Supplementary Information

for the article

Population Pharmacokinetic Comparability of CT-P16 versus EU-Avastin® and US-Avastin® in Healthy Subjects and Patients with Non-squamous Non-small Cell Lung Cancer

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Supplementary Table S1. Stepwise covariate evaluation: forward inclusion and backward elimination

Covariate selection followed a stepwise approach: forward inclusion ($p < 0.05$; $\Delta OFV \leq -3.84$) followed by backward elimination ($p < 0.01$; $\Delta OFV \geq +6.63$). One-at-a-time ΔOFV values relative to the base model (OFV = 29,168) and backward elimination results relative to the final model (OFV = 28,780) are summarised below.

Covariate	Parameter	Forward ΔOFV [a]	Backward ΔOFV [a]	Retained
Body weight	CL	−57.18	+59.0	**Yes**
Sex	CL	−60.14	+3,480.0	**Yes**
Disease / phase status	CL	−100.3	+40.0	**Yes**
Treatment group	CL	−30.3	0.0	**No** [b]
Sex	V_1	−151.3	+4,370.0	**Yes**
Albumin	V_1	−0.012	+0.01	**Yes** [c]
Disease / phase status	V_1	−100.3	+13.0	**Yes**
Treatment group	V_1	−30.3	−8.0	**No** [b]

[a] Forward ΔOFV: change relative to the base model (OFV = 29,168); inclusion threshold $\Delta OFV \leq -3.84$ ($p < 0.05$). Backward ΔOFV: change upon removal from the final model (OFV = 28,780); retention threshold $\Delta OFV \geq +6.63$ ($p < 0.01$). Positive backward ΔOFV indicates worsening of model fit upon covariate removal.

[b] Treatment group was evaluated during stepwise covariate modelling after accounting for disease/phase-related covariates and was not retained due to the absence of a meaningful reduction in OFV.

[c] Although albumin inclusion produced only a modest OFV change (ΔOFV = −0.012), its exclusion resulted in minimization failure (unsuccessful covariance step), whereas inclusion yielded successful minimization, indicating that albumin contributes to the numerical identifiability and stability of the final model. Bootstrap confidence intervals were relatively wide (0.007–0.485), reflecting limited albumin variability within the study population rather than parameter instability, and the direction of the effect was consistent across all bootstrap replicates. Albumin was therefore retained on the basis of model stability, mechanistic plausibility (FcRn-mediated IgG recycling and inflammation-driven catabolism), and consistency with prior bevacizumab and monoclonal antibody PopPK analyses, rather than on statistical strength alone.

Supplementary Table S2. Comparison of population pharmacokinetic models for reference bevacizumab and bevacizumab biosimilars

Parameter / characteristic	Lu 2008 (Avastin originator)	Han 2016 (Avastin originator)	Li 2020 (PF-06439535 / Zirabev®)	Owen 2023 (MYL-1402O / Abevmy®)	Hong 2026 (SCT510)	Present study (CT-P16 / Vegzelma®)	Position of present study
STUDY DESIGN							
Journal	Cancer Chemother Pharmacol	Cancer Chemother Pharmacol	Cancer Chemother Pharmacol	Eur J Drug Metab Pharmacokinet	Clin Drug Investig	**BioDrugs**	Adis-family target; PopPK absent from BioDrugs to date
Year	2008	2016	2020	2023	Jan 2026	**2026 (this study)**	Most recent biosimilar PopPK
Population	Mixed solid tumours	8 cancer types	NSCLC patients only	HV + NSCLC patients	HV + NSCLC patients	**HV + NSCLC patients**	Same as Hong/Owen integrated design
Reference product evaluated	EU-Avastin®	Pooled Avastin (multi-source)	EU-Avastin®	EU-Avastin® (+ small US-Avastin® in Phase 1)	Avastin (single source)	EU-Avastin® and US-Avastin®	ONLY study with full simultaneous EU + US comparison
N subjects	491	1,792	705	771	399	**834**	Largest among bevacizumab biosimilar PopPKs
N PK observations	4,629	8,943	8,632	8,724	2,647	**8,058**	Comparable to Li/Owen, ≈ 3× Hong
Phase 1 + Phase 3 integrated?	No	No	No (Ph3 only)	Yes	Yes	**Yes**	Same integrated approach
STRUCTURAL MODEL							
Model structure	2-CMT linear (FOCE)	2-CMT linear (FOCE)	2-CMT linear (FOCE-I)	2-CMT linear (FOCE-I)	2-CMT linear	**2-CMT linear (FOCE-I)**	Consistent with class
CLEARANCE (CL)							
CL — typical (mL/h)	8.63 (F, 0.207 L/d)	9.01 (70-kg)	11.3 (71-kg F)	10.9 (typical)	Reported	**6.43 (typical, before sex / LC multipliers)**	Lower base because sex / LC modelled as exp() multipliers
CL after Male + NSCLC adjustment (mL/h)	10.9 (M, F)	—	14.3 (71-kg M)	—	—	**≈ 11.0 (71-kg M, NSCLC)** [a]	Comparable to Lu 2008 male and Li 2020 male
BWT exponent on CL	0.368	0.589	0.354	0.726 (BSA)	—	**0.369**	Three-way numerical convergence with Lu 2008 / Li 2020

Parameter / characteristic	Lu 2008 (Avastin originator)	Han 2016 (Avastin originator)	Li 2020 (PF-06439535 / Zirabev®)	Owen 2023 (MYL-1402O / Abevmy®)	Hong 2026 (SCT510)	Present study (CT-P16 / Vegzelma®)	Position of present study
Sex effect on CL (M vs F)	+26%	Significant	+26.2%	Not significant	Significant	**+36% (exp(0.308))**	Magnitude slightly larger; Phase 1 (100% M) likely contributes
Albumin effect on CL	−19% if ALB < 29 g/L	Negative	Not significant	Negative power	Significant	**Not retained on CL (only V_1)**	Consistent with Li 2020
IIV CL (CV%)	26.0%	—	29.5%	≈ 30%	—	**32%**	Comparable range
CENTRAL VOLUME (V_1)							
V_1 — typical (L)	2.39 (F)	2.88	2.99 (71-kg F)	3.13	—	**1.87 (typical, before multipliers)**	Lower base because sex / LC modelled as exp() multipliers
V_1 after Male + NSCLC adjustment (L)	3.29 (M)	—	3.73 (71-kg M)	—	—	**≈ 3.27 (71-kg M, NSCLC) [a]**	Comparable to Lu 2008 male and Li 2020 male
BWT exponent on V_1	0.411	0.470	0.468	0.919 (BSA)	—	**Not retained (sex / ALB / LC retained)**	Different from Lu / Li — explained in Discussion
Sex effect on V_1 (M vs F)	Significant	Significant	+24.7%	Not significant	Significant	**+74% (exp(0.553))**	Magnitude larger than Li 2020; Phase 1 (100% M) likely contributes
Albumin effect on V_1	Negative	—	—	Negative	Significant	**+8% per g/L (modest, retained for stability)**	Direction physiologically plausible
IIV V_1 (CV%)	16.8%	—	34.2%	≈ 26%	—	**29%**	Mid-range
PERIPHERAL DISPOSITION							
V_2 (L)	—	2.57	6.09	1.61	—	**2.16**	Comparable to Han / Owen
Q (mL/h)	—	18.7	269	34.6	—	**27.4**	Comparable to Han / Owen
Terminal t½ (days)	19.9	19.6	—	20.6	—	**—**	Consistent IgG1 disposition
BIOSIMILARITY ASSESSMENT							
Drug-product effect tested?	N/A	N/A	Yes (vs EU-Avastin)	Yes (vs EU-Avastin)	Yes (vs Avastin)	**Yes (vs EU-Avastin AND US-Avastin)**	ONLY study with simultaneous EU + US bridging in unified model

Parameter / characteristic	Lu 2008 (Avastin originator)	Han 2016 (Avastin originator)	Li 2020 (PF-06439535 / Zirabev®)	Owen 2023 (MYL-1402O / Abevmy®)	Hong 2026 (SCT510)	Present study (CT-P16 / Vegzelma®)	Position of present study
Drug-product effect — result	—	—	Not significant; 95% CI of CL / V_1 effect contained 1.0	Not significant (P = 0.453 CL; P = 0.161 V_1)	Not clinically relevant	**Not retained (ΔOFV criteria not met)**	Aligned with Li / Owen / Hong conclusions
Disease status (HV vs patient) modelled as covariate?	No	No	No (Ph3 only)	Study factor only	Yes (subject type)	**Yes (LC covariate, mechanistically interpreted)**	Mechanistic interpretation absent in Hong 2026
EXPOSURE–RESPONSE LINKAGE							
External E-R benchmark used for simulation interpretation?	No (dosing-regimen simulation only)	No	No (PK only)	No (PK only)	No (PK only)	**Yes (89.1 µg/mL $C_{min,ss}$; SB8-derived PFS-90% anchor)**	ONLY biosimilar PopPK to anchor simulation to published E-R
Steady-state simulation performed?	Yes (Q2W vs Q3W)	No	No	Yes (AUC, t½, Cmax, Cmin tertiles)	Yes	**Yes (1,000 virtual subjects, 7 cycles, BWT × ALB × sex strata)**	Most granular subgroup-level simulation
Subgroup-by-subgroup E-R sufficiency reported?	No	No	No	No	No	**Yes**	UNIQUE

[a] Approximate values calculated from Equations 5 and 6 of the present manuscript. CL = 6.43 × (70.9/70.9)^0.369 × exp(0.308 × 1 + 0.226 × 1) ≈ 6.43 × 1.36 × 1.25 ≈ 11.0 mL/h for a typical 71-kg male NSCLC patient. V_1 = 1,870 × exp(0.553 × 1 + 0.632 × 1) ≈ 1,870 × 1.74 × 1.88 ≈ 3,265 mL (assuming the albumin term ≈ 1.0 at the population median).

ALB, baseline serum albumin; BSA, body surface area; BWT, body weight; CL, clearance; CV%, coefficient of variation expressed as a percentage; FOCE, first-order conditional estimation; FOCE-I, first-order conditional estimation with interaction; HV, healthy volunteers; IIV, inter-individual variability; LC, lung cancer; M, male; F, female; NSCLC, non-small cell lung cancer; PFS, progression-free survival; popPK, population pharmacokinetics; Q, intercompartmental clearance; Q2W, every 2 weeks; Q3W, every 3 weeks; V_1, central volume of distribution; V_2, peripheral volume of distribution.

References for Supplementary Table S2: Lu et al. 2008 [11]; Han et al. 2016 [10]; Li et al. 2020 [24]; Owen et al. 2023 [25]; Hong et al. 2026 [26]; present study.

Supplementary Table S3. Estimated drug-product effects on clearance (CL) and central volume of distribution (V_1) from forced-covariate analysis, with CT-P16 as the reference

Parameter	Comparator	Estimate (%)	95% CI (%) [c]
CL	EU-Avastin® [a]	+0.78	−3.28 to +5.97
	US-Avastin® [b]	−5.25	−10.06 to +0.79
V_1	EU-Avastin® [a]	+0.65	−4.14 to +6.72
	US-Avastin® [b]	+0.46	−4.82 to +7.63

Drug product (EU-Avastin® and US-Avastin®, with CT-P16 as the reference) was added to the final population pharmacokinetic model as a categorical covariate on CL and V_1. Estimates represent the fractional difference (%) relative to CT-P16. All confidence intervals encompassed zero and lay within the ±20% range conventionally regarded as clinically irrelevant for monoclonal antibody pharmacokinetics. Because US-Avastin® was administered only in the Phase 1 (healthy subject) study, its effect estimate is informed exclusively by healthy-subject data and carries wider uncertainty; the EU-Avastin® comparison, informed by data from both study phases, provides the more robust assessment of drug-product comparability. Notably, the US-Avastin® effect remained small with a confidence interval encompassing zero despite being estimated solely from healthy-subject data, suggesting that population-related differences (disease status, body weight, sex) were adequately accounted for by the covariate model.

[a] *EU-Avastin® was administered in both the Phase 1 and Phase 3 studies.*

[b] *US-Avastin® was administered in the Phase 1 study only; the estimated effect is therefore informed exclusively by healthy-subject data.*

[c] *95% confidence intervals were derived from non-parametric bootstrap analysis (1,000 replicates; 220 replicates retained after exclusion of runs with terminated minimization (751) or boundary estimates (29)). The reduced retention rate reflects the structural complexity of the forced-covariate model, which adds four additional drug-product parameters to a model that already incorporates a full OMEGA BLOCK(3) inter-individual variability covariance structure and ODE-based two-compartment disposition. Parameter estimates were consistent with independent sensitivity bootstrap analyses, supporting the reliability of the reported confidence intervals.*

CI, confidence interval; CL, clearance; V_1, central volume of distribution.

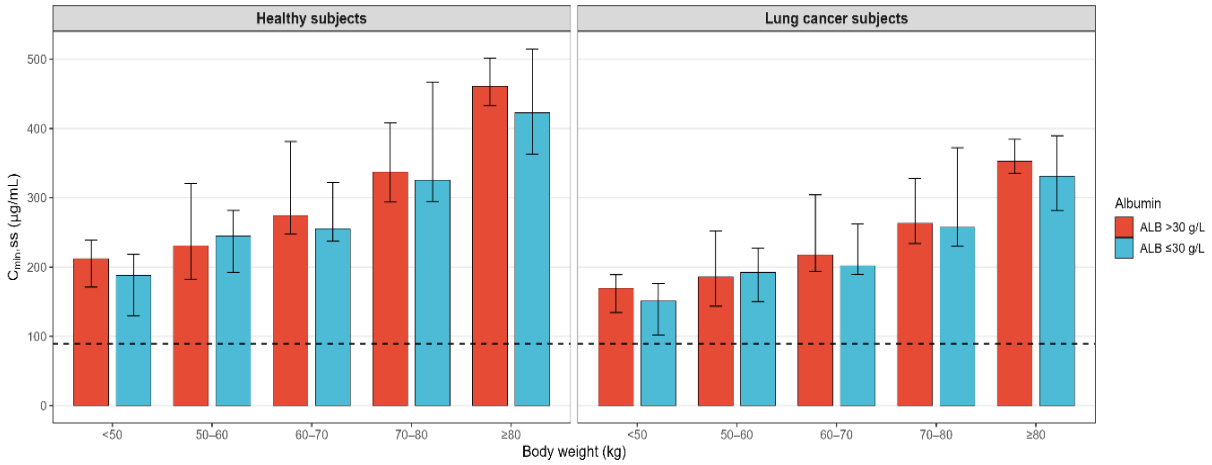
Supplementary Table S4. Sensitivity of simulated steady-state trough concentration ($C_{min,ss}$) to inter-individual variability (IIV) assumptions

IIV scenario	Median	5th percentile	Minimum	< 89.1 µg/mL (%)
Reference IIV	151.8	77.4	25.3	9.5
IIV ×1.5	151.2	68.2	18.8	13.6
IIV ×2.0	150.6	61.0	14.6	16.8

Population simulations were performed using the actual Phase 3 patient covariate distribution (N = 649 patients, 200 replicates per subject; 129,800 simulated observations per scenario) under the approved 15 mg/kg every-3-weeks regimen, with $C_{min,ss}$ evaluated as the pre-dose trough following the 7th cycle. Three IIV scenarios were compared: Reference IIV (final model OMEGA estimates: CL CV ≈33%, V CV ≈29%), and inflated IIV scenarios with OMEGA variance multiplied by 1.5 (CV ≈41/36%) and 2.0 (CV ≈48/42%). $C_{min,ss}$ values are presented in µg/mL. The 89.1 µg/mL benchmark represents a plateau-associated reference (corresponding to 90% of maximum progression-free survival effect) rather than a strict efficacy threshold.

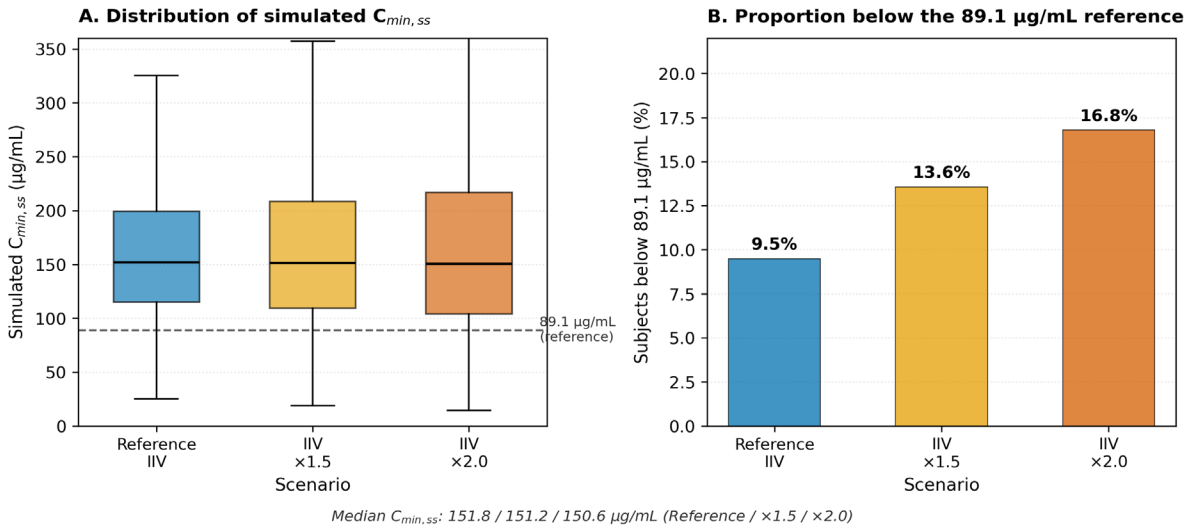
CV%, coefficient of variation expressed as percentage; IIV, inter-individual variability; OMEGA, variance/covariance matrix for IIV.

Supplementary Figure S1. Simulated steady-state $C_{min,ss}$ by disease status



Simulated steady-state trough concentrations of bevacizumab ($C_{min,ss}$) after 15 mg/kg every 3 weeks for 7 cycles are shown by disease status, body weight bin, and albumin group. Bars indicate median $C_{min,ss}$, and error bars indicate bootstrap 95% confidence intervals for the median based on 2,000 resamples. The dashed line represents the PFS 90% plateau-associated $C_{ss,trough}$ of 89.1 µg/mL and is provided for contextual comparison. These simulations were performed as a sensitivity assessment of disease-related covariate effects in the final population pharmacokinetic model; clinical interpretation of $C_{min,ss}$ was restricted to patients with NSCLC.

Supplementary Figure S2. Sensitivity of simulated $C_{min,ss}$ to inter-individual variability (IIV) assumptions



Population simulations using the actual Phase 3 patient covariate distribution (N = 649, 200 replicates each) under the approved 15 mg/kg every-3-weeks regimen, with $C_{min,ss}$ evaluated as the pre-dose trough following the 7th cycle. (A) Distribution of simulated $C_{min,ss}$ across IIV scenarios; boxes represent the interquartile range, horizontal lines the median, and whiskers the 5th–95th percentiles. The dashed line indicates the 89.1 µg/mL plateau-associated reference (corresponding to 90% of maximum progression-free survival effect). (B) Proportion of simulated subjects with $C_{min,ss}$ below the 89.1 µg/mL reference. Median $C_{min,ss}$ remained robustly above the reference across all scenarios (151.8, 151.2, and 150.6 µg/mL for the Reference, ×1.5, and ×2.0 IIV scenarios, respectively; approximately 1.7-fold the benchmark), while the proportion of individual simulated subjects below the benchmark increased modestly with greater assumed variability (9.5%, 13.6%, and 16.8%, respectively). IIV, inter-individual variability.

Declarations

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Competing Interests

Taekyung Kim, Keumyoung Ahn, Taehong Park, and Hyungseok Baek are employees of Celltrion, Inc., the manufacturer of CT-P16 (Vegzelma®). Min Jung Chang, Heungjo Kim, and Hongjae Lee have received research funding from Celltrion, Inc.