Electronic Supplementary Material

Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Atacicept in a Randomized Trial in Healthy Caucasian and Japanese Subjects

European Journal of Drug Metabolism and Pharmacokinetics

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Table S1 Patient inclusion and exclusion criteria

Inclusion criteria
• Healthy male and female subjects of Japanese or Caucasian origin, age 18–55 years (inclusive) at screening • Japanese subjects were required to: hold a valid Japanese passport; have been born in Japan with both parents and all four grandparents being Japanese; have lived outside of Japan for <5 years; and have a body weight of 45–90 kg (female) or 55–90 kg (male) and BMI of 18–29.9 kg/m^2 • Caucasian subjects had to have Caucasian parents and be matched one to one by sex, body weight at screening (±20% body weight [kg]), and height (±15% height [cm]), to each Japanese subject • Female subjects of childbearing potential had to be using a highly effective method of contraception for ≥4 weeks prior to randomization, agree to continue to practice highly effective contraception until 5 months after the study administration and have a negative serum pregnancy test at screening and on TD-1 (1 day before study drug administration) • Ability to communicate well with the investigator • Signed informed consent before the conduct of any trial-related procedure • Subjects willing to participate in the pharmacogenetic part of this trial had to sign a separate specific informed consent form

Exclusion criteria

- Positive hepatitis serology, (i.e. positive hepatitis B surface antigen, antibody to hepatitis B core antigen, or hepatitis C virus antibodies at screening)
- Positive HIV 1 or 2 serology at screening
- Known active clinically significant viral, bacterial (including known history of tuberculosis), or fungal infection, history of recurrent infection or any major episode of infection requiring hospitalization, or treatment with anti-infectives within 8 weeks before screening
- Serum IgG <6 g/L at screening
- Treatment with another investigational drug, antibody or biologic therapy, within 6 months or 5 half-lives (whichever was the longer) prior to TD-1
- Use of concomitant prescription or non-prescription medications (including herbals such as St John's Wort, vitamins and minerals), except occasional paracetamol/acetaminophen, and/or oral contraceptives at recommended doses, during the 14 days prior to screening, or anticipated need for such medication during the planned duration of the trial
- Vaccination with live or live-attenuated vaccines within 6 months prior to screening or plan for any such vaccination during the trial or within 5 months after end of trial
- History of any demyelinating disease
- Positive for urine alcohol/drug abuse at screening and TD-1; a history of alcohol/drug abuse or alcohol intake within 72 hours before the study administration
- Excessive caffeine consumption, defined as ≥800 mg/day
- Smoking more than 10 cigarettes per day or the inability to refrain from smoking

during hospitalization

- Pregnancy or breastfeeding
- Loss or donation of more than 400 mL of blood or blood products within 90 days prior to screening
- Chronic medical condition requiring prescription medications
- Clinically significant abnormal values for hematology, clinical chemistry, coagulation, or urinalysis, as judged by the investigator
- Known current or past history of significant vascular, pulmonary, gastrointestinal, endocrine, hematologic, rheumatologic, autoimmune, or metabolic disturbances as assessed by the investigator
- Any condition, including findings in the laboratory tests, medical history, or other screening assessments, that in the opinion of the investigator constituted an inappropriate risk or a contraindication for participation in the trial or that could interfere with the trial's objectives, conduct, or evaluation
- Clinically significant abnormality on ECG performed at screening, TD-1, or pre-dose
- Abnormal vital signs at the screening visit after 10 minutes resting in the semi-supine position (SBP >140 mmHg or <90 mmHg; DBP >90 mmHg or <50 mmHg; Pulse rate >100 bpm or ≤40 bpm; body temperature: >37.5 °C or <35 °C)
- Known or suspected hypersensitivity to any biologic medication
- Hypersensitivity to any of the components of the formulated atacicept
- Legal incapacity or limited legal capacity at screening
- Any circumstances or conditions which, in the opinion of the investigator, may have affected the safety of the subject, his or her full participation in the trial, or his or her compliance with the protocol

- Creatinine clearance <60 mL/min estimated by Cockcroft-Gault method.

BMI body mass index, *ECG* electrocardiogram, *DBP* diastolic blood pressure, *HIV* human immunodeficiency virus, *SBP* systolic blood pressure, *TD* trial day

Table S2 Baseline demographics

Demographic characteristic	Atacicept 25 mg	Atacicept 75 mg	Atacicept 150 mg	Placebo
Age in years, median (range)				
Japanese	*n* = 7	*n* = 6	*n* = 6	*n* = 6
	28.0 (20–48)	28.0 (22–38)	29.5 (27–34)	25.0 (22–31)
Caucasian	*n* = 6	*n* = 6	*n* = 6	*n* = 9
	30.0 (21–39)	31.0 (18–48)	31.5 (24–44)	27.0 (19–42)
Male gender, *n* (%)				
Japanese	*n* = 7	*n* = 6	*n* = 6	*n* = 6
	3 (42.9)	3 (50.0)	3 (50.0)	3 (50.0)
Caucasian	*n* = 6	*n* = 6	*n* = 6	*n* = 9
	3 (50.0)	3 (50.0)	3 (50.0)	5 (55.6)
Height in cm, median (range)				
Japanese	*n* = 7	*n* = 6	*n* = 6	*n* = 6
	161.5 (152.5–171.0)	160.5 (153.0–174.5)	165.0 (159.0–176.0)	171.25 (149.0–180.0)

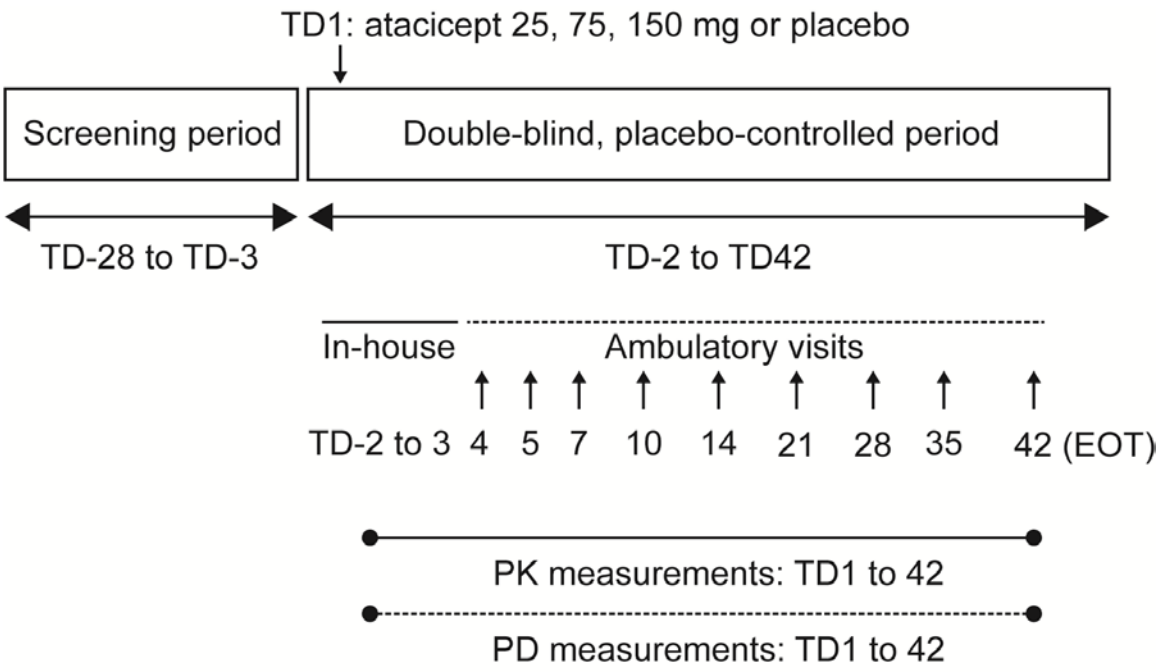
Caucasian	*n* = 6	*n* = 6	*n* = 6	*n* = 9
	170.8 (161.5–183.0)	170.0 (156.0–177.0)	169.0 (157.0–183.5)	166.0 (159.0–184.0)
Weight in kg, median (range)				
Japanese	*n* = 7	*n* = 6	*n* = 6	*n* = 6
	57.0 (53.4–61.2)	59.4 (45.4–78.9)	57.7 (48.5–77.1)	68.2 (48.9–76.6)
Caucasian	*n* = 6	*n* = 6	*n* = 6	*n* = 9
	66.1 (61.7–67.8)	66.1 (47.2–83.1)	66.9 (56.9–85.6)	68.1 (51.9–80.8)
Body mass index in kg/m^2, median (range)				
Japanese	*n* = 7	*n* = 6	*n* = 6	*n* = 6
	21.1 (19.4–26.3)	22.5 (19.4–27.3)	21.9 (18.4–27.5)	22.0 (19.4–27.3)
Caucasian	*n* = 6	*n* = 6	*n* = 6	*n* = 9
	22.9 (19.9–25.9)	23.9 (19.2–27.6)	23.4 (22.1–26.0)	22.8 (20.4–25.4)

Table S3 Atacicept dose-proportionality assessment: power model (PK analysis set)

Ethnicity	PK parameter		Point estimate	90% CI
Japanese	$AUC_{0\text{-}t}$, day·ng/mL	Slope	0.659	0.544, 0.774
n = 18		Intercept	8.945	8.247, 9.643
	C_{max}, ng/mL	Slope	0.996	0.783, 1.210
		Intercept	5.785	4.491, 7.079
Caucasian	$AUC_{0\text{-}t}$, day·ng/mL	Slope	0.547	0.432, 0.661
n = 18		Intercept	9.347	8.587, 10.11
	C_{max}, ng/mL	Slope	0.807	0.5952, 1.019
		Intercept	6.621	5.212, 8.030

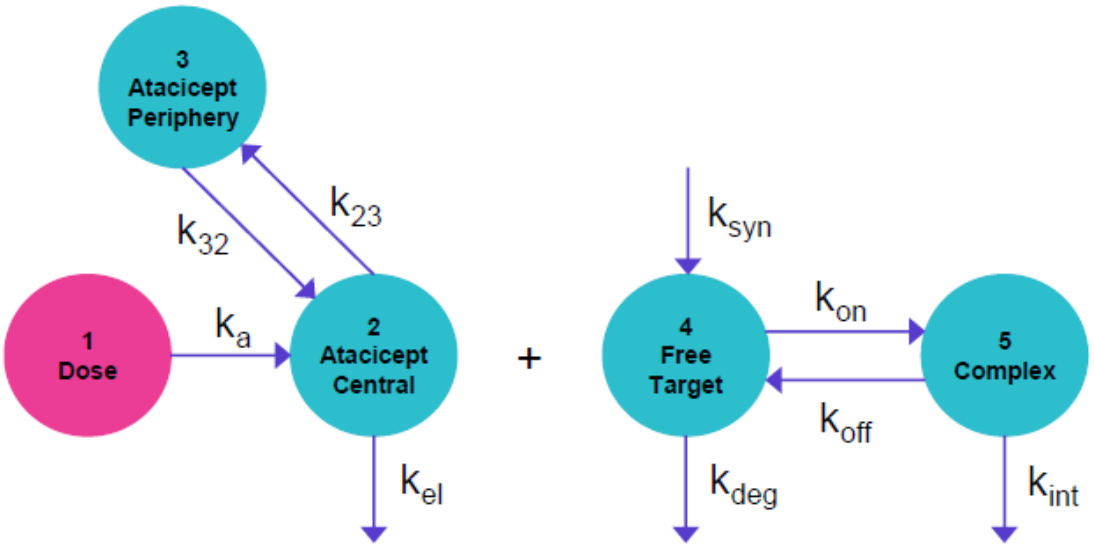
$AUC_{0–t}$ area under the serum concentration time curve from time zero to the last sampling point, *CI* confidence interval, *C_{max}* maximum observed serum concentration, *PK* pharmacokinetic

Fig. S1 Study design



EOT end of trial, *PD* pharmacodynamics, *PK* pharmacokinetics, *TD* trial day

Fig. S2 Schematic representation and differential equations of the two-compartment quasi steady-state target-mediated drug disposition binding model



$$C = \frac{1}{2}\left[C_{tot} - R_{tot} - Kss) + \sqrt{(C_{tot} - R_{tot} - Kss)^2 + 4.Kss.C_{tot}}\right]$$

$$Kss = \frac{Koff}{Kon} + \frac{K\,\mathrm{int}}{Kon}$$

$$\frac{dA_1}{dt} = -Ka.A_1$$

$$\frac{dC_{tot}}{dt} = Ka.A_1 - (K_{el} + K_{23}).C - \frac{K\,\mathrm{int}.C.R_{tot}}{Kss + C} + K_{32}\frac{A_3}{Vc}$$

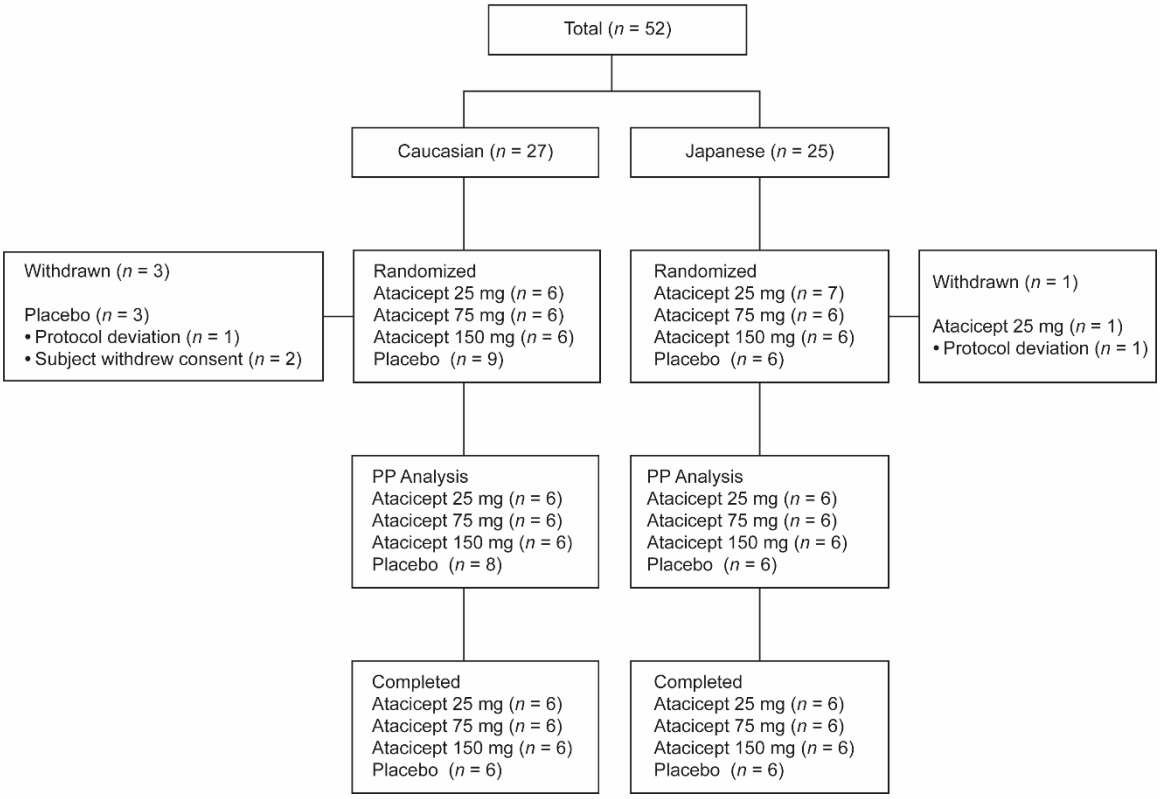
$$\frac{dA_3}{dt} = K_{23}.CVc - K_{32}.A_3$$

$$\frac{dR_{tot}}{dt} = Ksyn - K\,\mathrm{deg}.R_{tot} - (K\,\mathrm{int} - K\,\mathrm{deg}).\frac{R_{tot}.C}{Kss + C}$$

C free atacicept concentration, C_{tot} total atacicept concentration, R_{tot} total target concentration, K_{ss} steady-state constant, K_{off} dissociation rate constant, K_{on} binding rate constant, K_{int} drug-target complex elimination rate constant, K_a absorption rate constant, A_n amount in n^{th} compartment, K_{el} elimination rate constant, K_{23} transfer rate from central to peripheral compartments, K_{32} transfer rate from peripheral to central compartment,

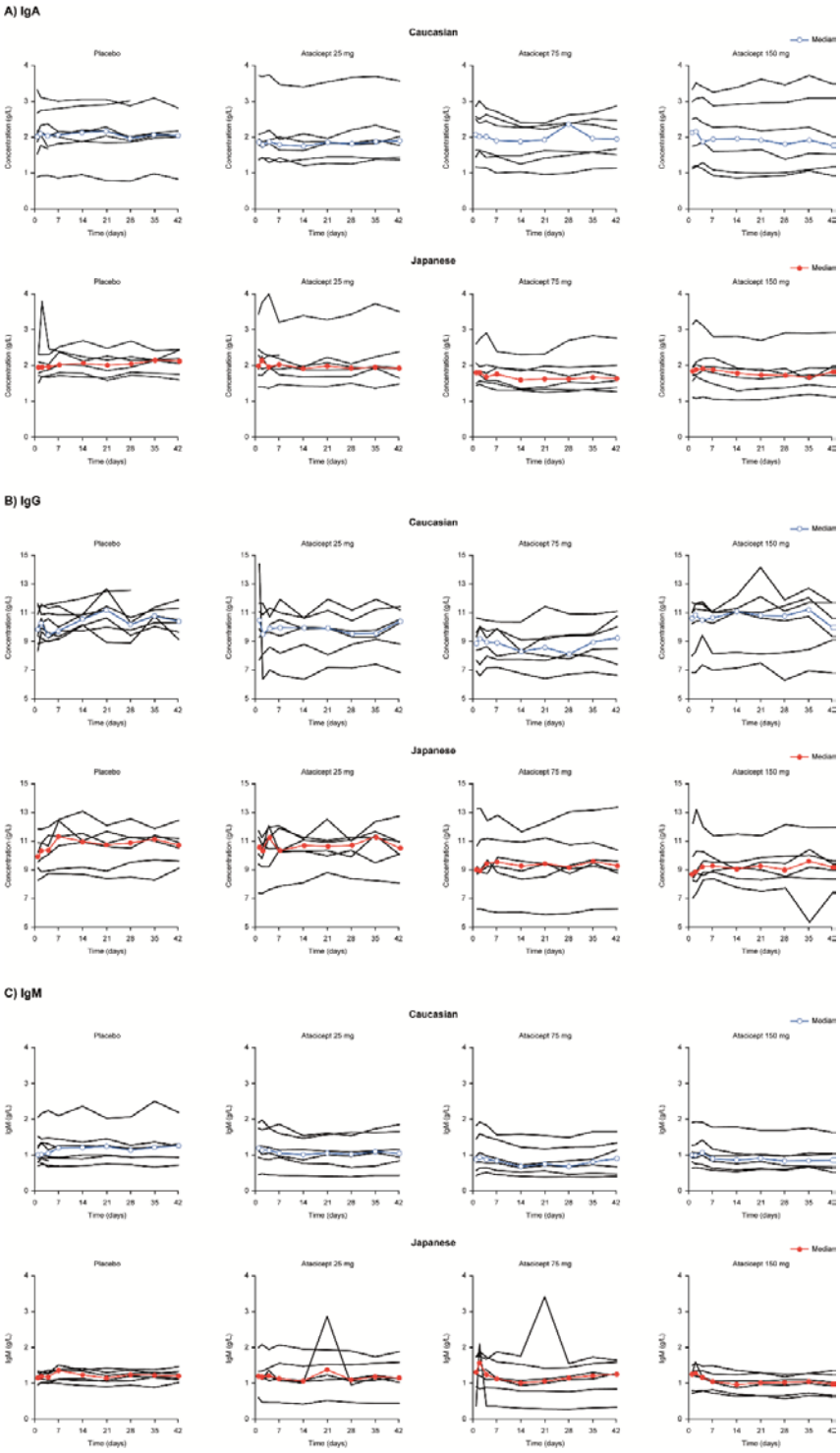
K_{syn} target production rate, K_{deg} target elimination rate constant, V_c volume of the central compartment

Fig. S3 Summary of subject disposition



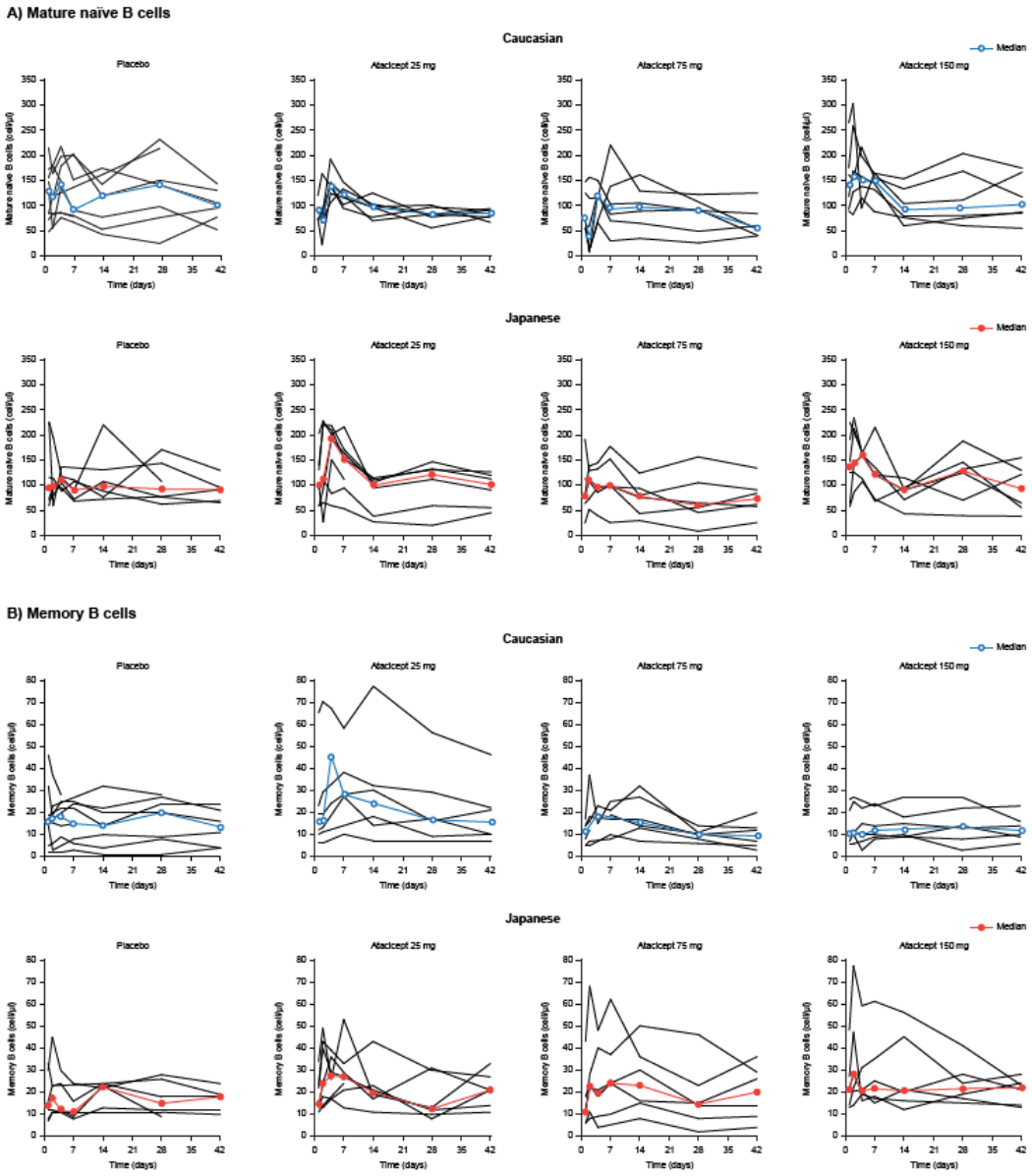
PP per protocol set: a subset of the safety analysis set including all subjects without any major protocol violations with respect to factors likely to affect the primary analysis; compliance with all entry criteria; absence of relevant protocol deviations and events or conditions with respect to factors likely to affect the comparability of PK results; and sufficient data available for determination of the primary target variables C_{max} and $AUC_{0-\infty}$ (or AUC_{0-t}; if the $AUC_{0-\infty}$ following atacicept administration is available for less than 80% of the subjects in one ethnic group, then AUC_{0-t} was to be used instead – the PK set was a subset of the PP set, excluding the placebo-treated groups)

Fig. S4 Individual and median **a.** IgA, **b.** IgG and **c.** IgM levels over time by treatment group and ethnicity (safety analysis set)



IgA normal range 0.70–4.00 g/L; IgG normal range 7.00–16.00 g/L; IgM normal range: 0.40–2.30 g/L. Median IgA, IgG, and IgM levels over time for each treatment group are indicated by the thicker (red and blue) curves.

Fig. S5 Individual and median a. mature naïve and b. memory B cell count over time by treatment group and ethnicity (safety analysis set)



Individual data and median mature naïve ($CD19^{+}CD20^{+}IgD^{+}CD27^{-}$) and memory B cell ($CD19^{+}CD20^{+}IgD^{-}CD27^{+}CD38^{-}$) count over time for each treatment group are indicated by the thicker (red and blue) curves.

Appendix S1 Assessment and reporting of adverse events

Adverse events (AEs) were assessed and reported in line with the U.S. Code of Federal Regulations for the safety reporting for investigational new drugs (§ 312.32 – IND safety reporting) [1].

Severity/intensity of AEs was assessed according to the Qualitative Toxicity Scale:

- Mild: the subject is aware of the event or symptom, but the event or symptom is easily tolerated
- Moderate: the subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity
- Severe: significant impairment of functioning; the subject is unable to carry out usual activities.

Serious AEs were defined as any AEs fulfilling the following criteria:

- AE results in death
- AE is life-threatening (this definition refers to an event in which the subject is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe)
- AE requires inpatient hospitalization or prolongation of existing hospitalization
- AE results in persistent or significant disability/incapacity
- AE is a congenital anomaly/birth defect
- AE is otherwise considered as medically important

References

1. U.S. Code of Federal Regulations. § 312.32 - IND safety reporting. 2019. https://www.govregs.com/regulations/expand/title21_chapterI_part312_subpartB_section312.32 (last accessed August 2019).

Appendix S2 Methodology of pharmacokinetics sample analysis

The ELISA used in this study for the quantitative determination of dissociated ('total') atacicept in human serum has been developed by Merck Serono, in line with EMA guidelines and a best-practice publication by Viswanathan CT, et al [1, 2]. Critical reagents included atacicept reference standard (Merck Serono), mouse monoclonal antibodies against human atacicept (clones 251.15.1.1.1.5 and 251.10.1.4.3.1 provided by Merck Serono), and normal human serum. Validated ranges for the bioanalytical method are 100 ng/mL (LLOQ) to 5000 ng/mL (ULOQ). Validation parameters included intra-run accuracy and precision, selectivity, ligand interference, robustness (inter-operator variability), extended range evaluation (sample dilution), benchtop stability (short term stability), freeze/thaw cycles stability.

References

1. European Medicines Agency (EMA). Guideline on Bioanalytical Method Validation. Committee for Medicinal Products for Human Use (CHMP), London, UK (01 February 2012).
2. Viswanathan CT, et al. Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays, The AAPS Journal 9(1):2007.

Appendix S3 Methodology of flow cytometry and cell subpopulations measured

Sampling

Whole blood samples (*n* = 354) were collected at the clinical site in acid-citrate-dextrose tubes for B cell and plasma (CD138) subset analysis and in CytoChex tubes for TBNK (T, B and natural killer cells) analysis. The draw tubes were filled completely, and tubes mixed immediately (without shaking) by gentle inversion 8 to 10 times. Samples were shipped at ambient temperature from the clinical site to the local Quintiles Central Laboratories for testing. Storage at the sites and at Quintiles was ambient.

Each sample was assayed undiluted in singlicate (200 µL for the B cell and plasma [CD138] subsets assay and 50 µL for the TBNK panel assay). All samples were analysed by a first and verified by a second analyst. Laboratory personnel remained blinded throughout sample analysis. For participant-safety reasons, tests were un-blinded to laboratory personnel if a critical alert value was met.

Testing was repeated if any of the following applied: enumeration of T-cell subsets, incomplete sample lysis, intra-panel failure or incomplete staining of antibody for the B cell and plasma (CD138) subsets assay; incomplete sample lysis and if an error/'need for review' message was displayed by the analysis software for the TBNK panel assay.

Evaluation of B and plasma cell (CD138) subsets in whole blood by flow cytometry

Two polystyrene falcon tubes were filled with cocktails of isotype controls (Tube 1) and test antibodies (Tube 2) (Table 1).

Table 1. Flow cytometry panel for B and plasma (CD138) cell subset analysis

Tube	FITC	PE	PerCP-Cy5.5	PeCy7	APC	APC-H7	BV421	V500
1 (Isotype*)	IgG1, 10 µL	CD19, 40 µL	IgG1, 5 µL	CD3, 10 µL	IgG1, 10 µL	CD20, 10 µL	IgG2a, 10 µL	CD45, 5 µL
2 (Test)	CD138, 10 µL	CD19, 40 µL	CD38, 5 µL	CD3, 10 µL	CD27, 10 µL	CD20, 10 µL	IgD, 10 µL	CD45, 5 µL

*Used to determine the level of background/non-specific antibody staining and the baseline level of fluorescence in that channel

To each tube, 200 µL of the appropriate whole blood sample or 100 µL of CD Chex Plus quality control material was added. The tubes were incubated in the dark at room temperature for 15 minutes. To all tubes, 4 mL of 1x BD FACS Lyse Solution was added and they were again incubated in the dark at room temperature for 15 minutes. All tubes were centrifuged at 500g for 5 minutes at room temperature. The supernatant was decanted and the tubes blotted on an absorbent paper towel. The cell pellet was re-suspended in residual volume and PBS (4 mL) was added to all tubes. All tubes were again centrifuged at 500g for 5 minutes at room temperature and the supernatant was decanted and the tubes blotted on an absorbent paper towel. The cell pellet was resuspended in 350 µL of PBS. The tubes were then transferred to the carousel on the FACS Canto II for analysis.

Analysis was carried out on a BD Canto II Flow Cytometer. The configuration 3-laser 8 colour (4-2-2) BV510.csv (visible at the top of the Diva window) was ensured. In the inspector window, the threshold was checked to ensure it was set to FSC = 15,000. In the Acquisition dashboard the stop gate was set to collect 200,000 Lymphocyte and Monocyte events or 300 seconds. The flow rate was set to Medium. Each sample was played through gating appropriately as outlined in the assay specific gating guides and results were calculated using a result calculation template.

Evaluation of TBNK subset in whole blood by flow cytometry

The analysis panel (Table 2) consisted of single tubes analysed with the CE/IVD 6 Color TBNK panel supplied by BD Bioscience using a lyse/no-wash method that allowed for the direct measurement of absolute cell counts using Cyto-Chex BCT (Streck 213386).

Table 2. Flow cytometry panel for TBKN subset analysis

Tube	**V450**	**V500**	**FITC**	**PE**	**PerCP-Cy5.5**	**PeCY7**	**APC**	**APC-H7**
1	–	–	CD3	CD16/CD56	CD45	CD4	CD19	CD8

One CD Chex Plus and one CD Chex CD4 Low quality control sample were processed per analytical run. The Low quality control is a College of American Professors (CAP) requirement.

BD Multitest 6-Color TBNK Reagent (20 µL) and subsequently control/sample (50 µL) were pipetted onto the grill at the bottom of each Trucount tube. The samples were vortex mixed and incubated at room temperature in the dark for 15 minutes.

FACSLyse 1 x solution was added (450 µL) and vortex mixed. Samples were again incubated at room temperature in the dark for 15 minutes. The tubes were then transferred to the carousel on the FACS Canto II for analysis.

Analysis was carried out on a BD Canto II Flow Cytometer. Each sample was played through gating appropriately as outlined in the assay specific gating guides and results were calculated using a result calculation template.

Cell subsets and quality control targets

B and plasma cell and TBNK subsets and quality control targets and acceptable ranges are shown in Table 3.

Table 3. B Cell and Plasma (CD138) cell and TBNK cell subsets

Cell subset	Cell types (CD nomenclature)	Target	Acceptable range	QC date
B and plasma cells				
Total B cell	CD45+CD3-CD19+ (%)	9.17	7.34–11.01	
Mature Naïve B cell	CD19+CD20+IgD+CD27- (%)	6.23	4.99– 7.48	
Memory B cell	CD19+CD20+IgD-CD27+CD38- (%)	1.16	0.93–1.39	14 Oct 13
Plasma cell	CD19+CD20-IgD-CD27+CD38hiCD138hi (%)	0.01	0.00–0.02	
Plasmablast	CD19+CD20-IgD-CD27+CD38hi (%)	0.01	0.00–0.02	
Total B cell	CD45+CD3-CD19+ (%)	10.22	8.18–12.26	
Mature Naïve B cell	CD19+CD20+IgD+CD27- (%)	7.34	5.87–8.81	
Memory B cell	CD19+CD20+IgD-CD27+CD38- (%)	1.34	1.07–1.61	8 Dec 13
Plasmacell	CD19+CD20-IgD-CD27+CD38hiCD138hi (%)	0.01	0.00–0.02	
Plasmablast	CD19+CD20-IgD-CD27+CD38hi (%)	0.01	0.00–0.02	
TBNK				

Total T cell	CD3+ (%)	76.0	72.2–79.8	
Cytotoxic T cell	CD3+CD8+ (%)	25.3	20.3–30.3	
Helper T cell	CD3+CD4+ (%)	49.0	44.0–54.0	14 Oct 13
NK cell	CD56+/CD16+ (%)	9.8	8.8–10.8	
B cell	CD19+ (%)	13.2	11.8 to 14.6	
Total T cell	CD3+ (%)	74.7	70.9–78.5	
Cytotoxic T cell	CD3+CD8+ (%)	25.1	20.1–30.1	
Helper T cell	CD3+CD4+ (%)	47.7	42.9–52.5	08 Dec 13
NK cell	CD56+/CD16+ (%)	10.9	9.9–11.9	
B cell	CD19+ (%)	13.6	12.2–15.0	
Total T cell	CD3+ (%)	75.7	71.9–79.5	
Cytotoxic T cell	CD3+CD8+ (%)	26.8	21.4–32.2	
Helper T cell	CD3+CD4+ (%)	45.6	41.0–50.2	2 Feb 14
NK cell	CD56+/CD16+ (%)	11.0	9.8–12.2	
B cell	CD19+ (%)	12.3	11.1–13.5	

Appendix S4 Atacicept-population pharmacokinetics model: goodness of fit and validation graphs

Fig. 1 Basic goodness-of-fit plots – all data

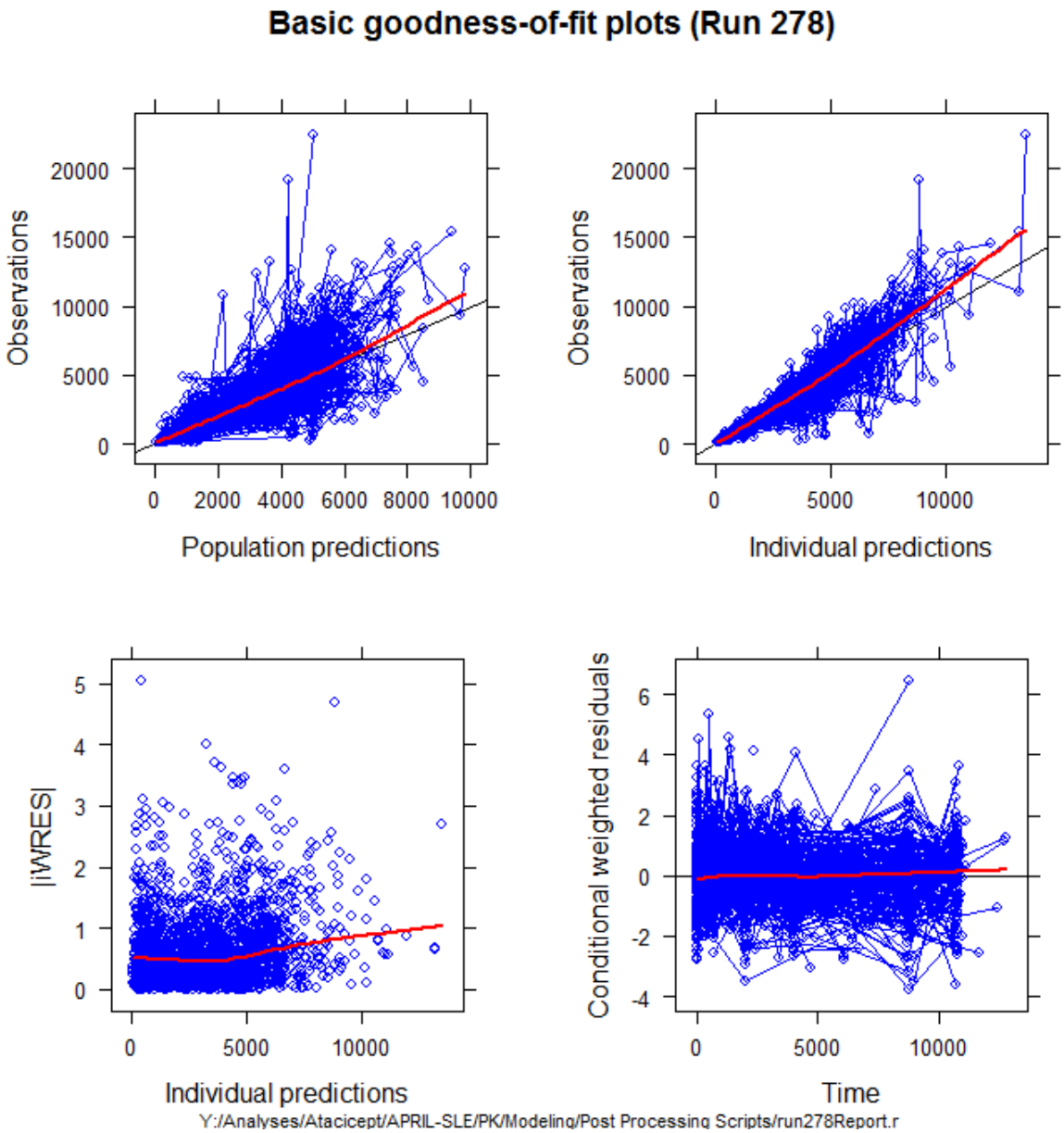


Fig. 2 Basic goodness-of-fit plots – 022 study

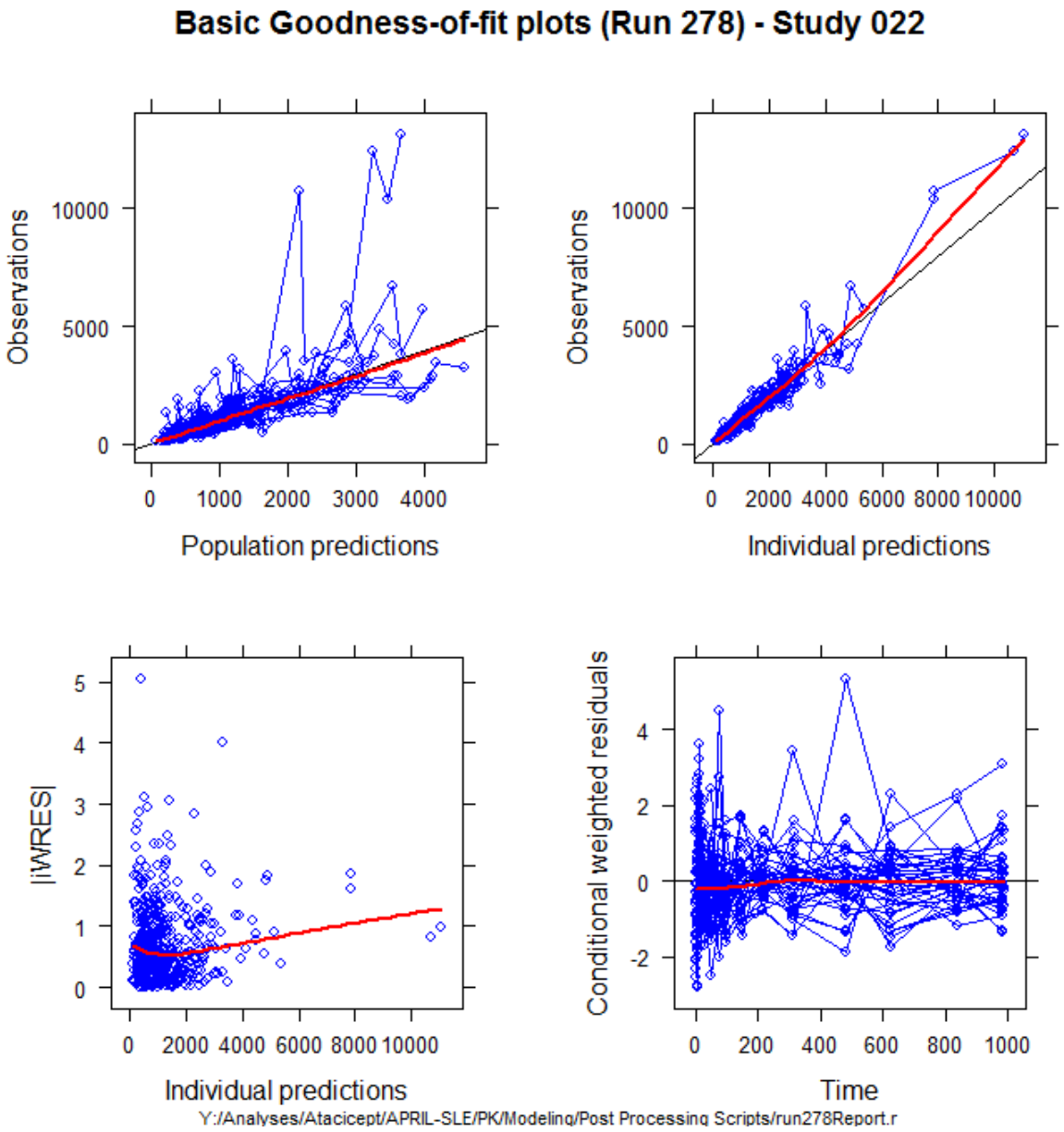


Fig. 3 Basic goodness-of-fit plots – APRIL-SLE study

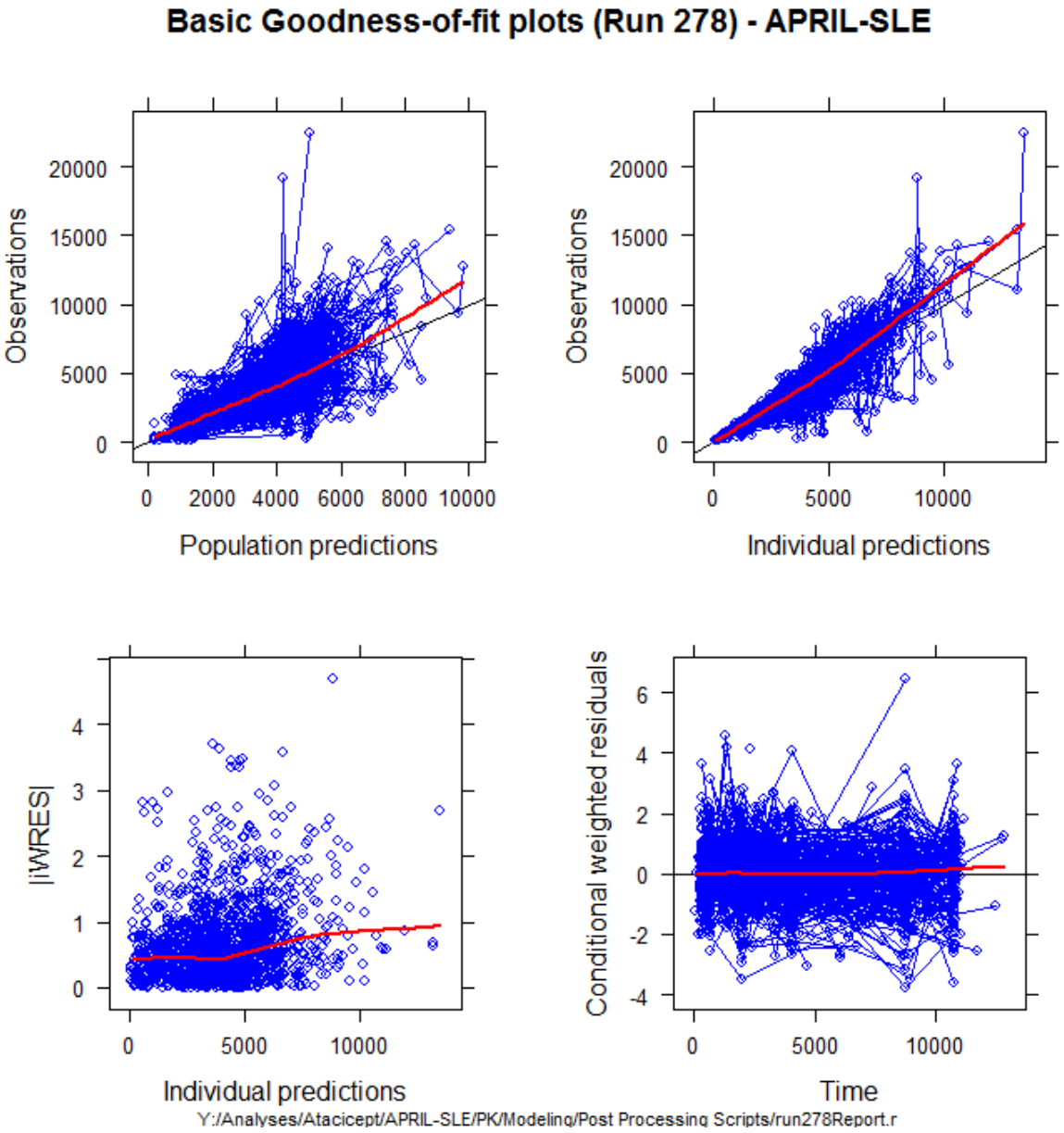


Fig. 4 Population (top left panel), individual (top right panel) and conditional (bottom) weighted residuals vs. time – 022 study

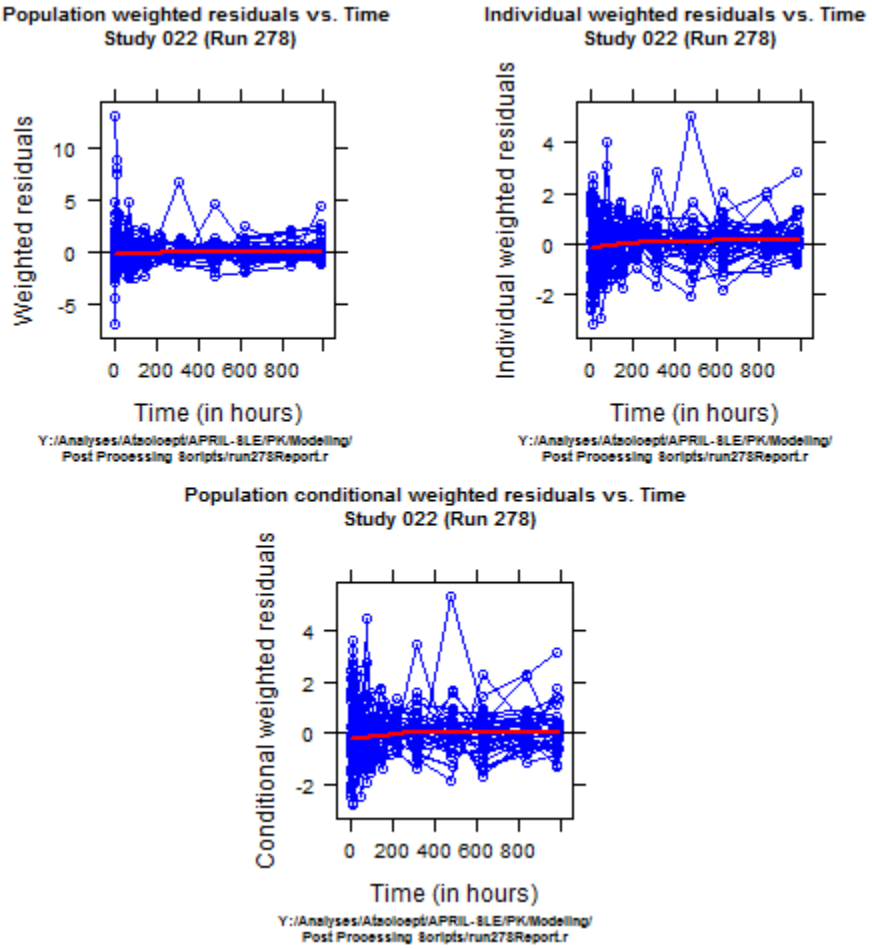


Fig. 5 Population (top left panel), individual (top right panel) and conditional (bottom) weighted residuals vs. time – APRIL-SLE study

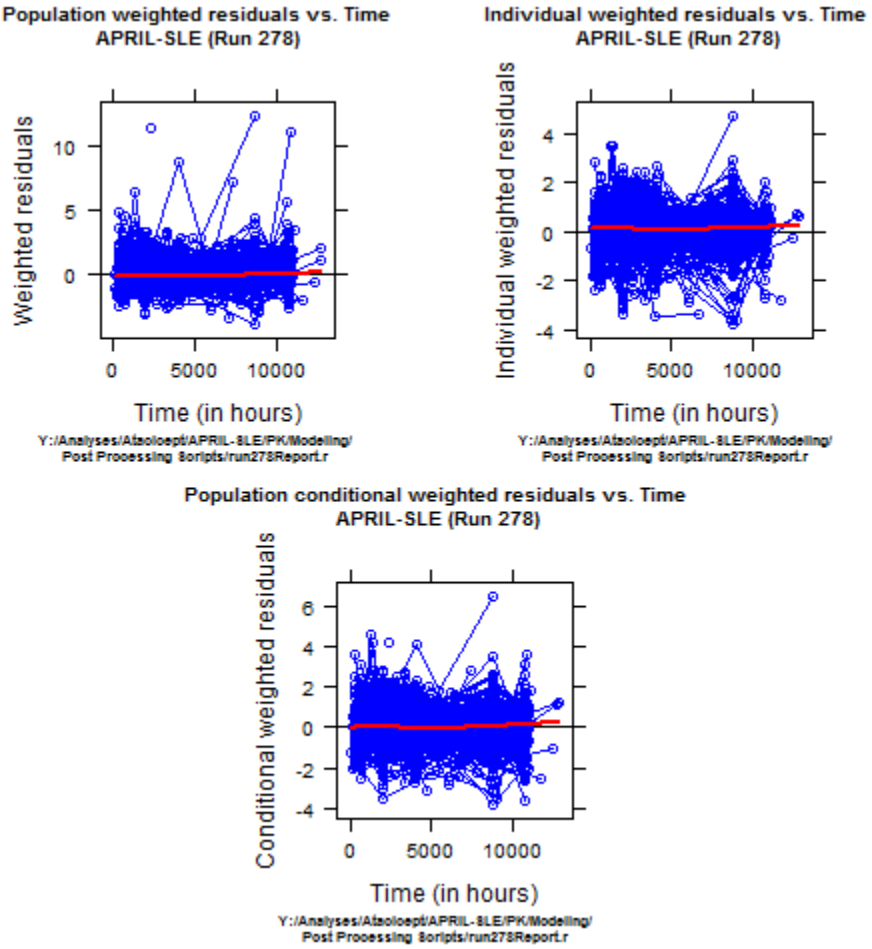


Fig. 6 Observations versus time with population and individual fits – 25 mg, 022 study

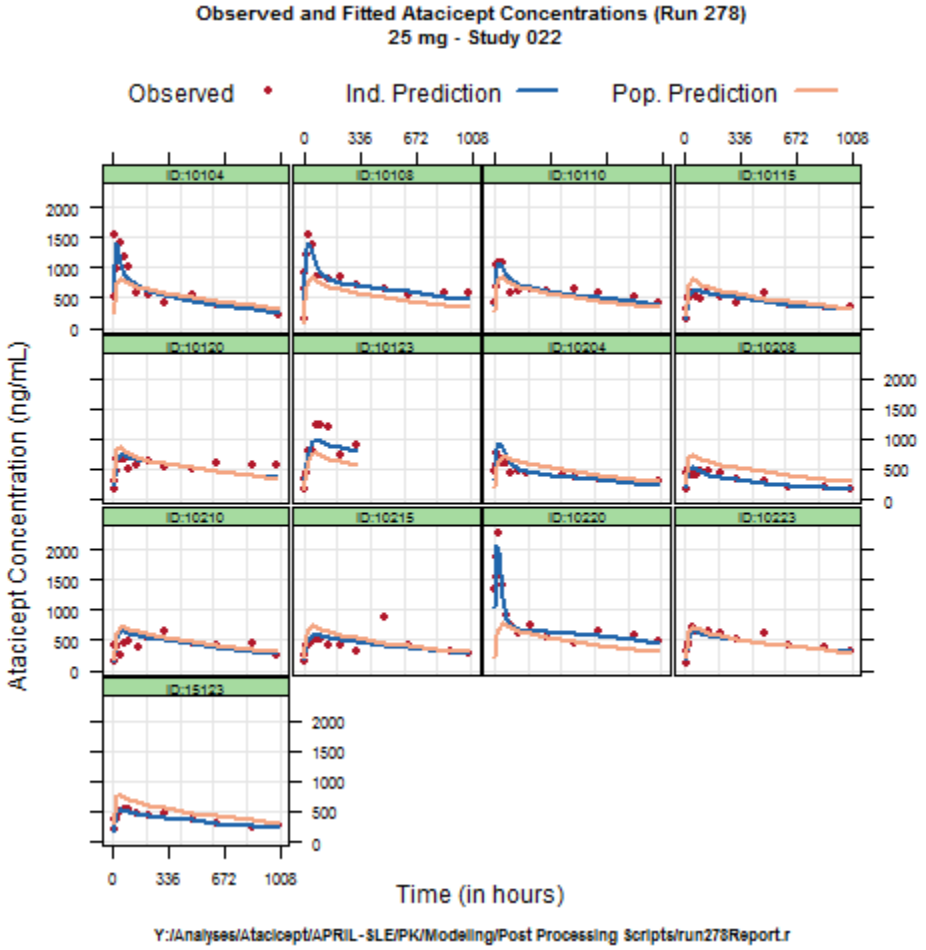


Fig. 7 Observations versus time with population and individual fits – 75 mg, 022 study

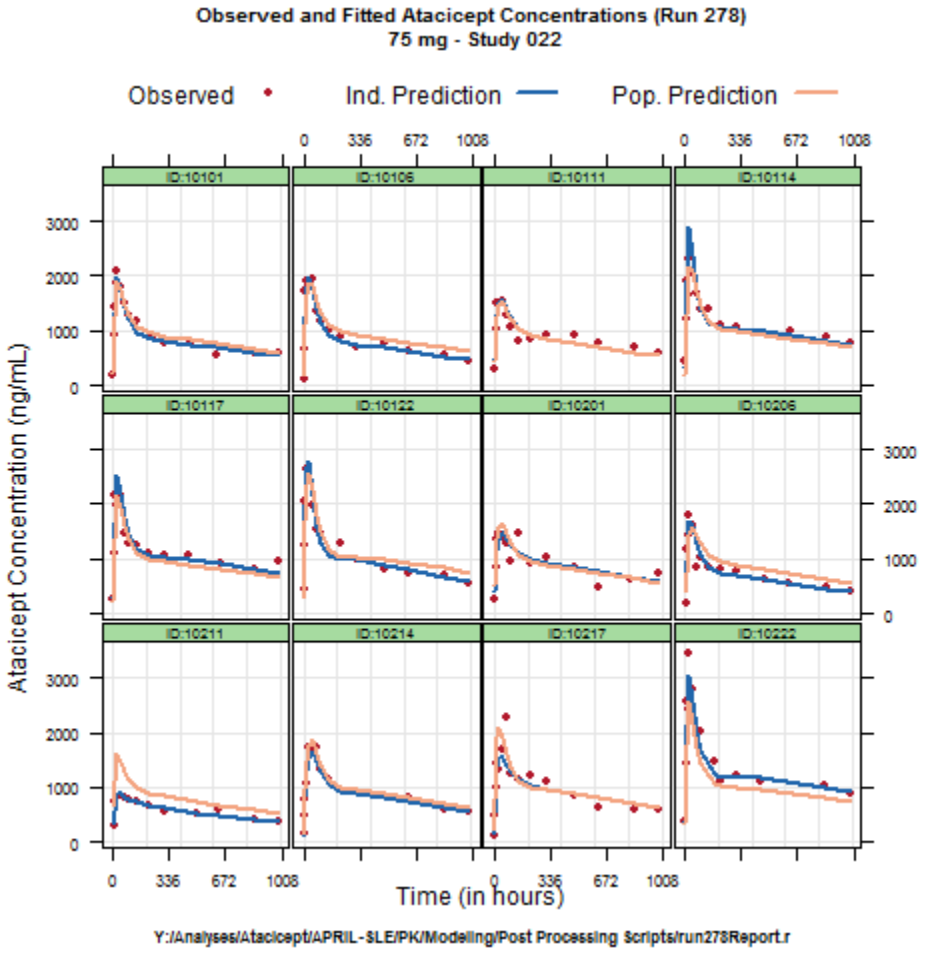


Fig. 8 Observations versus time with population and individual fits – 150 mg, 022 study

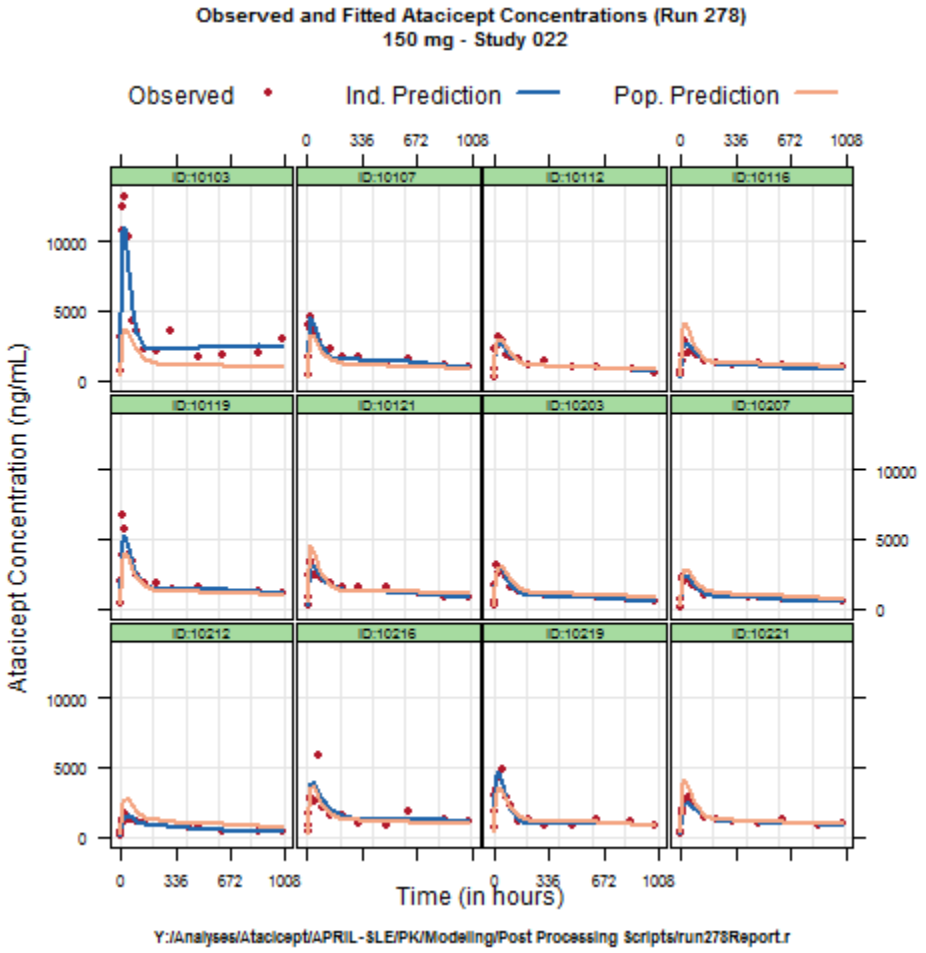


Fig. 9 VPC 022 study

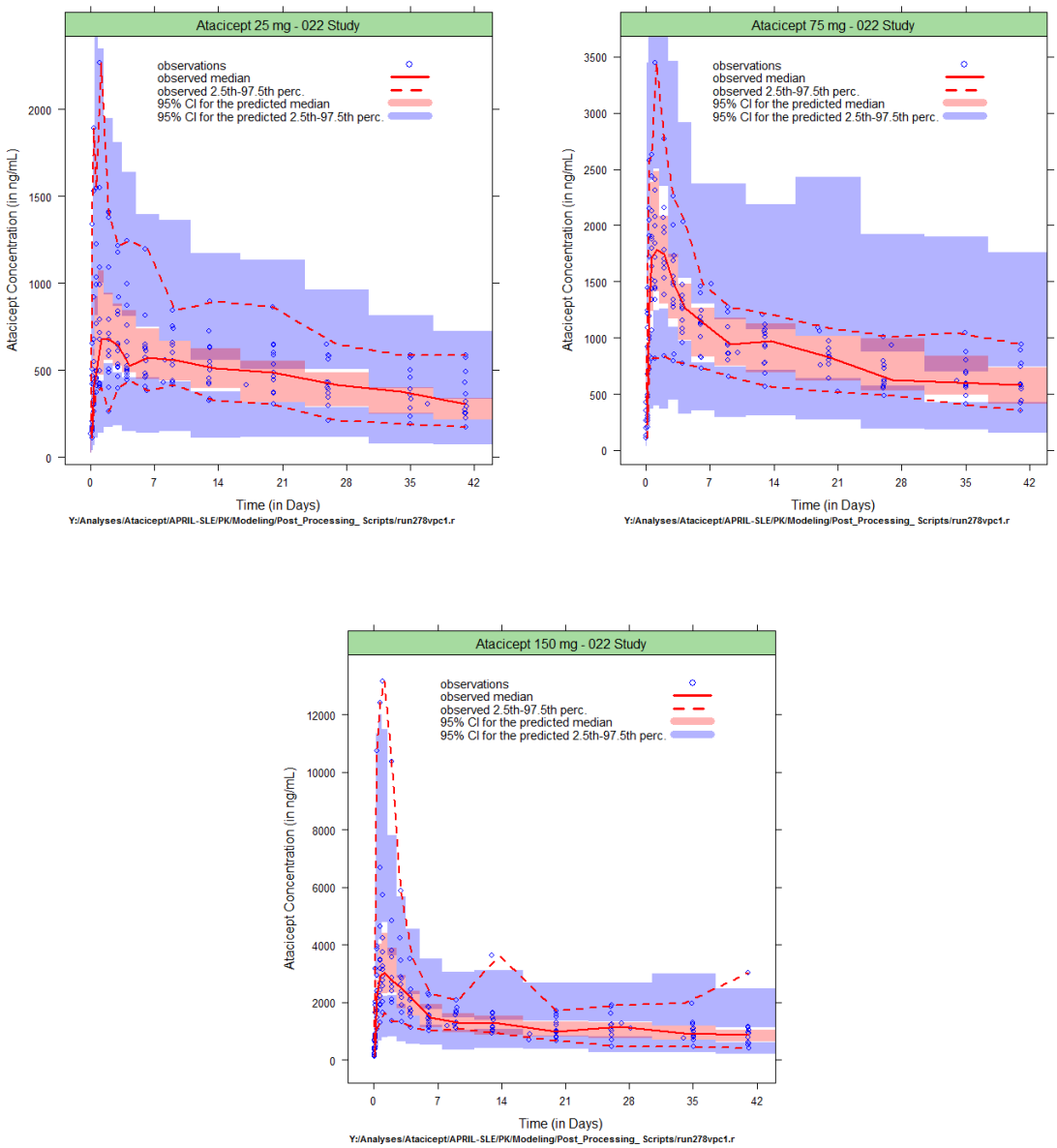


Fig. 10 VPC APRIL-SLE study