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Target-Mediated Modeling of Alirocumab in Adolescents and Children ≥8 to <12 Years of Age Using Phase II and III Data

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Abstract

A population pharmacokinetic (PK) covariate analysis was conducted utilizing data from adolescents and children ≥8 to <12 years of age with heterozygous familial hypercholesterolemia. One phase II and 1 phase III study were analyzed (121 patients on active treatment). A 2-compartment target-mediated model with linear and target-mediated elimination and transit compartments describing lag time in absorption was utilized. Weight and high-dose statins were statistically significant covariate. Except for the central volume, estimated population PK parameters describing linear kinetics were similar across pediatric patients and healthy adults. Coadministration of concomitant high doses of statins was associated with an increase in the production rate of proprotein convertase subtilisin/kexin type 9. The primary covariate model adequately described alirocumab PKs in the pediatric population. The analysis supports the recommended weight-adjusted subcutaneous dosing regimens for alirocumab in children with heterozygous hypercholesterolemia aged ≥8 years.

Keywords

Alirocumab, model, PCSK9, pediatric, pharmacokinetic

Elevated levels of low-density lipoprotein cholesterol (LDL-C) are associated with increased cardiovascular risk, and reduction in LDL-C by therapeutic means has been demonstrated to reduce the risk of cardiovascular events.[1] LDL-C is cleared from the circulation by low-density lipoprotein receptors (LDLRs) on the surface of hepatocytes. The number of available LDLRs is regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9), which promotes the degradation of LDLRs, thus limiting clearance of LDL-C. Monoclonal antibodies that bind to and inhibit PCSK9 lead to reduced degradation of LDLRs, resulting in an increase in LDLR numbers on the cell surface and hence a reduction in circulating LDL-C levels. Alirocumab, a human monoclonal antibody, is directed against PCSK9. The binding of alirocumab to PCSK9 results in blockade of the function of PCSK9.

Heterozygous familial hypercholesterolemia (HeFH) is an inherited genetic disorder that causes dangerously high LDL-C levels, which can lead to heart disease, heart attack, or stroke at an early age if left untreated. Alirocumab is approved in the USA, the EU, and other territories to: reduce the risk of myocardial infarction, stroke, and unstable angina in adults with established cardiovascular disease; reduce LDL-C, as adjunct to diet (alone or in combination with other LDL-C-lowering therapies) in adults with primary hyperlipidemia, including HeFH; reduce LDL-C, as an adjunct to other LDL-C-lowering therapies in adult patients with homozygous familial hypercholesterolemia; and reduce LDL-C, as an adjunct to diet and other

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LDL-C-lowering therapies, in children aged ≥8 years with HeFH.

Alirocumab has been studied in phase I through phase IV trials to evaluate the safety, tolerability, pharmacokinetics (PKs), pharmacodynamics, and immunogenicity of single subcutaneous (SC) and intravenous (IV) doses in participants from 8 years of age, as well as multiple SC doses in adults.

Previous analyses have described the nonlinear PK of alirocumab in adults. Martinez et al.[2] reported an analysis using a Michaelis-Menten approximation to describe total alirocumab concentration–time data in a large dataset containing results from phase I/II/III studies. Djebli et al.[3] developed a model based on the quasi-steady-state approximation of the target-mediated model to describe the PKs of total alirocumab and total PCSK9. These analyses showed an impact of covariates on population PK parameters (PPPs). Nicolas et al.[4] utilized this PK model to build a population pharmacodynamic model which provided insight into covariates of pharmacodynamics in adult patients.

Graphical representation of free PCSK9 and LDL-C concentrations[5,6] implied that when free PCSK9 levels were suppressed by at least 90% a pharmacodynamic effect achieved by PCSK9-lowering antibodies was visually indistinguishable from maximal.

In this analysis, we have extended the work of Martinez et al.[2] and Djebli et al.[3] to develop a full target-mediated PK model, utilizing the available observed total alirocumab and free PCSK9 data from pediatric patients, as well as data from densely sampled phase I studies in healthy adults. Such a model was used to describe the PKs of alirocumab in children ≥8 years old and to substantiate the choice of weight-based dosing regimens. This manuscript provides information on target-mediated population PKs of alirocumab and covariates of PPP in pediatric patients diagnosed with HeFH.

Insights into log-likelihood profiling (LLP) and shrinkage are provided. As with many pediatric populations, the limited sample sizes with sparse sampling complicate parameter estimates for pediatric patients and increase shrinkage. However, LLP allowed for the justification of fixing unstable parameters to adult values, and implemented measures of shrinkage of individual PK parameters in addition to shrinkage of random effects allowed for smaller estimates of shrinkage in a covariate model.

Methods

Study Design and Population

A population PK analysis was performed using pediatric data from a phase II study NCT02890992 (DFI14223) and a phase III study NCT03510884 (EFC14643). In addition, data from adult phase I studies NCT01026597 (R727-CL-0902) and NCT01074372 (R727-CL-0904) were also utilized; these studies have been published and described previously.[7–9] The studies presented here were performed in accordance with Good Clinical Practice guidelines and adhered to the Declaration of Helsinki. The study protocols and procedures were approved by the appropriate institutional review boards and ethics committees at each study site. All participants provided written informed consent before any study procedure was undertaken. There were 8 treatment cohorts (Table 1).

NCT02890992 was an 8-week, open-label, dose-finding phase II study to evaluate the efficacy and safety of alirocumab in children and adolescents with HeFH in 4 patient cohorts, with a total of 42 patients enrolled and having received an active treatment. Each patient cohort had 2 treatment regimens. Overall, there were 6 treatment regimens. Sparse samples were collected at baseline and weeks 4, 8, and 16.

Study NCT03510884 was a randomized, double-blind, placebo-controlled phase III study, followed by an open-label treatment period, in children and adolescents with HeFH to evaluate the efficacy and safety of alirocumab. A total of 150 participants were enrolled and 99 received an active treatment; 20 of the 99 participants had previously participated in study NCT02890992 and had undetectable concentrations of alirocumab at the beginning of the phase III study. Overall, 121 patients participated in 1 or both studies, and received active treatment. One patient did not have any PK observations and was not included in the population PK analysis set; another patient had only 1 post-dose observation of alirocumab and was excluded from the analysis. Overall, 119 participants remained in the analysis. Samples were collected at baseline and mostly at weeks 8, 12, and 24.

In the primary analysis, 390 alirocumab samples and 531 free PCSK9 measures were used. Samples of alirocumab collected before the first dose and samples in patients on placebo were not used in the analysis and were not counted. Concentrations below the lower limit of quantification (LLOQ) were used in the analysis.

After the screening/run-in period of study NCT02890992, patients were enrolled in an 8-week (12-week for cohort 4), open-label, dose-finding treatment period followed by an extension phase. Enrollment was sequential into 4 separate and independent cohorts of children and adolescents aged 8-17 years with HeFH; for each cohort, it was planned to enroll 10 patients with no fewer than 4 patients in each bodyweight category (weights of <50 kg and ≥50 kg). The cohorts and doses are presented in Table 1. The different weight groups received different treatment regimens. A treatment adjustment was allowed at the week

Table 1. Treatment Regimens by Study, Cohort Number, and Weight

Study	Cohort number	Weight (kg)	Dosing regimen	Dose adjustment possible from week 12
NCT02890992	1	<50	30 mg Q2W	
		≥50	50 mg Q2W	
	2	<50	40 mg Q2W	
		≥50	75 mg Q2W	
	3	<50	75 mg Q4W	
		≥50	150 mg Q4W	
	4	<50	150 mg Q4W	
		≥50	300 mg Q4W	
NCT03510884	1	<50	40 mg Q2W	75 mg Q2W
		≥50	75 mg Q2W	150 mg Q2W
	2	<50	150 mg Q4W	75 mg Q2W
		≥50	300 mg Q4W	150 mg Q2W

Q2W, every 2 weeks; Q4W, every 4 weeks.

12 visit. Overall, there were 8 potential sequences of the treatment regimens in the phase III study (Table 1). As many patients who participated in both phase II and III trials received different treatments, several additional sequences of treatment regimens were observed in the integrated dataset. Unlike descriptive analyses, the population PK approach allows for informative integration across many small treatment sequences.

Among children in the active treatment groups, the mean ± standard deviation (SD) age was 13.1 ± 2.86 years, weight was 52.0 ± 18.8 kg, and 42.5% of patients were males.

The same model was applied to pediatric and healthy adult data, with the goal of fixing unstable parameters in the pediatric model to the adult values.

Analytical Methods

Total alirocumab, total PCSK9, and free PCSK9 were measured using enzyme-linked immunosorbent assay-based bioanalytical assays. Alirocumab has 2 binding sites and can form complexes with 1 or 2 molecules of PCSK9. The total alirocumab bioanalytical assay quantitates free alirocumab, as well as alirocumab bound to 1 or 2 molecules of PCSK9. The LLOQ of total alirocumab is 0.0780 mg/L. The total PCSK9 assay measures free PCSK9 and PCSK9 bound to alirocumab, while the free PCSK9 assay measures only PCSK9 not bound to alirocumab. The LLOQs of total and free PCSK9 are 0.0780 and 0.0312 mg/L, respectively.

Anti-alirocumab antibodies are assessed in human serum samples using an electrochemiluminescence bridging immunoassay. The method potentially involves 3 steps in the evaluation of a human sample: screening; confirmation; and titer determination. The screening step identifies potential positive samples, the confirmation (drug specificity) step is used to exclude false positive samples, and the titer step is used to measure antidrug antibody (ADA) titers. In this report, titers were set to 0 if the screening test was negative, ADAs were not drug-specific, or the titers were confirmed negative. The analytical methods are described in greater detail elsewhere.[10–12]

A comparison of data from assays measuring free and total PCSK9 before the administration of alirocumab, which would be expected to yield similar results, indicated that predose concentrations of total PCSK9 were higher than those from the free PCSK9 assay. The interpretation of this observation is that the total assay captures PCSK9 bound to endogenous proteins, which are not measured in the free assay. However, this nonspecific binding is poorly understood, making any mathematical description somewhat speculative.

Population PK Analysis

Given the apparent differences in the observed free and total PCSK9 concentrations, a model that adequately describes both the free and total PCSK9 concentrations was not identified, as the inclusion of total PCSK9 data adversely affected the model. A decision was made to focus the analysis on a dataset that included only the total alirocumab and free PCSK9 observations in order to build a target-mediated drug disposition model.

A concentration-time profile of rich adult data was evaluated to accommodate the selection of a structural model for the pediatric population. As alirocumab concentration data exhibited a classic profile for a monoclonal antibody, with absorption, distribution (alpha), linear (beta), and target-mediated phases, full target-mediated and Michaelis-Menten models with parallel linear and target-mediated elimination were considered. After initial evaluations, a full target-mediated

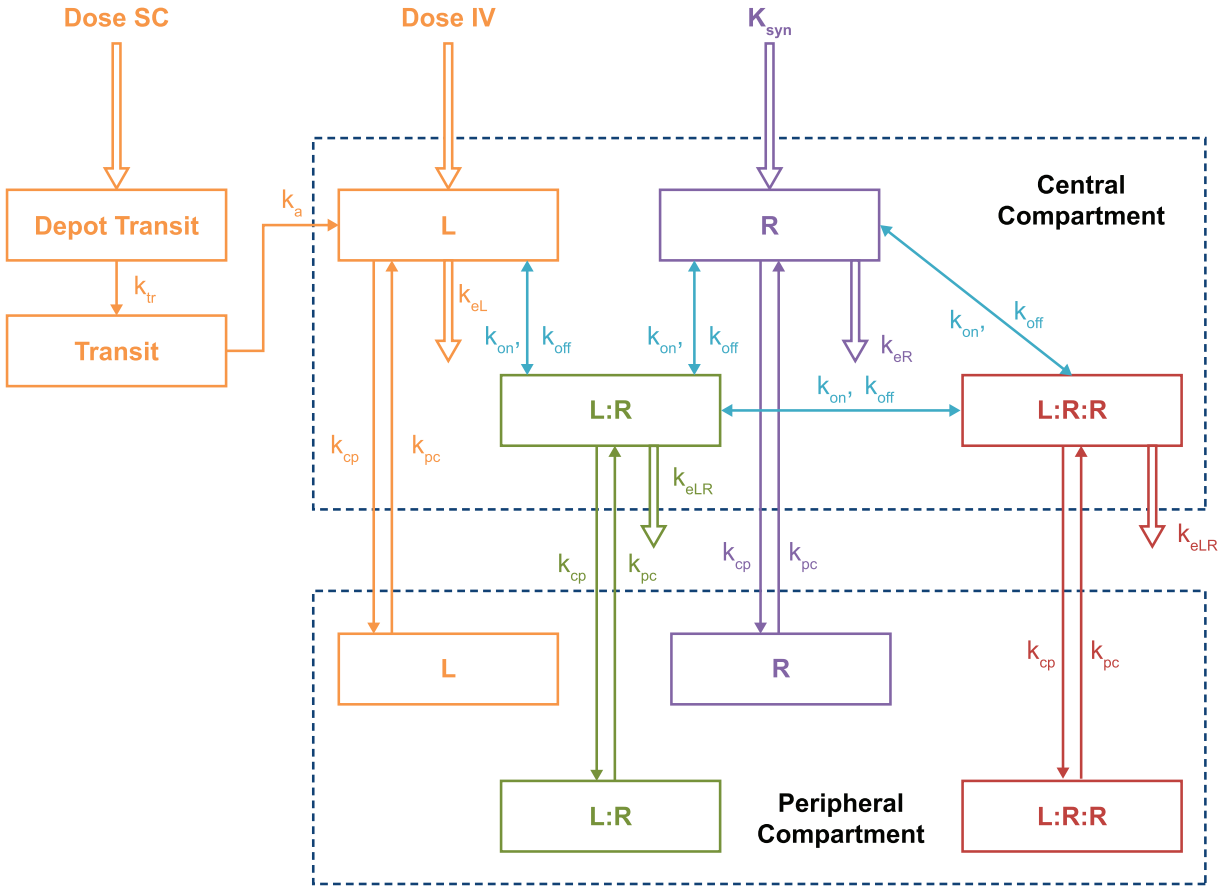

Figure 1. The bidirectional blue arrows depict binding and unbinding. The thick 1-directional arrows depict the change in mass of alirocumab and PCSK9 caused by alirocumab delivery (IV infusion or SC injection), synthesis of PCSK9, and elimination of alirocumab and PCSK9. The 1-directional arrows depict transit and inter-compartmental rates. IV, intravenous; k_a, absorption rate; k_{cp} and k_{pc}, central-to-peripheral and peripheral-to-central rates, respectively; k_{eL}, k_{eR}, k_{eLR}, and k_{eLRR}, elimination rates of L, R, L:R, L:R:R, respectively; k_{on}, association rate constant; k_{syn}, production rate of R; k_{tr}, transit rate; L, alirocumab (yellow); L:R, drug:target complex (green); L:R:R, drug:target:target complex (red); k_{off}, dissociation rate constant; R, PCSK9 (magenta); SC, subcutaneous.

model was selected to best describe the observed total alirocumab and free PCSK9 data. The full target-mediated model was used to take advantage of PCSK9 measures and to better describe the sparse pediatric data. A 2-step modeling approach was utilized, where parameters were first estimated in the adult population. Subsequently, certain parameters were fixed to the adult values to enable estimation of the remaining parameters in the pediatric population.

The structural PK model for alirocumab and PCSK9 is depicted in Figure 1.

A 2-compartment PK model was utilized to describe the distribution of alirocumab. As a lag time was observed after SC doses in adults, a transit-compartment model of absorption was utilized. Bioavailability (F), central-to-peripheral and peripheral-to-central intercompartmental rates (k_{cp} and k_{pc}), transit rate (k_{tr}), and absorption rate (k_a) were estimated in adults using rich data, and were subsequently fixed to the adult values in the pediatric model. The association and dissociation rate constants (k_{on} and k_{off}, respectively) were fixed to the values estimated in vivo utilizing Biacore technology.[13] The elimination rate of free PCSK9 was calculated as k_{eR} = production rate of PCSK9 (k_{syn})/baseline value of free PCSK9 (R_0). A 1- and 2-arm binding of alirocumab to PCSK9 was modeled. Distribution of free, 1-arm bound, and 2-arm bound alirocumab was implemented. The developed full target-mediated model was the same in adults and in the pediatric population; that is the model structure, differential equations, and model of residual error were identical. The only difference was that the adult model did not include high-dose statins as a covariate because adults were healthy and did not take high doses of statins.

The exponential model $Y_i = Y(\lambda_i)\cdot\exp(\eta_i)$ was explored as the primary model for the random effects, where Y_i is an individual PK parameter, $Y(\lambda_i)$ is a PPP adjusted for covariates, λ_i is a vector of covariates, η_i (estimate of the random effects $[ETA]_i$), representing

the random effects, accounts for a deviation between population and individual values, and i is a patient index. The individual vectors η_i are assumed to be independently and normally distributed around zeros with a covariance matrix Ω. The PK parameters had log-normal distribution in the models.

Interindividual variability was implemented on the central volume (V_c), the elimination rate of alirocumab (k_{eL}), and the elimination rate of alirocumab:PCSK9 complexes (k_{eLR}), R_0, and k_{syn}.

Estimates of shrinkage in ETAs and in individual parameters were provided. ETAs characterize the difference between individual PK parameters and PPPs when there are no covariates in the model. Shrinkage reflects the quality of individual Bayesian estimates (EBEs) and diagnostic plots where EBEs are used. EBEs "shrink" toward the PPPs when the data are sparse or otherwise insufficient to characterize each individual precisely. Shrinkage in ETAs is defined as a percentage decrease in the SD of estimated individual ETAs compared to a theoretical (population-predicted) estimate of the SD of ETAs.[14] Monolix software provided improved figures of shrinkage, reflecting shrinkage in individual parameters without calculating a metric reflecting the improvement. Shrinkage in individual parameters is defined as a percentage decrease in the SD of estimated individual parameters, incorporating the effect of covariates, compared to the SD of simulated theoretical (population predicted) distribution of individual parameters incorporating the effect of covariates. Similar to the shrinkage of ETAs, shrinkage in individual parameters is calculated as $(1 - SD_{estimated}/SD_{theoretical}) \times 100\%$. Monolix option "conditional mode (EBEs)" was always used during graphics generation and estimation of shrinkage. The shrinkage in individual parameters was calculated using datasets with individual estimated and individual simulated parameters generated by Monolix (datasets simulatedIndividualParameters.txt and estimatedIndividualParameters.txt). The shrinkage in individual parameters accounts for their potential improvement after the addition of covariates.

A model with high shrinkage is useful if research and conclusions are based on population rather than on individual parameter estimates.[14] When simulated PK concentration and diagnostic plots incorporating simulations are based on PPPs, they are mostly unaffected by shrinkage. As shrinkage informs on the quality of individual parameters, population model selection based on parameter shrinkage is not necessary.[14]

The combined proportional and additive model of error was explored: [observed concentration$_{ij}$] = [individual predicted concentration$_{ij}$] + (a + b·[individual predicted concentration$_{ij}$])·ε_{ij}, where ε_{ij} is a standardized error, i represents the patient, and j represents the observation.

The M4 method (as opposed to the M3 method)[15] of incorporating observations below the limit of quantitation (BLQ) values was utilized to avoid negative simulated concentrations in diagnostic plots.

A full target-mediated model from the Monolix model library was modified and utilized as a model prototype. Transit compartments describing the lag time of absorption, 2-arm binding of alirocumab to PCSK9, and distribution of alirocumab:PCSK9 complexes were implemented.

Variability in the objective function value (OFV, also abbreviated as –2LL) was evaluated during model building. The software estimates OFV after the model convergence is completed. The estimate of OFV can be imprecise or even unacceptable for model building even when PPPs are stable.[16–18] The variability in OFV can be inflated by a combination of sparse data, a steep target-mediated phase, and the presence of BLQs in an analysis.

The following log-linear model was used to test for continuous covariates:

$$Y(\lambda i) = Y \cdot \left[\frac{\lambda_i}{\text{Median}(\lambda_i)}\right]^{\theta}$$

where $Y(\lambda_i)$ is a PPP adjusted for covariate λ, λ_i is an individual value of a covariate, i is a patient index, Y is a PPP at the median or another selected level of λ, and θ is a parameter describing an effect of the covariate on the PPP. As pediatric patients were assigned to treatment groups according to weight (<50 and ≥ 50 kg), the median value of 50.1 kg in the formula was replaced with 50 kg.

The following multiplicative model was used to test for dichotomous covariates:

$$Y(\lambda_i) = Y \cdot e^{\theta \cdot \lambda_i}$$

where $Y(\lambda_i)$ is a PPP adjusted for the covariate, λ_i is an individual value of a covariate, i is a patient index, Y is a PPP when $\lambda_i = 0$, and θ is a parameter describing an effect of the covariate on the PPP; λ_i is equal to 0 or 1.

The log-linear and multiplicative models were chosen to avoid negative individual PK parameters.

The continuous covariates tested were weight, age, albumin, creatinine clearance, and body mass index (BMI); the categorical covariates tested were high-dose statins (HDS), ADAs at any time, sex, and ezetimibe.

Stepwise forward inclusion/backward elimination was used for covariate selection. Covariates were incorporated or rejected based on the following criteria:

- Forward inclusion: A parameter included in the model when $\alpha \leq 0.01$.
- Backward elimination: A parameter remains in the model when $\alpha \leq 0.001$.

The covariate model building was conducted using time-dependent weight, BMI, and age, and was then repeated using baseline covariates. Baseline covariates were utilized since high variability in OFV and signs of overparameterization were observed when time-dependent covariates were explored. Standard goodness-of-fit plots were evaluated for the base and covariate models.

The log-likelihood profile (LLP) was used to explore stability and evaluate confidence intervals (CIs) of PPP. LLP is a plot of the OFV versus PPP, and it is an established alternative to the bootstrapping model validation method. LLP allows for the exploration of stability of both estimated and fixed PPPs; however, a disadvantage is that it can be affected by variability in the OFV. Two hundred bootstraps were executed to confirm that bootstrap CIs around PPP were consistent LLP CIs.

The median regression option of the SAS QUANTREG procedure was used to create a regression line in dependent variable (DV) versus population predictions and DV versus individual predictions figures. The median regression repeatedly provided more meaningful approximations than default splines, presumably because it was less sensitive to outliers, high influence points, and deviations from normal distributions. Under some other circumstances, splines can be more informative.

The stability of the base and covariate models was evaluated using: (a) 10-model runs with large random changes in initial parameters; (b) the condition number; (c) relative standard error (%) of PPPs; (d) Wald *P*-values, and (e) LLPs. Unstable versions of the model were eliminated from further development.

The following sensitivity analyses were conducted as additional validation steps (Tables S2-S10): (a) the primary covariate model was executed with all outliers and patients in the analysis; (b) covariate models parameterized using elimination rate and clearance were compared; (c) PPPs in covariate models with bioavailability of 10%; and (d) PPPs in covariate models with bioavailability of 100% were estimated to demonstrate that OFV and PPPs other than V_c are not sensitive to such changes; (e) weight, age, and BMI were tested as time-dependent covariates (regressors) rebuilding the entire model to demonstrate similar PPP; (f) the primary covariate model was executed assuming that all patients across the phase II and III studies were unique to demonstrate that a change in baseline covariates had no meaningful impact on PPP; (g) the impact of the removal of inter-individual variability in k_{eLR} on PPPs and shrinkage was evaluated; (h) the impact of accounting for correlation between V_c and k_{eL} on PPPs, shrinkage, and exposure metrics of simulated concentrations was evaluated; and (i) the impact of both accounting for correlation between V_c and k_{eL}, and the removal of inter-individual variability in k_{eLR} on PPPs and shrinkage was explored. In Table S11, PPPs from the primary covariate model and the sensitivity analyses are presented together.

The population PK analysis was used to simulate concentrations of alirocumab and free PCSK9. Simulated PCSK9 concentrations based on the analyses provided in this manuscript helped to substantiate the efficacy of the selected doses, as well as the outcomes of some other pharmacometric analyses (data not shown). Monolix Suite 2024R1 was used to conduct population pharmacometric analyses.

Results

Estimates of PPPs for the pediatric covariate model are presented in Table 2; for comparison, PPPs of the adult model are also presented. Data from healthy adult studies with dense PK sampling (NCT01026597 and NCT01074372)[9] were used to estimate PPPs. HDS was a covariate in pediatric patients only, as healthy adults were not receiving HDS. Pediatric values for F, k_{cp}, k_{pc}, k_{tr}, and k_a were fixed to the estimates from adults. The adult and pediatric models demonstrated consistent results.

The initial PPPs of the model were randomly changed 10 times using the "assessment" option, and parameters were re-estimated. This analysis demonstrated a good stability of the base and the primary covariate models. Diagnostic plots demonstrated a good model fit (Figures 2, 3, S1, and S2). Diagnostic plots of the adult model are also presented (Figures S6-S9).

In the sensitivity analyses (a) through (f) defined in the Methods section and provided in Tables S2-S7, population PK parameters were similar to those in the primary covariate model (Tables 2 and S11).

Shrinkage of ETAs and individual parameters in the base and covariate models are presented in Tables S1 and 3, respectively, and also depicted in Tables S8-S10. Estimates of shrinkage in the sensitivity analyses are provided in Table 3 to demonstrate the impact of changes in the structure of the OMEGA matrix on shrinkage. Shrinkage in ETA of V_c was notably higher in the primary covariate model than in the base model (Table 3), while the SD of ETAs of V_c was more than 2-fold lower in the covariate model (SD of 0.108; Table 2) than in the base model (SD of 0.244; Table S1). The shrinkage in individual PK parameters affected by covariates (Table 3) in the primary covariate model was lower than the shrinkage in ETAs of the same parameters in the base and primary covariate models. The largest changes in shrinkage between base and covariate models were associated with parameter V_c; the strongest impact of a covariate (weight) was also related to this parameter.

Table 2. Primary Covariate Model: Population PK Parameters in Pediatric Patients and Healthy Adults

		Population estimate (bootstrap 95% CI)	
Type of parameter	Parameter name	Pediatric population	Adults
PK parameters	V_c (L)	1.64 (1.36, 1.95)	3.01 (2.67, 3.11)
	k_{eL} (1/day)	0.0800 (0.0641, 0.0953)	0.0698 (0.0622, 0.0883)
	k_{syn} (nM/day), HDS = "No"	5.76 (4.44, 7.14)	4.97 (3.55, 5.51)
	R_0 (nM)	2.43 (2.20, 2.62)	2.28 (2.16, 2.45)
	k_{eLR} (1/day)	0.372 (0.210, 0.522)	0.320 (0.248, 0.347)
	k_{cp} (1/day)	0.194 (—)	0.194 (0.158, 0.291)
	k_{pc} (1/day)	0.280 (—)	0.280 (0.236, 0.482)
	k_a (1/day)	0.333 (—)	0.333 (0.269, 0.461)
	k_{tr} (1/day)	22.5 (—)	22.5 (14.5, 43.2)
	F (unitless)	0.655 (—)	0.655 (0.488, 0.678)
Covariate coefficients	$V_c \sim$ weight	0.724 (0.589, 0.925)	0.787 (0.530, 1.29)
	$k_{syn} \sim$ HDS	0.576 (0.191, 1.00)	–
OMEGA matrix	SD(ln[V_c])	0.108 (0.0549, 0.171)	0.241 (0.141, 0.282)
	SD(ln[k_{eLR}])	0.356 (0.210, 0.522)	0.271 (0.178, 0.349)
	SD(ln[k_{syn}])	0.616 (0.386, 0.832)	0.354 (0.218, 0.420)
	SD(ln[R_0])	0.277 (0.136, 0.380)	0.214 (0.160, 0.261)
	SD(ln[k_{eL}])	0.164 (0.0965, 0.243)	0.242 (0.154, 0.430)
	SD(ln[k_a])	–	0.578 (0.263, 0.807)
	SD(ln[k_{tr}])	–	1.01 (0.563, 1.56)
Residual SD and OFV	SD(alirocumab)	0.247 (0.209, 0.278)	0.147 (0.124, 0.165)
	SD(PCSK9)	0.414 (0.387, 0.475)	0.249 (0.220, 0.273)
	OFV	1380.5	–
Derived parameters	k_{syn} (nM/day), HDS = "Yes"	10.3 (–)	–
	CL (L/day)	0.131 (–)	0.210 (–)
	Q (L/day)	0.318 (–)	0.584 (–)
	V_p (L)	1.14 (–)	2.09 (–)
	$t_{1/2beta}$ (day)	15.8 (–)	17.9 (–)

CI, confidence interval; CL, linear clearance; CV, coefficient of variation; F, bioavailability; HDS, high-dose statin; k_a, absorption rate; k_{cp}, central-to-peripheral rate; k_{pc}, peripheral-to-central rate; k_{eL}, elimination rate of alirocumab; k_{eLR}, elimination rate of alirocumab:PCSK9 complex; k_{syn}, synthesis rate of PCSK9; k_{tr}, transit rate; PCSK9, proprotein convertase subtilisin/kexin type 9; PK, pharmacokinetic; Q, intercompartmental clearance; R_0, baseline concentration of PCSK9; "–" = not calculated for fixed, derived, or excluded parameters; SD, standard deviation; SE, standard error; V_c, central volume; V_p, peripheral volume. The volumes are estimated at a weight of 50 kg in the pediatric population and 75 kg in the adult population.

LLPs for pediatric parameters fixed to adult values are presented in Figure S5. The bootstrap CIs of PPP are presented in Table 2. Both the LLPs of the estimated PPP and bootstrapping demonstrated acceptable CIs. As the LLP of estimated parameters demonstrated good stability, and LLP CIs were consistent with the bootstrap CIs, they added little to the bootstrap CIs and were not presented. The LLPs also demonstrated the instability of the parameters fixed to the adult values, confirming that these parameters have to be fixed.

Changes in the number of transit compartments of the adult model did not cause meaningful changes in PK parameters other than k_{tr}. The same modifications of the pediatric model also led to trivial changes in the remaining parameters, similar to those observed when the model was executed with slightly different initial values; there was no meaningful impact on OFV and visual predictive checks (VPCs) as well as primary results and conclusions.

The value of k_{syn} was slightly larger in pediatric patients who were on low or no doses of statin than in healthy adults; k_{syn} was 79% higher in pediatric patients who were on HDS than in pediatric patients not taking HDS; and k_{syn} in pediatric patients who were not taking HDS was only slightly higher than in adults. The association between statin consumption and k_{syn} is consistent with published data.[19] The baseline values of free PCSK9 in patients on low or no doses of statins and HDS were 0.176 and 0.208 mg/L, respectively. The difference was not particularly high, consistent with the notion that statins increase both production and elimination rates of PCSK9 and that the increase in production rate is somewhat larger than the increase in elimination rate. When HDS was used as a covariate of R_0 in addition to the covariate of k_{syn}, it was a statistical trend, and as a result this potentially important covariate did not remain in the model. Nineteen participants received HDS, and the power was not sufficient to keep

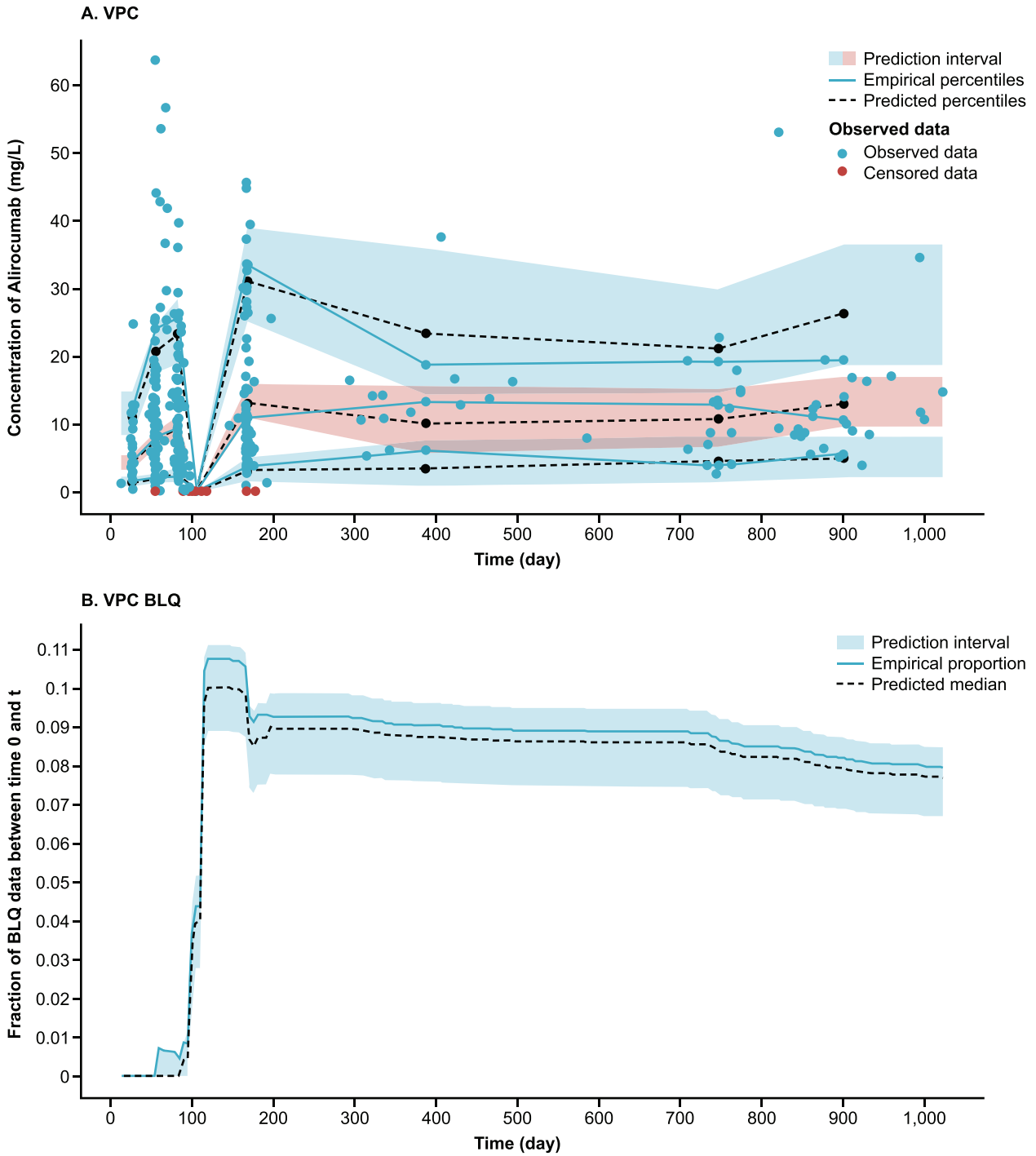

Figure 2. Visual predictive checks: (A) VPC of alirocumab; (B) VPC BLQ of alirocumab. BLQ, below the limit of quantitation; VPC, visual predictive check.

the HDS variable as a covariate of baseline free PCSK9 in the model.

The impact of ADAs was not statistically significant or clinically meaningful in both the pediatric and adult populations. There were no sudden and significant drops in the concentrations of alirocumab, which are usually caused by ADAs. The ADAs cannot be directly compared across the adult and pediatric populations due to substantially different study designs, including study duration and frequency of ADA assessments. An appraisal of ADAs across studies with comparable designs demonstrated 5.5% and 3% in adults and pediatric patients, respectively.

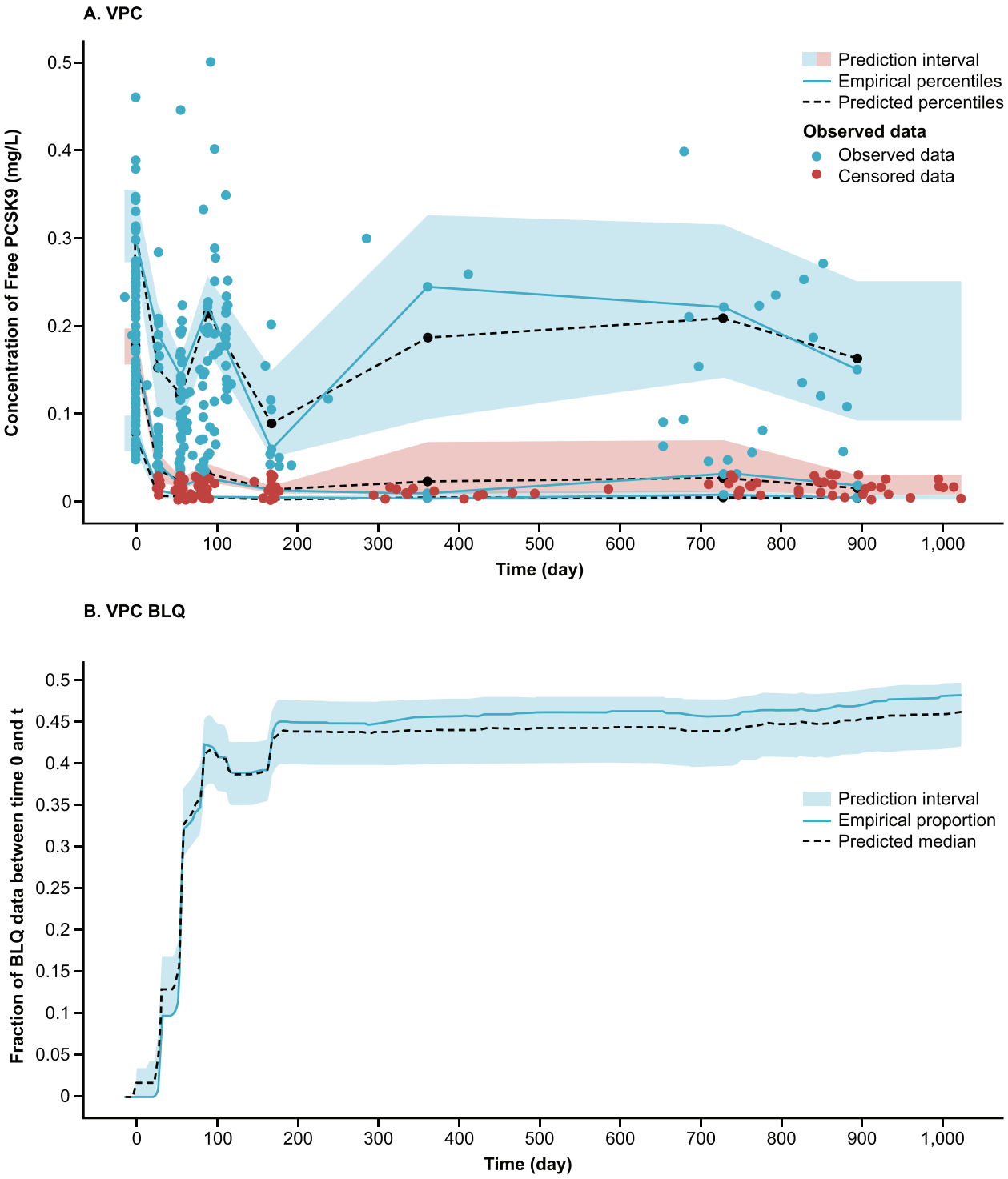

Figure 3. Visual predictive checks: (A) VPC of PCSK9; (B) VPC BLQ of PCSK9. BLQ, below the limit of quantitation; PCSK9, proprotein convertase subtilisin/kexin type 9; VPC, visual predictive check.

Simulations of studied dosing regimens demonstrated that free trough PCSK9 levels were suppressed by more than 90% in at least 1 treatment regimen among 2 treatments available for each pediatric weight cohort.

Discussion

While the limited sample sizes and sparse sampling in pediatric studies tend to limit precise parameter estimation for pediatric patients, the methods implemented in

Table 3. Covariate Model: Shrinkage in the SD of ETAs and the SD of Estimated Individual Parameters

	Shrinkage in SD of ETA (%)/shrinkage in the SD of estimated individual parameters (%)				
Parameter	Base model	Primary covariate model	Covariate model without OMEGA of k_{eLR}	Covariate model with correlation between V_c and k_{eL}	Covariate model with correlation between V_c and k_{eL} and without OMEGA of k_{eLR}
V_c	37.0/34.6	63.3/4.64	32.6/12.8	58.9/17.9	22.4/16.2
k_{eLR}	34.8/35.4	30.0/30.5	–	32.4/31.2	–
k_{syn}	31.0/31.5	27.3/23.5	29.7/24.8	27.5/22.7	29.9/28.0
R_0	39.2/36.1	39.5/37.4	35.7/35.3	44.0/42.2	33.0/32.6
k_{eL}	52.6/52.8	52.2/53.1	46.6/47.0	53.7/53.1	17.1/21.8

ETA, estimate of the random effects; k_{eL}, elimination rate of alirocumab; k_{eLR}, elimination rate of alirocumab:PCSK9 complex; k_{syn}, production (synthesis) rate of PCSK9; PCSK9, proprotein convertase subtilisin/kexin type 9; R_0, baseline concentration of PCSK9; SD, standard deviation; V_c, central volume.

this population PK approach improved the precision and model fit.

As densely sampled IV and SC data were not collected in the pediatric population, it was impossible to estimate F, k_{cp}, k_{pc}, k_{tr}, and k_a in the pediatric population alone. Since the addition of sparse data to rich datasets, particularly rich data from a different population, can lead to deviation of some of the listed parameters from their physiological values, it was not planned to combine rich adult and sparse pediatric data.[18] Imperfections of the log-linear approximations of parameter-covariate relationships can negatively affect the estimation of PPPs, particularly for the sparsely sampled data from pediatric patients. The inclusion of sparsely sampled data from adults and pediatric patients may unduly affect F, k_{cp}, k_{pc}, k_{tr}, and k_a, which can best be estimated from the densely sampled studies in adults. In addition, a negative impact of the inclusion of a large amount of sparsely sampled adult data can increase the presence of the target-mediated clearance and BLQ values.[15–18] Such data integration may lead to biased estimates, nonphysiological values, large standard errors, and instability, as well as an unacceptable variability in the OFV. As OMEGAs and shrinkage in pediatric and adult models differ due to the difference in populations and sparsity of the data, combining pediatric and adult data leads to uninterpretable OMEGAs, shrinkage, and simulations. Thus, the approach of using rich adult data to estimate these PPPs and subsequently fix them in the pediatric model[18] was considered to be a better option to obtain physiologically meaningful estimates for PPPs not informed by the pediatric dataset, allowing for better estimates of the remaining PPPs in the pediatric patients.

It is presumed that shrinkage in V_c from the covariate model was notably lower than shrinkage of ETAs of V_c from the base and covariate models and V_c of the base model because the estimate of shrinkage of individual PK parameters accounts for covariate impact.

In VPCs, predicted CIs of alirocumab and free PCSK9 concentrations covered median observed concentrations well (Figures 2A and 3A, respectively). The BLQ VPCs show a matching of predicted and observed frequencies of BLQ observations of alirocumab (Figure 2B), as well as free PCSK9 (Figure 3B). In the figures, the dashed black line represents a median predicted frequency of observations below the LLOQ levels, the blue area represents a CI around the predicted median, and the blue line is a median of observed frequencies of BLQ observations. The observed median stays within the model-predicted CI in both figures, demonstrating good model predictions.

The decrease in the variability of OFV and the higher stability of estimated parameters when baseline covariates rather than time-dependent regressors were

used can be explained by an implementation of a more advanced algorithm of covariate than regressor estimation, leading to the difference in stability.

PPPs of alirocumab, such as the impact of weight on V_c and parameters describing the linear part of PK, were consistent with published values for typical monoclonal antibodies in adult [16,17] and pediatric[18,20] populations.

The analyses of adult and pediatric data cross-validated each other, that is, the values of k_{eL}, k_{eLR}, R_0, and the covariate coefficient of V_c on weight were similar across the populations. The difference in k_{syn} between populations was well explained by the HDS taken by some pediatric patients. As the HDS intake in pediatric patients was not randomized, it is possible that not only did HDS affect k_{syn}, but also that patients on HDS had more severe disease and thus had higher k_{syn} before they were treated with HDS.

Weight was an important covariate on the PKs of alirocumab in both pediatric and adult patients, therefore patients weighing $\geq$50 kg received the high-dose regimen (ie, 300 mg Q4W) while those weighing $<$50 kg received the low-dose regimen (ie, 150 mg Q4W). The analyses and followed simulations substantiated the recommended weight-adjusted dosing regimens for alirocumab in children with HeFH aged $\geq$8 years by demonstrating that trough levels of free PCSK9 were suppressed by more than 90% in at least 1 treatment regimen among 2 treatments available for each pediatric weight cohort.

The administration of high-dose statins was an important covariate on k_{syn} for PSCK9 and was associated with an approximately 79% increase in synthesis rate. Statins reduce hepatic intracellular cholesterol, which activates the transcription factor sterol regulatory element binding protein-2 (SREBP-2), and the stimulation of SREBP-2 results in increased gene expression of LDLRs and PCSK9.[19] The effect of statins on increased PSCK9 expression has been shown to be dose-dependent, with higher doses resulting in greater circulating PSCK9 concentrations. For instance, 20 and 80 mg QD of simvastatin was shown to increase PSCK9 by 13% and 41%, respectively.[21] More than 90% of the pediatric patients in the studies of alirocumab received statins. However, the doses of atorvastatin, simvastatin, and atorvastatin administered to these pediatric patients were $\leq$20 mg QD since high-dose statins are generally not prescribed to pediatric patients due to an adverse effects profile, which includes hepatoxicity and myopathy, albeit rare.[22] In the phase III study, uptitration of alirocumab was permitted at week 12 for patients with an inadequate response such that patients weighing $<$50 and $\geq$50 kg had the dose regimen adjusted from 150 or 300 mg Q4W to 75 or 150 mg Q2W, respectively. These dose regimens of alirocumab were sufficient to reduce LDL-C with a least-square mean difference versus placebo of 43.3%.[9] Based on the integrated data from all dosing regimens tested in phase II and III trials, this clinically meaningful reduction in LDL-C was further supported by the simulations demonstrating a 90% reduction in free PSCK9.

The sensitivity analyses (Tables S2-S11) substantiated model assumptions and simulations, as well as confirmed the primary results and conclusions, including the selected doses. In all supplemental tables representing PPPs, shrinkage of ETAs is presented. Simulations of alirocumab concentrations with and without correlation between V_c and k_{eL} demonstrated similar outcomes, including the 5th and 95th percentiles of simulated exposure metrics, demonstrating that the change in OMEGA structure was mostly compensated by notable changes in the OMEGA values provided in Tables 2 and S9.

Overall, the approaches utilized in this study allowed improved parameter estimates and reduced reported shrinkage of parameters affected by implemented covariates in the relatively small pediatric population with sparse data. First, the full target-mediated model was implemented, allowing for enrichment of sparse information and substantiation of dosing regimens. Second, the model allowed for simulations of PCSK9, which is a good biomarker of a decrease in LDL-C. Third, unstable parameters were fixed to adult values and the exploration of LLPs supported this approach. Finally, shrinkage in individual parameters affected by implemented covariates was lower than shrinkage in ETAs as it accounted for improvements in the model after the implementation of covariates. The results of a full target-mediated modeling of alirocumab in the pediatric population have not been previously published.

Conclusions

The primary covariate model adequately described the PKs of alirocumab in the pediatric population. The PK profile of total alirocumab and concentrations of free PCSK9 in children were well explained by a 2-compartment target-mediated model, with parallel linear and nonlinear elimination, and with a transit compartment model of absorption accounting for the time lag in absorption of SC doses of alirocumab. The addition of PCSK9 data increased the stability of the model and the power of model-related decision-making.

Diagnostic plots demonstrated a good fit of the primary covariate model. LLPs demonstrated stability and narrow CIs of the estimated PPPs. The bootstrap CIs were also narrow. The empirical assessment of stability confirmed the model was stable. Sensitivity analyses substantiated the model assumptions and confirmed the results. With the exceptions of the PCSK9 synthesis

rate and V_c, the PPPs of the primary covariate model in pediatric patients were consistent with those estimated in healthy adults. Estimated PPPs describing a linear part of PKs were similar to values published for monoclonal antibodies of the immunoglobulin G4 isotype.

LLPs helped to substantiate fixing the unstable parameters to adult values.

In the pediatric population, the impact of weight on V_c and HDS on the synthesis rate of PCSK9 was statistically significant and remained in the model. V_c increased with weight. The coadministration of HDS was associated with a 79% higher production rate of PCSK9. The covariate analyses did not warrant a dose adjustment in addition to the adjustment for weight already implemented in the phase III study. Such analyses substantiated the efficacy and recommended weight-adjusted dosing regimens for alirocumab in children with HeFH aged $\geq$8 years.

As shrinkage in individual PK parameters accounted for the impact of covariates, it was similar to or substantially lower than shrinkage in ETAs of parameters affected by covariates, and it was an informative addition to the shrinkage of ETAs.

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Conflicts of Interest

P.K., L.H., J.M., Y.W., J.D.D., and A.T.D. are employed by and hold stock/options in Regeneron Pharmaceuticals, Inc.

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Data Availability Statement

Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form, and statistical analysis plan) that support the methods and findings reported in this manuscript. Individual anonymized participant data will be considered for sharing (1) once the product and indication have been approved by major health authorities (eg, FDA, EMA, PMDA, etc.) or development of the product has been discontinued globally for all indications on or after April 2020 and there are no plans for future development; (2) if there is legal authority to share the data; and (3) there is not a reasonable likelihood of participant re-identification. Submit requests to https://vivli.org/.

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Supplemental Information

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