Analysis of “On/Off” Kinetics of a CETP Inhibitor Using a Mechanistic Model of Lipoprotein Metabolism and Kinetics

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RG7232 is a potent inhibitor of cholesteryl-ester transfer protein (CETP). Daily oral administration of RG7232 produces a dose- and time-dependent increase in high-density lipoprotein-cholesterol (HDL-C) and apolipoproteinA-I (ApoA-I) levels and a corresponding decrease in low-density lipoprotein-cholesterol (LDL-C) and apolipoproteinB (ApoB) levels. Due to its short plasma half-life (~3 hours), RG7232 transiently inhibits CETP activity during each dosing interval (“on/off” kinetics), as reflected by the temporal effects on HDL-C and LDL-C. The influence of RG7232 on lipid-poor ApoA-I (i.e., pre-/β1) levels and reverse cholesterol transport rates is unclear. To investigate this, a published model of lipoprotein metabolism and kinetics was combined with a pharmacokinetic model of RG7232. After calibration and validation of the combined model, the effect of RG7232 on pre-/β1 levels was simulated. A dose-dependent oscillation of pre-/β1, driven by the “on/off” kinetics of RG7232 was observed. The possible implications of these findings are discussed.

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While epidemiological studies have long established the inverse relationship between high-density lipoprotein cholesterol (HDL-C) and cardiovascular disease (CVD) risk, therapies aimed at raising HDL-C have yet to demonstrate a positive CVD outcome.1 HDL is thought to have several antiatherogenic functions, among which is the mediation of reverse cholesterol transport (RCT), the process whereby cholesterol molecules from peripheral tissues are carried back to the liver for elimination.1,2 It has been hypothesized that the enhancement of RCT may decrease CVD risk by removing cholesterol from arterial lesions;2 however, this remains to be demonstrated. Although HDL-C has drawn much interest as a biomarker, there are growing data indicating that it may have a correlation rather than causal relationship with CVD risk.1 There have been experimental and modeling efforts to understand HDL measures and their relationship to disease.3–5 In particular, the relationships between HDL-C, the total ApoA-I (the main protein constituent of HDL particles), HDL particle concentration, and HDL size are complex and pharmacotherapies that affect the HDL profile may not increase the RCT rate.6,7 While pharmacokinetic/pharmacodynamic (PK/PD) modeling has been utilized for inferring the relationship between drug exposure and changes in lipid levels (e.g., ref. 8), the lack of a direct correspondence between the commonly measured plasma biomarkers and the in vivo RCT rate renders those models ill-suited for the task of assessing the potential efficacy of drugs that target the HDL pathway. Instead, physiological systems models9 are needed in order to assess whether the proposed drug mechanisms have a sound basis for affecting the underlying disease biology.

In the effort to better understand HDL-C-raising therapies, we propose the use of a systems pharmacology model to help quantify the multitude of effects brought about by drug action from a holistic perspective.10 There has been prior mechanistic, model-based analysis of the role of cholesteryl-ester transfer protein (CETP) inhibitors on lipoprotein profiles and as well as the associated changes in CVD risk;11 however, the details of the proprietary model used to derive the results shown in ref. 11 have not been described. In this work, we utilize a published model of lipoprotein metabolism and kinetics (LMK),12 combine it with a PK model for the CETP inhibitor (RG7232, Patent Cooperation Treaty No. WO200709074813), and refine parameter estimates using data from the multiple-ascending dose (MAD) study. We qualify the model for predicting changes in lipoprotein measures with the use of additional data that were not used in the calibration process.

CETP is a plasma protein that mediates the transfer of cholesteryl ester (CE) and triglyceride (TG) molecules between HDL and ApoB-containing particles (including very low density lipoprotein [VLDL] and low density lipoprotein [LDL]).14 Due to the fact that there is a net movement of CE from HDL to VLDL and LDL, the inhibition of CETP would increase HDL-C while decreasing LDL-C and it has been suggested that this would be antiatherogenic.14 However, the inhibition of CETP will lead to a number of effects on RCT, one of which is the impact on HDL remodeling. HDL particles are known to undergo extensive remodeling, by fusing with one another, or reducing their core size by lipid exchange with other...
lipoproteins, or lipid removal by the scavenger receptor class B1 protein (SR-BI). Such remodeling processes help to regenerate lipid-poor ApoA-I, which can subsequently be loaded with cholesterol by the ATP-binding cassette member 1 (ABCA1) transporter and participate in RCT.15 Due to the role that CETP plays in the removal of CE from the core of HDL particles, the inhibition of this pathway would block core shrinkage, resulting in enlarged HDL particles and potentially impacting the regeneration of lipid-poor ApoA-I. We remark that there are various ways to experimentally quantify lipid-poor ApoA-I; for instance, as pre-β1 using an electrophoresis technique,16 or as small LpA-I using size-exclusion chromatography17; for convenience, we will use the terminology pre-β1 interchangeably with lipid-poor ApoA-I.

Due to the important role pre-β1 plays in initiating RCT18,19 and the appreciation that complete CETP inhibition may adversely affect pre-β1 generation, it has been suggested that preserving the role of CETP in HDL remodeling may be the best approach.20,21 Ideally, CETP inhibitors should induce a significant LDL-C lowering without impairing HDL remodeling; we study this question by analyzing data generated with a CETP inhibitor having a short half-life. The potential benefit of a CETP inhibitor with such a property is that the presence of the “off” phase of the drug action may restore HDL remodeling and the regeneration of pre-β1. Although the effect of CETP inhibitors on pre-β1 generation and cholesterol efflux can be studied in vitro, a crucial limitation of such studies is that they are done in static cell culture systems and hence do not account for the dynamic processes of cholesterol movement and HDL remodeling that occur in vivo.19 Therefore, we utilize the LMK model to evaluate the hypothesis in question by performing in silico experimentation, based on the PK properties of the drug.

METHODS

Study design and population PK analysis
A combined single and multiple ascending dose study was conducted to investigate the safety, tolerability, PK, and PD of RG7232 in n = 58 healthy male subjects. This study was conducted in full conformance with the principles of the Declaration of Helsinki or with the laws and regulations of the country in which the research was conducted. A population pharmacokinetic analysis was conducted using nonlinear mixed-effect modeling (NONMEM v. 7.2, ICON Development Solutions, Ellicott City, MD). For additional details, refer to Supplementary Text 1.

Table 1 Parameter estimates for the population PK model of RG7232

| Parameter                          | Unit       | Population estimate | RSE (%) |
|-----------------------------------|------------|---------------------|---------|
| Clearance, CL/F                   | L/hour     | 9.38                | 3.1     |
| Central volume of distribution, V/F| L          | 25.2                | 2.3     |
| Peripheral volume of distribution, VP/F | L         | 15.2                | 8.3     |
| Rate of absorption, Ka            | 1/hour     | 1.36                | 12.9    |
| Intercompartmental clearance, Q/F | L/hour     | 0.587               | 5.7     |
| Lag time, ALAG                    | hour       | 0.984               | 0.1     |
| Relative bioavailability (only for 420 mg dose), F | unitless | 0.625               | 3.6     |

PK/PD sampling
Venous blood samples were collected to obtain the plasma concentrations of RG7232 as well as for PD assessments. Analyses of lipid profiles included total cholesterol, LDL-C, HDL-C, ApoA-I, Apo-B and were performed using standard assays. The NMR profiles were measured using the NMR LipoProfile and carried out by LipoScience. The schedule of PK/PD assessments is shown in Supplementary Table 1.

Model extension: ApoB dynamics
In the LMK model,12 ApoB dynamics are not described. However, it is known that under CETP inhibition ApoB levels can decrease.22 Unlike HDL particles, which have a variable number of ApoA-I molecules on them, LDL and VLDL particles each have a single molecule of ApoB per particle; hence, the decrease in their cholesterol ester content alone cannot explain the observed decrease in ApoB levels: a different mechanism is needed to explain the drop in ApoB under CETP inhibition. One plausible explanation for this phenomenon is provided by the experimental evidence suggesting that the affinity of LDL particles to LDL receptors may depend on the particle size (or, alternatively, the density).23 Motivated by these data, we implemented a composition-dependent contribution to LDL-C and ApoB clearances. That is, the elimination rate of LDL-CE is taken to be:

\[ k_{\text{out}, \text{total}}^{LDL} \times \frac{[\text{LDL-CE}]}{[\text{ApoB}]_{\text{LDL}}} = k_{\text{out}, \text{linear}}^{LDL}\times \frac{[\text{LDL-CE}]}{[\text{ApoB}]_{\text{LDL}}} + \frac{1}{1.12}, \]

where \( k_{\text{out}, \text{linear}}^{LDL} \) represents CE elimination by both SR-B1 as well as the composition-independent contribution of LDL-R receptor particle uptake, and the value 1.12 represents the nominal value of the ratio [LDL-CE]/[ApoB]_{LDL}. When the value of \( k_{\text{out}, \text{linear}}^{LDL} \) is negative (as turned out to be the case using the parameter estimation procedure), this implies that the smaller the LDL particles are (in an averaged sense), the faster they will be eliminated. This is consistent with in vitro data showing that the affinity of LDL to LDL receptors (LDL-R) is higher for smaller particles,24 hence providing support that the in vivo catabolism of small LDL may be higher than that of large LDL. We remark that although we used [LDL-CE]/[ApoB]_{LDL} as a surrogate measure for the average LDL size, this is different from the mass-weighted average LDL particle size as obtained from NMR measurements.25; the size heterogeneity of LDL particles needs to be taken into account when drawing conclusions on the average LDL size.
Due to the assumption that LDL particles are eliminated by LDL receptors in a manner dependent on their composition, the ApoB on LDL particles also need to have the same dependence. We describe ApoB dynamics via the following two-compartment model: denote ApoB on LDL and VLDL by \( \frac{1}{2} \text{ApoB}_{LDL} \) and \( \frac{1}{2} \text{ApoB}_{VLDL} \), respectively. ApoB is assumed to be synthesized at the rate \( r_{\text{ApoB}} \) and enters the VLDL compartment. This rate was estimated in five normolipidemic subjects as 24.8±6.5 mg/kg/day, which translates to the value \( r_{\text{ApoB}} = 55 \) mg/dL/day as given in Table 2. From the VLDL compartment, ApoB can either be eliminated by holoparticle clearance of VLDL (assumed to be at the rate of 1 pool/day), or go into an LDL compartment via lipolysis (assumed to occur at the same rate as CE transfer from VLDL to LDL, \( k_{VL} \)). Once in the LDL compartment, ApoB is eliminated in a composition-dependent manner via the LDL-R. To summarize, the following equations are used in describing ApoB dynamics:

\[
\frac{d}{dt} \frac{1}{2} \text{ApoB}_{VLDL} = r_{\text{ApoB}} - k_{VL} \times \frac{1}{2} \text{ApoB}_{VLDL} - \frac{1}{\text{day}} \times \frac{1}{2} \text{ApoB}_{VLDL},
\]

\[
\frac{d}{dt} \frac{1}{2} \text{ApoB}_{LDL} = k_{VL} \times \frac{1}{2} \text{ApoB}_{VLDL} - \left( k_{\text{ApoB}} + k_{\text{LDL-linear}} \times \frac{[\text{LDL-CE}]}{[\text{ApoB}]_{LDL}} - 1.12 \right) \times \frac{1}{2} \text{ApoB}_{LDL},
\]

where again we used the value 1.12 as representing the nominal value of \([\text{LDL-CE}]/[\text{ApoB}]_{LDL}\). Finally, the total ApoB concentration is given by the sum: \([\text{ApoB}] = [\text{ApoB}]_{VLDL} + [\text{ApoB}]_{LDL}\).

Bayesian parameter refinement
In this work, we use the MAD data to either refine the existing parameter estimates of the LMK model or estimate those that were added in the systems pharmacology model (including: drug effect parameters \( E_{\text{max}}, EC_{50} \); new parameters associated with ApoB dynamics, \( k_{\text{ApoB}} \), \( k_{\text{LDL-linear}}, r_{\text{ApoB}} \)). Please refer to Supplementary Text 2 for the technical details. The SimBiology toolbox of MathWorks (Natick, MA) was used to build the model and this implementation provided in the Supplementary File 1: LMK-CETPi-model.

RESULTS
PK of RG7232
The population PK dataset included 2,427 observations from 58 individuals for six dose groups (10, 30, 60, 80, 180, and 420 mg). A two-compartment model with first-order absorption and lag time showed the best fit to the data. The exposure of RG7232 increased linearly between 10 mg and 180 mg, while the increase was less than dose proportional from 180 to 420 mg; hence, for the 420 mg dose group the relative bioavailability was estimated. We utilized only population estimates (typical values) but did not include PK and PD variability as they were not essential for assessing the effects of the drug on lipid-poor ApoA-I/RCT dynamics. The final parameter estimates are given in Table 1. As an illustration of the ability of the model to describe the median PK data, we show in Figure 1 a comparison of the model simulation and data for the 180 mg dose group.

Systems pharmacology model and parameter refinement
By combining the drug model of RG7232 with the LMK model via the action of the drug on the activity of CETP, a systems pharmacology model was obtained (see Figure 2 for the schematic diagram). Due to the modular structure of the systems pharmacology model, the

*http://www.mathworks.com/
parameter estimation process is carried out in a two-step approach. First, the typical values of the PK parameters are obtained as described in the previous section. Subsequently, we consider the top three dose groups (80, 180, and 420 mg) and the mean values of PD variables (including HDL-C, LDL-C, ApoA-I, ApoB) were used to estimate parameters linking the drug concentrations to CETP activity, as well as to refine parameter estimates of the LMK model from their prior values. In particular, we model the effect of plasma concentration of RG7232 on CETP mediated CE flux rates by using the Emax expression: with the inhibition effect of RG7232 modeled as:

\[ ECETPi(t) = E_{\text{max}} \times \frac{C_{\text{RG7232}}(t)}{EC_{50} + C_{\text{RG7232}}(t)}; \]

the following rate constants are diminished under the time-dependent drug concentration:

- \[ k_{\text{HL}}^\text{CETP} = (1 - ECETPi(t)) \times k_{\text{HL}}^\text{CETP}; \]
- \[ k_{\text{LH}}^\text{CETP} = (1 - ECETPi(t)) \times k_{\text{LH}}^\text{CETP}; \]
- \[ k_{\text{HV}}^\text{CETP} = (1 - ECETPi(t)) \times k_{\text{HV}}^\text{CETP}. \]

Since we would like to explain the time-dependent data of ApoB, a model extension to ref. 12 for ApoB dynamics was implemented. In particular, a two-compartment model is used to describe the production of ApoB via VLDL particle synthesis, their lipolysis to LDL particles, and the respective holo-particle elimination pathways via LDL and VLDL receptors (refer to Figure 2 for the schematic diagram and the Methods section for more details). The maximum a posteriori (MAP) procedure\(^27\) was applied to the combination of systems pharmacology model and the MAD data, so that the model parameters are either refined or determined with the use of the study data; further details are available in the Methods section.

We note that the baseline PD variables are not the same across the three dose groups considered (80, 180, and 420 mg); in particular, HDL-C ranges from 44.78 to 50.95 mg/dL, ApoA-I from 123.6 to 134.8 mg/dL, LDL-C from 97.3 to 110.0 mg/dL, and ApoB from 69.6 to 79.9 mg/dL. In order to account for the differences in baselines, we allow the following three parameters to vary across dose groups: \( k_{\text{ABCA1}}, r_{\text{VLDL}} \) in \( \ldots \), \( r_{\text{ApoB}} \) in \( \ldots \). The first two parameters were chosen as they have the most prior uncertainty (refer to table 5 of ref. 12) and are known to strongly influence the baseline values of HDL-C, ApoA-I, and LDL-C, while the third parameter influences ApoB. Other than these three parameters, the remaining parameters in the model were jointly estimated, from data for all three dose groups. The parameter estimates inferred from data are given in Table 2; note that only point estimates are given, as the main goal of this work is to evaluate the dynamical aspects of the “on/off” drug kinetics, rather than quantify the precision of those estimates. From the list of inferred parameter values, we remark that the value \( E_{\text{max}} = 0.92 \) implies that RG7232 is a nearly full inhibitor of CETP. It is reassuring that most of the model parameters taken from ref. 12 do not need to undergo large changes in order to explain the new data.

The contributions to the objective functions from the data-misfit for the 80 mg, 180 mg, and 420 mg dose groups are 91, 162, and 81, respectively; hence, while the model fits the data from 80 mg and 420 mg dose groups comparably well, the data for 180 mg dose group is not well explained by the model.

\(^{1}\)This parameter was not in the original LMK model.\(^{12}\)
the fit to the 180 mg is poorer. The fit of the model to the data for the top dose group of 420 mg is illustrated in Figure 3. In particular, we see that the model can describe the dynamics of HDL-C rise due to CETP inhibition, as well as a drop in LDL-C due primarily to the decreased CETP mediated CE flux from HDL to VLDL and LDL. We note that as a consequence of the decline in CE flux from HDL to LDL particles, there is a reduction in the average CE per LDL particle. Due to the composition-dependent elimination rate of LDL particles as identified by the parameter fitting algorithm (refer to the Methods section), CETP inhibition leads to an increased elimination of ApoB from the LDL pool. We remark that since the kinetics of processes underlying the metabolism of ApoA-I are slower compared to that of HDL-C, the model predicts that a longer time is needed for ApoA-I to reach its steady state.

Model evaluation
In order to evaluate the predictive strengths of the model, we try to confirm one of the model outcomes using data that were not used in the calibration procedure. In the study, NMR methodology was used to examine the average HDL size at days 1 and 14 (for the experimental methodology employed, refer to the Methods section). The data shows that for the three dose groups (80, 180, and 420 mg) the HDL size increased from 9.22±0.13 nm, 8.83±0.08 nm and 9.04±0.05 nm to 9.69±0.13 nm, 9.68±0.06 nm and 9.97±0.06 nm, respectively (given as mean±SEM). As the NMR data are sparsely sampled, it was not used in the model calibration; instead, we used it as a validation data to test the model. The model predicts a dose-dependent increase in HDL size (refer to Figure 4). For the three dose groups (80, 180, and 420 mg), the HDL size data showed increases of 0.47±0.05 nm, 0.65±0.07 nm, and 0.93±0.07 nm, respectively, while the model predicted increases of 0.45, 0.58, and 0.7 nm, respectively. Thus, we see good agreement for the 80 mg dose group, while the 180 mg and 420 mg data showed larger-than-expected increases in HDL size for an unknown reason. Due to the model assumption of particle homogeneity, we expect to see some discrepancy between the mean HDL size derived from NMR measurements and those derived from HDL-C and ApoA-I. This can be checked using the HDL size estimates based on a linear fit of the HDL-C/ApoA-I ratio as given in ref. 28. Using this formula, the predicted increases in HDL size are 0.48, 0.61, and 1.1 nm, respectively, for the 80, 180, and 420 mg dose groups. In particular, for the 180 mg dose group the observed HDL size increase is 0.24 nm larger than expected using the HDL-C/ApoA-I formula, with the latter being close to the model results. Furthermore, for the 420 mg dose group the difference between the predicted and observed HDL size increase is 0.17 nm. These results are consistent with the level of precision (±0.4 nm28) in predicting HDL sizes using an estimation approach based on the HDL-C/ApoA-I ratio.

Model predictions
While CETP inhibition can give rise to significantly increased HDL-C and ApoA-I, whether or not there is an increased RCT rate is a controversial topic. In particular, despite the increase in total ApoA-I, it is not well appreciated how much of this is associated with the pool size of HDL versus that of lipid-poor ApoA-I. It is thought that the latter is responsible for the initiation of RCT and hence is the more relevant quantity to examine.12,29

By running the model on the three dose groups that have been studied, we give predictions on the dynamic profile of pre-β1, and hence the potential implications of the different doses on RCT rate (as quantified in ref. 12). As shown in Figure 5, due to the “on/off” kinetics of RG7232, the model predicts that there would be oscillatory dynamics in pre-β1 concentrations, of relatively larger amplitudes compared to those for total ApoA-I and HDL-C. In particular, the model predicts that pre-β1 levels drop during the “on”-phase of the drug (when CETP activity is inhibited), and rise during the “off”-phase of the drug (when CETP activity is restored). An observation that can be made from the simulation results is that the oscillatory amplitude appears to decrease with increasing dosage: however, this is only true for the three doses shown and in fact for sufficiently low dosages the relationship is reversed, whereby the amplitude increases with increasing dosage. One implication regarding the experimental verification of pre-β1 dynamics is that assessments need to be done at both the expected peaks and troughs: otherwise, the oscillatory dynamics could have been missed. An observation that has potential implication for the effect of the drug on changing RCT rate is that while pre-β1 levels rise and fall around the mean, there is a small

Table 2: Prior and posterior estimates of model parameters. The prior parameter values are as given in ref. 12

| Parameter          | Unit     | Prior (mean±SD) | Posterior (mean) |
|--------------------|----------|-----------------|------------------|
| $\beta_1^H$       | mg/dL/day| 28.46±1.13      | 28.93            |
| $k_{\text{sumV}}$ | pool/day | 2.42±0.78       | 2.57             |
| $k_{\text{sumC}}$ | pool/day | 170±191         | 181.89           |
| $\gamma$          | unitless | 10.17±2.19      | 8.58             |
| $k_{\text{CETP}}^{\text{pre}}$ | pool/day | 1.49±0.24       | 1.49             |
| $k_{\text{CETP}}^1$ | pool/day | 6.92±0.81       | 6.87             |
| $k_{\text{CETP}}^2$ | pool/day | 2.89±0.34       | 2.93             |
| $k_{\text{ApoB}}$ | pool/day | 7.70±0.84       | 7.71             |
| $k_{\text{VLDL}}$ | pool/day | 1.30±0.35       | 0.83             |
| $k_{\text{LDL}}$  | pool/day | 0.64±0.07       | 0.57             |
| $k_{\text{SRB1}}$ | pool/day | 0.60±0.08       | 0.65             |
| $k_{\text{HDL}}$  | pool/day | 0.13±0.022      | 0.14             |
| $k_{\text{apoB}}$ | pool/day | 50000±15440     | 50005            |
| $\lambda_{\text{out}}$ | 1/(mmol/dL) | –0.016±0.004 | –0.015           |
| $K_{\text{out}}$ | Å2/mol/Å³ | 55.11±6.5     | 43.78             |
| $K_{\text{apoB}}^\text{out}$ | pool/day | –               | 0.57             |
| $K_{\text{apoB}}^\text{out}$ | pool/day | –0.31           |                  |
| $K_{\text{apoB}}^\text{out}$ | pool/day | 95.18±15.73     | 80 mg : 100.01   |
| $K_{\text{apoB}}^\text{out}$ | pool/day | 1.50±0.45       | 80 mg : 1.10     |
| $K_{\text{apoB}}^\text{out}$ | pool/day | 1.50±0.45       | 180 mg : 2.41    |
| $K_{\text{apoB}}^\text{out}$ | pool/day | 1.50±0.45       | 420 mg : 1.42    |
| $K_{\text{apoB}}^\text{out}$ | pool/day | 1.50±0.45       | 80 mg : 43.61    |
| $K_{\text{apoB}}^\text{out}$ | pool/day | 1.50±0.45       | 420 mg : 39.55   |
| $E_{\text{max}}$ | unitless | –               | 0.91             |
| EC50              | ng/mL    | –               | 6.7              |
net drop compared to their baseline values (see the inset of Figure 5 and visually compare the areas below and above the respective dotted lines). The model simulation results suggest that there is no net increase in RCT rate during treatment, no matter which dose is chosen.

DISCUSSION

HDL metabolism involves the interplay of many processes, such that mechanisms that increase HDL-C may not increase the RCT rate.\textsuperscript{10} In particular, due to the roles of various plasma factors involved in HDL remodeling,\textsuperscript{30} it is challenging to predict the effects of CETP inhibition on the dynamics of lipid-poor ApoA-I regeneration.\textsuperscript{31} Based on the finding that the number of ApoA-I molecules per HDL particle\textsuperscript{28} increases with HDL size, the LMK model\textsuperscript{12} has been developed to give quantitative estimates on the rate of lipid-poor ApoA-I regeneration as a result of processes that shrink the particle core. In this work, we combine the dynamics of HDL remodeling with the kinetics of a CETP inhibitor having a short half-life, to derive the temporal behavior of lipid-poor ApoA-I and hence potentially the RCT rate. By using a systems pharmacology model, we address the following question: What is the effect of a short drug half-life on the regeneration of lipid-poor ApoA-I? This is challenging to answer without resorting to a quantitative systems model since feedback processes need to be taken into account, including the regeneration of lipid-poor ApoA-I via HDL remodeling. In contrast, a PK/PD model of HDL-C and ApoA-I data would enable the selection of dose regimen that achieves their target values, but would not be suitable for assessing the impact of dose regimen on quantities that have not been experimentally measured, such as pre-$\beta_1$.

Our model finding is that while the “on/off” kinetics of the CETP inhibitor results in the ability to increase pre-$\beta_1$ during the “off” portion of the dosing interval, there is a net decrease

Figure 3 Fit of the systems pharmacology model simulation to the MAD data (mean±SEM) for the 420 mg dose group.
when averaged over time. This outcome is consistent with our previous finding that CETP inhibition is not a target for increasing RCT rate via the ABCA1 pathway. While our simulations suggest that transient or static CETP inhibition is not likely to increase RCT rate via the ABCA1/preβ pathway, the observed data and model results nevertheless show that RG7232 lowers LDL-C and ApoB levels. Such effects should be beneficial in reducing CVD risk as they reduce the forward cholesterol transport into the arterial wall. This finding may provide a perspective on the outcomes of past and ongoing clinical trials of CETP inhibitors. The failure of torcetrapib is thought to be attributed to off-target effects. Dalce-trapib, which was terminated due to the lack of efficacy, was associated with minimal effects on LDL-C levels. The outcomes for other CETP inhibitors with ongoing studies, including anacetrapib, and their associations with the levels of LDL-C and ApoB lowering remain to be seen.

Via the insights gained through the analysis, we illustrate in the context of cholesterol metabolism the value of utilizing existing biological knowledge and data in deriving a mechanistic model together with prior parameter estimates, and how these parameter estimates can be further refined with the use of new clinical data. In particular, we demonstrate that the systems pharmacology model can be used to test
hypotheses on how drug kinetics might impact cholesterol metabolism, whose dynamics may be difficult to fathom. Furthermore, the incorporation of mechanistic details into the systems pharmacology model allows for the potential to translate these findings from healthy volunteers to dyslipidemic patients.

While our work aimed to address whether CETP inhibitor would increase pre-β1 levels and the RCT rate, we draw no conclusions on the link between whole-body RCT and the decrease in plaque volume. In order to be able to assess how therapies targeting lipid pathways may affect CVD risk, drug-disease models that combine our systems pharmacology model with mechanistic modeling efforts such as refs. 35,36 are crucially needed to further understand the links between plasma lipid levels, the downstream events including macrophage recruitment and foam cell formation, and eventually to the resulting changes in plaque size and geometry. These remain topics for future research.

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