Model-Informed Dose Selection for Xentuzumab, a Dual Insulin-Like Growth Factor-I/II—Neutralizing Antibody

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Over the past decade, the insulin-like growth factor (IGF)-signaling pathway has gained substantial interest as potential therapeutic target in oncology. Xentuzumab, a humanized IgG1 monoclonal antibody, binds to IGF-I and IGF-II thereby inhibiting the downstream signaling essential for survival and tumor growth. This pathway is further regulated by circulating IGF binding proteins (IGFBPs). In this work, a mechanistic model characterizing the dynamics and interactions of IGFs, IGFBPs, and Xentuzumab has been developed to guide dose selection. Therefore, *in vitro* and *in vivo* literature information was combined with temporal IGF-I, IGF-II, and IGFBP-3 total plasma concentrations from two phase I studies. Based on the established quantitative framework, the time-course of free IGFs as ultimate drug targets not measured in clinics was predicted. Finally, a dose of 1000 mg/week—predicted to reduce free IGF-I and free IGF-II at steady-state by at least 90% and 64%, respectively—was suggested for phase II.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?

☑ The insulin-like growth factor (IGF) pathway represents a promising target in cancer therapy due to its role in survival and tumor growth. Xentuzumab is a humanized monoclonal antibody currently under development capable to simultaneously bind IGF-I and IGF-II, therefore, inhibiting their downstream signaling.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ How can computational modeling be used to increase our understanding of biological systems to supporting drug development and dose selection.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ A mathematical framework has been developed to simultaneously account for the binary binding of IGFs (IGF-I and IGF-II) to IGF endogenous ligands and exogenous drug (Xentuzumab). The framework supports a competitive interaction mechanism between the involved entities and enables the quantitative understanding of the temporal course of free IGF-I and free IGF-II—ultimate targets in clinics in presence of a drug disturbing the IGF system—guiding dose selection.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY?

☑ We propose a mechanistic quantitative framework that can be reused to support the development of similar compounds. Moreover, the model could be expanded to include information regarding the interaction of IGF with their receptors and its downstream response to better understand the dose–response relationship.

The insulin-like growth factor (IGF)-signaling pathway has been shown to play a relevant role in the development, maintenance, and progression of cancer.[1] This complex IGF-signaling system comprises different cell receptors belonging to the kinase receptor family (namely IGF-1 receptor (IGF1-R), insulin receptors (IR), and hybrid forms of the previous two (Hybrid-R)),[2] two structurally related polypeptides (IGF-I and IGF-II) acting as receptor ligands, and 6 known types of IGF binding proteins (IGFBP-1-6).[3]

The IGF-signaling pathway is initiated when free circulating IGF-I or IGF-II bind to their respective receptors thereby activating the downstream signaling cascade with progrowth and prosurvival effects.[2,4,5] Secretion of IGF-I, but not of IGF-II, is subject to control from the pituitary gland via growth hormone (GH) release. Likewise, IGFBP-3 production has shown to be GH-dependent.[6] Upon secretion into the bloodstream, IGF-I and IGF-II can exist in at least three forms: unbound, in binary complexes with IGFBPs, and in ternary complexes with IGFBPs and

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a liver-derived glycoprotein known as acid-labile subunit (ALS). The IGFBPs modulate the activity of the IGF ligands by several mechanisms, including protection of IGFs from rapid proteolytic degradation, transportation of IGFs into tissues, and regulation of binding of IGFs to their receptors. It is estimated that about 99% of IGF-I is bound to IGFBPs,[7] mainly to IGFBP-3, the most abundant IGFBP and with the highest affinity to IGF-I and IGF-II. As IGF-II is three times more abundant than IGF-I and also tends to exhibit higher binding affinity to IGFBPs, it occupies the majority of binding sites on circulating IGFBPs.

Elevated IGF-I and IGF-II levels have been found in various cancer types and are often associated with a poor prognosis.[8] Similarly, IGF-1R overexpression has been described for different cancers.[6,9,10] Moreover, the IGF signaling pathway is known to be involved in resistance to clinically important cancer therapies.[2] These findings together indicate that the IGF pathway is a promising target in cancer therapy.

Xentuzumab is a humanized affinity optimized IgG1 monoclonal antibody (Ab) that targets the IGF-I and IGF-II ligands currently under clinical development (**Figure 1**). Such a therapy enables inhibition of different IGF-signaling pathways at the same time, while not affecting IR-B, which regulates glucose homeostasis. Based on the shown potential of Xentuzumab to interact with this pathway during preclinical studies,[11] two parallel first-in-human phase I studies were conducted. In these trials, Xentuzumab was well tolerable at all investigated doses. A dose-dependent increase in total IGF-I plasma concentrations measured as target engagement markers over repeated drug administrations was observed. At the same time, Xentuzumab had little impact on total levels of total IGF-II or IGFBP-3. The concentrations of free IGF-I and IGF-II could not be measured within the clinical phase I trials.

Given the complexity of the IGF system and its tight regulation, a quantitative framework describing in a mechanistic way the different involved binding processes among Xentuzumab, IGFs, and IGFBPs and the potential role of the GH might improve the understanding of the dynamics of IGFs and could serve as a useful tool to inform drug development and guide dose selection.

Based on the above considerations, the objectives of the current analyses were (i) to develop a mechanistic mathematical model describing the dynamics of the interaction among IGF-I, IGF-II, and IGFBPs in the absence and presence of Xentuzumab, allowing the prediction of free IGFs over time as ultimate drug targets not measured in clinics, and (ii) to support dose selection by simulations. To the best of our knowledge such quantitative approximation in the context of drug development of blocking agents of the IGF-signaling pathway has never been attempted.

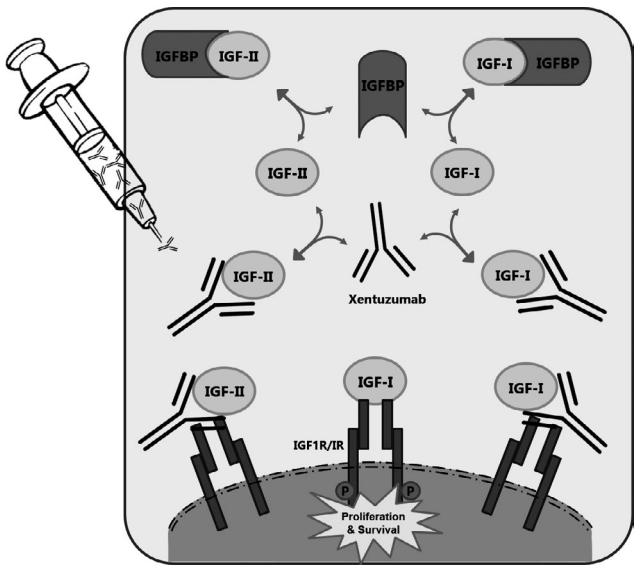

Figure 1 Schematic representation of the biological system under study. IGF1R, insulin-like growth factor-1 receptor; IGFBP, insulin-like growth factor binding protein.

RESULTS

Model development and evaluation

Xentuzumab pharmacokinetic model (drug pharmacokinetic model). A two-compartment model with linear elimination and proportional residual variability sufficiently described Xentuzumab pharmacokinetics (PKs) of all dosing scenarios (see **Figure 2** for external validation and **Figures S1 and S2** for internal validation and main goodness-of-fit). Interindividual variability (IIV) was implemented on drug clearance from the central compartment and central volume of distribution. No indications of nonlinear disposition were detected. Final PK parameter estimates are provided in **Table S1**.

Dynamic interaction model of IGFs and IGFBPs (IGF model). A good initial description of the literature data was obtained using the binding affinities of IGFs to IGFBP-3 reported by Vorwerk *et al.*[12] The data were overall better captured assuming a three times higher binding affinity of IGF-II to IGFBP-3 than IGF-I (and same synthesis rate constants). Slight modifications on the initial set of parameters extracted from the literature were required to successfully characterize all digitalized profiles: (i) a lower K_D (60% lower k_{off}), although still within the range of reported literature values, and (ii) increase in IGFBPs levels (implemented as a factor incrementing the initial pool of IGFBP-3 and assuming the same binding properties), which can be interpreted as IGFBPs other than IGFBP-3 also binding to IGFs. Two different values for the parameter characterizing the molar excess of all IGFBPs over IGFBP-3 (i.e., FACBP) were though needed to account for all concentration-time profiles: a value of 1.2 was required for Mizuno *et al.*[13] and Laron *et al.*[14] data, whereas for Boroujerdi *et al.*[15] data, a value of 1.5 was needed instead.

Dynamic interaction model of Xentuzumab, IGFs, and IGFBPs (drug-binding model). Combination of the drug PK model with the above-described IGF model in the absence of any type of calibration lead to some discrepancies (i.e., consistently lower levels of predicted than observed IGFBP-3 and IGF-I).

To account for these discrepancies, two negative feedback processes triggered by free IGF-I levels were implemented in agreement with the known physiology on the GH-dependent feedback[6] referring to (i) increased synthesis of IGF-I, when IGF-I levels

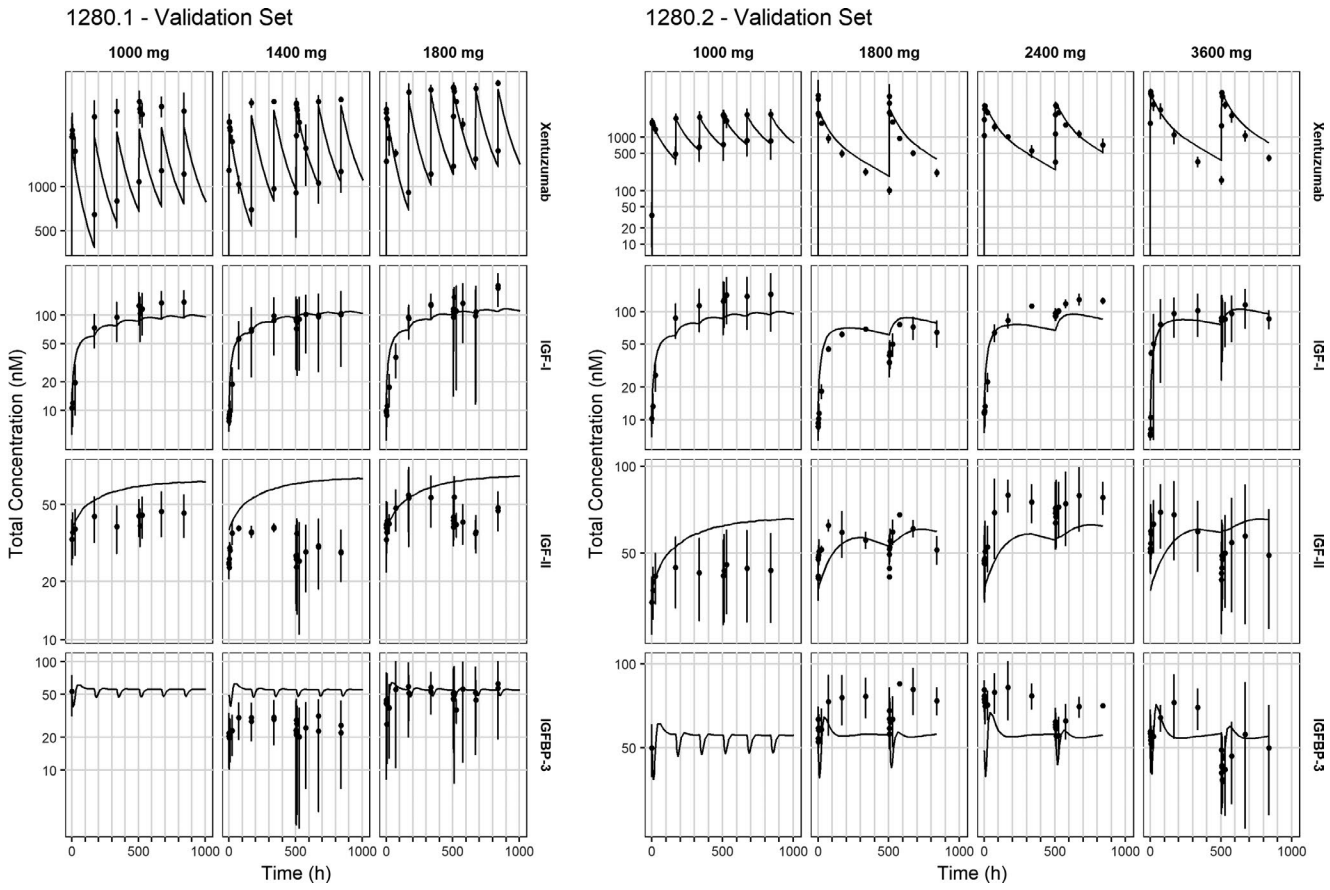

Figure 2 External model evaluation of the final drug binding model. Model predictions (lines) and observations (points and ranges) of Xentuzumab, insulin-like growth factor (IGF)-I, IGF-II, and insulin-like growth factor binding protein (IGFBP)-3 concentrations for different doses in the first two treatment cycles for study 1280.1 (weekly dose administration) and 1280.2 (doses administered every 3 weeks, except for 1,000 mg dose which followed a weekly dosing schema in 1280.2). Xentuzumab pharmacokinetic (PK) is also shown for the three high-dose groups of the 3 weeks schedule (1,800, 2,400, and 3,600 mg), although belonging to the development set for PK.

fall under baseline values and (ii) increased synthesis of IGFBP-3 through a series of delay compartments, when IGF-I levels fall under baseline values. A schematic representation of the final model can be found in **Figure S3**.

Finally, fine-tuning on the degradation rate constants of IGFs and the binding affinities of IGFs to IGFBPs were needed to adequately describe all data at this step. The described adaptations lead to an improvement in model performance reflected as a reduction of 14,543 points in the $-2x$ log likelihood. Tables with model parameters for the final drug binding model together with their sources are provided in **Supplementary Material S1**.

Overall, the final model was able to satisfactorily describe the levels of total IGF-I as well as those of total IGFBP-3 (**Figures S2**, **S4**, **and S5**) from the clinical development set. For total IGF-II levels—for which no measurements of its free entity were available in literature—an overprediction especially at high doses with the more frequent weekly administration schedule was observed. However, the overall performance was still considered acceptable. More important, the final model was able to adequately predict free, bound, and total biomarker entities from the literature (**Figure S6**) and from the clinical validation set (not used for model development purposes) as illustrated in **Figure 2**.

Impact of dosing regimens on target engagement

The integration of the Xentuzumab PK model into the mechanistic model describing the IGF system allowed us to explore the impact of different dose levels and dosing frequencies on the neutralization of free IGF levels *in silico*.

Simulations suggest that by a Xentuzumab dose of 1,000 mg weekly (i.e., 3,000 mg per cycle) a > 90% neutralization of free IGF-I at trough steady-state as compared with baseline was achieved (**Figure 3**). Simultaneously, a 64% inhibition of free IGF-II was predicted. When comparing results where different dosing regimens for the same total amount of drug is administered less frequently (e.g., 3,000 mg/cycle), reduced drug effects—in terms of percentage of inhibition of free IGFs—were observed. This estimate is in agreement with the lower trough Xentuzumab levels expected from prolonging the schedule of drugs exhibiting linear PK, whereas this prediction additionally accounts for the complex binding system of IGF. This indicates that overproportional doses would be required to achieve a similar drug effect by dosing every 2 weeks or every 3 weeks as compared with the weekly schedule.

Taken together, the results suggest that a high neutralization of both free IGF-I and IGF-II compared with baseline is achieved by a Xentuzumab administration of 1,000 mg/weekly.

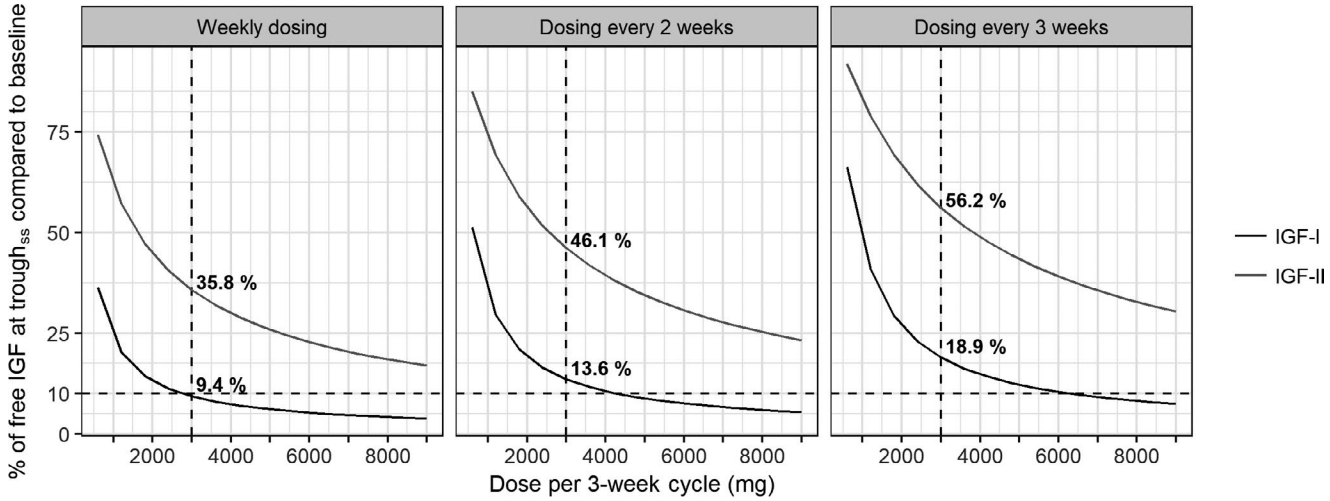

Figure 3 Xentuzumab neutralization of free insulin-like growth factor (IGF) levels. Percentage of free IGF at trough steady-state (1,008 hours after start of treatment) compared with baseline obtained for different total doses per cycle (i.e., dose in mg per 3 weeks) following different dosing regimens.

Sensitivity analysis

Results from sensitivity analysis (**Figure 4**) revealed that FACBP (i.e., relative ratio of total IGFBPs as compared with IGFBP-3 at baseline) is the only dominant parameter explaining by itself 87% (95% CI 83–91%) and 83% (95% CI 79–87%) of the variance of free IGF-I and free IGF-II at trough steady-state, respectively. Indeed, changes in parameter values by ± 10% had a little or modest impact on the model outcome except for FACBP, for which a drop of only 10% translated into more than double levels of predicted free IGF at trough steady-state. The impact was less pronounced for increasing amounts of IGFBPs at baseline than for decreasing amounts.

Additional evaluations referring to percentage of IGF inhibition (instead of absolute levels) revealed that FACBP values ranging from 1.1 to 1.5 translated into only a minor change in inhibition of free IGF-I and IGF-II at steady-state of 88.4–90.8% for free IGF-I, and 55.5–65.8% for free IGF-II for a dosing regimen of 1,000 mg administered weekly (**Figure 4b**). Increasing amounts of total IGFBPs translated into a lower predicted IGF neutralization due to a lower availability of free IGF at baseline (i.e., larger proportion of IGF bound to IGFBPs at baseline). In conclusion, the sensitivity analyses suggest model robustness regarding the influence of parameter values on the predictions of the percentage of inhibition of free IGF by Xentuzumab.

DISCUSSION

In this paper, we present a mechanistic model characterizing the time-dependent interaction between key elements of the IGF system (IGF-I, IGF-II, and IGFBPs) and Xentuzumab to inform drug development and to guide dose selection. The quantitative framework was developed combining existing *in vitro* and *in vivo* literature data on the IGF system, *in vitro* drug binding properties and concentration-time profiles of Xentuzumab, and IGF-I, IGF-II, and IGFBP-3 obtained from two phase I clinical studies.

A priori, some discrepancies between literature and clinical data were observed, with total concentrations of IGF-I, IGF-II, and IGFBP-3 at baseline from literature about twofold higher than those measured in the clinical trials. These discrepancies may arise from differences in the assays as well as reference substances used to quantify the different components. However, the ratios between the total concentrations of IGF-I, IGF-II, and IGFBP-3 were well consistent between literature and clinical trials.[7] The final mathematical model was able to successfully describe the mean tendencies of IGF-I and IGFBP-3 circulating levels for different scenarios (i.e., administration of recombinant IGF or different dosing regimens of Xentuzumab) from literature and clinical studies (including development set and validation set) in a mechanistic fashion. Nonetheless, the model provided an overprediction of IGF-II profiles at the higher doses of the weekly dosing schedule suggesting that the impact of Xentuzumab on IGF-II is overestimated. This misprediction was not as evident for the doses administered every 3 weeks, whereas a clearer trend (although still small) for a dose-dependent effect of Xentuzumab on IGF-II was observed as compared with the weekly schedule. Due to the limited knowledge available from literature on IGF-II concentrations over time, only few scenarios were tested to adapt IGF-II-related parameters and higher uncertainty was accepted. Extra information on the levels of free IGF-II would be needed to validate the model in terms of the relationship between total and free IGF-II levels, the impact of Xentuzumab on free IGF-II levels, and refine IGF-II-related parameters and hypothesis.

Additional model features leading to an improved description of the observed data, including the assumed GH-dependent feedback mechanism on total IGFBP-3 and IGF-I levels, were supported by the known physiology. On this regard, data obtained after repeated doses and multiple dose levels of Xentuzumab were essential to properly characterize the feedback mechanism.

When looking at total biomarker measurements from the clinical studies, only minor changes were observed for IGF-II and IGFBP-3 upon Xentuzumab treatment. In contrast to this, increased levels of total IGF-I over time were observed. This

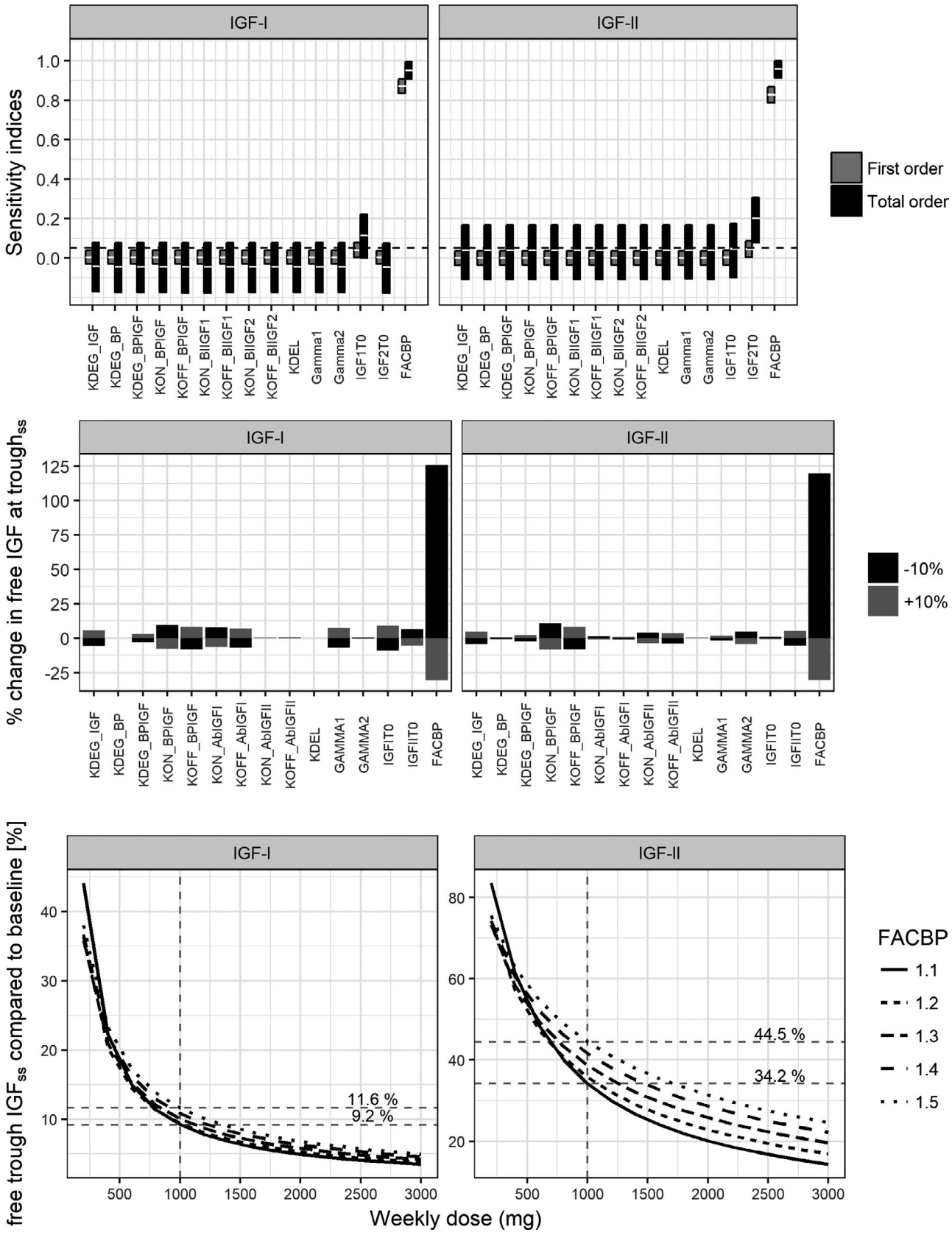

Figure 4 Sensitivity analysis. (**a**) First order and total order sensitivity indices from the Sobol analysis for insulin-like growth factor (IGF)-I and IGF-II trough steady-state levels (1,008 hours after start of treatment). (**b**) Changes on free IGF-I and free IGF-II concentrations at trough steady-state for a 10% change of each model parameter at a time. (**c**) Percentage of free IGF inhibition at trough steady-state (1,008 hours) compared with baseline for different values FACBP. In all cases, a weekly administration of 1,000 mg of Xentuzumab was used.

outcome can be interpreted from a quantitative system pharmacology perspective. Binding of Xentuzumab to IGFs leads to an extension of IGFs half-lives (lower degradation rate for bound than for free IGF). In turn, the reduction of free IGF-I triggers compensatory mechanisms, which increase IGF-I synthesis to restore homeostasis. Both processes lead to increments on total IGF-I, despite the reduction of free IGF-I levels. These effects are well in agreement with previous preclinical data in mice.[16] The lower predicted increase in total IGF-II can be explained by an assumed higher binding affinity of IGF-II to IGFBPs and a lower affinity of IGF-II to Xentuzumab than IGF-I. In addition, IGF-II synthesis is known to be independent of the GH-dependent feedback mechanism.

One of the main advantages of developing mechanistic models to characterize pharmacological systems is the possibility to explore not only measured variables, but also other relevant components or dosing scenarios not investigated in clinical studies. In our concrete case, only total PK and biomarker concentrations were measured in clinics, whereas the quantitative framework allowed us to explore the temporal behavior of the unmeasured entities of free IGF-I and IGF-II—ultimately responsible of activating the IGF-signaling pathway.[16] Such predictions would not have been possible based on a simpler data-driven PK/pharmacodynamic model in the absence of actual measurements.

Overall, weekly dosing administration provided the largest neutralization of free IGFs for a certain total dose per cycle compared with less frequent dose administrations (e.g., dosing every 2 or every 3 weeks), suggesting the convenience of the weekly administration to maximize efficacy. Weekly administration of 1,000 mg was predicted to induce an inhibition of around 88% of free IGF-I after the first week (shortly before the next drug administration), and higher than 90% over the entire dosing interval at steady-state (i.e., steady-state trough concentrations after 6 weeks of dose administration). Inhibition of free IGF-II was estimated to be at least 64% over the complete dosing interval at steady-state for the above-mentioned dosing schema. The lower inhibition as compared with IGF-I results from the lower affinity of Xentuzumab to IGF-II (i.e., higher K_D) than to IGF-I and an assumed higher affinity of IGF-II to IGFBPs. More important, the model predictions on percentage of IGF neutralization were only marginally influenced by changes on the most sensitive parameter, FACBP—accounting for the relative molar excess of IGFBPs compared with measured total IGFBP-3 values—suggesting model robustness and adequacy to support dose selection. Nevertheless, information on total IGFBPs at baseline in addition to IGFBP-3 would be useful, in order to further validate the model. It should be acknowledged though, that despite the consistent relative inhibition of free IGF, when assuming different levels of total IGFBPs, the absolute plasma concentrations of free IGF were predicted to substantially vary between the different scenarios.

In summary, a mathematical model has been developed to characterize the disposition of IGF-I, IGF-II, and IGFBP-3 in the absence or presence of Xentuzumab, a monoclonal antibody capable of neutralizing IGF-I and IGF-II. Due to the mechanistic nature of the model, it could be used to explore the impact of different dosing regimens on the neutralization of free IGF levels as ultimate drug targets not measured in clinics. The Xentuzumab dose of 1,000 mg weekly was estimated to substantially reduce both free IGF-I and IGF-II over time. These mechanistic modeling results were in agreement with statistical evaluations performed on biomarker and response data from two phase I studies (unpublished data), altogether supporting the selection of 1,000 mg Xentuzumab weekly as a biologically relevant dose for upcoming studies. As the model is capable of simultaneously describing different sources of data, it could further be used to quantitatively characterize other compounds with a similar mechanism of action to support their development. Finally, it could be extended (e.g., by incorporating quantitative information on the temporal evolution of IGF receptor levels), and even downstream signaling molecules, linking levels of free IGF to response, thus using the framework to personalize the dosing regimen needed to achieve sustained free IGF levels below a certain identified threshold.

MATERIALS AND METHODS

Literature data

A literature search was performed to identify published concentration time profiles of different forms (free, bound, and total) of IGF-I, IGF-II, and IGFBPs in plasma. Three publications were identified where total and free IGF-I, IGF-I bound to IGFBP-3 or other IGFBPs, and total IGFBP-3 plasma time profiles were measured at several time points after administration of recombinant IGF-I to healthy subjects.[13–15] Data were digitalized using GetData Graph Digitalizer software. No temporal profiles of total or free IGF-II were found. An overview of the available literature data is provided in **Table 1**.

Clinical Boehringer-Ingelheim data

Data from two phase I single agent studies, 1280.1 and 1280.2, in patients with advanced solid cancers were used for the analysis (NCT01403974 and NCT01317420). Both studies consisted of a dose escalation part (part I) to determine the maximum tolerated dose and/or relevant biological dose following a traditional 3 + 3 design and an expansion cohort (part II) using the selected dose from part I to assess antitumor activity. The studies were performed in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guideline for Good Clinical Practice. Approval was obtained from the independent ethics committees or institutional review boards of the participating sites. All patients provided written informed consent. A summary of the data can be found in **Table 1** and **Supplementary Material S1**.

Model development

Model building was performed in three steps: (i) population PK analysis of Xentuzumab (hereafter referred to as the Drug PK model), (ii) modeling of the biological IGF system representing the interaction among endogenous IGF-I, IGF-II, and IGFBPs in the absence of drug (hereafter referred to the IGF model), and (iii) integration of the two previous models to characterize perturbation of the IGF system in patients receiving Xentuzumab (hereafter referred to as the Drug binding model).

PKs were characterized using a data driven top-down approach, whereas for the IGF model the bottom-up paradigm was undertaken using mechanistic modeling on published data from literature. For integration of both models, the middle out approach was applied, in which the model predicted population PK profiles together with the mean IGF-I, IGF-II, and IGFBP profiles from mechanistic modeling were used to calibrate model structure and parameter values.

Table 1 Overview of literature and clinical phase I trial data from BI used in the analysis

Data source	Study design	No patients	Available entity (no. of samples)
Literature data			
Mizuno set	3-hour i.v. infusion of 15 µg/kg (127 nmol) 3-hour i.v. infusion of 30 µg/kg (248 nmol) 3-hour i.v. infusion of 60 µg/kg (498 nmol) 6-hour i.v. infusion of 120 µg/kg (947 nmol) s.c. administration of 60 µg/kg (482 nmol) s.c. administration of 120 µg/kg (1,075 nmol)	Mean concentrations from 5 healthy subjects	Free IGF-I (n = 78) Bound IGF-I (n = 73)[a]
Laron set	75 µg/kg bolus (434 nmol)	Mean concentrations from 8 healthy subjects	Total IGF-I (n = 7)
Boroujerdi set	3-hour i.v. infusion 60 µg/kg (665 nmol)	4 healthy subjects	Free IGF-I (n = 21) Total IGF-I (n = 63) IGF-I bound to IGFBPs in the 50 kDa fraction (n = 24)b IGF-I bound to IGFBPs in the 150 kDa fraction (n = 24)c Total IGFBP-3 (n = 49)
Clinical phase I trial data (BI) (data from all treatment cycles)			
1280.1	*Escalation phase* 1-hour infusion of 10 to 1,800 mg (66.7–12,000 nM) in weekly dosing schema over multiple cycles of 3 weeks each	48 patients with advanced solid tumors	Total Xentuzumab (n = 1438) Total IGF-I (n = 1,463) Total IGF-II (n = 1,493) Total IGFBP-3 (n = 1,486)
	Expansion phase 1-hour infusion of 1,000 mg (6,666.7 nM) in weekly dosing schema over multiple cycles of 3 weeks each	13 patients with advanced solid tumors	Total Xentuzumab (n = 416) Total IGF-I (n = 232) Total IGF-II (n = 234) Total IGFBP-3 (n = 13)
1280.2	*Escalation phase* 1-hour infusion of 10–3,600 mg (66.7–24,000 nM) administered every 3 weeks over multiple cycles of 3 weeks each	34 patients with advanced solid tumors	Total Xentuzumab (n = 640) Total IGF-I (n = 688) Total IGF-II (n = 688) Total IGFBP-3 (n = 688)
	Expansion phase 1-hour infusion of 1,000 mg (6,666.7 nM) in weekly dosing schema over multiple cycles of 3 weeks each	30 patients with advanced solid tumors	Total Xentuzumab (n = 616) Total IGF-I (n = 358) Total IGF-II (n = 360) Total IGFBP3 (n = 31)

BI, Boehringer-Ingelheim; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein.
[a]Free and total IGF-I initially measured, but graphs reported free and bound IGF-I instead.[b]Assumed to correspond to IGF-I bound to IGFBPs different than IGFBP-3.[c]Assumed to correspond to IGF-I bound to IGFBP-3.

Data for doses of 10 to 1,050 weekly and 10 to 3,600 every 3 weeks from the escalation phase part I were the basis for PK model development (n = 75 patients). The same data were used for the final drug binding model, except for three high-dose groups of the 3 weeks schedule (1,800, 2,400, and 3,600 mg), for which IGF biomarker data were not available at the time of model development (n = 66 patients). The rest of the data from the clinical trials was kept for model validation (50 and 59 patients for PK and IGF biomarkers, respectively). A workflow summarizing the model development strategy can be found in **Figure 5**.

Xentuzumab PK model (drug PK model). The antibody concentration–time profiles were analyzed by compartmental modeling using nonlinear mixed-effects modeling in NONMEM version 6.2 or higher (first-order conditional estimation method with interaction algorithm). The stochastic model and IIV were investigated. The IIV was modeled using exponential random effect models. For the residual variability, a proportional as well as an additive term was tested. IIV and residual variability were assumed to be symmetrically distributed around 0 with variances ω^2 and σ^2, respectively. Model selection was guided by change in objective function values, parameter precision, and goodness-of-fit plots. Model performance was assessed by prediction-corrected visual predictive checks based on 1,000 simulations. Summary statistics were calculated by bin (software PsN 4.6.0) and graphically represented. Covariate analysis was not pursued at this stage because the main goal was to support dose selection for the next clinical phase.

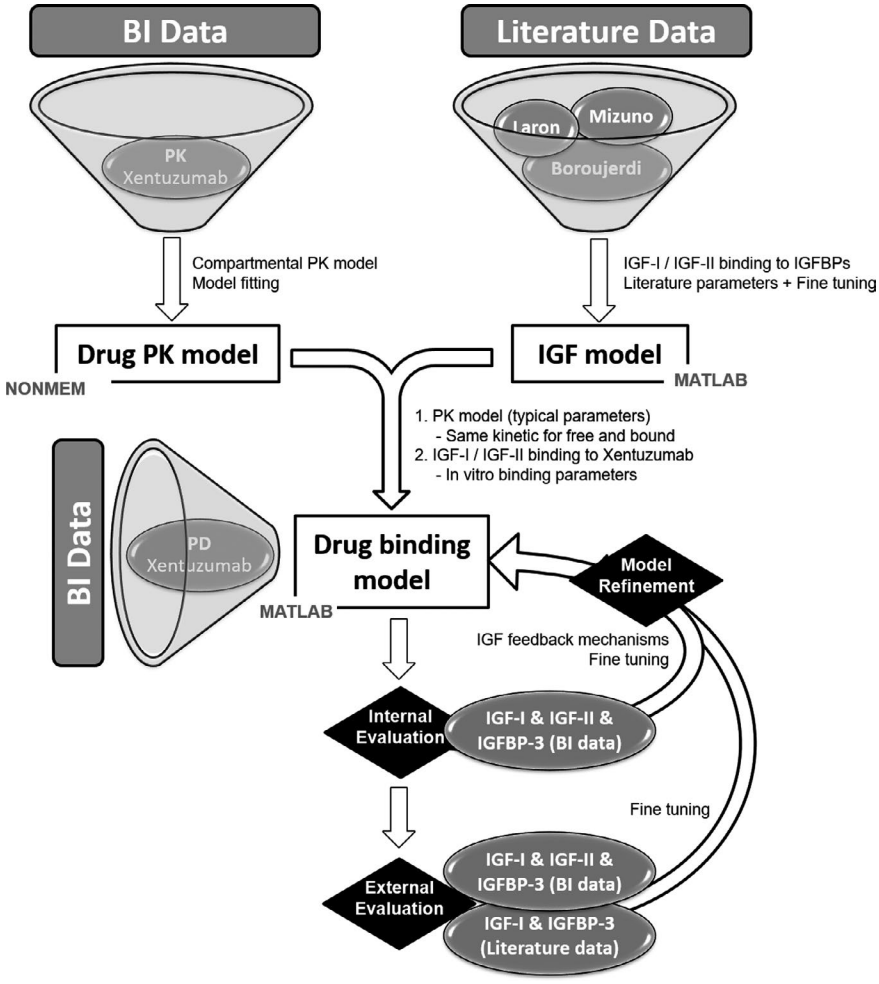

Figure 5 Workflow of the different steps followed during the model building process. BI, Boehringer-Ingelheim; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; PD, pharmacodynamic; PK, pharmacokinetic.

Dynamic interaction model of IGFs and IGFBPs (IGF model). Measurements of total or free IGF-I and IGFBPs after administration of recombinant IGF-I from the three identified publications[13–15] were used to establish the IGF model.

The IGF model considers that IGF-I and IGF-II bind in a reversible and competitive manner to IGFBP-3, main binding protein. Additional binding processes leading to ternary complexes (consisting of IGF, IGFBP-3, and ALS) were considered negligible considering the estimated substantially lower binding affinity (by 300-fold to 2,000-fold) of ALS to form ternary complexes than the constants resulting in binary complexes.[17]

Turnover processes of each of the proteins were considered by assuming zero order rate of synthesis and first order rate of degradation of free proteins represented by the k_{syn} and k_{deg} parameters, respectively. Degradation processes of bound proteins were initially not considered in the model.

Reversible binding followed the law of mass action, in which the k_{on} and k_{off} represent the second order rate constant of association and the first-order rate constant of dissociation, respectively.

Eqs. 1–3 characterize the kinetics of free IGF-I (IGFI), IGF-I bound to IGFBP-3 (BP3IGFI), and total IGF-I concentrations, respectively. Similar expressions were derived for IGF-II and IGFBP-3 (see Section 2 of **Supplementary Material S1** for the full binding model for illustration).

$$\frac{dIGFI}{dt} = Input_{hrIGF} + k_{syn\_IGFI} - k_{deg\_IGF} \times IGFI - k_{on\_BP3IGFI} \times IGFI \times BP3 + k_{off\_BP3IGFI} \times BP3IGFI \quad (1)$$

$$\frac{dBP3IGFI}{dt} = k_{on\_BPIGFI} \times IGFI \times BP3 - k_{off\_BP3IGFI} \times BP3IGFI \quad (2)$$

$$IGFI_T = IGFI + BP3IGFI \quad (3)$$

$Input_{hrIGF}$ represents the external administration of human recombinant IGF-I.

Values for the different model parameters were extracted from the literature[7,12,15,17–21] and used as starting points for model development. Model calibration was undertaken by visual comparison of available data from the literature with model predicted concentration-time profiles in Matlab software version 2010a or higher (ode23s solver) and adaptation of model parameters taking into account (the range of) published literature values.

During model development, the following additional scenarios were explored.

1. Assumption of equal or different binding affinities of IGF-I (K_{D1}) and IGF-II (K_{D2}) to IGFBP-3 as both options were reported in the literature[12,18,19,21,22]:
   - Same equilibrium binding constant (i.e., $K_{D1} = K_{D2}$). In order to maintain the known 3:1 ratio between circulating IGF-II and IGF-I levels,[7] synthesis of IGF-II was assumed to be 3 times larger than for IGF-I.

- Different equilibrium binding constant (i.e., 3 times higher affinity of IGF-II to IGFBPs than IGF-I ($K_{D1} = 3^{*}K_{D2}$)). In order to maintain the 3:1 ratio between circulating IGF-II and IGF-I, the same synthesis rate constants for IGF-I and IGF-II were assumed.

2. Increase of IGFBP-3 concentrations by a factor of 1.0 to 1.5 given the described molar excess of 50% for IGFBPs other than IGFBP-3[7] and their weaker affinity to IGFs.[21,22]

Dynamic interaction model of Xentuzumab, IGFs, and IGFBPs (Drug binding model). The Drug PK model was coupled to the IGF model by incorporating the association and dissociation processes between free IGFs and Xentuzumab.[16] In order to reuse parameters obtained during the PK analysis and maintain linear drug PKs, free and bound Ab concentrations were allowed to distribute to a peripheral (nonmeasurable) compartment and be eliminated assuming the same rate constants for both free and bound entities. A schematic representation of the drug binding model can be found in **Figure S1**.

The values of the binding constants (k_{on} and k_{off}) between Ab and IGF-I or IGF-II were obtained from *in vitro* studies (Biacore measurements performed at Boehringer-Ingelheim; data not shown). A detailed mathematical description of the final model, including initial conditions and parameter estimates can be found in **Supplementary Material S1**.

Model calibration was performed by comparing total IGF-I, IGF-II, and IGFBP-3 simulated concentration-time profiles to the clinical data. Thereby, different feedback mechanisms accounting for GH-dependent endogenous regulation of IGF-I and IGFBP-3 levels, as well as elimination of complexes between IGF and either IGFBPs or drug were explored. The final model was validated using the literature data from the previous step as well as data from the Boehringer-Ingelheim clinical trials not used for model development.

Dose simulation studies

The final model was used to predict the percentage of free IGF-I and IGF-II at trough after the first dose ($t = 168$) and at steady-state (after the sixth dose, $t = 1008$) for Xentuzumab doses ranging from 200 to 3,600 mg assuming different dosing schedules. The trough was selected, as the least neutralization of IGF during a dosing interval is expected at this critical time point.

Sensitivity analysis

To globally evaluate the impact of parameter variations on model output (i.e., free IGF at steady-state), a Sobol sensitivity analysis was performed using the SAFE Matlab toolbox,[23] which implements the approximation technique described by Saltelli *et al.*[24,25] In addition, the magnitude of the impact was assessed for increasing or decreasing one parameter at a time by 10% after administration of weekly Xentuzumab doses of 1,000 mg (selected as RBD for further drug development). Additional evaluations were performed for the most influential parameter (FACBP) by exploring its impact on the predicted percentage inhibition of free IGFs.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Figure S1. Xentuzumab PK model internal evaluation. Xentuzumab doses of 101,800 mg weekly or 103,600 mg every three weeks from the escalation phase of clinical studies 1280.1 and 1280.2 (development set) using a prediction-corrected visual predictive check (pcVPC) with 1,000 simulations and 15 time bins are represented.
Figure S2. Goodness-of-fit plots of the final model for both studies.
Figure S3. Mathematical representation of the final drug binding model.
Figure S4. Final drug binding model evaluation based on study 1280.1 (development set). Model predictions (lines) and observations (points and ranges) of total Xentuzumab, insulin-like growth factor (IGF)-I, IGF-II and IGF binding protein 3 (IGFBP-3) concentrations for the development set of study 1280.1 (weekly dose administration over 2 cycles of 3 weeks each).
Figure S5. Final drug binding model evaluation based on study 1280.2 (development set). Model predictions (lines) and observations (points and ranges) of total Xentuzumab, insulin-like growth factor (IGF)-I, IGF-II and IGF binding protein 3 (IGFBP-3) concentrations for the development set of study 1280.2 (dosing every 3 weeks over 2 cycles of 3 weeks each).
Figure S6. Final drug binding model performance using literature data. Model predictions (lines) for the different literature data scenarios versus observations (points) assuming different equilibrium binding constant (K_D). IGF, insulin-like growth factor; BP, IGF binding protein; BP3IGFI, IGF-I bound to BP-3 (assumed to mainly correspond to 150 KDa fraction in Boroujerdi *et al.*); BPIGFI, IGF-I bound to any BP; BPRIGFI, IGF-I bound to any BP except BP-3 (assumed to mainly correspond to 50 KDa fraction in Boroujerdi *et al.*).
Table S1. Xentuzumab pharmacokinetic model parameters.
Supplementary Material S1. Full dynamic interaction model of Xentuzumab, IGFs and IGFBPs (Drug binding model).

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Z.P.P.-G., U.S., and I.F.T. wrote the manuscript. Z.P.P.-G., U.S., A.J., M.F., and I.F.T. designed the research and analyzed the data.

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