

ORIGINAL ARTICLE

Target Mediated Drug Disposition Model of CPHPC in Patients With Systemic Amyloidosis

T Sahota^{1*}, A Berges¹, S Barton², L Cookson³, S Zamuner¹ and D Richards⁴

The amyloid deposits that cause disease in systemic amyloidosis always contain the normal plasma protein, serum amyloid P (SAP) component. SAP is the target of a novel immunotherapy approach now being developed to eliminate amyloid deposits. The treatment is enabled by, and critically depends on, the use of the drug (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC, GSK2315698, Ro 63-8695), which depletes circulating SAP almost completely but leaves some SAP in amyloid deposits for specific recognition by subsequently administered therapeutic anti-SAP antibodies. Herein, we report a mechanistic model that predicts, with clinically acceptable precision, the exposure-response relationship for CPHPC, both in healthy individuals and in patients with systemic amyloidosis. The model covariates are gender, renal function, total amyloid load, and presence of hepatic amyloid, all of which are known at baseline. The model is being used to predict individualized dosing regimens in an ongoing, first-in-human study with anti-SAP antibodies.

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Systemic amyloidosis is a rare (prevalence not more than ~2 per 10,000 in developed countries) but serious and usually fatal disease caused by extracellular deposition of amyloid in visceral parenchyma, connective tissues, and blood vessel walls, damaging their structure and function.¹ Amyloid is usually extensive at the time of diagnosis and is responsible for severe morbidity and mortality, but no current treatments for systemic amyloidosis directly target the amyloid deposits themselves. Deposits are complex, composed of amyloid fibrils (insoluble misfolded fragments of globular proteins), heparin and dermatan sulfate, proteoglycans and glycosaminoglycan chains, and the normal plasma glycoprotein, serum amyloid P (SAP).² A novel approach to elimination of amyloid deposits from the tissues is in development, and capitalizes on the fact that CPHPC produces rapid and almost complete depletion of circulating SAP.³ Substantial depletion of plasma SAP inevitably reduces the amount of SAP bound in amyloid deposits,^{3,4} because the blood is the only source of such bound SAP.⁵ Nevertheless, some SAP always remains in the deposits⁴ as a specific marker for amyloid of all types.⁵ Plasma SAP concentrations in healthy subjects and in patients with amyloidosis are in the range of 7–55 mg/L⁶ and therefore anti-SAP antibodies cannot be safely or effectively administered to patients without prior SAP depletion. However, after treatment with CPHPC, immunoglobulin G anti-SAP antibodies can be safely administered and can then reach and bind to the amyloid deposits where they trigger very effective, clinically silent, complement dependent, macrophage-mediated clearance of the amyloid.⁷

The potent depletion of plasma SAP by CPHPC is well established,^{3,4} but SAP dynamics are radically different between healthy subjects and patients with amyloidosis with different whole body amyloid loads.⁸ The plasma and extracellular fluid together contain about 50–100 mg of soluble SAP in all subjects, regardless of the presence of amyloid. In patients with amyloidosis, there is another SAP pool containing between a few hundred to more than 20,000 mg of SAP, which are avidly but reversibly bound to amyloid fibrils and are in dynamic equilibrium with the soluble SAP in the plasma and extracellular fluid.⁸ For optimal clinical use of CPHPC before administration of anti-SAP antibodies, the swiftest and most complete depletion of circulating SAP is required and the duration of SAP depletion must be known. We have therefore characterized the exposure-response relationship between CPHPC and plasma SAP concentration, both in healthy volunteers and in patients with systemic amyloidosis, and used the results to develop a robust pharmacokinetic-pharmacodynamic (PK-PD) model that predicts suitable dosing regimens for CPHPC in individual subjects.

RESULTS

We generated PK (total plasma CPHPC concentrations) and PD (total plasma SAP concentration) data from studies in healthy volunteers (CPH113776) and patients with amyloidosis (CPH114527) with varying amyloid loads and renal function (see **Table 1**).

First, an exploratory data analysis was performed to summarize PK and PD profiles for a variety of dosing regimens.

¹Clinical Pharmacology Modelling and Simulation, GlaxoSmithKline, UK; ²Clinical Statistics, GlaxoSmithKline, UK; ³Clinical Pharmacology, Science, and Study Operations, GlaxoSmithKline, UK; ⁴Academic DPU, GlaxoSmithKline, UK. *Correspondence: T Sahota (tarjinder.z.sahota@gsk.com)
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Table 1 Summary of disease characteristics in study cohorts

Study	Renal function	Amyloid load
CPH113776	Normal renal function	None
CPH114527	Cohort 1	Normal renal function / mild renal impairment (estimated CRCL >60 mL/min)
	Cohort 2	Normal renal function / mild renal impairment (estimated CRCL >60 mL/min)
	Cohort 3	Moderate renal impairment (CRCL >30 mL/min)
	Cohort 4	Moderate-severe renal impairment

CRCL, creatinine clearance.

Based on these data, knowledge of SAP physiology, and pharmacology of CPHPC, our model was developed with the final model parameters summarized. Model diagnostics were used to assess the model's ability to describe SAP depletion for all studied dosing regimens and patient disease characteristics. Finally, simulations were performed with the final model to show predicted SAP depletion in various disease states.

Data exploration

Individual measurements of total plasma concentrations of CPHPC and SAP are illustrated in **Figure 1**. Session 1 in CPH113776 showed rapid SAP depletion after the 1-hour infusion regimens. Sessions 2 and 3, with dosing infusions extended to 24 hours, showed further reduction of SAP concentrations below those in session 1. SAP profiles seemed to approach steady state equilibrium after 24-hour infusion (see middle left-hand panel). This implies that a predictive model of SAP depletion for short and extended infusion durations must account for time delays to reach steady state SAP equilibrium. The PK and SAP profiles were broadly similar in patients and healthy volunteers, but SAP profiles in patients did not seem to reach a steady state, even after 48 hours of CPHPC infusion. Postinfusion, subcutaneous dosing of 60 mg every 8 hours seemed to be sufficient to prevent recovery of SAP concentrations (see middle right-hand panel).

The graphical assessment of the impact of patient characteristics (covariates) on the PK and PD results is illustrated in **Figure 2**. The top row shows a clear correlation between the CPHPC clearance (derived as the ratio between the infusion rate at 24 hours and CPHPC concentration at the end of infusion) and the baseline creatinine clearance (CRCL). The subsequent rows show the impact of liver involvement (AMLIVER) and overall amyloid load at baseline (AMLOAD) on SAP profiles. In subjects with a small amyloid load and no liver involvement (black lines in the left-hand panels), there was substantial SAP depletion to less than 1 mg/L with recovery to baseline by 28 days post-dose, whereas in patients with a large amyloid load with liver involvement (gray lines in the right-hand panels), SAP depletion recovery was still incomplete at day 28. Our findings robustly confirm and extend the previous reports of these phenomena.^{3,9}

Model development

PK and PD parameters were estimated simultaneously with the aggregated CPH113776 and CPH114527 datasets. First, the base model (i.e., the structural model and random

effects model) was developed, followed by covariate testing to describe impact of disease status on physiological processes.

The structural model of choice was a simplified representation of the biology of SAP and pharmacology of CPHPC. We used a target-mediated drug disposition (TMDD) model to characterize SAP depletion in terms of receptor-ligand binding and subsequent elimination of bound SAP (see **Figure 3**).

Final model parameter estimates obtained with the importance sampling algorithm (IMP) were found to be more stable under perturbations of initial parameter estimates at the initial model development stage compared with those obtained with the first order conditional estimation method. IMP was therefore selected for model building. Because IMP retained acceptable numerical stability until final model development, first order conditional estimation was not retested for stability. **Table 2** shows the final model parameter estimates with covariate model results included. Final parameter estimates were found to be insensitive to perturbations of initial parameter estimates. The least precisely estimated structural model parameter in terms of relative standard error (RSE) was intercompartmental clearance of CPHPC (RSE of 38%). All other structural model parameters were estimated with high precision (RSE \leq 19%). The least precisely estimated covariate/random effects parameter was between-subject variability on Q4, intercompartmental clearance of SAP (RSE = 51%). All other covariate/random effects were estimated with RSE \leq 40%.

CPHPC PK

The PK model describes the rapid distribution and elimination of free CPHPC from the central compartment. The typical value of CPHPC clearance (TVCL) was modelled as a function of baseline creatinine (CRCL) in the base model, using Eq. 1. The clearance value (6.85 L/h = 114 mL/min) in individuals with healthy renal functioning (CRCL >80 mL/min) was in line with normal glomerular filtration rates. The inclusion of a peripheral compartment was necessary to capture the biphasic elimination observed in the CPHPC data. The low value of intercompartmental clearance compared to renal clearance was consistent with distribution-limited kinetics (i.e., the first phase being clearance limited and the second phase being distribution limited). Because of limited sampling immediately after subcutaneous (s.c.) dosing, the s.c. absorption parameters (KSC and F) were fixed

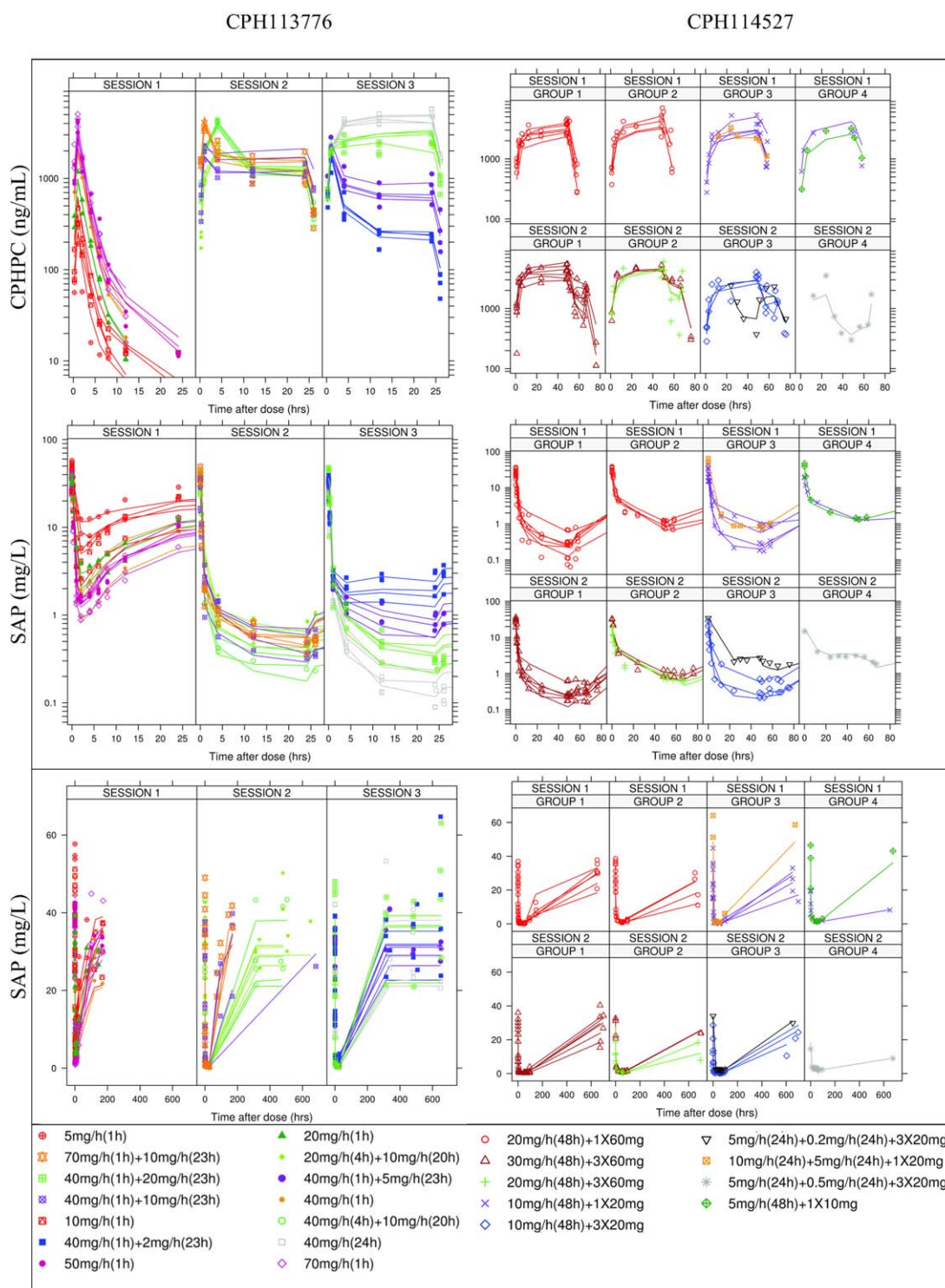


Figure 1 Pharmacokinetic-pharmacodynamic (PK-PD) individual profiles in the two adaptive studies. Points represent observed data and lines represent individual predictions using the final model. Healthy volunteer data are in the left column and patient data are in the right column. Top row displays the PK data, middle row displays PD data up to 24 hours (CPH113776) and 80 hours (CPH114527) after first dose, and the bottom row displays all PD data. All data are split by session and cohorts, and the treatment regimens are color-coded. Legend convention: infusion rate (infusion dur.) + infusion rate (infusion dur.) + number of s.c. doses x s.c. dose levels. SAP, serum amyloid P.

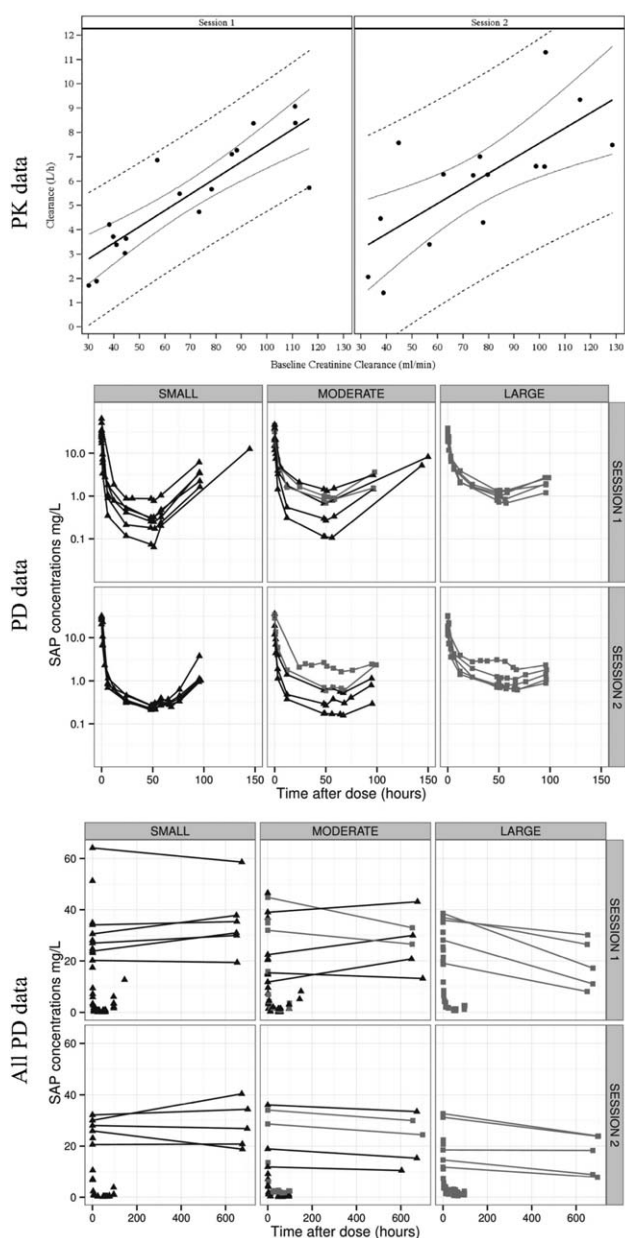


Figure 2 Covariate plots for creatinine clearance, amyloid load, and liver involvement for study CPH114527. Top row displays the pharmacokinetic (PK) covariate plots split by session for CPHPC clearance vs. creatinine clearance. The points are the individual values, the black line is the linear regression line, the dotted and solid grey lines outline, respectively, the 95% prediction limits and the 95% confidence limits of the linear regression analysis. Middle and bottom rows display the pharmacodynamic (PD) covariate plots split by session and overall amyloid load at baseline for serum amyloid P (SAP) profiles from 0–6 days (middle row) and from 0–28 days (bottom row). Grey and black colors indicate, respectively, the presence (or absence) of liver amyloid at baseline.

to the previously estimated values (see Methods section).

Eq. 1 covariate relationship between CRCL and CPHPC clearance:

$$CRCL_EFF = (1 + THETA(15) * (CRCL - THRESH))$$

$$TVCL = CRCL_EFF * EXP(THETA(2))$$

where THRESH is the CRCL threshold value of 80 mL/min, characterizing the normal renal function. CRCL was itself truncated to THRESH when $CRCL > THRESH$. TVCL (L/h) is the typical value of CPHPC clearance, CRCL_EFF (dimensionless) is the change in TVCL because of the CRCL lower than the threshold, THETA(15) (min/mL) is change in TVCL per unit change in CRCL below the threshold and THETA(2) is the TVCL constant value for CRCL greater than or equal to the threshold.

SAP turnover and distribution

At baseline, free SAP was assumed to be in equilibrium between production and elimination in the central compartment, as previously shown directly with radiolabelled SAP.⁸ The estimated baseline SAP parameter values were consistent with the established reference range for circulating SAP concentrations in healthy normal adults: mean (SD; range) 21 mg/L (8; 8–55) in women and 32 mg/L (7; 12–50) in men.⁶ The estimated elimination rate constant was also consistent with these studies^{8,10} ($KOUT = 0.046 h^{-1}$ vs. $0.056 h^{-1}$).

The inclusion of the peripheral compartment was necessary to describe the observed disease-dependent differences in SAP depletion and recovery and the impact of amyloid load on apparent volume of distribution in the periphery was tested via covariate analysis (see Eq. 2).

Binding and elimination for SAP-CPHPC complex

Although the complex of the pentameric SAP molecule with CPHPC comprises two SAP molecules crosslinked by five drug molecules when sufficient drug is available, very rapid clearance of circulating SAP starts when there is just one mole of drug available per mole of pentameric SAP.³ For the purposes of the model, both 2:5 and 1:1 molar binding ratios between SAP and CPHPC, were tested, with complex formation assumed to occur in well stirred conditions in a common central compartment ($V1 = V3$) and the complex eliminated from the same compartment. Both ratios gave similar diagnostic results, and parameter values (except for association rates and the elimination rate of the complex) but the 1:1 value yielded greater numerical stability and was therefore adopted. The estimated value of the volume of distribution (16.15 L) is consistent with unpublished GlaxoSmithKline preclinical studies where volume of distribution in preclinical species was consistent with extracellular water space (~15 L in humans). The SAP:CPHPC complex is known to be very stable *in vitro* and *in vivo* and the clearance of the complex by the liver is extremely fast.³ Therefore, the model assumes that the elimination rate constant of the complex from the central compartment, KINT, is much faster than its dissociation. Mathematically, this assumption is equivalent to setting the dissociation rate constant, KOFF, to zero. The predicted clearance rate was consistent with the liver blood flow rate in humans ($KINT * V3 = 93 L/h$ vs. $90 L/h$).

Covariate model building

Because there were multiple confounding covariates related to disease status, we restricted testing of covariate

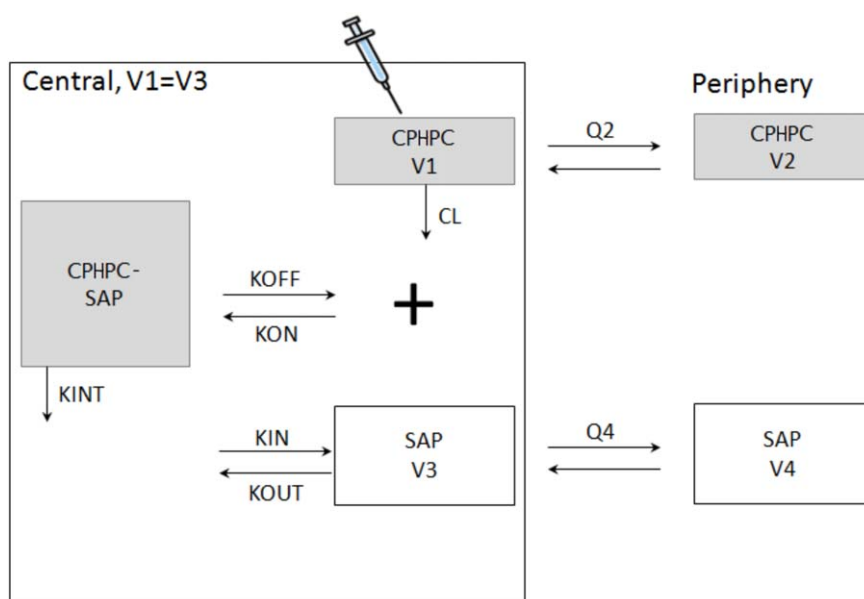


Figure 3 Schematic of the target-mediated drug disposition (TMDD) model. Clearance is the elimination clearance of free CPHPC from the central compartment and Q2 is the intercompartmental clearance of free CPHPC between central and periphery. V1 and V2 are the volumes of distribution of CPHPC in the central and in the peripheral compartments. KIN is the serum amyloid P (SAP) production constant and KOUT is the SAP elimination rate constant. At baseline, the plasma SAP concentration (SAP_BASE) can be derived by the ratio KIN/KOUT. Q4 is the intercompartmental clearance of free SAP between central and periphery. V3 and V4 are the volumes of distribution of SAP in the central and peripheral compartments. V3 is assumed to be equal to V1. KON and KOFF are the association and dissociation rate constants of the complex CPHPC-SAP and KINT is the elimination rate constant of this complex.

Table 2 Summary of PK-PD model parameters based on the final PK-PD model

	Parameters (units)	Estimates (%RSE)	BSV (%RSE)
GSK2315698	Clearance (L/h) ^a	6.85 (4%)	21.93% (22%)
	CRCL ~ clearance ^b (ml/min ⁻¹)	0.015 (5%)	
	Central volume (V1:L)	16.15 (5%)	30.23% (22%)
	Intercompartmental clearance (Q2:L/h)	1.72 (38%)	15% FIX
	Peripheral volume (V2:L)	17.57 (16%)	15% FIX
	S.c. absorption rate (KSC:1/h)	1.5 FIX	15% FIX
	SAP	SAP baseline (SAP_BASE:mg/L)	31.10 (4%)
	Gender ~ SAP baseline ^c	-0.30 (23%)	
	SAP elimination rate (KOUT:1/h)	0.046 (13%)	41.09% (22%)
	Central volume (V3:L)	= V1	
	Intercompartmental clearance (Q4:L/h)	2.84 (19%)	52.24% (51%)
	Amyloid liver ~ Q4 ^c	4.01 (26%)	
	Peripheral volume (V4:L)	12.15 (17%)	60.48% (30%)
	Amyloid load ~ V4 ^c	moderate = 6.39 (39%) large = 26.39 (26%)	
GSK-SAP	Association rate (KON:1/Mxh)	1.94+E06 (12%)	15% FIX
	Complex elimination (KINT:1/h)	5.78 (11%)	15% FIX
	Residual error (%)		PK: 28.62 (28%) SAP: 27.10 (16%)

BSV, between-subject variability; CRCL, creatinine clearance; PK, pharmacokinetic; RSE, relative standard error; SAP, serum amyloid P.

^aClearance for subjects where CRCL >80 mL/min.

^bRelationship between CRCL and CPHPC clearance are available in Eq. 1.

^cRelationships from the covariate model building are available in Eq. 2.

AMLIVER is the binary covariate indicating the presence or absence of amyloid in the liver.

AMLOAD is the categorical variable indicating the whole body amyloid load.

relationships to physiologically plausible relationships (see Methods section) and used a greedy search algorithm based on log-likelihood ratio testing, as implemented by the stepwise covariate method. The stepwise covariate method procedure identified the three relationships shown on Eq. 2 between covariates and model parameters related to SAP.

Eq. 2 significant covariates-parameter relationship from stepwise covariate method

Binary relationship between Q4 and AMLIVER

IF (AMLIVER.EQ.0) Q4 AMLIVER = 1
 IF (AMLIVER.EQ.1) Q4 AMLIVER = (1 + THETA_{AMLIVERQ4})
 TVQ4 = Q4 AMLIVER * TVQ4

Binary relationship between SAP_BASE and SEX

IF (SEX.EQ.1) SAP_BASE_SEX = 1
 IF (SEX.EQ.2) SAP_BASE_SEX = (1 + THETA_{SAP_BASE_SEX})
 TVSAP_BASE = SAP_BASE_SEX * TVSAP_BASE

Binary relationship between V4 and AMLOAD

IF (AMLOAD.EQ.0) V4AMLOAD = 1
 IF (AMLOAD.EQ.1) V4AMLOAD = 1
 IF (AMLOAD.EQ.2) V4AMLOAD = (1 + THETA_{AMLOADV42})
 IF (AMLOAD.EQ.3) V4AMLOAD = (1 + THETA_{AMLOADV42}
 + THETA_{AMLOADV43})
 TVV4 = V4AMLOAD * TVV4

where TVQ4, TVSAP_BASE and TVV4 represent the typical value of the model parameters and Q4AMLIVER, SAP_BASE_SEX and V4AMLOAD represent the impact of each category of covariates on the model parameters. To ensure a monotonic relationship between AMLOAD and V4, a positive new theta was added to V4AMLOAD at each increment of AMLOAD covariate.

The parameter estimates related to the covariate-parameter relationship are available in **Table 2** and showed that the estimated gender effect reduction in SAP baseline (30% reduction) was in line with a study by Nelson *et al.*⁶ (which reported a 25% reduction). Intercompartmental SAP clearance (Q4) in patients with hepatic amyloidosis was estimated to increase by fivefold compared with patients with no hepatic amyloid deposits. The value of V4 increased by 7.4-fold and 33.78-fold in patients with moderate and large amyloid loads, respectively, compared with those with small amyloid loads or healthy controls without amyloidosis.

Model diagnostics

Figure 4 shows normalized prediction distribution error (NPDE) plots for model diagnosis that measure prediction discrepancies between observations and model predictions

by regimen and disease state. Overall, the NPDEs for both PK and PD observations seem to fall within the 95% prediction intervals. Healthy volunteer PK predictions showed some misspecification for some 24-hour infusion regimens, but those misspecifications did not translate to significant misspecification with respective PD observations.

Model simulations

Simulations were performed with the final model and are shown in **Figure 5**.

DISCUSSION

The anti-SAP approach to clearing amyloid deposits has the potential to transform the treatment of systemic amyloidosis. There is a requirement for extensive depletion of circulating SAP before administration of the anti-SAP antibodies, which actually mediate the therapeutic effect. It was therefore essential for clinical use to characterize robustly the optimal CPHPC regimen to provide the swiftest and most complete depletion of plasma SAP. An iterative approach with two dose-adaptive studies was used to construct a TMDD model, assisted by the existing detailed understanding of human SAP metabolism in health and in amyloidosis,⁸ and the molecular mechanism of action of CPHPC.³

Systemic amyloidosis is both rare and extremely complex with wide variations between patients in amyloid load, organ distribution, and effects on organ function. The small numbers of patients available for study coupled with their diverse baseline features might have made PK-PD prediction very problematic. However, the small number of baseline clinical parameters we selected was sufficient to characterize robust and reliable individual PK-PD plasma profiles of CPHPC and SAP. The model-based simulations illustrated in **Figure 5** clearly show the impact of the selected covariates on predicted plasma profiles of CPHPC and SAP.

Precisely as originally reported,³ the amyloid load has a major effect on both the extent of plasma SAP depletion by CPHPC in patients with systemic amyloidosis, and the rate of plasma SAP recovery after stopping CPHPC treatment. For example, after 48-hour infusion of CPHPC in a typical patient with large amyloid load, the plasma SAP was reduced to approximately 2 mg/L compared to 0.5 mg/L in a patient with no/small amyloid load (dashes vs. continuous line on the middle-left panel). After stopping CPHPC treatment, the plasma SAP concentration in this patient returned to its baseline value after more than 600 hours compared with 200 hours in a patient with no/small amyloid load (dashes vs. continuous line on the bottom-left panel). The amyloid load, however, had no effect on the PK profile of CPHPC, consistent with the fact that the drug, relative molecular mass M_r 340, was in vast molar excess over SAP, M_r 127,310, which implies that CPHPC concentrations are in the micromolar range during tested infusions when SAP is in the nanomolar range.

Also consistent with previous observations,⁴ CPHPC plasma exposure was substantially increased in patients with reduced CRCL, resulting in prolonged SAP depletion. CPHPC plasma concentrations were fourfold higher in a typical subject with severe renal impairment compared with

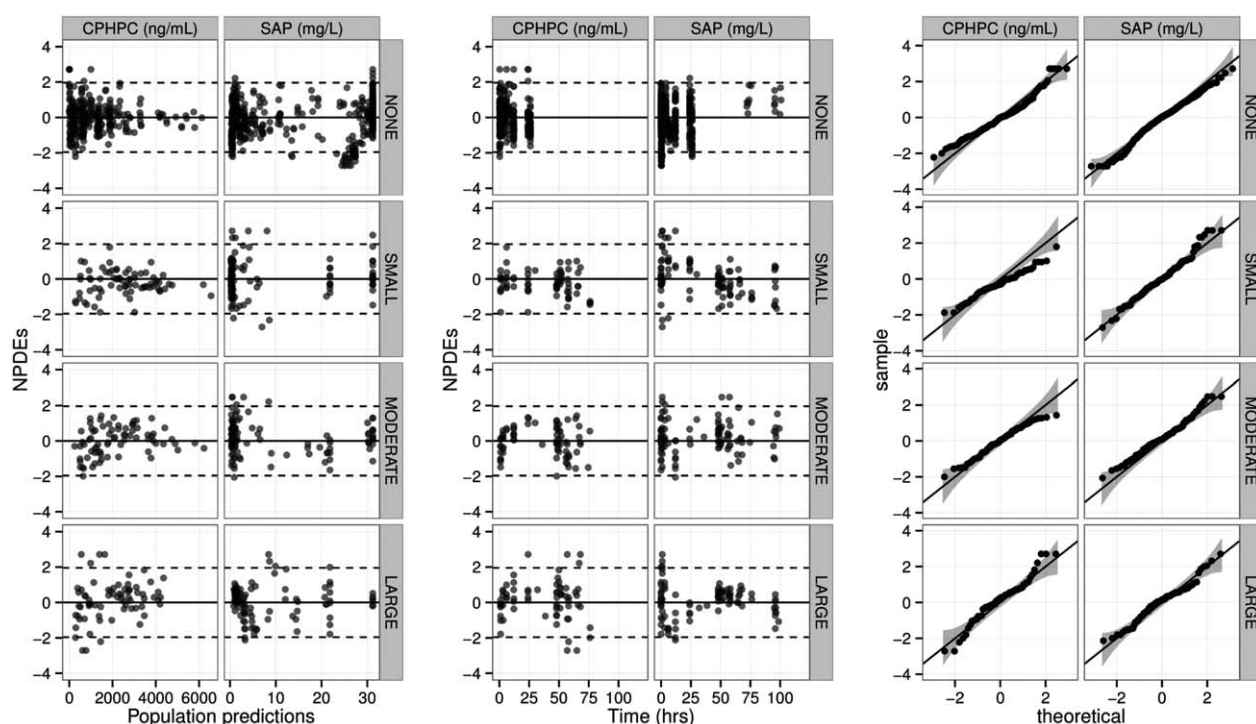


Figure 4 Normalized prediction distribution error (NPDE) plots for pharmacokinetic (PK) and pharmacodynamic (PD) observations are shown grouped by amyloid load. Left panel shows NPDEs vs. population predictions, middle panel shows NPDEs vs. time, and right panel shows Q-Q-plots of NPDEs. Lines of identity and prediction intervals (95%) of NPDEs are depicted by solid line and by the grey shaded area, respectively. A correctly specified model should have NPDEs randomly distributed around zero but within prediction intervals for 95% of NPDEs (shaded areas and dashed lines) and show no significant trends with population predictions and independent variables (time).

subjects with normal kidney function (dashes vs. continuous line on the top-right panel).

The final structural model adequately predicted study data and was consistent with parameters derived from historical studies. It was therefore considered an adequate approximation of the underlying pharmacology. However, several limitations in the data needed to be overcome during model development: most importantly there was limited SAP sampling in the recovery phase and, because no assay exists for the SAP-CPHPC complex itself, only plasma concentrations of total SAP and total CPHPC were measured.

Although the ratio of the rates of SAP synthesis, KIN, and clearance, KOUT, was informed by baseline concentrations, the lack of recovery phase data meant that there was no direct information on specific SAP synthesis and clearance rates. These values were thus dependent on other components of the model and were therefore sensitive to model misspecification. With an empirical two-compartment indirect response model,¹¹ KIN and KOUT values were more than fourfold higher than the published values for SAP synthesis and plasma clearance rates, whereas the physiological TMDD model was entirely consistent with them. This suggests that modeling of the binding between CPHPC and SAP was an important component in minimizing model misspecification. Such modeling also provides increased confidence in predictions of plasma SAP concentration during the

recovery phase after stopping CPHPC administration. Although warfarin is an early example of application of TMDD modeling in small molecules,¹² subsequent TMDD modeling has primarily focused on biological molecules. The present study shows that TMDD may have wider utility in describing the PK-PD of small molecules.

The assumption that the complex elimination rate was much faster than the dissociation rate was consistent with the rapid elimination of SAP, indicating rapid association rate, KON, and KINT relative to KOFF. The approximation was necessary for model identifiability and enabled estimation of the binding association process despite only having values for the total plasma concentration of the analytes. Other commonly used TMDD approximations were investigated¹³ and gave similar fits but resulted in SAP synthesis and elimination parameters that were not consistent with the published values measured directly using radiolabeled SAP.⁸ To the authors' knowledge, this is the first instance of this approximation being used to aid in model identifiability and may have use for describing the PK and PD of other compounds with rapid conjugate elimination.¹⁴

Limitations

The behavior of plasma SAP concentrations in response to CPHPC is significantly influenced by the presence of

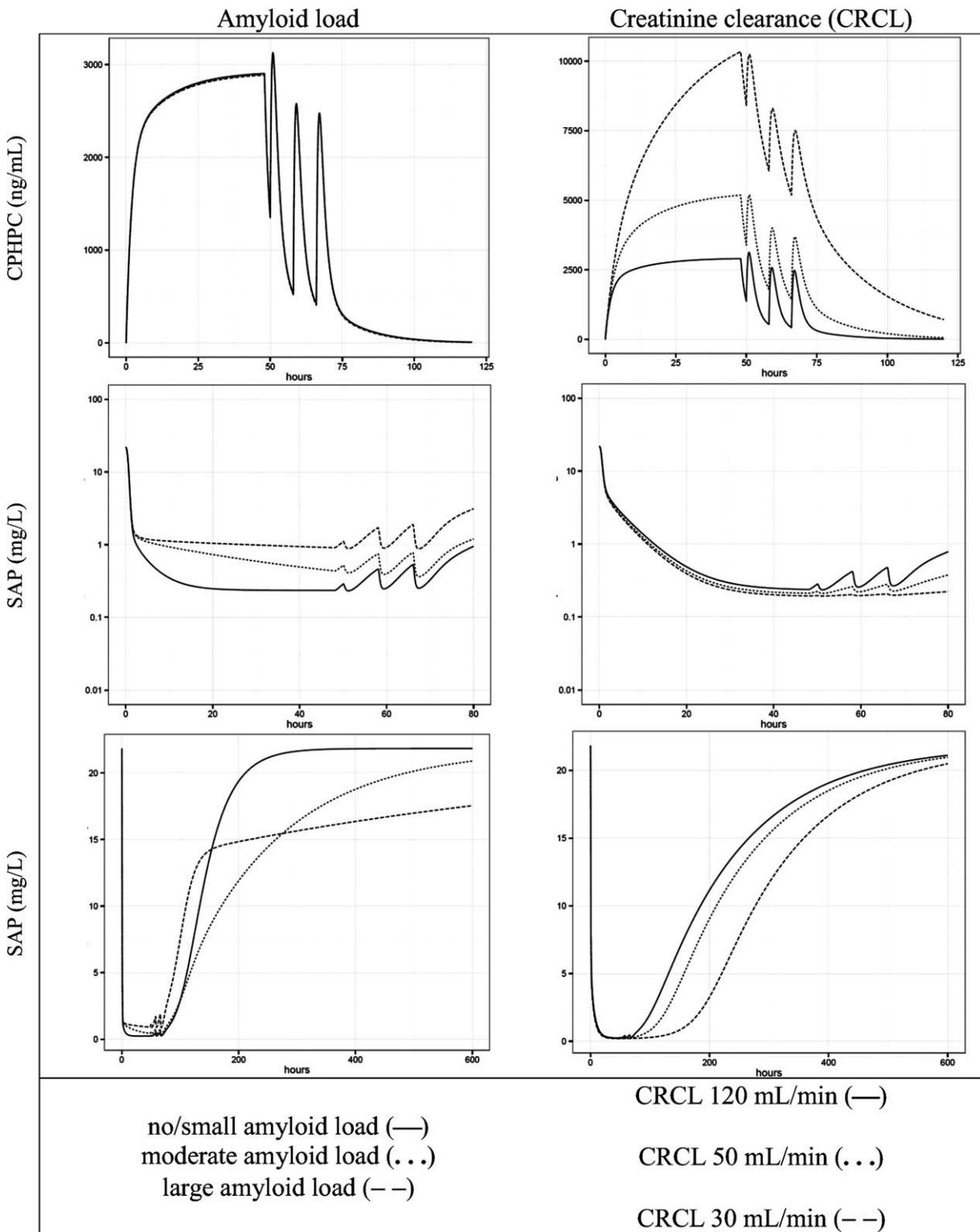


Figure 5 Model-predicted impact of disease status on CPHPC and serum amyloid P (SAP) profiles of a typical man. Simulations across levels of amyloid loads are in the left column and across levels of creatinine clearances are in the right column. Top row displays the pharmacokinetic (PK) data, middle row displays pharmacodynamic (PD) data up to 80 hours, and the bottom row displays all PD data. The dosing regimen of CPHPC used is 20 mg infusion for 48 hours followed by three subcutaneous doses of 60 mg, three times a day.

systemic amyloid deposits that may contain many thousands of milligrams of SAP in contrast to the total of 50–100 mg, which are present in the plasma and extracellular fluid of individuals without amyloidosis.⁸ The SAP in amyloid deposits is tightly but reversibly bound to the insoluble amyloid fibrils and is therefore in equilibrium with the free soluble SAP that is available to bind to the drug.⁸ However, it is not possible to estimate reliably the amount of SAP in amyloid in living subjects, imposing an unavoidable limitation on prediction of plasma SAP depletion efficacy. Nevertheless, the model performed very well, reproducibly indicating drug regimens, which delivered the desired SAP depletion. Although the model could, in theory, be used to predict the SAP content of the peripheral amyloid compartment, the amyloid covariates are all confounded and tissue estimates from the model cannot be considered reliable at this stage.

The model assumes a 1:1 molar ratio in the binding of CPHPC by SAP, which is known to be sufficient to initiate SAP clearance from the plasma *in vivo*, even though when sufficient drug is available, as it always is in practice, the actual molar ratio is 5:2. However, using 5:2 did not improve the model and pragmatically the 1:1 assumption is therefore acceptable. The estimated association rate and the elimination rate of the complex, however, may not numerically represent the true underlying rates.

SUMMARY AND CONCLUSIONS

We have developed a model encompassing the principal determinants of PK-PD for CPHPC in patients with systemic amyloidosis: gender, renal function, amyloid load, and whether amyloid involves the liver, all factors that are known before treatment with CPHPC is started. The model can predict a suitable individualized dosing regimen. The model will now be applied in a first-in-human study with anti-SAP antibodies in order to remove the need for patients to be admitted for test regimens of CPHPC before administration of anti-SAP antibodies.

METHODS

Data acquisition

Adaptive PK-PD studies. CPH113776 was an open label, dose escalation study assessing safety and pharmacokinetic/pharmacodynamic parameters of CPHPC in healthy volunteers.¹⁵ A total of 21 male subjects attended up to three sessions: session 1 was a single constant-rate i.v. infusion over 1 hour to confirm the safety of a single dose of CPHPC over a wide dose range (5–70 mg), sessions 2 and 3 investigated i.v. infusion regimens over 24 hours (induction phase followed by maintenance phase), with total dose ranging from 86–960 mg. The dosing regimen was adjusted adaptively to optimize the evaluation of PK-PD.

CPH114527 was a phase 1, open label, dose characteristic study to investigate the pharmacokinetics, pharmacodynamics, safety, and tolerability of i.v. and s.c. doses of CPHPC in patients with systemic amyloidosis.¹⁶ Subjects attended for 2 sessions: session 1 was a 48-hour i.v. infusion of CPHPC

ranging from 240–960 mg followed by a single s.c. dose ranging from 10–60 mg. Session 2 was a 48-hour i.v. infusion of CPHPC ranging from 124.8–1440 mg followed by three s.c. doses over 24 hours (3 x 20 or 60 mg).

In both studies, multiple plasma samples for assay of CPHPC (PK) and SAP (PD) were taken from baseline up to day 5 since session start. CPHPC was determined in human plasma using HPLC-MS-MS (limits of quantification: 10–10,000 ng/mL, |%bias| <10%, precision [%CV] within-run <16.2%). CPHPC was extracted from 50 μ l of human plasma by protein precipitation using acetonitrile, containing an isotopically labeled internal standard (2H-CPHPC). Extracts were analyzed by HPLC-MS-MS using a Turbolon-Spray interface and multiple reaction monitoring. SAP was measured in plasma using an anti-human SAP enzyme-linked immunosorbent assay based on the sandwich principle (Hycult Biotech, Uden, The Netherlands). The limits of quantification for the concentration are 0.2–50 mg/mL (|%bias| <10%, precision [%CV] within-run <9%). Samples with a concentration above this range were diluted appropriately before assay. Plasma SAP concentration was also measured at about days 7 and 28. Dosing regimens and patient selection were adjusted iteratively, based on emerging results, so that the predictive PK-PD model could be refined. The studies were conducted in accordance with International Conference on Harmonisation Good Clinical Practice and received approval from national research ethics committees. Participant demographics and adverse effects are shown in **Supplementary Materials S1 and S2**.

Dataset production

Results from each study were separately compiled in a single analysis-ready dataset. Covariate data items were added to both datasets as time-independent data items. CRCL was calculated from the baseline serum creatinine concentration using the modification of diet in renal disease formula.¹⁷ The whole body amyloid load covariate, AMLOAD, was a categorical score: 0 for no amyloid in healthy volunteers, 1 for small, 2 for moderate, and 3 for large. A derived binary covariate was used to indicate the presence (or absence) of amyloid in each specific organ: AMLIVER for liver, AMSPLEEN for spleen, and AMHEART for heart.

Previous studies to support model development and evaluation

Results were available from previously published^{3,4} and unpublished studies (P.N. Hawkins and M.B. Pepys). These small academic clinical studies, in healthy volunteers and systemic amyloidosis patients, investigated various doses of CPHPC (0.1–6 mg/kg) and different routes of administration (i.v. bolus, i.v. infusion, and s.c.). The estimated value of bioavailability with s.c. administration was 1.06 +/- 0.18 (unpublished results). We therefore assumed complete bioavailability for s.c. doses. Results from a PK-PD study in two carriers of the Ala60 transthyretin variant, who did not have amyloidosis,³ and from three patients with amyloidosis in the long-term treatment study⁴ were analyzed in NONMEM software (version 7.1.2 ICON Solutions) to aid identification of s.c. absorption parameters. Robust published values are available for the circulating SAP concentration reference range in

healthy adults⁶ and for SAP turnover, synthesis, and metabolism in systemic amyloidosis patients and healthy controls.^{8,10}

Model development and evaluation

The software packages NONMEM¹⁸ and PsN (Perl Speaks NONMEM, version 3.2.4)^{19,20} were used for the development of the nonlinear mixed effects PK-PD model. The first order conditional estimation with interaction and IMP estimation methods, as implemented in NONMEM 7, were considered as possible estimation methods for model development. A recent analysis of the bias, precision, and robustness of different estimation methods in NONMEM 7 has shown that IMP yields the least biased and most precise estimates of all of NONMEM's estimation methods.²¹ Choice of estimation method was determined in the initial model development phase by assessment of numerical stability of the convergence algorithm and by assessing sensitivity of final estimates to initial estimates.

Because of the TMDD of CPHPC, the PK and PK-PD relationships were modeled simultaneously. All values below/above the limit of quantification were excluded from the analysis (0.75% of CPHPC observations and 2.7% of SAP observations were excluded). The CRCL covariate relationship affecting CPHPC clearance was incorporated into the base model. Subsequent covariate modeling would then account for baseline patient characteristics, resulting in a final model. The NONMEM control stream of the final model is available in **Supplementary Material S3**.

The following relationships between the model parameters and patient characteristics (or covariates) at baseline were tested:

- Q4 as a function of AMLOAD and/or AMLIVER
- V4 as a function of AMLOAD and/or AMLIVER
- V4 as a function of AMLIVER
- KIN as a function of AMLOAD and/or AMLIVER
- SAP_BASE as a function of AMLIVER and/or GENDER and/or CRCL
- KINT as a function of AMLOAD

where Q4 is the intercompartmental clearance between central and periphery, V4 is the volume of distribution of the peripheral compartment, KIN is the SAP production constant, SAP_BASE is the baseline level of plasma SAP, and KINT is the elimination rate constant of the complex.

The stepwise covariate method procedure was applied to the covariate selection ($\alpha = 0.05$ for forward step, and $\alpha = 0.01$ for backward step).¹⁹ A graphical assessment of the respective impact of each selected covariate on the profiles of CPHPC and SAP in plasma was performed in R software (version 3.0.1).

Model evaluation

Model performance was judged by convergence status, covariance estimation, parameter estimation precision, parameter correlation, final objective function gradients (first order conditional estimation only), standard goodness-of-fit plots, and concordance of estimated parameters with previously reported literature values. Model selection was judged by performance on the above model assessment criteria and drop in objective function value for nested models

($\alpha = 0.05$). The NPDE weighted residual data item in NONMEM was used to generate NPDE plots.¹⁸ These were used to visually assess model adequacy and detect notable misspecifications in the structural, statistical, and covariate model. They were stratified by amyloid load.

Model simulation

The final model with the estimated population parameter values was translated into an R code using the deSolve package for a post hoc assessment of model predictions.²² A series of simulated mean profiles were thus generated for different sets of covariates. For each set, one covariate was varied while keeping the others fixed to typical values in amyloidosis.

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Conflict of interest. T.S., A.B., L.C., D.R., S.B., and S.Z. are employees of GlaxoSmithKline and are stockholders and/or have stock options in the company.

Author contributions. T.S., A.B., and D.R. wrote the manuscript. T.S., A.B., S.B., L.C., S.Z., and D.R. designed the research. T.S., A.B., and S.B. analyzed the data.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

- ✓ Treatment of amyloidosis is enabled by the use of the drug (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC, GSK2315698, Ro 63-8695), which depletes circulating SAP but leaves some SAP in amyloid deposits for specific recognition by subsequently administered therapeutic anti-SAP antibodies. The presence and location of amyloid deposits is known to heavily influence SAP kinetics.

WHAT QUESTION DID THIS STUDY ADDRESS?

- ✓ How can we select individualized dosing regimens of CPHPC in both healthy volunteers and in patients with systemic amyloidosis?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

- ✓ Sufficient understanding of the principal determinants of PK-PD for CPHPC in patients with systemic amyloidosis to predict, with a high degree of confidence, the plasma SAP concentrations in individual subjects.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

✓ The primary factors influencing the PK-PD of CPHPC are known before treatment with CPHPC is started. This allows for individualized dosing of CPHPC to aid subsequent study design. This illustrates the potential value of obtaining population PK-PD models in target populations early in drug development.

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