

## Onartuzumab (MetMab): Using Nonclinical Pharmacokinetic and Concentration–Effect Data to Support Clinical Development

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### Abstract

**Purpose:** We characterized the pharmacokinetics of onartuzumab (MetMab) in animals and determined a concentration–effect relationship in tumor-bearing mice to enable estimation of clinical pharmacokinetics and target doses.

**Experimental Design:** A tumor growth inhibition model was used to estimate tumoristatic concentrations (TSC) in mice. Human pharmacokinetic parameters were projected from pharmacokinetics in cynomolgus monkeys by the species-invariant time method. Monte Carlo simulations predicted the percentage of patients achieving steady-state trough serum concentrations ( $C_{\text{trough ss}} \geq \text{TSC}$ ) for every 3-week (Q3W) dosing.

**Results:** Onartuzumab clearance (CL) in the linear dose range was 21.1 and 12.2 mL/d/kg in mice and cynomolgus monkeys with elimination half-life at 6.10 and 3.37 days, respectively. The estimated TSC in KP4 pancreatic xenograft tumor-bearing mice was 15  $\mu\text{g/mL}$ . Projected CL for humans in the linear dose range was 5.74 to 9.36 mL/d/kg with scaling exponents of CL at 0.75 to 0.9. Monte Carlo simulations projected a Q3W dose of 10 to 30 mg/kg to achieve  $C_{\text{trough ss}}$  of 15  $\mu\text{g/mL}$  in 95% or more of patients.

**Conclusions:** Onartuzumab pharmacokinetics differed from typical bivalent glycosylated monoclonal antibodies with approximately 2-times faster CL in the linear dose range. Despite this higher CL, xenograft efficacy data supported dose flexibility with Q1W to Q3W dose regimens in the clinical setting with a TSC of 15  $\mu\text{g/mL}$  as the  $C_{\text{trough ss}}$  target. The projected human efficacious dose of 10 to 30 mg/kg Q3W should achieve the target TSC of 15  $\mu\text{g/mL}$ . These data show effective pharmacokinetic/pharmacodynamic modeling to project doses to be tested in the clinic. *Clin Cancer Res*; 19(18); 5068–78. ©2013 AACR.

### Introduction

The importance of pharmacokinetic and pharmacodynamic modeling and simulation in all phases of drug development has been recognized (1, 2). In particular,

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

N. L. 'Shasha' Jumble, M. Romero, and A. Peterson were employed by Genentech during their involvement in this study.

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target concentration approaches using nonclinical studies during drug development to support safe starting dose and therapeutically relevant dose escalation for human oncology studies, have been reported in the recent publications (3–5). However, limited work has been carried out for mAbs in the oncology field (6). Because inability to show efficacy is a leading reason for failure of phase III trials, exploration of the role of translational pharmacokinetics/pharmacodynamics to support dose and regimen selection in the clinic would improve risk management during drug development (7). This article describes how such an approach has been used in the development of onartuzumab (MetMab).

MET is a receptor tyrosine kinase with known specificity for a single ligand, the hepatocyte growth factor (HGF), also known as scatter factor (SF; ref. 8). HGF/SF binding to MET leads to receptor dimerization and autophosphorylation of MET at multiple tyrosines on its intracellular kinase domain, and subsequent phosphorylation of its C-terminal docking site and juxtamembrane domain (9). These phosphorylation events enable activation of multiple

### Translational Relevance

Nonclinical pharmacokinetics and efficacy data are typically generated to guide clinical testing of therapeutic monoclonal antibodies (mAbs). This approach was particularly useful during the early development of onartuzumab (MetMab) to predict whether the estimated efficacious target concentration of 15  $\mu\text{g}/\text{mL}$  was achievable with a once-every-3-week (Q3W) dosing strategy. There are three unique aspects of this article, which supports its translational relevance. First, it is the first article reporting pharmacokinetics in animals for an aglycosylated, monovalent mAb produced in *Escherichia coli*. Second, the pharmacokinetic data from cynomolgus monkeys are confirmed to be scalable to humans with the species-invariant time method used widely for other mAbs. Finally, the dose of 15 mg/kg Q3W in humans, which targeted steady-state trough serum concentrations of 15  $\mu\text{g}/\text{mL}$  or more (a concentration that was tumoristatic in the hepatocyte growth factor/MET autocrine KP4 xenograft tumor model) has been effective in phase I and phase II clinical trials.

downstream effector proteins such as the adaptor proteins Grb2 and Gab1, leading to activation of the PI3K, Ras/RAF/MEK/ERK, PLC- $\gamma$ , STATs, and FAK signaling pathways. MET plays a key role in a variety of cellular processes such as proliferation, survival, motility, and invasion in normal and tumor cells.

MET is expressed in normal tissues by cells of epithelial origin (10, 11) and MET overexpression or mutation has been linked to tumorigenesis and malignancy in a number of human cancers (8). High levels of either MET and/or HGF are correlated with poor prognosis in a wide variety of human cancers (8, 9, 12). These data provide a compelling rationale for targeting HGF/MET signaling as a therapeutic strategy in multiple tumor types.

A number of approaches have been explored to block HGF/MET signaling as a therapeutic strategy including small-molecule inhibitors and antibodies against either HGF or MET (13). For antibody therapeutics, 3 anti-HGF mAbs that disrupt HGF/MET signaling pathways are in clinical development: AMG 102 (SCH 900105, rilotumumab; refs. 14, 15), L2G7 (TAK701; ref. 16), and AV-299 (ficlatuzumab; ref. 17). Among them, AMG 102 is the most advanced with multiple clinical trials underway. However, no nonclinical concentration-effect relationships have been reported for any of these mAbs. There are no bivalent mAbs against MET currently in clinical development.

Onartuzumab is a monovalent (one-armed) humanized mAb produced in *Escherichia coli*, which binds to the Sema domain on the extracellular part of MET to block HGF binding (18–21). The novel monovalent design of onartuzumab, achieved using the "knobs-into-holes" method to minimize formation of the bivalent antibody configuration (22), prevents MET dimerization—an event that

leads to subsequent pathway activation and that occurs with some bivalent anti-c-MET mAbs (23). The inhibition of HGF-induced MET activation is expected to prevent cancer cell growth, survival, and metastasis. Onartuzumab has shown antitumor activity in multiple human tumor xenograft models (18–20). Onartuzumab is being clinically evaluated in multiple cancer types, including lung (adenocarcinoma and squamous cell: NCT01456325; NCT01519804; NCT01496742), colon (NCT01418222), breast (NCT01186991), stomach (NCT01590719), and brain (NCT01632228). Importantly, a complete response was observed in phase I studies (24, 25) and an overall survival (OS) benefit was observed in a phase II trial in non-small cell lung cancer (NSCLC; ref. 26), where onartuzumab was administered in combination with erlotinib.

A special consideration when developing onartuzumab was to understand the potential impact of its unique structure on safety, efficacy, and pharmacokinetics without prior clinical experience. Here, we describe an approach that combines pharmacokinetics from multiple species with a concentration-effect relationship derived from xenograft mice to project effective doses and regimens in humans. Specifically, we characterized the pharmacokinetics of onartuzumab in mice and cynomolgus monkeys, predicted clinical pharmacokinetics, and determined whether dose schedule had any impact on efficacy in the HGF/MET autocrine KP4 pancreatic xenograft tumor model. Furthermore, we applied the tumoristatic concentration (TSC) from concentration/response modeling in mice to estimate once-every-3-week (Q3W) doses in humans to achieve TSC at steady-state trough serum concentrations ( $C_{\text{trough ss}}$ )

## Materials and Methods

### Cell lines

The KP4 pancreatic ductal carcinoma cell line was obtained from the Riken BioResource Center Cell Bank (Cell line #: RCB1005). The cell line has been tested and authenticated at Genentech, Inc. by high-throughput single nucleotide polymorphism genotyping using the Illumina GoldenGate Genotyping Assay. Each new batch banked is tested for ancestry by the same method. An authenticated vial was used for studies described in this article.

### Antibody reagents

Onartuzumab (MetMab), produced in *E. coli* at Genentech, Inc., was provided at a nominal concentration of 20 mg/mL (5.5 mL/vial) in a formulation buffer comprising 10 mmol/L histidine succinate, 106 mmol/L trehalose dihydrate, and 0.02% polysorbate 20 at pH 5.7. Before its use in the study, the test material was stored at 2 to 8°C.

### Pharmacokinetic studies in athymic nude mice and cynomolgus monkeys

The pharmacokinetic study in mice was approved by Institutional Animal Care and Use Committee (IACUC) and conducted at Genentech, Inc.. The pharmacokinetic study in cynomolgus monkeys was approved by IACUC and

conducted at Preclinical Services Nevada, Charles River Laboratories.

The pharmacokinetics after a single intravenous dose was evaluated in athymic nude (nu/nu) mice and cynomolgus monkeys. Female nude (nu/nu) mice were given a single intravenous bolus dose of onartuzumab at 3, 10, or 30 mg/kg ( $n = 9$ /group) to alternate the blood collection at each time point ( $n = 3$ /time point/group). Serum samples were collected at predose, 15 minutes; 1, 4, 8, and 24 hours; and 3, 7, 14, and 21 days. Cynomolgus monkeys ( $n = 4$ /group) were given a single intravenous dose of onartuzumab at 0.5, 3, 10, or 30 mg/kg. Serum samples were collected at predose, 10 and 45 minutes; 2, 4, 7, 12, and 24 hours; and 2, 3, 5, 7, 10, 14, 21, 28, and 35 days. All serum samples were stored at  $-70^{\circ}\text{C}$  until analysis by ELISA as described below. Cynomolgus monkey serum was also used to measure anti-therapeutic antibodies (ATA) using a bridging electrochemiluminescence assay (ECLA) described below.

Serum samples were also collected from a single intravenous dose-response study from KP4 tumor-bearing athymic nude (nu/nu) mice. Three onartuzumab doses were investigated (1, 7.5, and 30 mg/kg) with 5 animals in each group at 2 hours and 3, 7, and 12 days post-dose.

#### Bioanalysis of onartuzumab serum concentration in mice and cynomolgus monkeys

A sandwich ELISA with human MET-Fc fusion protein (Genentech, Inc.) as a capture agent and goat anti-human F(ab')<sub>2</sub>-conjugated horseradish peroxidase (HRP; Jackson ImmunoResearch Laboratories, Inc.) as a detection agent was used to measure onartuzumab serum concentrations in mice. The linear range of the calibration curve was 0.16 to 40 ng/mL. The minimum dilutions of mouse serum samples were 1/100. Therefore, the lower limit of quantitation (LLOQ), corrected for dilution, in this assay was 16 ng/mL.

Diluted serum samples were assayed for onartuzumab concentrations in cynomolgus monkeys using a sandwich ELISA. In this particular assay, a recombinant histidine tagged human MET extracellular domain receptor (MET-ECD-8x-His; Genentech, Inc.) was used as the capture reagent and F(ab')<sub>2</sub> fragmented, goat anti-human immunoglobulin G (IgG) Fc antibodies conjugated to HRP were added for detection. The linear range of the calibration curve was 1.0 to 32.0 ng/mL. The minimum dilution of samples was 1/50 and the LLOQ (corrected for dilution) was 50 ng/mL for cynomolgus monkey serum.

The inter- and intra-assay precision [coefficient of variation% (CV%)] values for all the assays were within an acceptable level of 20%.

#### Bioanalysis of antitherapeutic antibodies in cynomolgus monkeys

To determine whether ATAs have an effect on onartuzumab concentrations and to gain insight into the potential immunogenic portions of the therapeutic, a bridging ECLA was used to detect the presence of ATAs in cynomolgus monkey serum. The conjugate reagents (onartuzumab conjugated to biotin and onartuzumab conjugated to BVTAG

label; BioVeris Corporation), were used at an in-plate concentration of 1.0  $\mu\text{g/mL}$ . Diluted serum samples and conjugates were incubated together. Streptavidin-coated magnetic beads (BioVeris Corporation) were added to the plates before reading on a BioVeris M384 analyzer (BioVeris Corporation). Samples that were determined to be positive for ATAs to onartuzumab were further characterized to determine whether the response was primarily toward the complementarity determining regions (CDR) or framework of onartuzumab. The minimum detectable response for the assay was 1.4 titer units.

#### Mouse and cynomolgus monkey pharmacokinetic data analysis

Pharmacokinetic parameters were estimated using WinNonlin Enterprise Version 5.0.1 (Pharsight Corp.) by non-compartmental analysis (NCA) for mice and cynomolgus monkeys. The nominal dose administered for each group was used for analysis. Group average serum concentrations at the same time point ( $n = 3$ /time point/group) were used in the mouse to provide one estimate for each dose group along with the SE of the fit for each pharmacokinetic parameter, whereas in the monkey, each animal was analyzed separately and results for each dose group were summarized as mean  $\pm$  SD. The following pharmacokinetic parameters were reported for NCA: predicted maximum serum concentration ( $C_{\text{max}}$ ), total onartuzumab exposure ( $\text{AUC}_{\text{inf}}$ ) defined as area under the concentration-time curve extrapolated to infinity, clearance (CL), volume of distribution under steady-state conditions ( $V_{\text{ss}}$ ), and elimination half-life ( $t_{1/2}$ ).

To further characterize the nonlinear pharmacokinetics in cynomolgus monkeys, a model comprising parallel linear and nonlinear clearance (CL) pathways was used to estimate onartuzumab pharmacokinetic parameters using NONMEM software (ICON Development Solutions). The following pharmacokinetic parameters were estimated for this analysis: linear (or nonspecific)  $\text{CL}_L$ , distributional CL ( $\text{CL}_d$ ), volume of distribution of the central compartment ( $V_1$ ), volume of distribution of the peripheral compartment ( $V_2$ ), maximum elimination rate for saturable CL ( $V_{\text{max}}$ ), and the concentration at 50%  $V_{\text{max}}$  ( $K_m$ ).

In addition, a two-compartment model was used to fit data from all mouse studies (3, 10, and 30 mg/kg groups) using NONMEM software. Population estimates for CL,  $\text{CL}_d$ ,  $V_1$ , and  $V_2$  were used for subsequent pharmacokinetic/pharmacodynamic modeling of tumor progression data.

#### Xenograft mice efficacy studies

The efficacy studies in mice were approved by IACUC and conducted at Genentech, Inc.. Antitumor xenograft mouse efficacy studies were conducted with the KP4 HGF/MET autocrine pancreatic tumor model (19) to determine the concentration-effect relationship for onartuzumab. Tumors were established as described previously (19) by subcutaneously inoculating female nude (nu/nu) mice (ages 6–8 weeks) with  $5 \times 10^6$  KP4 cells suspended in  $1 \times$  Hank's balanced salt solution. All treatment studies began when tumors reached volumes of between 150 and

250 mm<sup>3</sup>. Two xenograft efficacy studies were conducted. First, a dose-response study was carried out using single intravenous doses of onartuzumab at 0, 1, 3, 7.5, 15, 30, 60, or 120 mg/kg ( $n = 10/\text{group}$ ). Second, a dose-time fractionation study was carried out in which mice were given total onartuzumab doses of 2.5, 7.5, or 30 mg/kg fractionated into once-weekly (Q1W), once-every-2-week (Q2W), or Q3W regimens ( $n = 10/\text{group}$ ). For example, a 30 mg/kg total dose was given as 10 mg/kg Q1W, 15 mg/kg Q2W, or 30 mg/kg Q3W for a total of 3 weeks. Tumor volumes were approximated using digital calipers (Fred V. Fowler Company, Inc.) and the formula  $(L \times W \times W)/2$ , where  $L$  = tumor length, and  $W$  = tumor width.

#### Immunoprecipitation and immunoblotting to measure phosphorylated and total MET in KP4 tumors

KP4 tumors from animals treated with a single intraperitoneal dose of onartuzumab at 15 mg/kg were collected to measure phosphorylated MET (p-MET) and total MET levels in tumors at predose, 1, 3, 6, 12 hours post-dose and 1, 2, 3, 5, 7, and 14 days post-dose. The immunoprecipitation and immunoblotting were conducted as previously described (20).

#### Estimation of tumoristatic concentrations in KP4 xenograft mice

A tumor growth inhibition model (Supplementary Fig. S1A) using NONMEM software was used to describe onartuzumab antitumor activity in KP4 xenograft mice using pharmacokinetic data from mice pharmacokinetic studies and efficacy data from the antitumor efficacy studies.

In this model, the population pharmacokinetic parameter estimates for  $CL$ ,  $CL_d$ ,  $V_1$ , and  $V_2$  were fixed from the pharmacokinetic modeling analysis and were used to generate onartuzumab concentrations that drove tumor response. Pharmacodynamic parameters of net tumor growth rate in absence of drug (KGN), half maximal tumor growth inhibitory concentration of onartuzumab ( $IC_{50}$ ), and maximal tumor growth inhibition effect constant by onartuzumab ( $I_{Max}$ ) were estimated by the model. The TSC is the predicted onartuzumab concentration at which there is no net tumor growth and was calculated using the differential equation describing tumor mass (Supplementary Fig. S1B), where  $dTM(t)/dt = 0$ . The TSC for each mouse in the dataset ( $n = 177$ ) was calculated using the individual parameter estimates for  $IC_{50}$  and  $I_{Max}$  and then the median TSC value was derived.

Pharmacokinetic and pharmacokinetic/pharmacodynamic model building was conducted using the first-order conditional estimation method with INTERACTION using NONMEM 7 (27). Tumor mass data were log-transformed. Log-normal parameter distributions were used for IIV, where the parameter for an  $i$ th individual was represented by parameter <sub>$i$</sub>  = typical value  $\cdot \exp(\eta_i)$ , where  $\eta_i$  represents the IIV. The residual error was modeled as a proportional error plus additive error. The objective function value (OFV) was used for the comparison of hierarchical models, using the log-likelihood ratio test. A difference in OFV of less than

3.84, corresponding to a significance level of  $P$  less than 0.05, was used for discrimination between two nested models that differed in one parameter.

#### Estimation of human pharmacokinetic profile and clinical target dose range

Clinical onartuzumab serum concentration-time profiles in humans were projected by the species-invariant time method (28) using serum concentrations in cynomolgus monkeys at doses of 0.5, 3, 10, or 30 mg/kg. Scaling exponents of 0.75, 0.85, or 0.9 were used for  $CL$  and a scaling exponent of 1 was used for the volume in the equations below (28, 29).

$$\text{Time}_{\text{human}} = \text{Time}_{\text{cyno}} \left( \frac{\text{Bodyweight}_{\text{human}}}{\text{Bodyweight}_{\text{cyno}}} \right)^{\text{Exponent}_{\text{volume}} - \text{Exponent}_{\text{clearance}}}$$

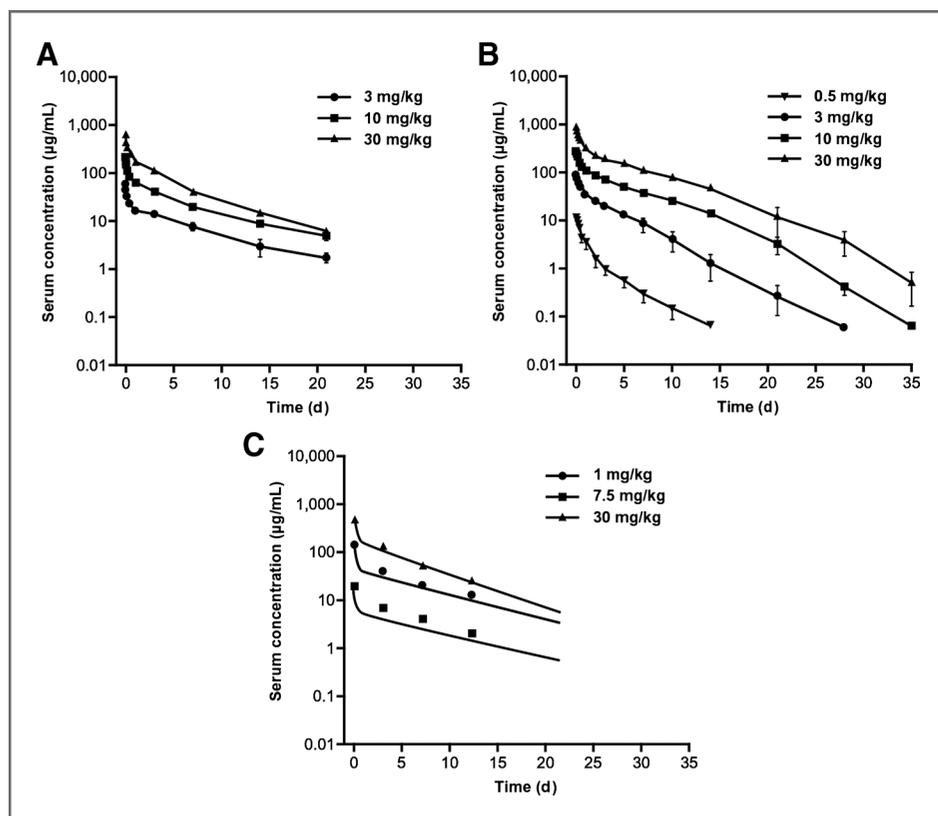
$$\text{Concentration}_{\text{human}} = \text{Concentration}_{\text{cyno}} \left( \frac{\text{Dose}_{\text{human}}}{\text{Dose}_{\text{cyno}}} \right) \times \left( \frac{\text{Bodyweight}_{\text{cyno}}}{\text{Bodyweight}_{\text{human}}} \right)^{\text{Exponent}_{\text{volume}}}$$

The projected human serum concentration-time data obtained from this method were analyzed by both NCA and the same model comprising parallel linear and non-linear  $CL$  pathways as described for the cynomolgus monkey pharmacokinetic analysis using NONMEM software. The estimated human onartuzumab pharmacokinetic parameters were used for simulations to determine the percentage of patients achieving  $C_{\text{trough ss}} \geq \text{TSC}$  with a Q3W regimen at various doses. For the simulations, 30% interindividual variability on  $V_{\text{max}}$ ,  $CL$ , and  $V_1$  for pharmacokinetics was incorporated on the basis of what is generally observed in humans for mAbs (30).

## Results

### Pharmacokinetics in mice and cynomolgus monkeys

The serum concentration-time profile for onartuzumab was determined from pharmacokinetic studies using single intravenous bolus doses in mice and cynomolgus monkeys (Fig. 1A and B). The pharmacokinetic parameter estimates from NCA indicate that within the linear-dose exposure range (3–30 mg/kg for mice and 10–30 mg/kg for cynomolgus monkeys) mean  $CL$  values were approximately 21.1 and 12.2 mL/d/kg in mice and cynomolgus monkeys with  $t_{1/2}$  at 6.10 and 3.37 days, respectively (Table 1). The maximum predicted serum concentration ( $C_{\text{max}}$ ) and  $AUC_{\text{inf}}$  were proportional to dose. However, in cynomolgus monkeys at doses of 0.5 and 3 mg/kg, onartuzumab  $AUC_{\text{inf}}$  was not dose proportional with fastest  $CL$  at the lowest dose tested (0.5 mg/kg). Analysis using a nonlinear two-compartment model indicated that the nonspecific  $CL$  in cynomolgus monkeys is 9.81 mL/d/kg and estimated  $V_1$  approximated serum volume (Table 2). These data suggest that in the linear dose range, the  $CL$  of onartuzumab is approximately two times faster than that observed for typical bivalent glycosylated mAbs (28).



**Figure 1.** Mean ( $\pm$ SD) serum concentration–time profiles following a single intravenous bolus dose of onartuzumab in mice and cynomolgus monkeys. A, mice ( $n = 3$ /time point). Animals were dosed at 3 (circle), 10 (square), or 30 (upright triangle) mg/kg and serum collected at the selected time points for pharmacokinetic analysis. B, cynomolgus monkeys ( $n = 3$ –4/group). Animals were dosed at 0.5 (inverted triangles), 3 (circle), 10 (square), or 30 (upright triangle) mg/kg and serum collected at the selected time points for pharmacokinetic analysis. C, observed (symbols) and predicted (lines) serum concentrations after a single intravenous dose of onartuzumab at 1, 7.5, or 30 mg/kg in KP4 tumor-bearing mice ( $n = 5$ /time point) and nontumor-bearing mice, respectively. Lines are predicted onartuzumab concentrations using pharmacokinetic parameters generated from serum concentration in nontumor-bearing mice in A. Error bars are  $\pm 1$  SD.

The possible impact on pharmacokinetics by ATAs was assessed in cynomolgus monkeys. Three monkeys dosed with 10 mg/kg onartuzumab and two monkeys dosed with 30 mg/kg onartuzumab tested positive for ATAs on day 35 (with a total incidence of positive ATAs in five of 16 treated cynomolgus monkeys). ATAs were determined to be directed toward the onartuzumab CDR and the titer values ranged from 1.95 to 2.34 in the 10 mg/kg dose group and from 2.59 to 2.86 in the 30 mg/kg dose group. The presence of ATAs seemed to affect onartuzumab serum concentrations on day 35 for these animals. However, the presence of ATAs had minimal impact on total onartuzumab exposure in these monkeys and had little effect on our ability to estimate

individual and group mean pharmacokinetic parameters (data not shown).

Onartuzumab serum concentrations from KP4 tumor-bearing mice were similar to the nontumor-bearing mice (Fig. 1C), indicating that the presence of the KP4 tumor did not affect the pharmacokinetics profiles within the dose range of 1 to 30 mg/kg. Therefore, the more robust results from the pharmacokinetic analysis in nontumor-bearing mice were deemed appropriate for concentration–effect modeling.

#### Antitumor efficacy in KP4 tumor-bearing mice

Both autocrine and paracrine tumor efficacy studies have been used to evaluate the inhibition of tumor growth by

**Table 1.** Summary of pharmacokinetic parameters for onartuzumab in mice and cynomolgus monkeys with noncompartmental analysis

Species	Dose (mg/kg)	$C_{max}$ ( $\mu$ g/mL)	$AUC_{inf}$ ( $d \cdot \mu$ g/mL)	CL (mL/d/kg)	$V_{ss}$ (mL/kg)	$t_{1/2}$ (d)
Athymic nude mouse ( $n = 3$ /time point)	3	59.9	162	18.6	146	5.95
	10	203	514	19.4	156	7.22
	30	669	1,180	25.4	131	5.13
Cynomolgus monkey ( $n = 4$ )	0.5	11.7 $\pm$ 1.59	13.1 $\pm$ 3.15	34.8 $\pm$ 8.59	81.2 $\pm$ 17.4	2.53 $\pm$ 0.278
	3	91.8 $\pm$ 3.08	190 $\pm$ 27.3	15.3 $\pm$ 2.62	55.6 $\pm$ 3.79	2.39 $\pm$ 0.300
	10	300 $\pm$ 86.5	786 $\pm$ 88.8	11.9 $\pm$ 1.31	64.5 $\pm$ 5.50	3.21 $\pm$ 0.489
	30	876 $\pm$ 107	2,330 $\pm$ 131	12.4 $\pm$ 0.659	73.8 $\pm$ 7.69	3.53 $\pm$ 0.819

**Table 2.** Summary of pharmacokinetic parameters for onartuzumab in cynomolgus monkeys and projected pharmacokinetic parameters in humans with nonlinear two-compartment model analysis

	Observed for cynomolgus monkeys		Projected pharmacokinetic parameters for humans					
			Scaling exponent for CL					
	Estimate	(% RSE)	Estimate	(% RSE)	Estimate	(% RSE)	Estimate	(% RSE)
$V_{max}$ ( $\mu\text{g}/\text{d}/\text{kg}$ )	152	(51.4)	107	(64.8)	90.3	(14.0)	63.8	(4.40)
$K_m$ ( $\mu\text{g}/\text{mL}$ )	4.89	(56.9)	4.80	(43.1)	4.75	(36.4)	4.66	(0.670)
$CL_L$ ( $\text{mL}/\text{d}/\text{kg}$ )	9.81	(15.7)	7.16	(53.5)	6.12	(6.14)	4.46	(5.54)
$V_1$ ( $\text{mL}/\text{kg}$ )	36.9	(2.93)	36.9	(20.8)	36.9	(2.79)	36.9	(0.854)
$CL_d$ ( $\text{mL}/\text{d}/\text{kg}$ )	39.8	(11.6)	28.8	(12)	24.6	(11.7)	17.8	(13.3)
$V_2$ ( $\text{mL}/\text{kg}$ )	50.8	(6.16)	50.6	(17.9)	50.5	(8.32)	50.4	(0.685)

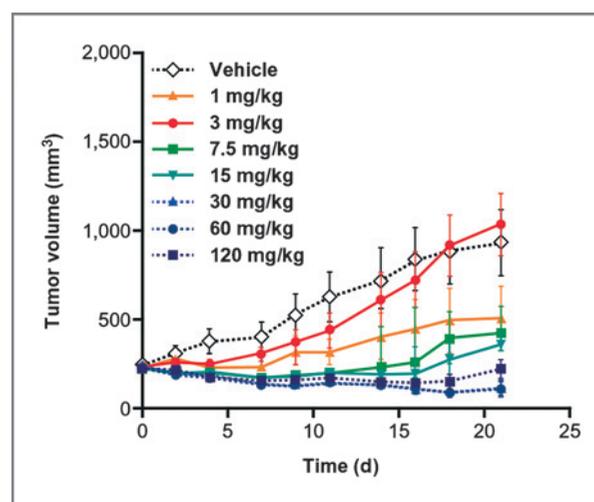
RSE = relative SE.

onartuzumab in mice. Because of the ease of use of auto-crine models, the HGF/MET autocrine ductal pancreatic cancer cell line, KP4, was used as a representative model to study the concentration–effect relationship of onartuzumab. This choice was supported by previous studies in paracrine tumor models. Similar efficacy was observed with onartuzumab in the BxPC-3 pancreatic xenograft model where paracrine human HGF was provided by osmotic pump (19). In addition, onartuzumab had similar efficacy in human-HGF-transgenic C3H-SCID mice (hHGFTg-C3H-SCID) bearing NCI-H596 tumors (31).

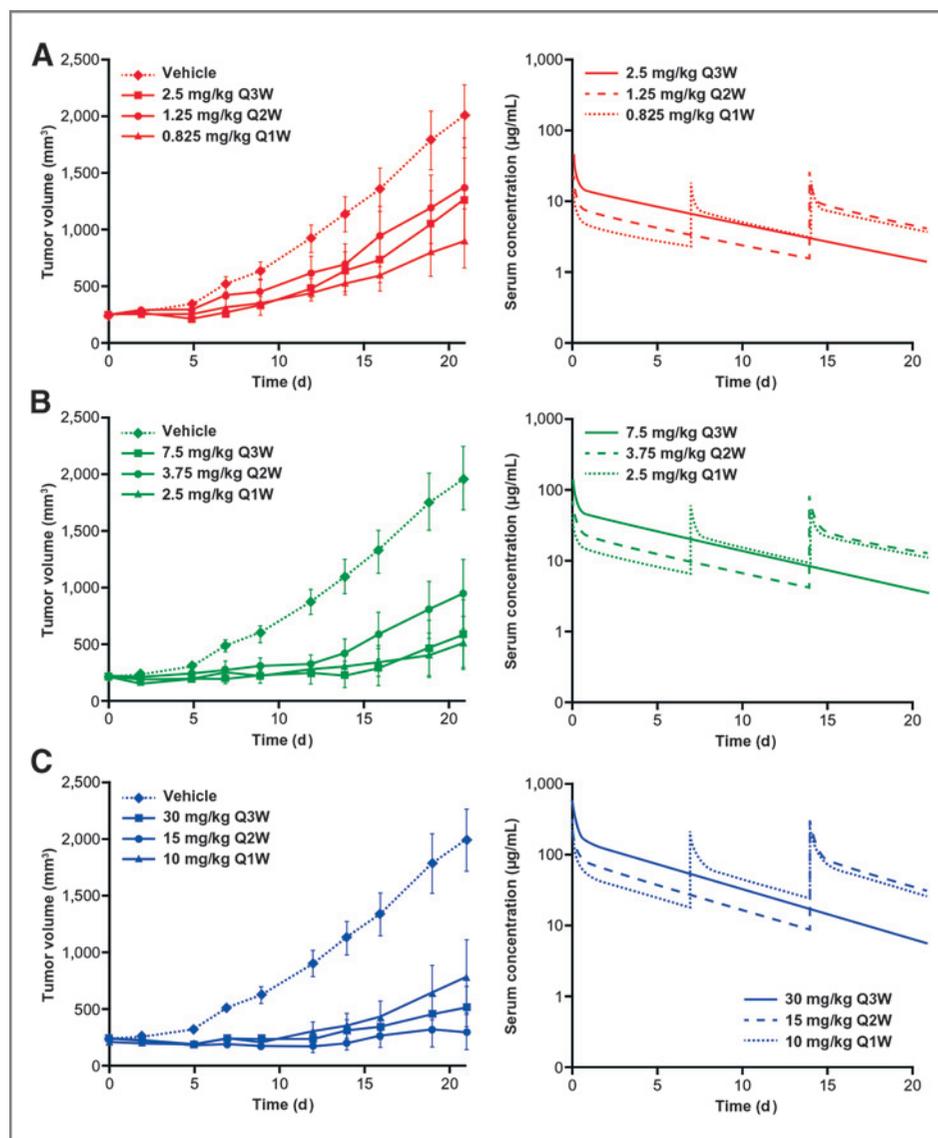
Onartuzumab inhibited tumor growth in a dose-dependent manner at doses ranging from 1 to 120 mg/kg (Fig. 2) except at doses 1 and 3 mg/kg. At these doses, the serum concentration was lower than TSC of 15  $\mu\text{g}/\text{mL}$  most of the time. Given the large variability in  $IC_{50}$  among mice, these doses are such that antitumor responses are also highly variable, which may explain the outliers in these groups. The group mean tumor volume on day 21 was used to generate a dose–response profile (data not shown) to select doses to be tested in a dose–time fractionation study. The dose–time fractionation study tested minimal, median, and maximal efficacious doses (2.5, 7.5, and 30 mg/kg, respectively) with different dose regimens to evaluate the impact on efficacy in the KP4 model. The study indicated that at low doses (total dose of 2.5 mg/kg), the Q1W dose schedule, which has a higher  $C_{trough}$ , had a greater effect on tumor growth inhibition than the other two-dose regimens. This suggested that there may be a threshold onartuzumab concentration that needs to be maintained to show tumor inhibition (Fig. 3A). However, at higher total doses (7.5 or 30 mg/kg), all dose levels and schedules showed similar degrees of efficacy, indicating that, at a similar total efficacious dose, the schedule of administration within a 3-week range has minimal impact on efficacy (Fig. 3B and C). The simulated serum concentration–time profile data for doses at 2.5, 7.5, and 30 mg/kg (Fig. 3A–C, respectively) indicated that AUC was similar for each total administered dose regardless of regimen. In addition, the  $C_{max}$  decreased with

more frequent dosing (Q3W>Q2W>Q1W), whereas  $C_{trough}$  increased with more frequent dosing (Q1W>Q2W>Q3W). Because the efficacy was similar for doses at 7.5 and 30 mg/kg with Q1W to Q3W dose regimen, these data imply that  $C_{max}$  is not the main driver for efficacy.

The effects of onartuzumab on p-MET and total MET levels in KP4 tumors were assessed using a single intraperitoneal dose at 15 mg/kg (Fig. 4), which had similar exposure as intravenous dosing due to the 100% bioavailability for onartuzumab (data not shown). Consistent with what was reported in the U-87 MG tumor model (20), onartuzumab



**Figure 2.** Group mean ( $\pm$ SE) tumor volume–time profiles in KP4 xenograft mice. Following a single intravenous bolus dose of onartuzumab with doses from 1 to 120 mg/kg in KP4 tumor-bearing nude mice; tumor volume was measured on the indicated days and is reported as the mean ( $\pm$ SE) for each group.  $n = 10$  per group. A few mice from the vehicle, 1 and 3 mg/kg groups had tumors that grew too large and these animals had to be removed from the study on the following days: two mice on day 12, one on day 16, and one on day 18 in the vehicle group; one on day 14 and one on day 18 in the 1 mg/kg group; one on day 14, one on day 16, and two on day 18 in the 3 mg/kg group. The last tumor measurement was carried forward for these mice to calculate group means.



**Figure 3.** Group mean ( $\pm$ SE) tumor volume–time and corresponding estimated serum concentration–time profiles following an intravenous bolus dose of onartuzumab with different dosing regimens in KP4 xenograft mice. A, tumor volume–time profile and estimated serum concentration using pharmacokinetic parameters obtained from pharmacokinetic study in nontumor-bearing mice following a total intravenous onartuzumab dose at 2.5 mg/kg with Q1W, Q2W, or Q3W dosing. B, tumor volume–time profile and estimated serum concentration–time profile using pharmacokinetic parameters obtained from pharmacokinetic study in nontumor-bearing mice following a total intravenous onartuzumab dose at 7.5 mg/kg with Q1W, Q2W, or Q3W dosing. The vehicle group is the same as in A. C, tumor volume–time profile and estimated serum concentration–time profile using pharmacokinetic parameters obtained from pharmacokinetic study in nontumor-bearing mice following a total intravenous onartuzumab dose at 30 mg/kg with Q1W, Q2W, or Q3W dosing. The vehicle group is the same as in A. The pharmacokinetic parameters used for above simulations are:  $CL = 21.6$  mL/d/kg,  $CL_d = 190$  mL/d/kg,  $V_1 = 48.8$  mL/kg, and  $V_2 = 90.7$  mL/kg. Tumor volume was measured on indicated days and is reported as the mean ( $\pm$ SE) for each group. Animals were dosed on day 0 for Q3W dosing; days 0 and 14 for Q2W dosing; and days 0, 7, and 14 for Q1W dosing.  $n = 10$  per group. One animal from vehicle control and one from 1.25 mg/kg Q2W groups had tumors that grew too large and the animals were taken off study on day 19. The tumor volume on day 19 was carried forward to day 21 to calculate group mean tumor volume.

induced inhibition of MET phosphorylation as early as 3 hours post-dose, which lasted at least 7 days. However, at the latest time point tested (14 days), p-MET levels started to rebound (Fig. 4). In a parallel efficacy study using a single dose of 15 mg/kg onartuzumab, tumors remained static through day 14 and began to grow between day 14 and 21. Therefore, the initial inhibition of p-MET is correlated with efficacy.

#### Estimation of tumoristatic concentrations in KP4 xenograft mice

A tumor growth inhibition model was used to describe onartuzumab antitumor activity in KP4 xenograft mice using pharmacokinetic data from nontumor-bearing mice and antitumor activity from dose–response and dose–time fractionation studies (Supplementary Fig. S1A). The pharmacokinetic parameter estimates used

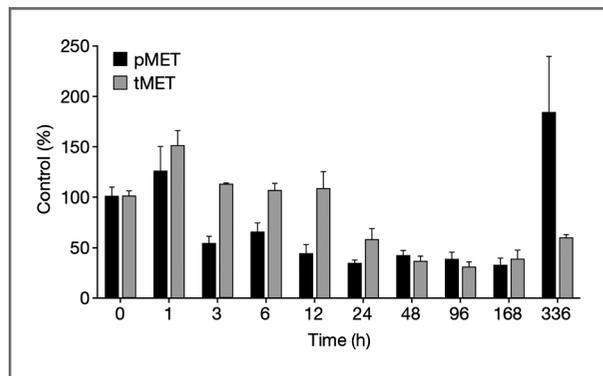


Figure 4. Onartuzumab effectively decreases p-MET and total MET in KP4 tumors. Immunoprecipitation-immunoblot analysis of p-MET (IP MET and IB p-Try) and total MET (IP and IB MET) in KP4 tumors collected at indicated time points following a single intraperitoneal dose of onartuzumab at 15 mg/kg.  $N = 4$ /time; pMET = p-MET; tMET = total MET.

were from a two-compartment modeling analysis of nontumor-bearing mouse pharmacokinetic data:  $CL = 21.6$  mL/d/kg,  $CL_d = 190$  mL/d/kg,  $V_1 = 48.8$  mL/kg, and  $V_2 = 90.7$  mL/kg. As pharmacokinetic samples were collected from different mice at different time points in the pharmacokinetic studies, the data were pooled and only a single set of pharmacokinetic parameters was calculated. From the pharmacokinetic/pharmacodynamic model, the parameter estimates with inter-individual variability were:  $KGN = 0.016$ /day (33.2%),  $I_{Max} = 1.86$  (28.3%), and  $IC_{50} = 13.2$   $\mu$ g/mL (236%). The relative SE for each parameter was 7.6% and 28%, respectively.  $KGN = 0.016$ /day was fixed to 0.016 based on a separate analysis of the model fit only to the control group. Basic goodness-of-fit plots and representative fits are presented in Supplementary Figs. S2, S3, and S4. As expected, 1 mg/kg was an outlier due to the unexpected original data (Fig. 2 and Supplementary Fig. S4). The median TSC value derived from all mice was approximately 15  $\mu$ g/mL with the TSC distribution presented in Supplementary Fig. S5. Theoretically, a continuous intravenous infusion maintaining a 15  $\mu$ g/mL onartuzumab concentration would achieve tumor stasis in the typical tumor-bearing KP4 mouse. Therefore, a dose maintaining  $C_{trough\ ss} \geq 15$   $\mu$ g/mL is a conservative target for clinical development.

#### Projection of disposition in human and clinical target dose

A species-invariant time method was used to scale onartuzumab serum concentration-time profiles from the single intravenous dose pharmacokinetic study in cynomolgus monkeys to humans. The estimated human serum concentration-time data were analyzed by both NCA and a nonlinear two-compartment pharmacokinetic model with parallel linear (nonspecific) and specific (target-mediated) CL. All pharmacokinetic parameters were estimated with reasonable precision with relatively small CV% for each parameter. Onartuzumab pharmacokinetic parameter esti-

mates by nonlinear two-compartment model analyses for humans are summarized for CL exponents at 0.75 (slow CL), 0.85 (median CL), and 0.9 (fast CL) in Table 2. The application of different scaling exponents for CL provides a range of estimated CL in humans. The estimated CL range by NCA in the linear dose range for humans was 5.74 to 9.36 mL/d/kg, which is approximately two times faster than a typical human IgG1 with the Genentech IgG backbone (28). The nonlinear two-compartment model analysis indicated that nonspecific CL in humans ranged from 4.46 to 7.16 mL/d/kg. Estimated values for the  $V_{max}$  and  $K_m$  for the target-mediated CL pathway were 63.8 to 107  $\mu$ g/d/kg and 4.66 to 4.80  $\mu$ g/mL, respectively.

Simulations were conducted to estimate the percentage of patients achieving  $C_{trough\ ss} \geq TSC$  (15  $\mu$ g/mL) using the three sets of pharmacokinetic parameters from 0.75, 0.85, and 0.9 scaling exponents of CL (Supplementary Table S1). On the basis of the simulations, 10 to 30 mg/kg onartuzumab Q3W are predicted to achieve  $C_{trough\ ss}$  of 15  $\mu$ g/kg in 95% of patients. The projected target dose range is based on the potential clinical pharmacokinetic variability, in the absence of an estimate of variability for clinical pharmacodynamics.

#### Discussion

Our study indicates that onartuzumab has linear CL in mice; a species in which onartuzumab does not bind host MET. In cynomolgus monkeys where onartuzumab binds host MET, CL is nonlinear and saturable. Preclinical pharmacokinetic and pharmacokinetic/pharmacodynamic modeling provided projected human PK and a target TSC estimated from xenograft mice. An efficacious onartuzumab dose range was determined and used in the onartuzumab clinical development program.

The elimination mechanism of an antibody comprises target-mediated (via specific antibody-ligand binding) and target-independent (i.e., nonspecific Fc-receptor mediated elimination) pathways (30, 32). The model-derived pharmacokinetics parameter  $V_{max}$  reflected on the abundance of target. The  $V_{max}$  of onartuzumab in cynomolgus monkeys at  $152 \pm 54.4$   $\mu$ g/d/kg is consistent with its modest pharmacokinetic nonlinearity and its expression in normal tissues (10, 11, 33, 34). The target-independent CL pathway should be similar for antibodies with the same Fc framework and similar binding affinity to FcRn. The CL of onartuzumab is approximately two times faster than that of typical bivalent glycosylated antibodies with the Genentech consensus sequence framework (28). The characteristics of aglycosylation, size, and "knob" and "hole" mutations within the  $C_H3$  domain of the Fc of onartuzumab are the main structural differences between onartuzumab and a typical Genentech IgG1 that could contribute to its faster CL in animals. There are no publications that clearly implicate aglycosylation as the reason for the rapid CL observed in animals. Glomerular filtration can affect CL of small proteins, but not onartuzumab, with a molecular weight of 99 kDa (35). "Knob" and "hole" mutations

within the C<sub>H</sub>3 domain might be expected to affect CL if they enhanced ATA production or altered antibody-binding affinity to FcRn. The ATAs developed against onartuzumab in cynomolgus monkeys had minimal impact on the overall estimation of pharmacokinetic parameters. Furthermore, there was no evidence that ATAs were directed against the "knob" or "hole" regions of onartuzumab, rather ATAs were directed against the onartuzumab CDR. Onartuzumab binds to FcRn with relative affinity similar to that of trastuzumab (20). Finally, because of its unique structure and production characteristics it is not possible to comment on whether onartuzumab has a regular pharmacokinetic profile.

The cynomolgus monkey has been identified as a suitable species to scale mAb CL and volume of distribution in humans as they have similar disposition and elimination pathways (28, 29). The selection of cynomolgus monkeys was also favored because onartuzumab binds to human and cynomolgus monkey MET with similar affinity (1.5 and 4.4 nmol/L, respectively), but does not bind to rodent MET (data not shown). Consequently, linear and nonlinear pharmacokinetics were observed in rodents and cynomolgus monkeys, respectively. For these reasons, pharmacokinetics in humans was projected using pharmacokinetic data from only the cynomolgus monkeys. Pharmacokinetic data in humans indicated that, in the linear dose range with doses equal to 4 mg/kg or more, the actual mean CL values were in the range of 6.8 to 9.9 mL/d/kg (36), which overlapped with the projected human CL values; thus confirming that the species-invariant time method from cynomolgus monkey data is an appropriate scaling method. In addition, the observed mean ( $\pm$ SD) human pharmacokinetic data from the phase I study was within the range estimated from cynomolgus monkey data using the scaling exponent of CL at 0.75 and 0.9 (Supplementary Fig. S6) including the lowest doses tested, 1 mg/kg, with significant target-mediated CL (36). More examples will be needed to confirm the general suitability of this model for monovalent aglycosylated antibodies.

The underlying purpose of performing nonclinical efficacy studies is to translate the observed activity of a therapeutic candidate molecule to humans with reasonable accuracy and confidence to support clinical development. To achieve this, the first step is to understand the driver of efficacy in animal models. For example,  $C_{\text{trough}}$  values for both bevacizumab and trastuzumab-DM1 were targeted on the basis of the understanding of their mode of action (6, 38). The assumptions underlying the use of the mouse TSC value as a target trough serum concentration for clinical dosing with onartuzumab were that:

1. There is a concentration–response relationship for onartuzumab in the KP4 model. Hence, the  $C_{\text{trough}}$  is an important factor in efficacy outcomes for onartuzumab.
2. The clinical tumor response relationships between onartuzumab exposure and antitumor activity would

be similar to what was observed in the KP4 athymic xenograft mouse.

3. Onartuzumab pharmacokinetics in humans can be predicted from cynomolgus monkey pharmacokinetics using a species-invariant time scaling method.

The following points were considered to support the first assumption. A concentration–response relationship was observed for onartuzumab in the KP4 model and is consistent with the receptor saturation–efficacy hypothesis. However, total drug exposure is another important determinant of the efficacy of onartuzumab in the KP4 model. Basing the target on  $C_{\text{trough}}$  versus AUC for the clinical program is a more conservative approach to maintain target receptors fully occupied irrespective of occupancy and turnover times. Although TSC was estimated from only one tumor model, the selection of this model is considered valid as similar efficacy was observed at the same dose level with other tumor models tested for onartuzumab (19, 31). There are limitations for the second assumption. It is expected that the tumor type, the nature of autocrine and paracrine tumors, and the location of tumors in patients will affect the perfusion of onartuzumab in these tumors, which will contribute to their sensitivities to onartuzumab. Therefore, we projected to have 95% of patients to achieve TSC of 15  $\mu$ g/mL at  $C_{\text{trough ss}}$  as a conservative approach.

On the basis of these assumptions and pharmacokinetic variability observed clinically for mAbs (i.e., 30% inter-individual variability on  $V_{\text{max}}$ , CL, and  $V_1$ ; ref. 30), an onartuzumab dose in the range of 10 to 30 mg/kg was projected to achieve a  $C_{\text{trough ss}}$  above the TSC of 15  $\mu$ g/mL in 95% of patients with a Q3W dose regimen (Supplementary Table S1). Therefore, 1 to 30 mg/kg Q3W was selected as the dose range in a phase I trial to determine which dose would achieve the  $C_{\text{trough ss}}$  target in 95% of patients (25). Phase I pharmacokinetic data indicated that 15 mg/kg Q3W would achieve  $C_{\text{trough ss}}$  in more than 90% of patients. Ninety-five percent of these patients achieved AUC observed at 30 mg/kg in mice after first dose. Therefore, 15 mg/kg Q3W was selected for testing in the phase II study (36, 37).

In the phase II study in patients with advanced NSCLC where onartuzumab was administered with erlotinib, onartuzumab 15 mg/kg Q3W resulted in  $C_{\text{trough ss}} \geq 15 \mu$ g/mL in 90% or more of patients (36, 37). Importantly, this dose was well tolerated and resulted in statistically significant and clinically meaningful improvements in both progression-free survival and OS in the MET-positive subgroup (26). However, there are no dose-ranging studies in the clinic to confirm that the TSC obtained from mice translates directly to clinical tumor response. The primary reason for using nonclinical data to guide dose selection for therapeutic mAbs relies on observations that (i) efficacy is rarely observed in phase I and (ii) toxicity profiles may not be limiting for ultimate dose selection. Thus, using nonclinical pharmacokinetics and efficacy data to support dose and

regimen selection in the clinic has value in therapeutic mAb development.

Taken together, the nonclinical studies used to determine the target dose and schedule paired with the encouraging clinical observations serve as a pertinent example of the use of nonclinical data to support dose and regimen selection for both pharmacokinetics and efficacy projection.

### Disclosure of Potential Conflicts of Interest

H. Xiang is employed as a scientist in Genentech, Inc/Roche and has ownership interest (including patents) in the Genentech, Inc/Roche stock. A. E. Reyes II is employed as a senior research associate in Genentech. M. Merchant is employed as a scientist in Genentech, Inc. and has ownership interest (including patents) in Roche. I. Nijem is employed as a senior research associate in Genentech and has ownership interest (including patents) in Roche stock. E. Mai is employed as a senior research associate and has ownership interest (including patents) in Roche stock. A. Peterson was employed and has ownership interest (including patents) in Roche/Genentech. No potential conflicts of interest were disclosed by the other authors.

### Authors' Contributions

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**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** M. Merchant, M. Romero, I. Nijem

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** H. Xiang, B.C. Bender, A.E. Reyes II, M. Merchant, N. 'Shasha' Jumbe, J. Young, A. Peterson, L.A. Damico-Beyer

**Writing, review, and/or revision of the manuscript:** H. Xiang, B.C. Bender, A.E. Reyes II, M. Merchant, N. 'Shasha' Jumbe, I. Nijem, E. Mai, J. Young, A. Peterson, L.A. Damico-Beyer

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** H. Xiang, E. Mai

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