Supplementary Material

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Characterization of the Pharmacokinetic and Pharmacodynamic Profile of Apraglutide, a Glucagon-Like Peptide-2 Analog, in Healthy Volunteers

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Appendix 1. Pharmacokinetic/Pharmacodynamic Model Description

Population pharmacokinetic (PK)/pharmacodynamic (PD) parameters were estimated using the stochastic approximation of expectation-maximization algorithm implemented in Monolix Suite 2019 R1 (Lixoft SAS; https://lixoft.com/download/win64-monolix-suite-2019r1/). Observations that were below the lower limit of quantification (LLOQ) were included in the estimation of the population parameters using the M3 method [1]. For study GYM-P3-698, observations after intravenous administration were excluded from the analysis because the subcutaneous (SC) route is the planned route of administration. Only pre-dose citrulline observations were considered for modeling. Apraglutide was administered in the morning after overnight fasting, thereby excluding any food effects from the analysis. Standard errors of parameter estimates were derived from the Fisher information matrix using stochastic approximation. For individual parameters, conditional means and standard deviations were computed. The −2 log-likelihood (−2LL) was computed using importance sampling. Rsmlx 2.0.2 was used to build the covariate model [2]. For both studies, baseline body weight and clinical biochemistry observations from the screening visit were used for the covariate analysis.

Population Pharmacokinetic/Pharmacodynamic Structural Model

The structural model used to describe apraglutide PK was 1-compartmental with a volume of distribution $V1$ and linear clearance Cl. Apraglutide absorption was modeled as a zero-order process from the SC depot parameterized by the absorption duration $Tk0$ (Figure S2). Plasma citrulline concentrations were described with a

turnover model with a stimulatory effect on the citrulline production rate. For the stimulatory effect, a sigmoid E_{max} model was used:

$$\frac{dR(t)}{dt} = k_{syn}\left(1 + E_{max} \cdot \frac{C(t)^{\gamma}}{C(t)^{\gamma} + EC_{50}^{\gamma}}\right) - k_{deg}R(t),$$

where $R(t)$ is the citrulline concentration at time t with $R(0)$ describing the pre-dose citrulline baseline in µg/mL, and $C(t)$ is the apraglutide plasma concentration at time t in ng/mL. The synthesis and degradation rates of citrulline are represented with k_{syn} in µg/mL/day and k_{deg} in 1/day, respectively. The unitless E_{max} describes the maximal effect, EC_{50} the half-maximal effective apraglutide concentration in ng/mL, and the unitless γ the Hill coefficient.

Population Pharmacokinetic/Pharmacodynamic Stochastic Model

To determine the between-subject variability (BSV) of the PK and PD parameters, individual parameters were modeled using log-normal distributions. The equation for an individual parameter was

$$\varphi_i = \varphi_{pop}\, e^{\eta},$$

where φ_{pop} is the population typical parameter, and η is a random variable with mean 0 and standard deviation ω. Continuous covariates were modeled with the equation

$$\varphi_i = \varphi_{pop}\left(\frac{\Omega_i}{\Omega_{pop}}\right)^{\beta} e^{\eta},$$

where Ω_i is the covariate of subject i, Ω_{pop} is the reference value, and β is the estimated covariate coefficient. Categorical covariates were modeled with the equation

$$\varphi_i = \varphi_{pop}\, e^{(\beta \Omega_i)} e^{\eta},$$

where $\Omega_i = 1$ if the individual covariate is in the category and $\Omega_i = 0$ otherwise, and β is the estimated covariate coefficient. A combined error model inclusive of additive and proportional errors was used to model the PK/PD observations:

$$y_{obs} = y_{pred} + \left(a_i + b_i y_{pred}\right)\varepsilon,$$

where y_{obs} is the observed apraglutide or citrulline concentration, y_{pred} is the model prediction, ε is an independent random variable normally distributed with mean 0 and variance 1, and a_i and b_i are the parameters of the error model, with $i = 1$ representing the apraglutide error model and $i = 2$ representing the citrulline error model.

Model Development

The population PK model with the covariate effects was developed independently of the PD data. In a second step, the PK/PD data were combined, and a full PK/PD model was developed while maintaining the previously determined structural and statistical properties of the PK model. To build the PK covariate model, conditional sampling for the stepwise approach based on correlation tests (COSSAC) with the Rsmlx library was used [2]. Model selection with COSSAC was based on a statistically significant difference in the −2LL (chi-squared test) at a level of significance of $p = 0.01$. For the covariate search, the typical PK covariates of age, sex, body weight, and ethnicity were included. Baselines of albumin concentration, alanine aminotransferase concentration, total bilirubin concentration, and creatinine clearance were additionally tested as covariates. Further, dose was included in the

covariate testing. The reference values for the continuous covariates were 5 mg for the dose, 35 years for age, 70 kg for body weight, 47 g/L for albumin, 16 U/L for alanine aminotransferase, 8 µmol/L for total bilirubin, and 100 mL/min for creatinine clearance. For the categorical covariates, the reference values were male for sex and White for ethnicity. Dose, age, sex, body weight, and ethnicity were tested on all parameters (Tk0, Cl/F, V1/F). Albumin was tested on V1/F and Cl/F. Alanine aminotransferase and total bilirubin were tested on V1/F, while creatinine clearance was tested on Cl/F. No covariates were tested on the PD parameters.

Model Selection

To evaluate the model, goodness of fit (GoF) plots and the relative standard error of the parameter estimates were used as generated by Monolix. GoF plots were constructed using random sampling from the conditional distributions of the individual parameters [3]. Data below the limit of quantification (BLQ) were included in the diagnostic plots by sampling the BLQ predictions from the conditional distribution.

Pharmacokinetic/Pharmacodynamic Simulations

Simulations were performed in R using the mlxR library [2]. The simulations included the covariate effect but not BSV. Simulations were also performed for body weights of 40, 50, 60, 70, 80, 90, 100, 110, and 120 kg with subcutaneous apraglutide at doses of 2.5, 5, and 10 mg. Week 6 AUC_T was calculated for each body weight using the PKNCA library [4].

Table S1. Baseline Characteristics of Subjects in the Present Study (TA799-002) by Treatment Arm

	Apraglutide				All Subjects
	1 mg (N=6)	**5 mg (N=6)**	**10 mg (N=6)**	**Placebo (N=6)**	**(N=24)**
Male, n (%)	3 (50)	3 (50)	4 (67)	3 (50)	13 (54)
Age (mean ± SD), y	26.7±6.3	24.5±4.0	27.7±7.8	29.2±7.5	27.0±6.4
Height (mean ± SD), cm	178.02±8.11	178.52±11.39	175.48±8.29	175.67±11.19	176.92±9.31
Weight (mean ± SD), kg	75.68±12.54	67.55±11.07	72.15±14.24	71.23±12.38	71.65±12.12
BMI (mean ± SD), kg/m^2	23.72±2.02	21.13±2.41	23.22±2.22	22.98±2.30	22.76±2.32
American Indian or Alaska Native/Asian/Mixed/White, n	0/0/1/5	1/1/0/4	0/1/0/5	0/0/0/6	1/2/1/20
Baseline citrulline concentration (LSM), µg/mL	5.65	6.60	6.97	5.34	5.46 (2.6–8.2)
Alanine aminotransferase (mean ± SD), U/L	18.3±4.7	19.7±8.9	20.0±6.1	21.8±14.8	16.0 (11.0–39.0)
Albumin (mean ± SD), g/L	47.8±2.9	47.7±3.9	47.8±3.2	49.2±1.7	47.0 (42.0–52.0)
Creatinine (mean ± SD), µmol/L	83.2±13.6	74.2±14.0	80.3±14.5	78.3±13.1	110.8 (81.3–172.4)
Total bilirubin (mean ± SD), µmol/L	10.8±6.0	11.8±8.7	9.2±4.9	12.0±4.6	8.0 (4.0–23.0)

LSM=least-squares mean.

Table S2. Treatment-Emergent Adverse Events

	Treatment Arm							
	1 mg Apraglutide (N=6)		**5 mg Apraglutide (N=6)**		**10 mg Apraglutide (N=6)**		**Placebo (N=6)**	
Relatedness	*Related*	*Unrelated*	*Related*	*Unrelated*	*Related*	*Unrelated*	*Related*	*Unrelated*
System organ class/preferred term	n	n	n	n	n	n	n	n
Any events	**1**	**5**	**3**	**7**	**6**	**7**	**3**	**3**
Eye disorders	-	-	-	-	-	-	-	1
Blepharitis	-	-	-	-	-	-	-	1
Gastrointestinal disorders	1	1	1	-	2	-	3	-
Abdominal pain lower	-	-	1	-	-	-	1	-
Abdominal pain upper	1	-	-	-	2	-	2	-
Breath odor	-	1	-	-	-	-	-	-
Constipation	-	-	-	-	1	-	1	-
Diarrhea	-	-	-	-	1	-	-	-
Nausea	-	-	-	-	2	-	1	-
Vomiting	1	-	-	-	1	-	-	-
General disorders and administration site conditions	-	-	-	1	3	2	-	-
Administration site erythema	-	-	-	-	1	-	-	-
Administration site hematoma	-	-	-	-	-	1	-	-
Administration site rash	-	-	-	-	1	1	-	-
Influenza-like illness	-	-	-	1	-	-	-	-

Injection site pain	-	-	-	-	1	-	-	-
Infections and infestations	-	2	-	2	-	2	-	1
Folliculitis	-	-	-	-	-	1	-	-
Nasopharyngitis	-	1	-	1	-	1	-	1
Pharyngitis	-	1	-	1	-	-	-	-
Injury, poisoning, and procedural complications	-	1	-	1	-	-	-	2
Blister	-	-	-	-	-	-	-	1
Muscle hemorrhage	-	-	-	-	-	-	-	1
Muscle strain	-	1	-	-	-	-	-	-
Skin injury	-	-	-	1	-	-	-	-
Investigations	-	-	2	-	1	-	-	-
Liver function test abnormal	-	-	2	-	1	-	-	-
Musculoskeletal and connective tissue disorders	-	-	-	-	-	1	-	-
Bone pain	-	-	-	-	-	1	-	-
Tendon pain	-	-	-	-	-	1	-	-
Nervous system disorders	-	1	-	1	1	1	-	1
Dizziness	-	-	-	-	1	-	-	-
Headache	-	1	-	1	-	-	-	1
Hypoesthesia	-	-	-	-	-	1	-	-
Vertigo	-	-	-	-	-	1	-	1
Reproductive system and breast disorders	-	-	-	1	-	-	-	-
Premenstrual cramps	-	-	-	1	-	-	-	-
Respiratory, thoracic, and mediastinal disorders	-	-	-	1	-	-	-	-

Nasal congestion	-	-	-	1	-	-	-	-
Skin and subcutaneous tissue disorders	-	1	-	1	-	1	-	2
Dermatitis contact	-	-	-	1	-	-	-	-
In-growing nail	-	-	-	-	-	1	-	-
Rash pruritic	-	-	-	-	-	-	-	1
Scratch	-	1	-	-	-	-	-	-
Sunburn	-	-	-	-	-	-	-	- 1

TEAE=treatment-emergent adverse event.

Unrelated TEAEs include all TEAEs considered unrelated or unlikely related to study treatment; related TEAEs include all TEAEs possibly or probably related to study treatment.

Table S3. Predicted Plasma Apraglutide and Citrulline Levels per Kilogram Body Weight

		Plasma Apraglutide		Plasma Citrulline
Body Weight, kg	**Dose, mg**	**AUC_T, ng·day/mL**	**C_{max}, ng/mL**	**C_{trough}, µg/mL**
40	2.5	420.2 (145.4, 947.1)	177.5 (56.01, 467.0)	7.726 (2.228, 24.01)
	5	865.9 (244.0, 2678)	346.7 (102.7, 1106)	8.671 (2.019, 31.77)
	10	1714 (596.3, 4576)	635.8 (209.8, 1709)	8.907 (2.822, 31.38)
50	2.5	295.4 (88.33, 705.3)	122.8 (30.47, 383.0)	7.608 (2.159, 22.38)
	5	571.1 (200.3, 1725)	220.6 (67.05, 767.9)	8.601 (2.851, 37.42)
	10	1120 (443.0, 2641)	406.5 (137.9, 1267)	9.164 (2.846, 28.09)
60	2.5	205.2 (75.51, 542.5)	83 07 (24.69, 215.1)	7.025 (2.349, 22.43)
	5	411.9 (130.0, 1173)	158.1 (49.06, 517.3)	8.170 (2.185, 29.31)
	10	866.9 (285.2, 2270)	313.8 (93.92, 850.4)	8.672 (2.560, 28.78)
70	2.5	158.2 (56.01, 417.7)	63.90 (19.00, 181.3)	6.999 (1.640, 19.34)
	5	309.8 (102.4, 924.8)	117.5 (36.76, 384.7)	7.829 (2.385, 24.25)
	10	636.8 (208.6, 1661)	225.7 (76.63, 708.2)	8.771 (2.170, 26.67)
80	2.5	124.7 (40.12, 331.3)	49.36 (16.50, 138.6)	6.782 (1.886, 19.90)
	5	257.2 (90.01, 616.2)	96.66 (30.92, 226.2)	8.023 (1.744, 36.89)
	10	496.5 (195.3, 1229)	175 (57.83, 509.1)	8.668 (1.619, 47.82)
90	2.5	101.1 (35.88, 272.0)	39.68 (13.80, 108.3)	6.894 (1.887, 19.31)
	5	200.5 (62.71, 453.7)	74.39 (21.42, 182.5)	7.385 (1.986, 24.26)
	10	404.4 (121.6, 1466)	141.6 (38.57, 549.8)	8.313 (2.223, 25.59)
100	2.5	83.51 (27.36, 236.8)	32.42 (9.053, 91.20)	6.744 (1.256, 20.17)
	5	165.1 (41.84, 547.4)	60.82 (18.19, 217.8)	7.325 (2.139, 24.41)
	10	330.4 (98.94, 800.6)	114.8 (25.78, 332.4)	8.353(2.066, 32.97)
110	2.5	69.86 (22.12, 181.5)	27 (8.728, 85.21)	6.426 (1.277, 20.96)
	5	140.9 (50.22, 359.6)	51.64 (17.85, 148.6)	7.148 (2,416, 24.79)
	10	280.6 (93.34, 646.7)	96.44 (27.86, 269.5)	7.642 (1.747, 26.29)
120	2.5	59.64 (18.94, 163.4)	22.9 (6.698, 61.03)	6.376 (2.061, 19.52)
	5	120.7 (46.14, 296.8)	43.9 (16.03, 108.9)	6.676 (1.814, 28.11)
	10	237.1 (84.70, 577.1)	81.24 (21.24, 225.5)	7.771 (2.187, 22.2)

AUC_T=area under the curve during a dosing interval (in this case, week 6); C_{max}=maximum concentration; C_{trough}=trough concentration; SC=subcutaneous.

Values are shown as mean (min, max) to 4 significant digits.

Week 6 AUC_T and C_{max} for plasma apraglutide and week 6 C_{trough} for plasma citrulline were calculated from 500 individuals per dose and per body weight group after weekly SC administrations of 2.5, 5, or 10 mg apraglutide.

Figure S1. Subject disposition.

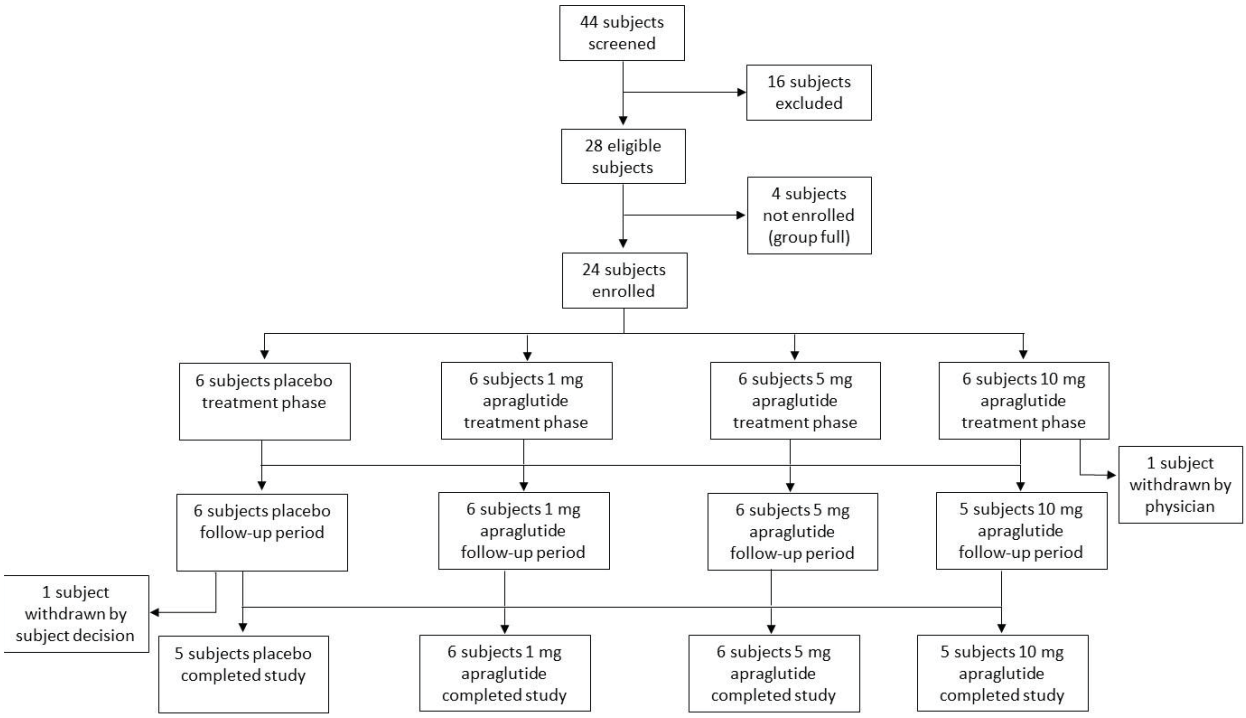


Figure S2. Population pharmacokinetic/pharmacodynamic model structure and parameter definitions. Cl/F, clearance; EC_{50}=half-maximal effective concentration; E_{max}=maximum effect; γ=Hill coefficient; R_0=baseline value; k_{deg}=degradation rate; k_{syn}=endogenous synthesis rate; SC=subcutaneous; Tk0, absorption time of the subcutaneous administration; V1/F, volume of the central compartment.

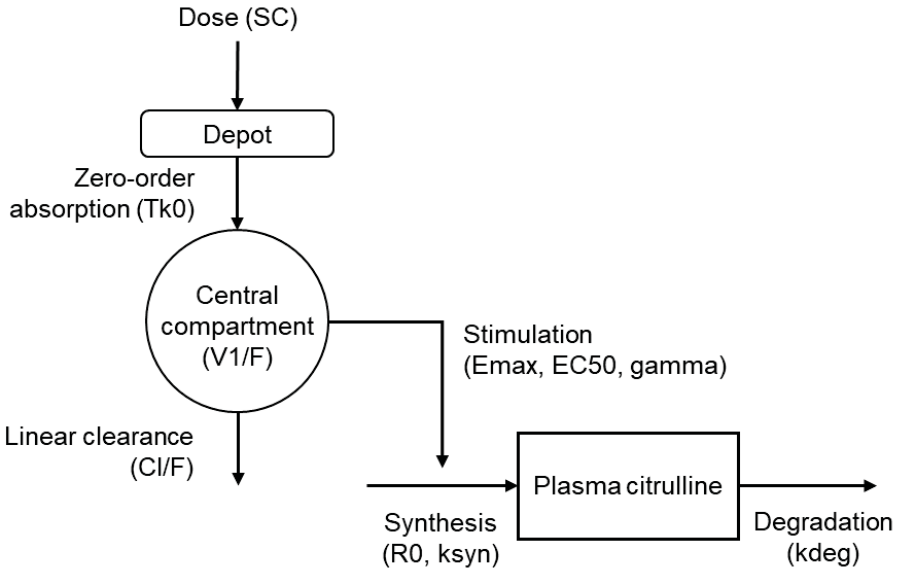
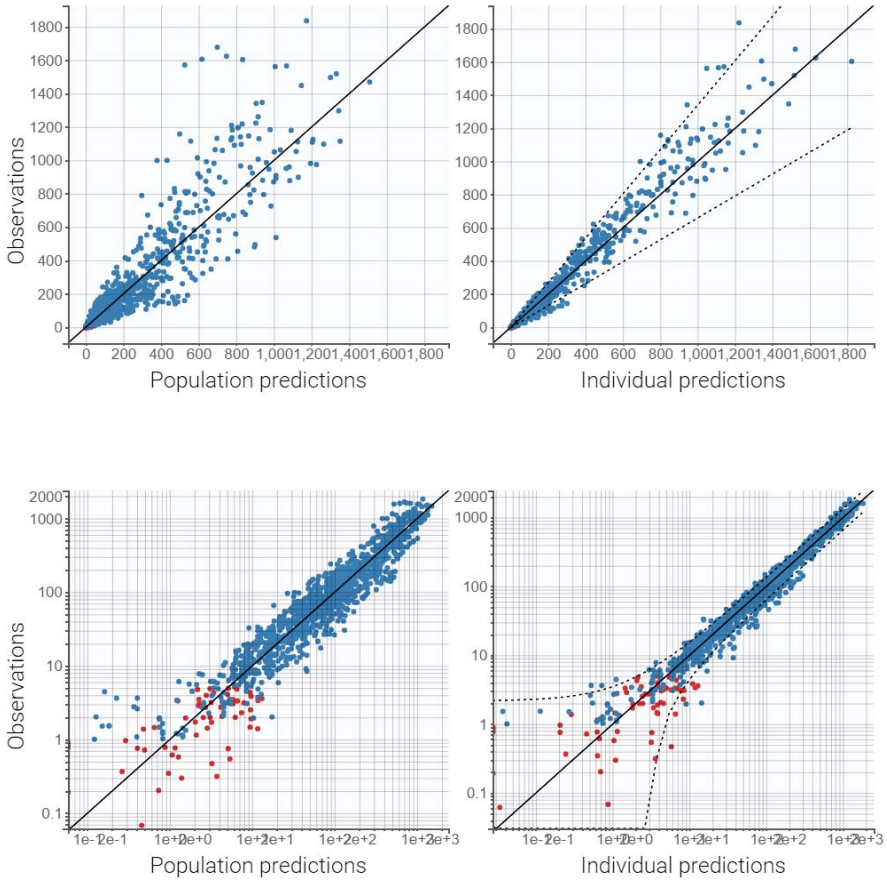
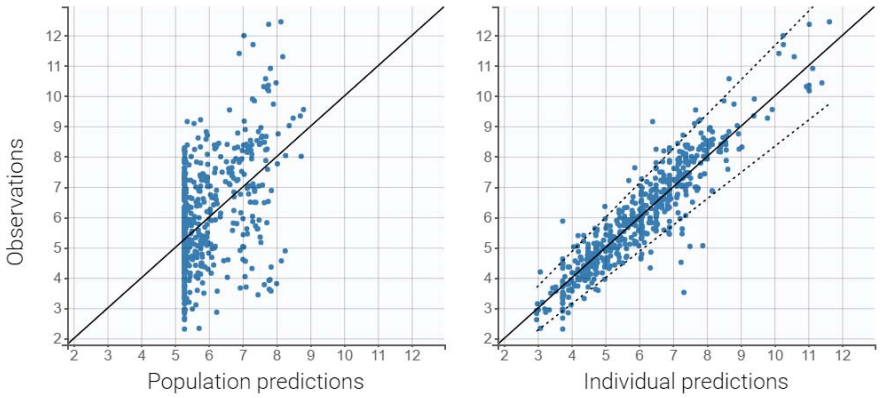


Figure S3. Observed versus population (left) and individual (right) predicted apraglutide plasma concentrations on linear (top) and log10 scales (middle), and plasma citrulline (bottom). A 1-compartmental model with zero-order absorption and linear clearance was developed using data from this study and the first-in-human study [5] as described in Appendix 1. To evaluate the model, GoF plots and the relative standard error of the parameter estimates were used as generated by Monolix. GoF plots were constructed using random sampling from the conditional distributions of the individual parameters [3]. BLQ data (shown in red) were included in the diagnostic plots by sampling the BLQ predictions from the conditional distribution. The 90% prediction interval is shown as dashed lines. GoF=goodness of fit; BLQ=below the limit of quantification.




Observations
Population predictions
Individual predictions
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