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ARTICLE

Mechanistic modeling of a human IgG_4 monoclonal antibody (tralokinumab) Fab-arm exchange with endogenous IgG_4 in healthy volunteers

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Abstract

Therapeutic IgG_4 antibodies engage in Fab-arm exchange with endogenous human immunoglobulin G4 (IgG_4) to form monovalent hybrid molecules. A mechanistic population model was developed to quantitatively characterize the dynamic Fab-arm exchange of tralokinumab, a human IgG_4 monoclonal antibody currently being developed for the treatment of atopic dermatitis, with endogenous IgG_4 in healthy volunteers. The estimated pharmacokinetic parameters for IgG_4 were similar to those of immunoglobulin G1 or immunoglobulin G2 in humans. However, the mechanistically modeled clearance of half molecules is 21-fold higher, likely due to the loss of avidity for the neonatal Fc receptor. Half molecules of tralokinumab randomly associate with those of endogenous IgG_4 to form monovalent hybrid molecules, which became the dominant form of tralokinumab within 1 day postdose in healthy volunteers. As the potency of monovalent tralokinumab is comparable with that of bivalent tralokinumab, the IgG_4 Fab-arm exchange with endogenous IgG_4 is not expected to affect the potency of neutralization of interleukin-13 in vivo.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Therapeutic immunoglobulin G4 (IgG_4) antibodies engage in dynamic Fab-arm exchange with endogenous IgG_4 to form monovalent hybrid molecules.

WHAT QUESTION DID THIS STUDY ADDRESS?

We developed a mechanistic pharmacokinetic (PK) model to characterize the in vivo Fab-arm exchange of tralokinumab, a monoclonal IgG_4 antibody, with endogenous IgG_4 in healthy volunteers.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Based on the mechanistic modeling, half molecule (~75 kD) is cleared 21-fold faster than IgG_4. Half molecules of tralokinumab randomly associate with those of endogenous IgG_4 to form monovalent hybrid molecules, which became the dominant form of tralokinumab within 1 day postdose. The IgG_4 Fab-arm exchange is not expected to affect the in vivo neutralization of interleukin-13, as

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the potency of monovalent tralokinumab is comparable with that of bivalent tralokinumab.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The mechanistic modeling approach facilitated evaluation of human PK predictability of therapeutic IgG$_4$. Variation in Fab-arm exchange related to interindividual differences in baseline endogenous IgG$_4$ concentration was accounted for by the model.

INTRODUCTION

Tralokinumab (CAT-354) is a human immunoglobulin G4 (IgG$_4$) monoclonal antibody currently being developed for the treatment of moderate to severe atopic dermatitis (AD).[1] It potently and selectively neutralizes interleukin (IL)-13,[2] a key cytokine associated with the severity of AD.[3,4] In a randomized, double-blind, placebo-controlled phase IIb study (NCT02347176), a significant improvement in Eczema Area and Severity Index (EASI) score was seen in adults with moderate to severe AD receiving 300 mg tralokinumab every 2 weeks, and a greater percentage of participants achieved an Investigator's Global Assessment Response.[1]

As with some other therapeutic antibodies such as reslizumab, ibalizumab, and natalizumab,[5–7] tralokinumab was engineered as an IgG$_4$ to avoid potential complement activation and antigen cross-linking while retaining a long pharmacokinetic (PK) half-life.[8–10] In healthy volunteers, the PK of tralokinumab was typical for an IgG, with mean systemic clearance (CL) of 0.188 L/d and a PK half-life of 21.4 days.[11] After tralokinumab entered clinical development, it was reported that unlike other IgG subclasses, IgG$_4$ antibodies are in a dynamic Fab-arm exchange with each other.[12] In vitro and animal studies further demonstrated that therapeutic IgG$_4$ antibodies engaged in Fab-arm exchange with endogenous human IgG$_4$, raising potential PK uncertainty concerns for these molecules.[13] The mechanism of IgG$_4$ Fab-arm exchange was further investigated by kinetic studies using a sensitive real-time fluorescence resonance energy transfer assay.[14]

To fully characterize the PK property of tralokinumab and the dynamic Fab-arm exchange of IgG$_4$ in humans, serum PK samples collected from healthy volunteers receiving a single intravenous (i.v.) infusion of 150 mg tralokinumab were analyzed using three different immunoassays. A mechanistic Fab-arm exchange model was developed to describe the observed serum concentrations of intact (bivalent) tralokinumab, total (bivalent, half molecule, and monovalent hybrid) tralokinumab, and total IgG$_4$ in healthy volunteers.

METHODS

Study design

In a randomized, single-dose, open-label PK study, 30 male healthy volunteers received a 30-min i.v. infusion of 150 mg tralokinumab or a subcutaneous (s.c.) injection at the 150 or 300 mg dose level (n = 10 per group, NCT00638989). Blood samples were withdrawn predose, at the end of infusion or immediately following injection, and at various timepoints postdose up to Day 56 for PK evaluation. The study was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki, the International Conference on Harmonisation Guidance for Good Clinical Practice, and the US Code of Federal Regulations Title 21.

Bioanalysis

Serum PK samples from participants receiving a single i.v. infusion of tralokinumab were analyzed using three different assays developed and validated by MedImmune.

A double-bridging immunoassay was used to quantify the intact (bivalent) tralokinumab in serum. Biotinylated CAT-375 (antibody against the idiotypic region of tralokinumab) was used as both capturing and detecting reagent. The lower limit of quantitation of this double-bridging assay was 1.0 μg/ml, with ≤20% coefficient of variation (CV) for both intra-assay precision and interassay precision.

The second type of immunoassay was developed to measure the serum concentration of total tralokinumab, which includes bivalent intact tralokinumab, half molecules, and monovalent hybrid with endogenous IgG$_4$. The bioassay was performed on a Gyrolab assay platform (Gyros AB), with biotinylated CAT-375 captured on streptavidin-coated columns of the Gyros compact disc as the capture reagent, and a sheep anti-human IgG$_4$ antibody labeled with Alexa Fluor® 647 (Invitrogen) as the detecting reagent. The assay has a

lower limit of quantification (LLOQ) of 0.30 μg/ml, with ≤20% CV for both intra-assay precision and inter-assay precision.

The third assay quantified the total IgG_4 in serum (Human IgG Subclass Kit, The Binding Site Group), including bivalent tralokinumab, endogenous IgG_4, the half molecules, and hybrid monovalent IgG_4. This was a commercial kit, with an LLOQ of 4 μg/ml with ≤2.5% CV for both intra-assay precision and interassay precision.

Mechanistic IgG_4 Fab-arm exchange model

A mechanistic PK model was constructed to describe the disposition of intravenously administered tralokinumab, de novo production and degradation of endogenous IgG_4, dissociation of IgG_4 to single-arm half molecules, and reassociation to form the monovalent hybrid molecule (Figure 3).

The double-bridging immunoassay quantified the intact, bivalent tralokinumab ( ) in serum, the central compartment. The second immunoassay with anti-idiotypic capturing and sheep anti-human IgG_4 detection format measured the total serum concentration of bivalent tralokinumab ( ), dissociated single-arm molecule ( ), and monovalent hybrid IgG_4 ( ). Lastly, the universal IgG_4 immunoassay detected all species in serum ( ).

The equilibrium dissociation constant (K_d) of IgG_4 was fixed at 3.8 nM, a value determined in vitro at 37°C.[15] As such, k_{off} is imputed as $k_{on} \cdot K_d$, with the association rate constant k_{on} to be estimated by modeling. The zero-order production rate of endogenous IgG_4 is computed from steady-state constraints as

$$k_{syn} = \left(\frac{CL}{V_c} + k_{off} \right) \cdot eIgG_{4_{baseline}} - k_{on} \cdot eHF^2_{baseline}$$

where $eIgG_{4_{baseline}}$ and $eHF_{baseline}$ represent the baseline concentrations of endogenous IgG_4 ( ) and half molecules ( ) in serum, respectively. This equation was derived from eq. 1 in the Supplement Materials under steady-state condition assumption. Furthermore, as the half molecule concentration was not directly measured by these immunoassays, distribution parameters (peripheral volume of distribution ([V_p] and intercompartment clearance [Q]) of the half molecule were assumed to be the same as those of IgG_4 to avoid model overparameterization. The differential equation system for the mechanistic model and initial conditions (including $eHF_{baseline}$) are provided in the Supplemental Material.

Data analysis

Serum concentration data of intact and total tralokinumab and total IgG_4 from all participants receiving a single i.v. administration of 150 mg tralokinumab were log-transformed and simultaneously modeled using the pharmacostatistical software package NONMEM (Version 7.2; ICON). The first-order conditional estimation method with interaction, as implemented in NONMEM, was used for model development. An additive residual error model was used when the logarithm transformed concentration data were analyzed (it approximates a proportional residual error model in linear scale). Model stability and performance were assessed by bootstrapping and visual predictive check (VPC). Because of the small sample size, demographic covariate analysis was not conducted.

Intact IgG_4 was used as the assay standard for the total IgG_4 immunoassay, which detected the Fc component of a molecule. As such, molar concentration of the half molecule (or) was halved for computation of the total IgG_4 concentration (ie, 1 nM of half molecule is associated with an assay signal equivalent to 0.5 nM of intact IgG_4). Similarly, the predicted molar concentration of half-tralokinumab ( ) from total tralokinumab assay was also halved. Molar concentration of the hybrid molecule ( ) was unadjusted for the calculation of total IgG_4 ( ) while a scaling factor was introduced to account for potential variation in total tralokinumab assay ( ).

RESULTS

Subjects and data set

In a randomized, single-dose, open-label PK study, 30 male healthy volunteers received a 30-min i.v. infusion of 150 mg tralokinumab or an s.c. injection at the 150 or 300 mg dose level ($n = 10$ per group). All participants received the intended dose (150 mg i.v., 150 mg s.c., or 300 mg s.c.).[11]

Serum PK samples from 10 participants in the i.v. dose group were analyzed using three different immunoassays measuring intact tralokinumab, total tralokinumab, and total IgG_4. The PK data set contained 424 quantifiable tralokinumab or IgG_4 concentration data points. The mean age of these 10 participants was 30 years, and the mean body weight was 78.7 kg. A majority (80%) of these participants were White. Baseline endogenous IgG_4 concentration was unquantifiable in one subject. In nine other healthy volunteers, the median endogenous IgG_4 at baseline was 304 μg/ml (range, 58–1383 μg/ml).

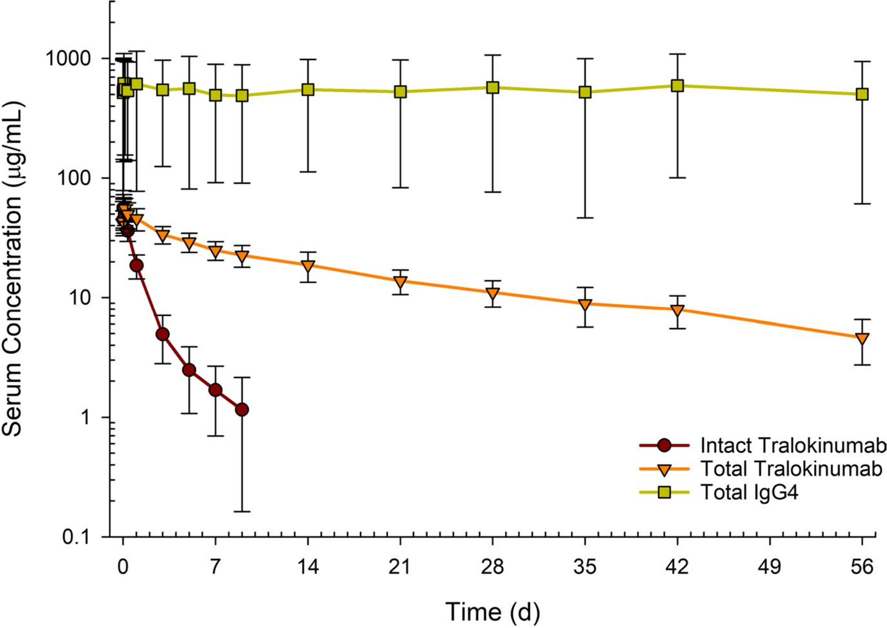
Total tralokinumab PK data from s.c. dose groups were previously reported.[11] Because of the potential flip-flop kinetics of intact tralokinumab, greater variability, and confounding absorption process, PK data from participants who received an s.c. injection of tralokinumab were not used for mechanistic model development.

PK profiles of tralokinumab and IgG$_4$

Mean serum concentration–time profiles of tralokinumab and IgG$_4$ as measured by three different immunoassays are shown in Figure 1. To facilitate interpretation of the assay results, bivalent intact tralokinumab, half molecule, monovalent hybrid tralokinumab, and endogenous IgG$_4$ are denoted hereafter as , , , and , respectively. The PK profile of total tralokinumab ( ) in nine participants with quantifiable endogenous IgG$_4$ at baseline was typical for IgG (Figure 1a). The mean concentration of intact tralokinumab ( ), as determined by a double-bridging immunoassay, fell below the assay lower quantification limit (1.0 μg/ml) after Day 10. Serum concentrations of total IgG$_4$ ( ) as measured by a universal IgG$_4$ assay were much higher. However, the observed tralokinumab and IgG$_4$ profiles in one participant with unquantifiable endogenous IgG$_4$ at baseline differed from those in other participants (Figure 1b).

Noncompartmental analysis

Noncompartmental PK parameter values are summarized in Table 1. The CL of bivalent intact tralokinumab ( ) increased with baseline endogenous IgG$_4$ concentration (Figure 2).



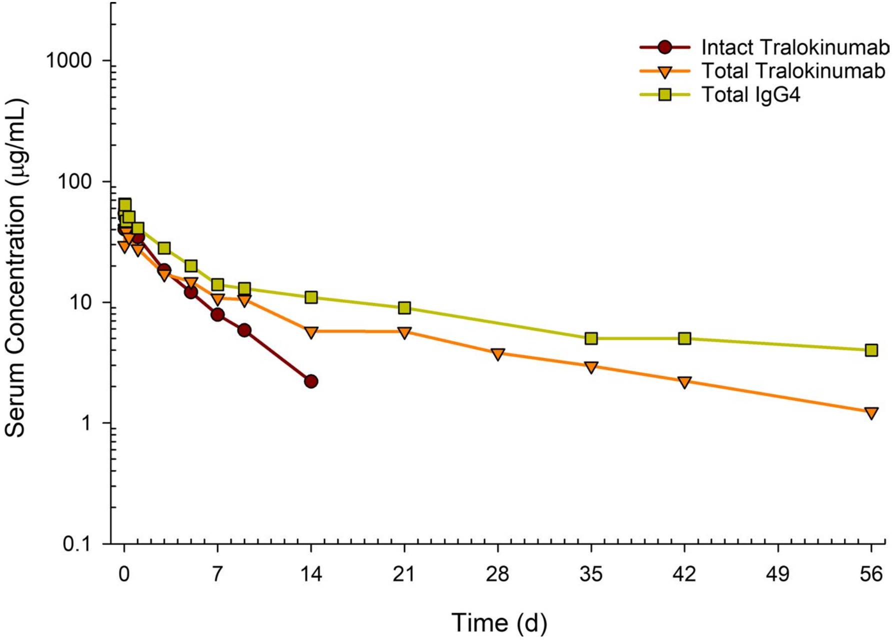


FIGURE 1 Observed serum concentration–time profiles of bivalent intact tralokinumab, total tralokinumab, and total IgG$_4$ in healthy volunteers. The assay lower limit of quantification was 1.0 μg/ml for intact tralokinumab, 0.30 μg/ml for total tralokinumab, and 4 μg/ml for total IgG$_4$. IgG$_4$, immunoglobulin G4

TABLE 1 Noncompartmental pharmacokinetic parameter summary for tralokinumab and IgG_4 in healthy volunteers receiving single intravenous infusion of 150 mg tralokinumab

	Intact tralokinumab		**Total tralokinumab**		**Total IgG_4**	
	With quantifiable $IgG_{4baseline}$	**No quantifiable $IgG_{4baseline}$**	**With quantifiable $IgG_{4baseline}$**	**No quantifiable $IgG_{4baseline}$**	**With quantifiable $IgG_{4baseline}$**	**No quantifiable $IgG_{4baseline}$**
n	9	1	9	1	9	1
C_{max} (μg/ml)	56.9 (10.8)	54.4	60.2 (13.8)	41.0	706 (555)	65.0
AUC[a] (μg × d/ml)	81.2 (26.1)	190	951 (236)	367	29,900 (25,300)[a]	552[a]
CL (L/d)	2.02 (0.67)	0.79	0.166 (0.038)	0.409	NA	NA
V_{ss} (L)	5.35 (1.98)	3.64	4.46 (0.78)	8.26	NA	NA
$t_{1/2}$ (d)	3.93 (2.38)	3.63	20.5 (2.67)	16.6	NA	NA

Note: Parameters are shown as mean (standard deviation).

Abbreviations: AUC, area under the concentration-time curve; CL, systemic clearance; C_{max}, maximum observed concentration; IgG_4, immunoglobulin G4; NA, not applicable given flat terminal phase (continuous endogenous IgG_4 production); n, number of subjects; $t_{1/2}$, half-life; V_{ss}, steady-state volume of distribution.

[a]AUC from time zero to 56 days postdose.

Mechanistic modeling of IgG_4 Fab-arm exchange

The structure of the IgG_4 Fab-arm exchange model is shown in Figure 3. Upon i.v. administration, bivalent intact tralokinumab dissociates in blood to form half molecules that randomly associate with other half molecules to form either intact tralokinumab or a monovalent hybrid IgG_4. Parameters k_{on} and k_{off} are the rate constants for association and dissociation of IgG_4 molecules, respectively. CL and CL_{hf} are the systemic clearance of intact IgG_4 and single-arm half molecules, respectively. In this diagram, the central compartment represents serum with a distribution volume (V_c). Peripheral tissue distribution of IgG_4 and half molecules is characterized by V_p and Q. The de novo production of endogenous IgG_4 is denoted by a zero-order input function k_{syn}.

Intact tralokinumab, total tralokinumab, and total IgG_4 data from 10 healthy volunteers were simultaneously modeled using a population approach. Estimated population PK parameters, interindividual variability, and residuals are listed in Table 2. The estimated CL of half molecules is 20.9-fold higher than that of IgG_4. Although one healthy volunteer had no quantifiable IgG_4 at baseline, from population modeling the mechanistic model could be used to estimate the endogenous IgG_4 concentration in this subject, 21.6 nM or 3.24 μg/ml, slightly below the LLOQ of total IgG_4 assay (4.0 μg/ml). A scaling factor (0.685) was also incorporated in the model and estimated to account for monovalent hybrid and bivalent intact tralokinumab in the total tralokinumab assay.

Except for baseline IgG_4 concentration, the interindividual PK variability was relatively small in healthy volunteers (15%–32% CV). Furthermore, the estimated residual variability of these three immunoassays (12%–16% CV) agreed well with the parameters from the assay validation criterion.

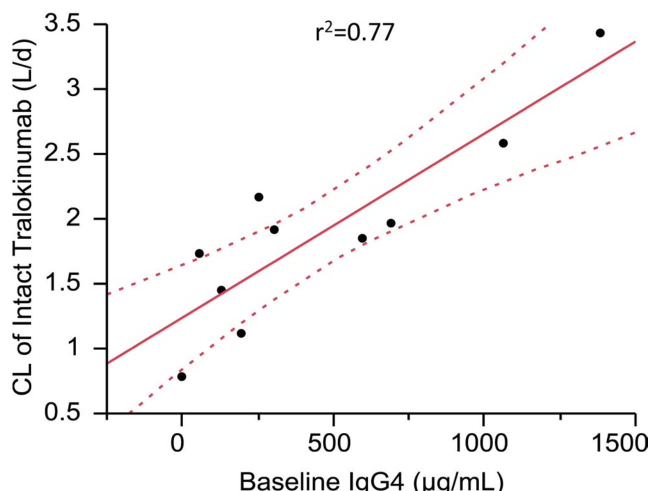


FIGURE 2 Correlations of systemic clearance of intact tralokinumab with baseline endogenous IgG_4. The dotted curves represent 95th percentile confidence limits. CL, systemic clearance; IgG_4, immunoglobulin G4

Model evaluation

Predominantly, the medians of PK and variance parameters from bootstrapping runs were close to the values originally estimated from the model (Table 2). Basic goodness-of-fit plots and individual/typical profiles of tralokinumab and IgG_4 are presented in Figures S1–S3. VPC plots are shown in Figure 4. Symbols represent the

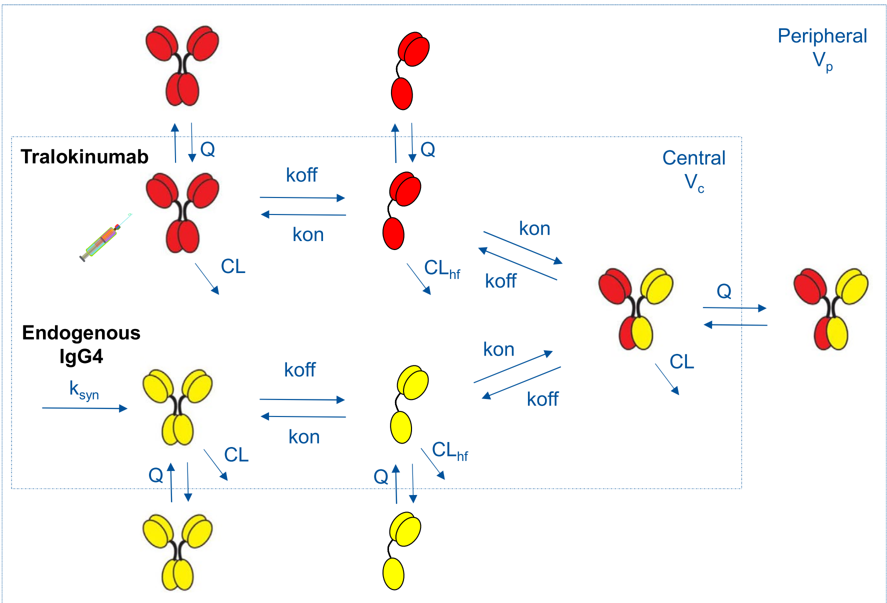


FIGURE 3 Mechanistic model structure for IgG$_4$ Fab-arm exchange in humans. CL, systemic clearance; CL$_{hf}$, clearance of half IgG$_4$ molecules; IgG$_4$, immunoglobulin G4; k_{off}, dissociation rate constant of IgG$_4$ molecules; k_{on}, association rate constant of IgG$_4$ molecules; k_{syn}, de novo production of endogenous IgG$_4$; Q, intercompartment clearance; V_c, central volume of distribution; V_p, peripheral volume of distribution

observed serum concentrations. The assay LLOQ was 1.0 μg/ml for intact tralokinumab. Concentrations below the LLOQ were not plotted. The shaded bands in VPC plots for nine participants with quantifiable baseline IgG$_4$ concentrations reflect the interindividual and residual variability (Figure 4a). As there was only one subject with unquantifiable endogenous IgG$_4$ at baseline, the shaded bands in Figure 4b only correspond to residual variability of assays.

DISCUSSION

IL-13 is secreted predominantly by cluster of differentiation 4$^+$ (CD4$^+$) T helper-2 (Th2) cells, which may play a central role in the pathogenesis of AD.[3,4,16–18] Tralokinumab is a recombinant fully human monoclonal antibody that potently and specifically neutralizes IL-13. It was engineered as an IgG$_4$ antibody to avoid complement system activation and sensitization of mast cells. In vitro and in vivo animal studies demonstrated potent inhibition of IL-13 induced effects and no evidence of drug-related toxicity.[2] With a 3-week half-life, the PK of tralokinumab was typical for an IgG in healthy volunteers.[11] A phase II clinical trial demonstrated that tralokinumab treatment achieved clinically significant improvements in EASI scores in adults with moderate to severe AD.[1]

When tralokinumab had just entered clinical development, it became known that IgG$_4$ molecules engaged in a dynamic Fab-arm exchange.[12] In this process, bivalent IgG$_4$ molecules dissociate to form half molecules, which then randomly combine with other half molecules to form monovalent hybrid IgG$_4$. Such IgG$_4$ Fab-arm exchange was further characterized in vitro and in animal studies, with kinetic models built to delineate the random dissociation–association process.[13,14] There had been concerns, however, with the implications of IgG$_4$ Fab-arm exchange for clinical efficacy and human PK predictability of therapeutic IgG$_4$ molecules.[13] In this investigation, three different immunoassays were deployed to analyze PK samples from healthy volunteers receiving a single 150 mg intravenous infusion of tralokinumab. A mechanistic population PK model was developed to characterize the in vivo Fab-arm exchange of tralokinumab with endogenous IgG$_4$ in humans (Figure 3).

The PK profile of total tralokinumab (bivalent, half molecule, and monovalent) was typical for an IgG (Figure 1a), with a mean CL of 0.166 L/d and an elimination half-life of 20.5 days from noncompartmental analysis (Table 1). The faster clearance of bivalent tralokinumab, as measured by a double-bridging immunoassay, reflected the conversion/Fab-arm exchange into monovalent molecules. Serendipitously, one of these 10 subjects had no quantifiable endogenous IgG$_4$ at baseline, and the PK profiles in this subject were dramatically different from others (Figure 1b). The apparent CL of bivalent intact tralokinumab increased with baseline endogenous IgG$_4$, reflecting augmented formation of hybrid monovalent IgG$_4$ in subjects with high endogenous IgG$_4$ (Figure 2).

The Fab-arm exchange of tralokinumab with endogenous IgG$_4$ in healthy volunteers was adequately described by a mechanistic PK model (Figure 3). The structure and variance parameter estimates from the population model were close to the medians of bootstrapping (Table 2), and there was no apparent trend in conditional weighted residuals when plotted against population predicted concentrations or time (Figure S1). In VPC plots, the observed

TABLE 2 Population pharmacokinetic structure and variance parameters estimated from mechanistic modeling

Parameter	Original estimate	Bootstrap ($n = 711$)[a]	
		Median	95% CI
CL (L/d)	0.151	0.151	0.102–0.185
V_c (L)	2.94	2.95	2.63–3.28
Q (L/d)	0.559	0.547	0.514–0.588
V_p (L)	3.18	3.20	2.58–4.07
CL_{hf}/CL	20.9	20.8	10.0–43.7
$IgG_{4baseline}$ (nM)	2330	2290	1280–4,320
$IgG_{4baseline,ID10}$ (nM)	21.6	20.9	20.1–21.7
k_{on} ($nM^{-1}d^{-1}$)	0.260	0.260	0.226–0.283
K_d (nM)	3.8 fixed[b]	–	–
Scaling factor (hybrid)	0.685	0.680	0.633–0.735
Interindividual variability[c]			
η_{CL}	15.0	13.4	2.0–20.1
η_{Vc}	17.1	15.8	7.1–23.3
η_{Vp}	32.4	32.5	0.3–43.3
$\eta_{IgG4baseline}$	91.2	86.0	57.9–108
Residual variability[c]			
Intact tralokinumab assay	12.2	12.1	10.1–14.7
Total tralokinumab assay	15.0	15.0	11.7–19.0
Total IgG_4 assay	15.5	15.3	12.6–18.4

Note: Scaling factor (hybrid), a parameter to account for assay signals of bivalent and monovalent tralokinumab in the total tralokinumab assay.

Abbreviations: CI, confidence interval; CL, clearance; CL_{hf}/CL, ratio of half molecule clearance and IgG_4 clearance; IgG_4, immunoglobulin G4; $IgG_{4baseline}$, serum level of endogenous IgG_4 at baseline; K_d, equilibrium dissociation constant of IgG_4; k_{on}, association rate constant of half molecules; Q, intercompartmental clearance; V_c, central volume of distribution; V_p, peripheral volume of distribution.

[a]Among 1000 bootstrapping runs, 711 converged with a number of significant figures ≥2.

[b]Fixed to a value as determined by Förster resonance energy transfer assay.[15]

[c]Shown as percent coefficient of variation (%CV).

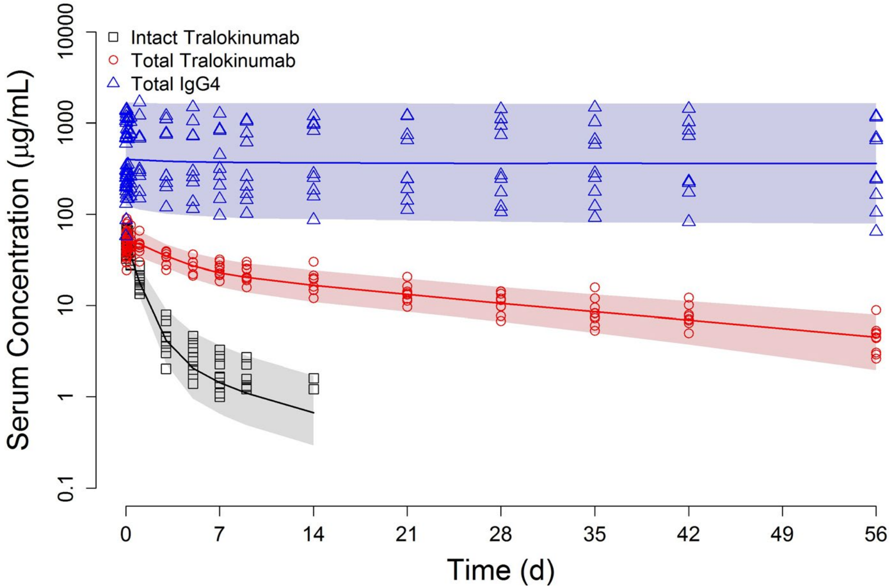
concentrations were evenly distributed across the simulated median curves and enclosed within the shaded 5th–95th percentile range (Figure 4).

The population estimates of PK parameters for IgG_4 were similar to those of immunoglobulin G1 (IgG_1) or immunoglobulin G2 (IgG_2) in humans, with a typical CL of 0.151 L/d. However, the estimated CL of half molecules is 21-fold higher (Table 2). The low CL of IgG by the reticuloendothelial system is due to neonatal Fc receptor (FcRn)–mediated intracellular recycling of endocytosed IgG. The effective FcRn-mediated recycling requires bivalency of the Fc dimer to decelerate the dissociation in the endosome.[19] Without such avidity effect, the monomeric form of Fc fused with nonspecific Fab fragment was cleared 30-fold faster than keyhole limpet hemocyanin–derived antibody in mice.[15] As such, the estimated substantially faster clearance of IgG_4 half molecules in healthy volunteers may be associated with the loss of avidity for FcRn in endosomes, resulting in enhanced intracellular degradation in lysosomes. The smaller molecular size of IgG_4 half molecules may also contributed to the rapid clearance, as demonstrated in studies of antibody fragments in FcRn knockout mice.[20]

The observed rapid decline of intact (bivalent) tralokinumab concentration in serum is according to the model due to its dissociation to half molecules with subsequent hybrid molecule formation. The K_d of IgG_4 was fixed to 3.8 nM, as determined by in vitro monitoring of Förster resonance energy transfer (FRET) assay signals.[14] From mechanistic modeling, the estimated association rate constant k_{on} was 0.260 $nM^{-1}\ d^{-1}$, corresponding to a k_{off} of 0.988 d^{-1} or a dissociation half-life of 0.70 days for IgG_4 in humans (Table 2). The k_{off} of IgG_4 from the in vitro FRET assay was $1.2 \times 10^{-3}\ s^{-1}$ ($1.7\ d^{-1}$).[14] The slower dissociation of IgG_4 in vivo compared with the in vitro system could be attributed to the variation in local redox potentials.

One subject had no quantifiable endogenous IgG_4 concentration at baseline. The estimated CL of IgG_4 at 0.149 L/d from mechanistic modeling was not much different from that in other subjects. After the faster clearance of half molecules and endogenous IgG_4-dependent formation of hybrid molecules were taken into account, the interindividual variability in the CL and V_c of IgG_4 were low in healthy volunteers (15% CV for CL and 17% CV for V_c). On the contrary, the endogenous IgG_4 was highly variable among these subjects (91% CV).

Circulating IL-13 is a monomeric Th2 cytokine with a molecular weight of approximately 15 kD. As such, the avidity effect is irrelevant in the neutralization of IL-13 signaling by tralokinumab. Indeed, from reporter gene assay or an in vitro assay using human umbilical vein endothelial cells, the bioactivity of tralokinumab was not impacted by the Fab exchange.[2,21] Extensive preclinical and clinical studies have demonstrated potent and specific IL-13 neutralizing activity of tralokinumab. Despite the dynamic Fab-arm exchange with endogenous IgG_4, in the absence of target-mediated clearance, the reported PK half-lives of therapeutic IgG_4 were 3–4 weeks, similar to those of IgG_1 and IgG_2.[11,22,23] Based on overall evaluation of PK, in vitro potency, and clinical efficacy, the Fab-arm exchange of tralokinumab with endogenous IgG_4 is not expected to affect its potential use for the treatment of AD. Given the smaller molecular size, the peripheral distribution, in particular the intercompartmental clearance of the half molecules, could be different from IgG_4.[23] In the absence of half-molecule PK data, distribution parameter values (V_p and Q) of half molecules were



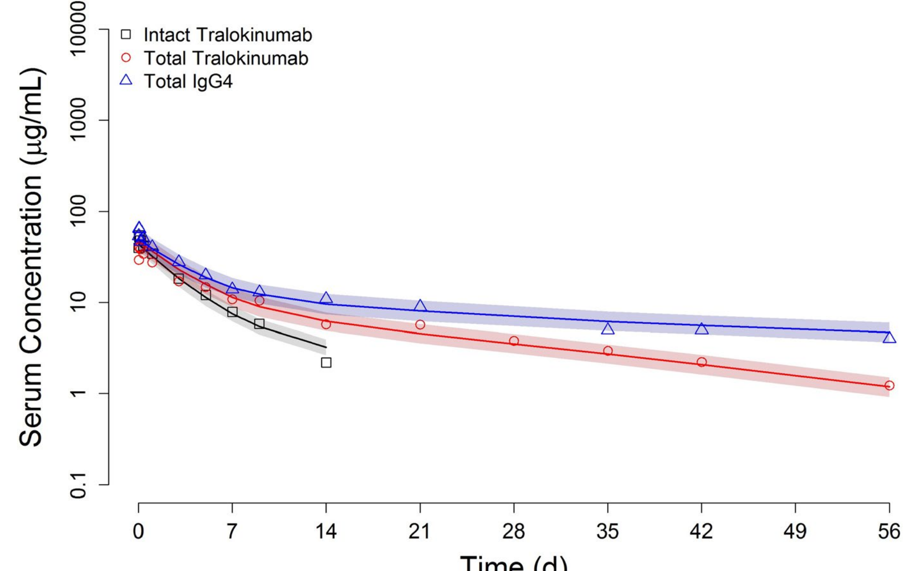


FIGURE 4 Visual predictive check for IgG$_4$ Fab-arm exchange model: symbols, observed concentrations; solid curve, median of 1000 simulated profiles; and shaded area, range between the 5th and 95th percentiles. The assay lower limits of quantification were 1.0 µg/ml for intact tralokinumab, 0.30 µg/ml for total tralokinumab, and 4 µg/ml for total IgG$_4$

assumed the same as IgG$_4$ to avoid model overparameterization. Although the overall data fitting was reasonably well, this assumption poses a main limitation of the mechanistic model.

In summary, a mechanistic model was developed to characterize the Fab-arm exchange of tralokinumab with endogenous IgG$_4$ in healthy volunteers. Upon i.v. administration, bivalent tralokinumab dissociates into half molecules with a dissociation half-life of 0.7 days. The clearance of half molecules is 21-fold higher than IgG$_4$, due to less efficient FcRn-mediated intracellular recycling. Half molecules of tralokinumab randomly associate with those of endogenous IgG$_4$ to form monovalent hybrid molecules, which became the dominant form of tralokinumab within 1 day post-i.v. administration in healthy volunteers. As the potency of monovalent tralokinumab is comparable with that of bivalent tralokinumab, the IgG$_4$ Fab-arm exchange with endogenous IgG$_4$ is not expected to affect the potency of in vivo neutralization of IL-13 in patients with asthma.

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CONFLICT OF INTEREST

B.W. is a former employee of AstraZeneca. L.K.R. and J.G. are current employees of AstraZeneca. All authors own AstraZeneca stocks.

AUTHOR CONTRIBUTIONS

B.W., J.G., and L.K.R. designed the research, performed the research, analyzed the data, and wrote the manuscript.

REFERENCES

1. Wollenberg A, Howell MD, Guttman-Yassky E, et al. Treatment of atopic dermatitis with tralokinumab, an anti-IL-13 mAb. *J Allergy Clin Immunol*. 2019;143:135-141.
2. May RD, Monk PD, Cohen ES, et al. Preclinical development of CAT-354, an IL-13 neutralizing antibody, for the treatment of severe uncontrolled asthma. *Br J Pharmacol*. 2012;166:177-193.
3. Ungar B, Garcet S, Gonzalez J, et al. An integrated model of atopic dermatitis biomarkers highlights the systemic nature of the disease. *J Invest Dermatol*. 2017;137:603-613.
4. Khattri S, Shemer A, Rozenblit M, et al. Cyclosporine in patients with atopic dermatitis modulates activated inflammatory pathways and reverses epidermal pathology. *J Allergy Clin Immunol*. 2014;133:1626-1634.
5. Salfeld JG. Isotype selection in antibody engineering. *Nat Biotechnol*. 2007;25:1369-1372.
6. Rispens T, Leeuwen AV, Vennegoor A, et al. Measurement of serum levels of natalizumab, an immunoglobulin G4 therapeutic monoclonal antibody. *Anal Biochem*. 2011;411:271-276.
7. Dumet C, Pottier J, Gouilleux-Gruart V, Watier H. Insights into the IgG heavy chain engineering patent landscape as applied to IgG4 antibody development. *mAbs*. 2019;11:1341-1350.
8. van der Zee JS, van Sweiten P, Aalberse RC. Inhibition of complement activation by igg4 antibodies. *Clin Exp Immunol*. 1986;64:415-422.
9. Jefferis R. Antibody therapeutics: isotype and glycoform selection. *Expert Opin Biol Ther*. 2007;7:1401-1413.
10. Schuurman J, Van Ree R, Perdok GJ, Van Doorn HR, Tan KY, Aalberse RC. Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. *Immunology*. 1999;97:693-698.
11. Oh CK, Faggioni R, Jin F, et al. An open-label, single-dose bioavailability study of the pharmacokinetics of cat-354 after subcutaneous and intravenous administration in healthy males. *Brit J Clin Pharmacol*. 2010;69:645-655.
12. van der Neut Kolfschoten M, Schuurman J, Losen M, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic fab arm exchange. *Science*. 2007;317:1554-1557.
13. Labrijn AF, Buijsse AO, van den Bremer ETJ, et al. Therapeutic IgG4 antibodies engage in fab-arm exchange with endogenous human IgG4 in vivo. *Nat Biotechnol*. 2009;27:767-771.
14. Rispens T, Ooijevaar-de Heer P, Bende O, Aalberse RC. Mechanism of immunoglobulin G4 fab-arm exchange. *J Am Chem Soc*. 2011;133:10302-10311.
15. Ishino T, Wang M, Mosyak L, et al. Engineering a monomeric Fc domain modality by N-glycosylation for the half-life extension of biotherapeutics. *J Biol Chem*. 2013;288:16529-16537.
16. Moreno AS, McPhee R, Arruda LK, Howell MD. Targeting the T helper 2 inflammatory axis in atopic dermatitis. *Int Arch Allergy Immunol*. 2016;171:71-80.
17. Hamilton JD, Ungar B, Guttman-Yassky E. Drug evaluation review: dupilumab in atopic dermatitis. *Immunotherapy*. 2015;7:1043-1058.
18. Simpson EL, Flohr C, Eichenfield LF, et al. Efficacy and safety of lebrikizumab (an anti-IL-13 monoclonal antibody) in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical corticosteroids: a randomized, placebo-controlled phase II trial (TREBLE). *J Am Acad Dermatol*. 2018;78:863-871.
19. Tesar DB, Tiangco NE, Bjorkman PJ. Ligand valency affects transcytosis, recycling and intracellular trafficking mediated by the neonatal Fc receptor. *Traffic*. 2006;7:1127-1142.
20. Li Z, Krippendorff B-F, Shah DK. Influence of Molecular size on the clearance of antibody fragments. *Pharm Res*. 2017;34:2131-2141.
21. Carlsson M, Braddock M, Li Y, et al. Evaluation of antibody properties and clinically relevant immunogenicity, anaphylaxis, and hypersensitivity reactions in two phase III trials of tralokinumab in severe, uncontrolled asthma. *Drug Saf*. 2019;42:769-784.
22. Industries TP. Cinqair (reslizumab) injection prescribing information; 2016.
23. van Hartingsveldt B, Nnane IP, Bouman-Thio E, et al. Safety, tolerability and pharmacokinetics of a human anti-interleukin-13 monoclonal antibody (CNTO 5825) in an ascending single-dose first-in-human study. *Br J Clin Pharmacol*. 2013;75:1289-1298.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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