F1 = 1

;;; Estimate AUC (in µM*h) - using ADOS column - based on AMT
AUCA   = ADOS * 1000 / CL * (1000/4113.6)
AUC   = DLVL * 1000 / CL * (1000/4113.6)
CAVG   = AUC/168

$ERROR
IPRE = F
W = F
IRES = DV - IPRE
IWRES = IRES/W
Y = F + W*EPS(1)
$EST METHOD=1 INTERACTION PRINT=5 MAX=9999 MSFO=msf
```