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ORIGINAL PAPER

Translational pharmacokinetic and pharmacodynamic modelling of the anti-ADAMTS-5 NANOBODY® (M6495) using the neo-epitope ARGS as a biomarker

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Abstract

M6495 is a first-in-class NANOBODY® molecule and an inhibitor of ADAMTS-5, with the potential to be a disease modifying osteoarthritis drug. In order to investigate the PK/PD (pharmacokinetic and pharmacodynamic) properties of M6495, a single dose study was performed in cynomolgus monkeys with doses up to 6 mg/kg, with the goal of understanding the PK/PD properties of M6495. The neo-epitope ARGS (Alanine-Arginine-Glycine-Serine) generated by cleavage of aggrecan by ADAMTS-5 was used as a target-engagement biomarker. A long-lasting dose-dependent decrease in serum ARGS could be observed after a single dose of M6495 in cynomolgus monkeys. The serum biomarker ARGS decreased to levels below the limit of quantification of the assay in animals which received doses of M6495 of 6 mg/kg and higher, indicating a strong inhibition of ADAMTS-5. Data from the single-dose PK/PD study was combined with data from a multiple dose study, and a non-linear mixed effects model was used to explore the relationship between plasma concentrations of M6495 and the reduction of serum ARGS. The model was subsequently used to inform the clinical phase 1 study design and was successful in predicting the human clinical pharmacokinetics and pharmacodynamics of M6495. In addition to having enabled a Phase 1 trial with M6495, this is the first PK/PD model describing the pharmacodynamics of the neo-epitope ARGS after ADAMTS5 inhibition. It is expected that in the future, this model can be used or adapted to explore the PK/PD relationship between M6495 serum concentrations and the ARGS serum biomarker.

Keywords M6495 · ARGS · ADAMTS-5 · Aggrecan · Osteoarthritis · Nanobody · Pharmacokinetics

Introduction

ADAMTS-5 (a disintegrin and metalloproteinase with thrombospondin motifs) is a metalloprotease which is thought to play an important role in the pathogenesis of osteoarthritis by degrading aggrecan. Blocking ADAMTS-5 represents a potential strategy to halt osteoarthritis disease progression by preventing or slowing down aggrecan degradation. Osteoarthritis is considered a serious disease with a high unmet medical need, and there are currently no disease modifying osteoarthritis drugs (DMOADS). on the market Studies suggest that blocking ADAMTS-5 in vivo could lead to protection of the cartilage via reduction of aggrecan loss [1, 2].

M6495 is a multi-domain NANOBODY® construct, with a molecular weight of 28 kDa, which binds and inhibits ADAMTS-5 as a strategy to reduce aggrecan degradation.

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In addition, another domain of M6495 binds to human serum albumin in order to extend its half-life and have "antibody-like" pharmacokinetics [3].

ADAMTS-5 inhibitors for osteoarthritis are currently under clinical development, such as the molecule described in this study. The results of two phase 1 studies in humans with M6495 were recently published and demonstrated the potential of M6495 to inhibit ADAMTS-5 with long-lasting pharmacodynamic effects [4].

In this article, we share the pre-clinical experimental and translational pharmacokinetic and pharmacodynamic (PK/PD) modelling work that was performed to understand the PK/PD of M6495 through the pharmacodynamic biomarker neo-epitope ARGS (Alanine-Arginine-Glycine-Serine) and inform the design of the Phase I clinical studies.

The neo-epitope ARGS, used in this study as a target engagement or pharmacodynamic marker, is a product of aggrecan cleavage by ADAMTS-5. ARGS has been shown to be involved in osteoarthritis and to be a promising biomarker in this disease [5, 6]. However, so far, it has not been shown clinically or preclinically that ARGS modulation is indirectly, or directly linked to pain benefit in osteoarthritis [7].

It is of high value to understand the preclinical pharmacokinetics and pharmacodynamics of M6495, to predict human PK/PD, mitigate risks and increase the success rate of first-in human studies. For this purpose, translational biomarkers like the neo-epitope ARGS, that can be measured in both preclinical species as well as humans, are of great interest.

A commonly used approach to predict human PK/PD of a drug is to first predict its pharmacokinetics in humans and use a "human pharmacokinetic model" to relate PK with a pharmacodynamic model. The pharmacodynamic model that is linked to the PK model is typically based on data generated in a relevant pre-clinical in vivo model.

While there are several examples in the literature on the scaling of clearance and prediction of human pharmacokinetics for monoclonal antibodies (mAbs), there are to date no reports of a human PK or PK/PD prediction for half-life extended NANOBODY® therapeutic compounds, or other albumin binding scaffolds in published, peer-reviewed literature. There have been reports on inter-species allometric scaling for albumin-binding NANOBODY® molecules, DARPins or VNARs, however, a comparison to measured human clinical pharmacokinetics has to the best of our knowledge not yet been reported [3, 8, 9].

In order to evaluate the pharmacokinetics and pharmacodynamics of M6495, cynomolgus monkeys were selected in this case due to similar binding of M6495 to ADAMTS-5 and serum albumin, as well as to similarities in the neo-epitope of proteolytically cleaved human ARGS [5].

This article, (i) describes the pre-clinical PK/PD properties of M6495, (ii) describes the methodology for scaling to human including the relationship between the pharmacokinetic model and the pharmacodynamic model and, (iii) discusses the accuracy and usefulness of this model and its predictions versus the observed PK/PD profile of M6495 in the Phase I trial.

Methods

M6495 pharmacokinetics and ARGS modulation in cynomolgus monkeys

All in vivo studies were performed in accordance with the local guidelines of the Institutional Animal Care and Use Committee. Two studies were performed in cynomolgus monkeys, a single dose PK/PD study to study M6495 pharmacokinetics and pharmacodynamics and a single and multiple-dose safety study, where the biomarker ARGS was also measured. The main route of administration was s.c., and an i.v. group (1 mg/kg) was included to assess the absolute bioavailability of M6495. PK and PD samples were taken up to 672 h (28 days) in the multiple dose study and up to 1512 h (63 days) in the single dose PK/PD study. A summary of study design, doses and sample size is presented in Supplementary Tables 1 and 2. All samples were analyzed for M6495 and ARGS using immunoassay sandwich methods. The lower limit of quantification (LLOQ) for the detection of M6495 in serum was 3.2ng/mL as determined using qualified assay and validated assays.

As described in the introduction, the neo-epitope ARGS, which is a product of the cleavage of aggrecan by M6495, was used as a pharmacodynamic marker. A ligand binding assay was used for the detection of the concentrations of ARGS in serum. This assay employs a biotinylated mAb (monoclonal antibody) targeting the G2 domain of aggrecan as a capture reagent, and a horse-radish peroxidase conjugated mAb targeting the ARGS neo-epitope as detection reagent (the assays were developed and validated at Nordic Bioscience, Denmark). The assay used for assessing ARGS concentrations in the single and multiple-dose safety was characterized with a lower and an upper limit of quantification of 0.08 nM and 1.8 nM, respectively. This assay was further analytically validated in support of the PK/PD single dose study, resulting in a LLOQ (lower limit of quantification) of 0.064 and a ULOQ (upper limit of quantification) of 0.5 nM. Samples above the quantitative range were diluted into the quantitative range, supported by the dilution recovery (parallelism) evaluation of the assay as assessed during the analytical validation, and the parallelism was considered fit-for-purpose.

Pharmacokinetic and pharmacodynamic data from the single dose PK/PD study and the safety study in cynomolgus monkeys were pooled for the population PK/PD model building using nonlinear mixed effect methods. Data below the LLOQ were treated as missing in the modelling dataset, or set to 0 for graphical presentation.

Modelling of M6495 PK/PD in cynomolgus monkeys

Nonlinear mixed-effects modelling was performed using NONMEM 7.3 (ICON Development Solutions) and the software tool PsN version 4.6.0. The data and various model outcomes of the NONMEM model outputs were explored graphically using R version 3.3.1.

A PK model was developed based on the subcutaneous data, and the intravenous administration data were added to the dataset allowing the estimation of the bioavailability. This PK model was subsequently used as a starting point for simultaneously fitting the PK (M6495) and PD (ARGS) data.

For describing M6495 pharmacokinetics, a 2-compartment model with parallel linear and non-linear clearance was used, as described by the following differential equations (1 to 4):

$$Kel = \frac{CL}{V1}; k12 = \frac{Q}{V1}; k21 = \frac{Q}{V2}; \text{C1} = \text{A1/V1} \tag{1}$$

$$\frac{dA}{dt} = -Ka * A \tag{2}$$

$$\frac{d\text{A1}}{dt} = Ka * F * A - k12 * \text{A1} + k21 * \text{A2} - kel*\text{A1} - Vmax * \frac{\left(\frac{\text{A1}}{\text{V1}}\right)}{Km + \left(\frac{\text{A1}}{\text{V1}}\right)} \tag{3}$$

$$\frac{dA2}{dt} = k12 * A1 - k21 * A2 \tag{4}$$

The differential equations for the modelling in NONMEM were parameterized with *A*, *A1* and *A2*, representing the amounts in the depot compartment and in the central and peripheral compartments, respectively, and with micro-constants *kel*, *k12* and *k21*, described in the Eq. 1. The parameters *ka*, *F*, *Q*, *CL*, *Vmax* and *Km* represent the absorption rate constant, bioavailability, inter-compartmental flow, clearance, maximal rate of non-linear clearance and the concentration at which the non-linear clearance is 50% of the maximum value, respectively. The Michaelis Menten parameter Km was described in concentration units, and the equation expressed in amount divided by the volume of distribution (*A1/V1*, Eq. 3). The concentration of M6495 in the central compartment (*C1 = A1/V1*) was fitted to the PK data.

The pharmacokinetics and pharmacodynamics of M6495 were modelled simultaneously. M6495 pharmacodynamics were described with an indirect response model as illustrated by Eq. (5), where ARGS is the concentration of ARGS measured in serum in nM. The parameters *kin*, *kout*, *Imax and IC50* represent, respectively, the synthesis rate of ARGS, the elimination rate constant of ARGS, the maximal inhibition of ARGS synthesis and the concentration at which 50% of the maximal inhibition is achieved.

$$\frac{dARGS}{dt} = kin\left(1 - \frac{Imax * C1}{IC50 + C1}\right) - kout * ARGS \tag{5}$$

The synthesis rate of ARGS was defined as described by Eq. 6, assuming steady state at time = 0, where $\text{ARGS}_{\text{baseline}}$ is the baseline concentration of ARGS, which is a model parameter.

$$kin = ARGS_{baseline} * kout \tag{6}$$

Also at time = 0, as initial condition for the model,

$$ARGS = ARGS_{baseline} \tag{7}$$

The first-order conditional estimation with η-ε interaction in NONMEM 7.3 was employed for all model runs. Inter-individual variability on the parameters was expressed as an exponential error model based on the assumption of a log-normal distribution. Imax was parameterized using a box-cox transformation (Eq. 8 to 9) on the interindividual variability parameter (η) for Imax (η_{Imax}) in order to restrict the value to between 0 and 1 [10]. Imax was therefore defined by its typical population value, Θ_{Imax}, and the transformed η (Eq. 10).

$$\Phi = e^{\eta_{\text{Imax}}} \tag{8}$$

$$\eta_{\text{transformed}} = \frac{\Phi^{\text{box-coxparameter}} - 1}{\text{box} - \text{coxparameter}} \tag{9}$$

$$\text{Imax} = \ominus_{\text{Imax}} * e^{\eta_{\text{transformed}}} \tag{10}$$

The model validation was based on graphical analyses and a prediction corrected visual predictive check shown in Supplementary Fig. 2 to 5 (pcVPC). Assessment of model adequacy was driven by the data and guided by goodness of fit criteria [11]. Parameter estimates were reported with a measure of estimation uncertainty, the standard error of the estimates.

Predicting M6495 human pharmacokinetics and pharmacodynamics

The parameters obtained from the model in cynomolgus monkey were used to predict M6495 pharmacokinetics and pharmacodynamics in humans after subcutaneous administration of M6495 at different dose levels, and to anticipate the possible doses in human based on the reduction of ARGS levels. The Pharmacokinetic parameters *V1*, *V2*, *Q*, *CL*, *Vmax and Ka* were scaled allometrically from the cynomolgus monkey model according to the following relationship, using the exponents 1, 1, 0.75, 0.75, 0.75 and −0.25, respectively:

$$Parameter_{human} = Parameter_{Cyno} * \left(\frac{BW_{human}}{BW_{cyno}}\right)^{Exponent} \quad (11)$$

The parameters *F*, *Km*, *Imax*, *IC50 and kout* were assumed to be the same as in the cynomolgus monkey model. The baseline ARGS concentration parameter ($ARGS_{baseline}$) in the cynomolgus monkey model was replaced by data generated in-house for the ARGS concentration in human serum and respective variability (0.14 nM, 46% CV), which were available from serum samples from 48 healthy humans. These scaling assumptions are summarized in Table 2 and in more detail in Supplementary Table 3. The exponential interindividual variability in the linear PK parameters *CL* and *Ka* was increased to a variance of 0.1 to account for the higher pharmacokinetic variability anticipated in a human clinical trial (this would correspond to ~30% interindividual variability). A similar approach of inflating variability in clearance to predict human pharmacokinetics was suggested and used successfully by Deng et al. [12]. The interindividual variability in the parameter accounting for non-linear clearance *Vmax* was kept the same as in the cynomolgus monkey. The interindividual variability in the pharmacodynamic parameter *Imax* was assumed to be the same as in the cynomolgus monkey, while the ARGS baseline parameter was replaced by own data, as described above.

The pharmacokinetic and pharmacodynamic profiles for 500 virtual subjects were simulated based on the previously described model and scaled parameters, (Fig. 4).

The maximal individual decrease in the ARGS biomarker simulated through the human scaled model was calculated as percentage from baseline and summarized with descriptive statistics over the simulated dose range (Fig. 5). While ARGS levels expressed as concentration values (nM) was appropriate for both the monkey PK/PD model and the human predictions, the values were subsequently converted to percentage decrease from baseline in order to simulate the dose range for the human Phase I trial, and to allow comparison with the metric used to evaluate ARGS in the human trial [4].

The simulations of the scaled PK/PD model for predicting human PKPD shown in this publication were performed in R version 4.1.0 using the RxODE package (1.0.9), and the code is included as a supplementary information for reproducibility in an open-source format, and for further use for research in this field.

Results

Pharmacokinetics and pharmacodynamics of M6495 in cynomolgus monkeys

The concentration-time profiles of M6495 after a single-dose administration of M6495 in cynomolgus monkeys are shown in Fig. 1. The M6495 concentrations increased with dose and the pharmacokinetic profile was typical of a drug showing target mediated drug disposition (TMDD) [13]. The overlapping profiles of M6495 at 1 mg/kg after i.v. and s.c. administration indicate a high bioavailability of M6495 after s.c. administration (Fig. 1), which was also confirmed by the modelling which determined a bioavailability of 100% (F = 1).

In the single dose PK/PD study, a dose-dependent reduction of the ARGS biomarker (Fig. 1) could be demonstrated, with the highest decrease in ARGS serum concentrations being shown at the highest dose of 6 mg/kg. A recovery in ARGS serum concentrations back to baseline could be observed, also in a dose-dependent manner. At the highest dose-level of 6 mg/kg, ARGS levels remained low for over 30 days.

Consistent with the single-dose administration, higher doses of M6495 ranging from 6 to 150 mg/kg in a multiple-dose study, resulted in ARGS levels remaining low (below the LLOQ) for the majority of the 4-week study period in several study animals (Supplementary Fig. 1). Due to the large percentage of samples below the limit of ARGS quantification, evaluation of the complete extent of ARGS inhibition remained limited (> 30%, Supplementary Table 2).

Samples were also analyzed for the presence of anti-drug antibodies (ADAs) and it was confirmed that the presence of ADAs did not have any significant effect on PK or PD (data not shown).

Non-linear mixed effects modelling of M6495 pharmacokinetics and pharmacodynamics

Graphical analysis of the M6495 concentration data over time demonstrated a distinct elimination phase at lower concentrations indicating target-mediated drug disposition

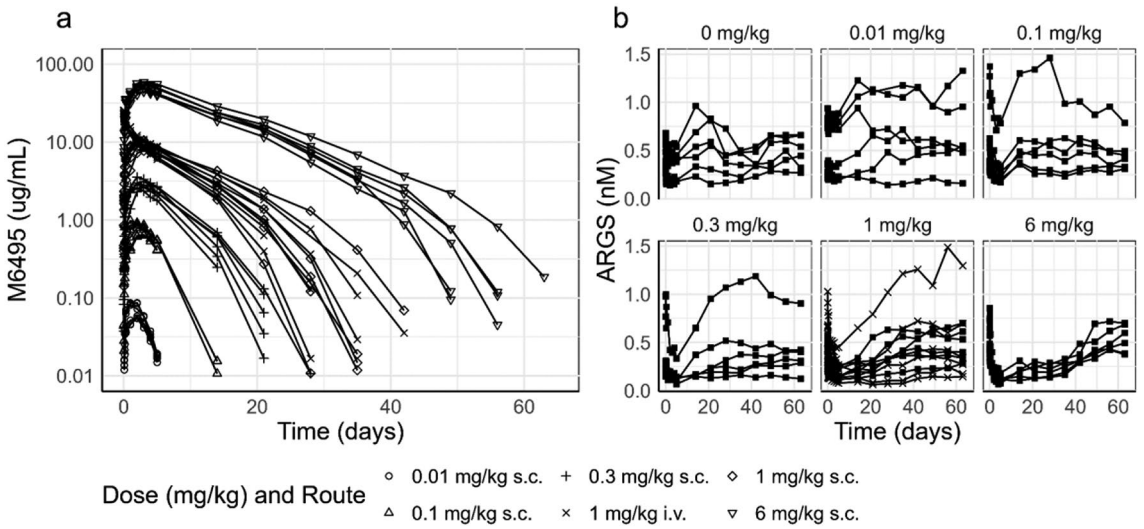

Fig. 1 (**a**) Concentration-time profiles of M6495 after a single s.c. (0.01, 0.1, 0.3, 1 and 6 mg/kg) and i.v. (1 mg/kg) dose of M6495 in cynomolgus monkeys. (**b**) Concentration-time profiles of the biomarker ARGS after a single s.c. (0.01, 0.1, 0.3, 1 and 6 mg/kg) and i.v. (1 mg/kg) dose of M6495 in cynomolgus monkeys. The 1 mg/kg i.v. and s.c. doses are presented in the same panel, with the i.v. dose labelled differently (×). The vehicle group (0 mg/kg) is also included in the figure

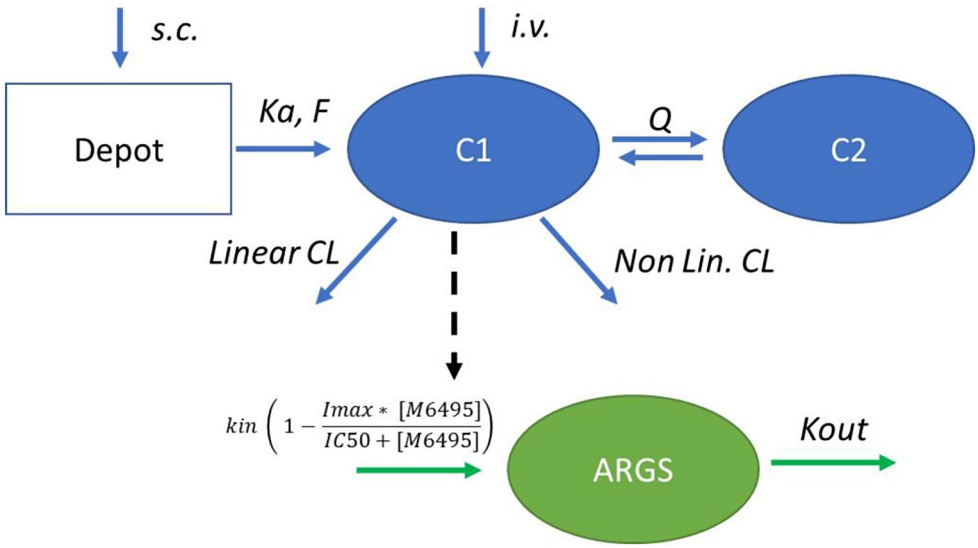

Fig. 2 Graphical representation of the compartmental PK/PD model used to describe the concentration-time profiles of M6495 and the biomarker ARGS in cynomolgus monkeys. The PK model is represented by the blue boxes, with the subcutaneous (s.c.) compartment in white. The PK model is connected to the PD model, shown in green, with an indirect response model on the production rate (kin) of ARGS

(TMDD), requiring the addition of a nonlinear clearance process to the PK model. The nonlinear clearance was described using the Michaelis-Menten equation and both i.v. and subcutaneous data were fitted to determine bioavailability through the model. A visual description of the PK model is shown on Fig. 2.

After confirming good estimates for the PK parameters, a simultaneous PK/PD modelling step was implemented in which the changes in ARGS concentrations (PD model) were described using an indirect response model as described by the Eq. 5 to 7 in the methods section and visually depicted in Fig. 2. The PK/PD model was implemented using the FOCEI method (First-order conditional estimation with interaction). Inter-individual variability was expressed as an exponential error model. The residual error was expressed as 2 proportional errors, one for the PK data and one for the PD data. A box-cox transformation was applied in the final model for limiting Imax between 0 and 1 (refer to Methods). The final PK and PD parameters and the main output of the model are shown in Table 1.

This model adequately described the M6495 dose dependent changes in the ARGS concentrations over time in cynomolgus monkeys, including the return to baseline. The main goodness of fit plots are shown in Supplementary Fig. 2 to 5. The CWRES (conditional weighted residuals) appeared log-normally distributed, and scatter plots of CWRES vs. population predicted concentrations and of CWRES vs. time show the CWRES to be evenly distributed, indicating the

Table 1 Final parameters of the PK/PD non-linear mixed effects model of M6495 in Cynomolgus monkeys

Parameter (units)	Fixed Effects Estimate (Relative Standard Error %)	IIV (CV%) (Relative Standard Error %)	Shrinkage (%)
CL (L/kg/h)	0.000251(7.3%)	16.2% (17.4%)	36%
V1 (L/kg)	0.046(2.8%)	0 FIX	
Q (L/kg/h)	0.00163(26.9%)	0 FIX	
V2 (L/kg)	0.0311(10.6%)	0 FIX	
Vmax (mg/kg/h)	0.000417(11.4%)	19.3% (23.1%)	38%
Km (mg/L)	0.265(14.3%)	0 FIX	
Ka (/h)	0.0394(7.6%)	28.1% (14%)	30%
F	1 (6.3%)	15.1% (17.8%)	26%
Kout (/h)	0.0401(10.8%)	0 FIX	
Imax	0.725(3.4%)	20.8% (23.3%)	29%
IC50 (mg/L)	1.17(27.6%)	0 FIX	
BASE (nM)	0.36(9.8%)	54.2% (8.4%)	25%
Boxcox parameter[1]	-3.16(57%)		
Residual proportional error	Estimate (%) (precision)		
RE1 (PK)	15.2(7.9%)		8.6%
RE2 (PD)	22.1(5.8%)		3.6%

validity of the selected structural and random effects model for both PK and PD. The shrinkage was in the 25–38% range and considered acceptable (Table 1). Overall, there was a good correspondence between the model predicted and the observed concentrations. A pcVPC (prediction-corrected VPC) was performed in which the variability due to binning across independent variables was removed by normalizing the observed and simulated dependent variable based on the typical population prediction for the median independent variable in the bin [14]. Overall, the observed PK and PD data appear to be symmetrically distributed around the median, with limited observations distributed outside the 90% prediction interval, indicating that the model adequately describes the observed M6495 concentrations. The median concentration of ARGS return to baseline after ~100 h (Supplementary Fig. 4, right panel) was outside of the prediction interval. This was found to be related to a study effect on the baseline ARGS concentrations between the two different studies (multiple vs. single dose). Inclusion of the effect of study on baseline ARGS as a covariate resulted in a clear improvement in the goodness of fit plots as made evident by the pcVPC (Supplementary Fig. 5). Although the pcVPC showed an improvement, all parameter estimates were very similar to the base model, except for the baseline ARGS value (Supplementary Table 4). It was noted that in the PK/PD study, the baseline values in a subset of the animals were higher than observed in the multiple dose study. However, for the human simulations, actual values are used rather than the estimated ARGS baseline, as described in the methods sections (Table 2, 0.14 nM, 46% CV). Therefore, the base model was considered adequate to describe the data and for further human predictions. For visualization purposes, the individual PK and ARGS model-predicted median, the 90% prediction interval and the observed data were plotted for all individuals as well as for the single dose PK/PD study as shown in Fig. 3.

Scaling of the PK/PD model and human dose prediction

The PK/PD model was scaled using allometric scaling, as described in the methods section and summarized in Table 2. The simulated human pharmacokinetics and pharmacodynamics of M6495 at different dose levels are shown in Fig. 4, overlayed with the observations in the human single ascending dose Phase I study, as discussed in the next section. In addition, as described in the methods section, the maximal percentage decrease of ARGS from baseline to be expected was calculated for different dose levels and is shown in Fig. 5. The effect of M6495 on the ARGS biomarker response was predicted to be minimal at doses in the range of 1 to 5 mg in humans, while the maximal effect was predicted to be achieved at doses above 75 mg. The results from this PK/PD modelling approach were used to inform the design of the first in human, single ascending dose (SAD), clinical study of M6495 (clinicaltrials.gov, NCT03224702). The doses of 1, 5, 20, 75, 150 and 300 mg, were suggested by the model to cover the dose-effect curve (Figs. 4 and 5) and were applied in the Phase I single ascending dose study.

Comparison of the pre-clinical model predictions to the observed clinical data

The Phase I studies for M6495 were recently published [4] and this paper evaluates how well the cynomolgus monkey PK/PD model, scaled to humans, predicted the PK and PD of M6495 and how useful it was, retrospectively, in supporting the design of the Phase I study.

The pharmacokinetic dataset from the single ascending dose Phase I study [4] was used to visually assess the performance of the allometric scaling method used to scale the pharmacokinetics of M6495 from the cynomolgus monkey to humans (Fig. 4a). Overall, the model was successful in predicting the non-linear human PK of M6495 at different dose levels, confirming the applicability of the allometric scaling method employed here.

The published pharmacodynamic data of the Phase I study [4] was compared with the scaled model predictions for human PK/PD of M6495 (Fig. 4b). While the predictions from the scaled pharmacodynamic model were not as accurate as for the pharmacokinetic model, with some tendency for underestimation (Fig. 4b), the model was considered

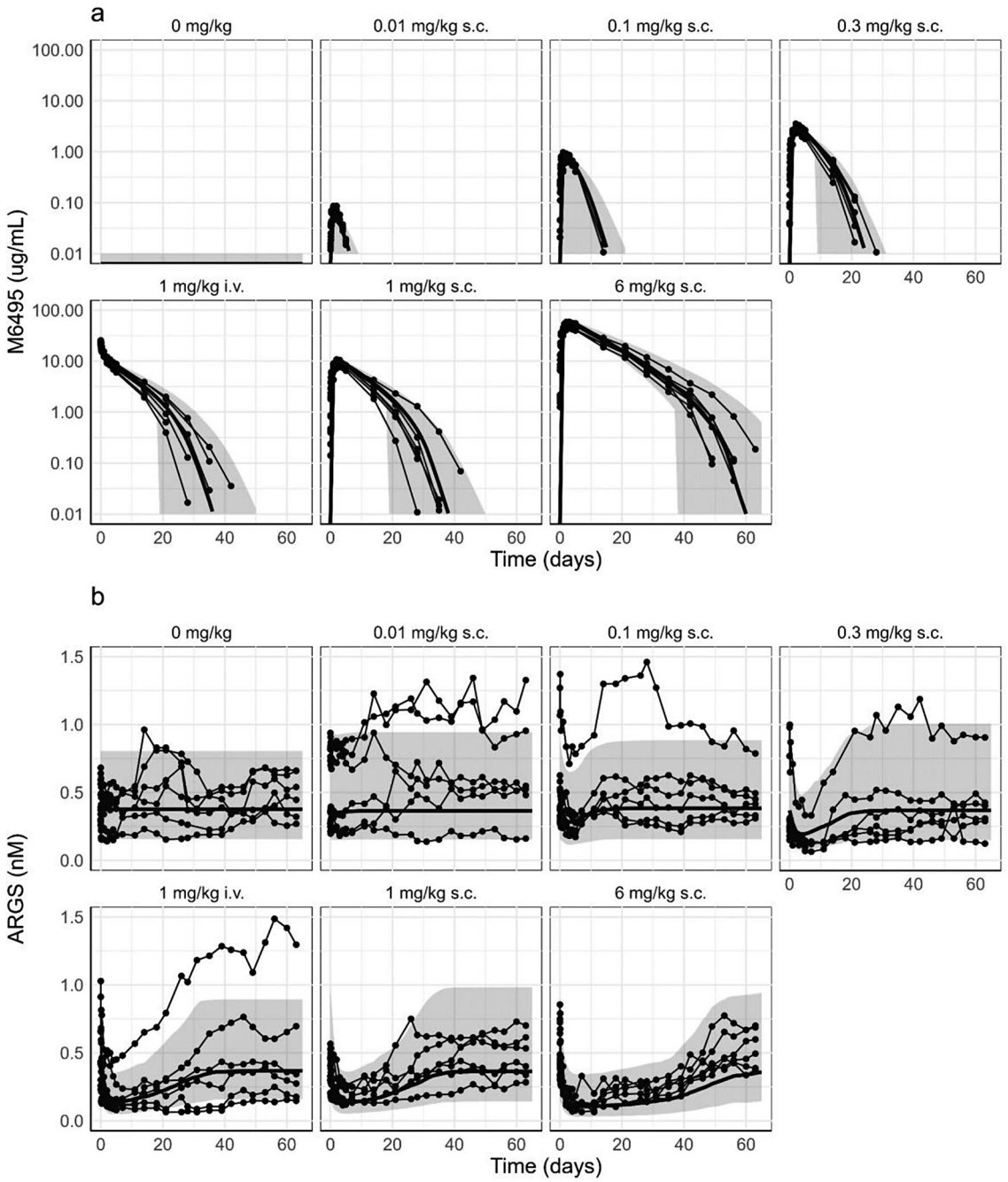

Fig. 3 Graphical representation of the model-based simulation of the concentration-time profiles of M6495 and ARGS in cynomolgus monkeys. The figure shows the model median (solid lines) and the 90% prediction interval (grey area) overlayed with the actual measurements of the concentration-time profiles of M6495 and ARGS in cynomolgus monkeys

useful for predicting human PK/PD. The PK/PD model predicted minimal effects on ARGS at the dose of 1 mg in humans, as observed in the Phase I study. At 5 mg, the quick onset of ARGS inhibition after dosing was well captured, however, while the model would anticipate a return to baseline, similar to what was observed for other dose levels, the data from the Phase I study does not reflect this, specifically for the 5 mg dose level (Fig. 4b). Time ~ 5–10 days). From doses of 20 mg onwards, the predictions of the scaled model were remarkably accurate, suggesting an ARGS inhibition above 70% for the highest doses studied in the Phase I study. In summary, the PK/PD model scaled from cynomolgus monkeys predicted well the overall dose and maximal effect relationship of M6495 on ARGS levels in the Phase I study (Fig. 5). As with the human clinical study [4], the ARGS assay used in the cynomolgus monkey study was limited

Table 2 Overview of cynomolgus monkey model parameters and scaled human parameters

	Parameter (unit)	Parameter estimates for a 3.9 kg Cynomolgus Monkey	Allometric scaling factor	Parameters for a 70 Kg Human
PK Model Parameters	Ka (/h)	0.0394	-0.25	0.0191
	F	1	NA	1
	CL (L/h)	0.00098	0.75	0.0085
	V1 (L)	0.194	1	3.22
	Q (L/h)	0.0064	0.75	0.0558
	V2 (L)	0.12	1	2.177
	Vmax (mg/h)	0.00163	0.75	0.0142
	Km (mg/L)	0.265	NA	0.265
PD Model Parameters	Kout (/h)	0.0401	NA	0.0401
	IMax	0.725	NA	0.725
	IC50 (mg/L)	1.17	NA	1.17
	BASE (nM)	0.36	(measured in human samples)	0.14

by its LLOQ, with a high percentage of samples below the limit of quantification (Overall 7,6% of samples and > 30% of the samples for the higher dose groups, Supplementary Fig. 1 and Supplementary Table 2). Therefore, it cannot be excluded that the determination of maximal inhibitory effect (see discussion) was affected by the LLOQ.

While the full extent of ARGS inhibition may have been limited by the lower limit of quantification, the clinical observations so far have confirmed the usefulness of this translational PK/PD modelling approach to inform the Phase I study design.

Discussion

The main goal of this study was to investigate the pre-clinical pharmacokinetics and pharmacodynamics of M6495 in order to predict human PK/PD and guide first in human clinical study design. This was achieved through a thorough pre-clinical PK/PD evaluation and the implementation of the translational biomarker ARGS in a modelling and simulation strategy.

There are several reports of human PK prediction for monoclonal antibodies which have established single-species allometric scaling based on cynomolgus monkey PK [12, 15, 16]. Importantly, this is the first report in a peer-reviewed publication of the prediction of human pharmacokinetics for a NANOBODY® immunoglobulin single variable domain or an albumin-binding scaffold, comparing these predictions with actual clinical pharmacokinetic data.

The single-exponent approach using allometric exponents of 0.75 and 1 was selected for both clearance (target-mediated and linear) and volume of distribution, respectively. This choice was based on institutional knowledge of pharmacokinetic scaling of other NANOBODY® albumin-binding constructs, and has also been suggested for antibody-based therapeutics [17]. Our approach led to an accurate prediction of human PK at all dose levels (Fig. 5). As more pharmacokinetic data on albumin binding scaffolds becomes publicly available, more thorough evaluations of the optimal allometric exponents for prospectively predicting human pharmacokinetics for albumin binding scaffolds, from cynomolgus monkey data, should be performed.

Although the assumption of bioavailability equal to 100%, as in the cynomolgus monkey, cannot be confirmed from the human data since the human clinical study was designed to only include subcutaneous administrations, the fact that the pharmacokinetic profiles were well predicted, suggests that the combination of bioavailability and clearance parameters estimated for the human prediction were appropriate.

While in this study the pharmacokinetics of M6495 were successfully described with a non-mechanistic TMDD model using the simplified Michaelis-Menten approximation [18], if data on direct M6495-ADAMTS5 target occupancy would have been available, a more mechanistic TMDD model would also have been an option. However, since only concentration data was available, a TMDD model with more parameters would have been over-parameterized. A mechanistic TMDD model would have also allowed for a direct scaling of the TMDD model parameters. In this work, the approach taken was based of the work of Dong et al. [19], showing that allometric scaling of Vmax, while keeping Km the same as in the cynomolgus monkey model can be used to predict human pharmacokinetics of antibodies showing non-linear pharmacokinetics. Furthermore, the pharmacokinetic model used was considered fit-for-purpose, since it was describing the pharmacokinetic profile, in order to drive the pharmacodynamic model.

M6495 shows TMDD and the model and scaling approach used were able to accurately predict human pharmacokinetics from monkey pharmacokinetics. TMDD has mostly been reported for membrane bound targets, however, it has also been observed for molecules targeting soluble targets. It can be hypothesized that the mechanism of target mediated clearance is due to M6495 being internalized together with its target ADAMTS-5, through LRP-1, and thereafter degraded [20]. A similar mechanism of target mediated drug clearance has also been proposed for mAbs targeting soluble targets, for example for the anti-C5 antibody eculizumab [21]. f.

While an allometric scaling approach of model parameters was used for this study, an alternative method that has not been investigated, is the species-invariant.

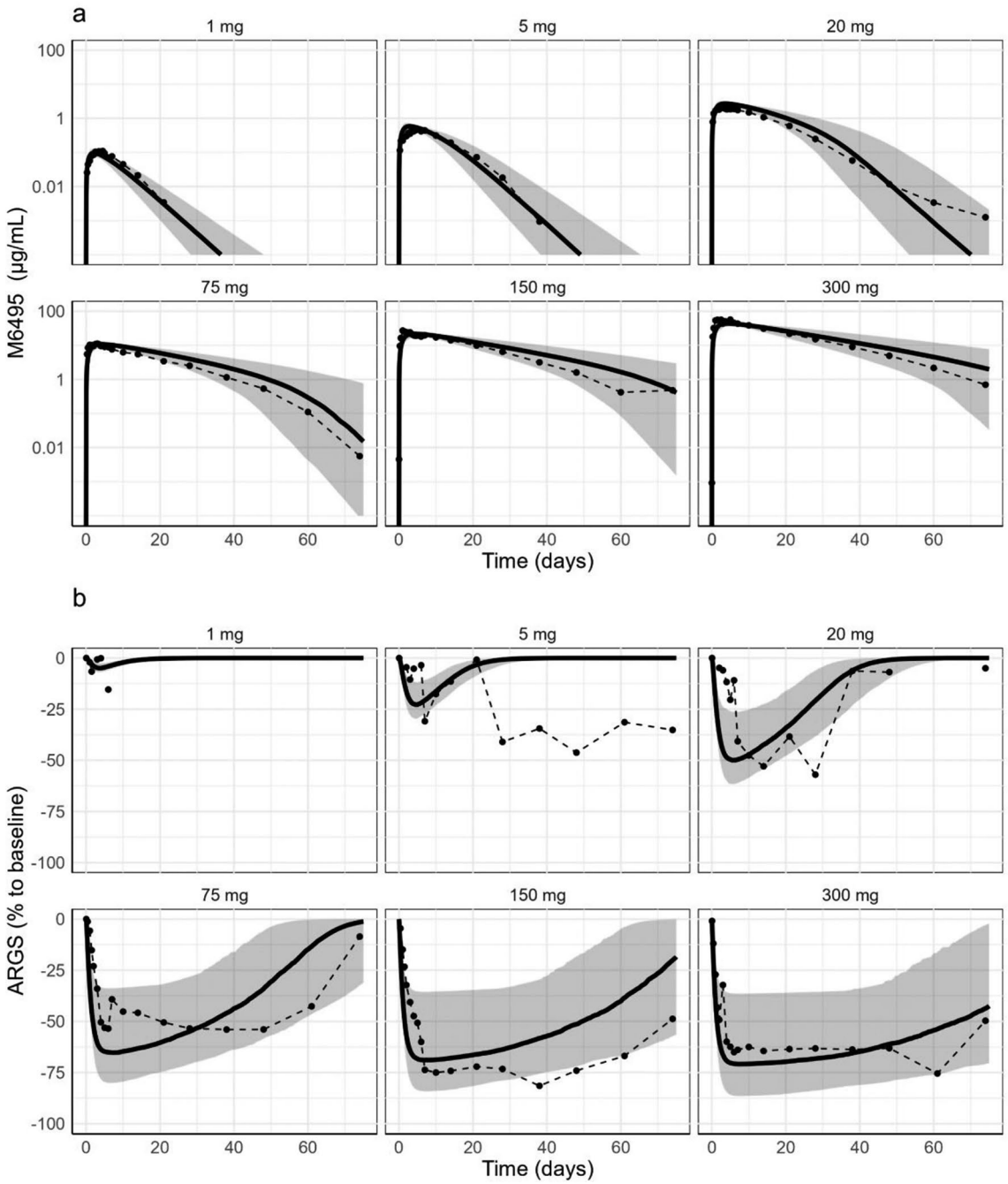

Fig. 4 Model based simulation of the predicted concentration-time profiles of M6495 and ARGS (nM) for a 75 kg human using the scaled PK/PD model. The simulations show the predicted median (solid line) and 90% prediction interval (shading), overlayed with the mean PK and PD (circles and dashed lines) profiles obtained in the Phase I single ascending dose study [4]

time method, also discussed in Deng et al. [12] to scale the entire concentration-time profile in cynomolgus monkeys to human and use the resultant predicted human concentration-time to obtain the predicted human PK parameters. While this method has been applied successfully for the scaling of pharmacokinetic data, it is not known whether it could also be applied for longitudinal pharmacodynamic data.

In a further step, the scaled PK/PD model was used to select the dose-range for the Phase I clinical study (1, 5, 20, 75, 150 and 300 mg), and predict ARGS inhibition at those dose levels. In the Phase I study, M6495 demonstrated a clear dose-dependent inhibition of ARGS, with substantial inhibition observed at dose levels of 20 mg and higher [4]. This was anticipated by our PK/PD model, which predicted an inhibition of ARGS above 70% at the highest planned

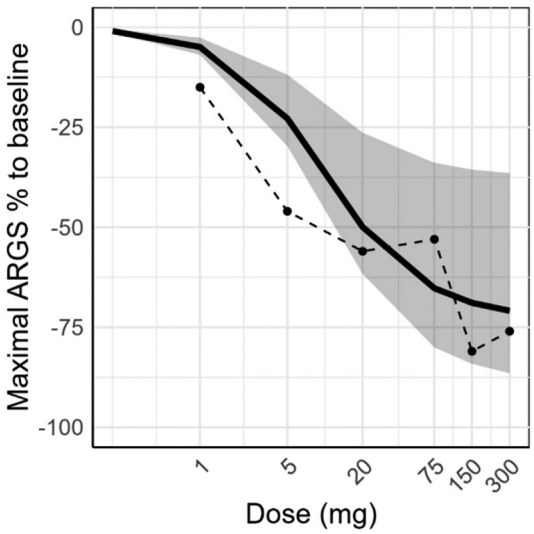

Fig. 5 Calculation of the predicted maximal ARGS percent inhibition from baseline for each dose level in humans from the human scaled model vs. the mean maximal inhibition of ARGS in humans observed for each dose level. The figure shows the predicted median (solid line) and 90% prediction interval (shading) for the dose range 0.2 to 150 mg

clinical doses of 150 mg and 300 mg. As presented in the results sections, remarkably good predictions of the potential for ARGS inhibition in humans were achieved with the model, at doses higher than 20 mg in humans (Figs. 4 and 5). While the prediction based on the model scaled from the PK/PD model in cynomolgus monkey did not describe accurately the time-dependency of ARGS inhibition at all dose levels (in particular at 75 mg, Fig. 4b), overall, it successfully captured the extent and duration of ARGS inhibition and its return to baseline, which was a requirement in order to decide on the duration of the Phase I study. The predictions were considered reliable enough to include this PK/PD model in the planning of a Phase I clinical study. However, both the Phase I study in humans [4] and this preclinical PK/PD study in cynomolgus monkeys were limited by the LLOQ of the assay. In the higher, multiple dose groups of our study in cynomolgus monkeys (Supplementary Fig. 1), approximately 30% of the samples fell below the LLOQ (vs. 3.5% in the PK/PD study), which limited our ability to quantify the full extent of ARGS inhibition. It is expected that an updated PK/PD model of the human data with an updated, more sensitive, ARGS assay, will provide more insights about the PK/PD properties of M6495.

Different approaches can be used to accommodate data below the LLOQ including, omitting values below the LLOQ, setting values below the LLOQ to LLOQ/2, or the M3 method, which estimates the likelihood that a sample is below the limit of quantification [22]. The first two methods are associated with either a slight positive bias (underestimation of drug effect), or negative bias (overestimation of drug effect) when applied to turnover models [22]. In this study, it was selected to omit values below the LLOQ, since in the single dose PK/PD study, which constituted the majority of our dataset (Supplementary Table 2), the number of samples below the LLOQ was very limited (~3.5%). The M3 method [14] was also evaluated (data not shown), however, no covariance step could be obtained in NONMEM, although parameter estimates were similar.

Within the context of estimating the dose range for a Phase I study, the major objective for the selection of a safe starting dose is the ARGS inhibition at lower dose levels, which is not limited by the LLOQ, and could hence be adequately predicted (no effect at 1 mg). Furthermore, this molecule was planned for subcutaneous development, with a maximal feasible dose of 150 to 300 mg based on the formulation used at the time these studies were conducted. Therefore, a prediction model that slightly underestimates the maximal drug effect provides a worst-case estimate of achieving a high inhibition of ARGS at the maximal feasible subcutaneous doses of 150 and 300 mg. The authors consider it very important to evaluate the context-of-use of PK/PD models, when deciding on the best approaches to accommodate data below the limit of quantification.

Although limited by the LLOQ, the model was able to describe well the data and its inter-individual variability. Moreover, the Imax was determined with a high precision of 0.725 and a relative standard error of 3.4% (Table 1), which was attributed to an appropriate design of the in vivo phase of the PK/PD study in the cynomolgus monkey. However, as previously mentioned, it cannot be excluded that with a more sensitive assay, a stronger inhibition or the ARGS biomarker may have been observed in the multiple dose study and would have influenced the Imax parameter.

Despite these challenges, the evaluation of the simulated human pharmacodynamics from the pre-clinical PK/PD model aligns well with the clinical observations. ARGS was considered an excellent biomarker of high translational value for integration in a PK/PD modelling approach within its context of use [23], and a more sensitive assay for determining ARGS inhibition in humans is currently under development [4].

Conclusions

Overall, this translational model described the in vivo pharmacokinetics and pharmacodynamics of M6495 in cynomolgus monkeys and allowed for a successful design of a Phase I study leading to the clinical investigation of M6495 as a potential disease modifying treatment for osteoarthritis. Furthermore, despite the sensitivity limitations of the currently available ARGS-assay, the ARGS neo-epitope

is considered an excellent serum biomarker for both preclinical and clinical evaluation of M6495 pharmacology in a longitudinal manner. The inclusion of translational longitudinal biomarkers in clinical studies and drug development programs, combined with PK/PD models, improves clinical study design, and increase their safety and success rate, to the benefit of clinical trial participants and patients.

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Author contributions JNSP wrote the main manuscript. JNSP and IO performed the work and prepared figures and tables. BS, CL and SL contributed to data generation. HG, CL, BS, IO and SL designed the in vivo study.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests JNSP, SL and HG are employees of Merck Healthcare KGaA, Darmstadt, Germany. IO was a past employee of Sanofi Ghent, Belgium. BS is an employee of Sanofi Ghent, Belgium. CL is a past employee of Merck Healthcare KGaA, Darmstadt, Germany.

References

1. Miller RE, Tran PB, Ishihara S et al (2016) Therapeutic effects of an anti-ADAMTS-5 antibody on joint damage and mechanical allodynia in a murine model of osteoarthritis. Osteoarthr Cartil 24:299–306. https://doi.org/10.1016/j.joca.2015.09.005
2. Siebuhr AS, Werkmann D, Bay-Jensen AC et al (2020) The anti-ADAMTS-5 nanobody® M6495 protects cartilage degradation ex vivo. Int J Mol Sci 21:1–14. https://doi.org/10.3390/ijms21175992
3. Hoefman S, Ottevaere I, Baumeister J, Sargentini-Maier M (2015) Pre-clinical intravenous serum pharmacokinetics of albumin binding and Non-half-life Extended Nanobodies®. Antibodies 4:141–156. https://doi.org/10.3390/antib4030141
4. Bihlet AR, Balchen T, Goteti K et al (2024) Safety, Tolerability, and Pharmacodynamics of the ADAMTS-5 Nanobody M6495: two phase 1, Single-Center, Double-Blind, randomized, placebo-controlled studies in healthy subjects and patients with osteoarthritis. ACR Open Rheumatol 6:205–213. https://doi.org/10.1002/ACR2.11610
5. Sharma N, Drobinski P, Kayed A et al (2020) Inflammation and joint destruction may be linked to the generation of cartilage metabolites of ADAMTS-5 through activation of toll-like receptors. Osteoarthr Cartil 28:658–668. https://doi.org/10.1016/j.joca.2019.11.002
6. Germaschewski FM, Matheny CJ, Larkin J et al (2014) Quantitation of ARGS aggrecan fragments in synovial fluid, serum and urine from osteoarthritis patients. Osteoarthr Cartil 22:690–697. https://doi.org/10.1016/j.joca.2014.02.930
7. Cowan KJ, Kleinschmidt-Dörr K, Gigout A et al (2020) Translational strategies in drug development for knee osteoarthritis. Drug Discov Today 25:1054–1064
8. Müller MR, Saunders K, Grace C et al (2012) Improving the pharmacokinetic properties of biologics by fusion to an anti-HSA shark VNAR domain. MAbs 4:673–685. https://doi.org/10.4161/mabs.22242
9. Kovaleva M, Ferguson L, Steven J et al (2014) Shark variable new antigen receptor biologics-A novel technology platform for therapeutic drug development. Expert Opin Biol Ther 14:1527–1539. https://doi.org/10.1517/14712598.2014.937701
10. Petersson KJF, Hanze E, Savic RM, Karlsson MO (2009) Semiparametric distributions with estimated shape parameters. Pharm Res 26:2174–2185. https://doi.org/10.1007/s11095-009-9931-1
11. Nguyen THT, Mouksassi MS, Holford N et al (2017) Model evaluation of continuous data pharmacometric models: Metrics and graphics. CPT Pharmacometrics Syst Pharmacol 6:87–109. https://doi.org/10.1002/psp4.12161
12. Deng R, Iyer S, Theil FP et al (2011) Projecting human pharmacokinetics of therapeutic antibodies from nonclinical data: what have we learned? MAbs. 3:61–66. https://doi.org/10.4161/mabs.3.1.13799
13. Peletier LA, Gabrielsson J (2012) Dynamics of target-mediated drug disposition: characteristic profiles and parameter identification. J Pharmacokinet Pharmacodyn 39:429–451. https://doi.org/10.1007/s10928-012-9260-6
14. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO (2011) Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J 13:143–151. https://doi.org/10.1208/s12248-011-9255-z
15. Oitate M, Masubuchi N, Ito T et al (2011) Prediction of human pharmacokinetics of therapeutic monoclonal antibodies from simple allometry of monkey data. Drug Metab Pharmacokinet 26:423–430. https://doi.org/10.2133/dmpk.DMPK-11-RG-011
16. Oitate M, Nakayama S, Ito T et al (2012) Prediction of human plasma concentration-time profiles of monoclonal antibodies from monkey data by a species-invariant time method. Drug Metab Pharmacokinet 27:354–359. https://doi.org/10.2133/dmpk.DMPK-11-SH-059
17. Mahmood I (2009) Pharmacokinetic allometric scaling of antibodies: application to the first-in-human dose estimation. J Pharm Sci 98:3850–3861. https://doi.org/10.1002/jps.21682
18. Gibiansky L, Gibiansky E, Kakkar T, Ma P (2008) Approximations of the target-mediated drug disposition model and identifiability of model parameters. J Pharmacokinet Pharmacodyn 35:573–591. https://doi.org/10.1007/S10928-008-9102-8/METRICS
19. Dong JQ, Salinger DH, Endres CJ et al (2011) Quantitative prediction of human pharmacokinetics for monoclonal antibodies:

retrospective analysis of monkey as a single species for first-in-human prediction. Clin Pharmacokinet 50:131–142. https://doi.org/10.2165/11537430-000000000-00000/FIGURES/4
20. Yamamoto K, Troeberg L, Scilabra SD et al (2013) LRP-1-mediated endocytosis regulates extracellular activity of ADAMTS-5 in articular cartilage. FASEB J 27:511–521. https://doi.org/10.1096/fj.12-216671
21. Wijnsma KL, ter Heine R, Moes DJAR et al (2019) Pharmacology, Pharmacokinetics and Pharmacodynamics of Eculizumab, and possibilities for an Individualized Approach to Eculizumab. Clin Pharmacokinet 58:859–874
22. Bergstrand M, Karlsson MO (2009) Handling Data Below the Limit of Quantification in Mixed Effect Models. AAPS J 2009 112 11:371–380. https://doi.org/10.1208/S12248-009-9112-5
23. Goodman J, Cowan KJ, Golob M et al (2020) Update to the European Bioanalysis Forum recommendation on biomarkers assays; bringing context of use into practice. Bioanalysis 12:1427–1437. https://doi.org/10.4155/BIO-2020-0243