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ORIGINAL ARTICLE

Preclinical pharmacokinetics of MFGR1877A, a human monoclonal antibody to FGFR3, and prediction of its efficacious clinical dose for the treatment of t(4;14)-positive multiple myeloma

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Abstract
Purpose MFGR1877A is a human IgG1 monoclonal antibody that binds to fibroblast growth factor receptor 3 (FGFR3) and is being investigated as a potential therapy for relapsed/refractory FGFR3+ multiple myeloma. The purpose of these studies was to characterize the pharmacokinetics (PK) of MFGR1877A in mouse, rat, and monkey and predict its human PK and efficacious dose.
Methods PK of MFGR1877A was determined in athymic nude mice, Sprague–Dawley rats and cynomolgus monkeys after administration of single intravenous doses. Human PK profiles were projected from monkey PK profiles using a species-invariant time method, and human population PK parameters were estimated using a non-linear, two-compartment model comprising specific (target-mediated) and non-specific clearance pathways. The anti-tumor efficacy in mice bearing human tumor xenografts was used in conjunction with inhibitory activity in cell proliferation assays and human PK projections to estimate clinical efficacious dose.
Results The PK of MFGR1877A in mice was non-linear in the dose range of 1–50 mg/kg, while in rats and monkeys, PK was non-linear in the dose range of 1–10 mg/kg and linear at doses ≥10 mg/kg. The predicted non-specific clearance range in humans was 2.6–4.4 mL/day/kg. Doses ranging from 2 to 3 mg/kg weekly to 6–10 mg/kg every 4 weeks were predicted to achieve the target exposure in ≥90% of multiple myeloma patients.
Conclusions The predicted non-specific clearance of MFGR1877A in humans is similar to typical human IgG1 antibodies and will be verified in a Phase 1 study. The projected human efficacious dose and regimen appear to be achievable in patients.

Keywords FGFR3 · Multiple myeloma · Pharmacokinetics · Xenograft model

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Introduction

Fibroblast growth factor receptor 3 (FGFR3) is a cell surface receptor tyrosine kinase that regulates cell proliferation, survival, and migration [1, 2]. Aberrant expression or mutational activation of FGFR3 is implicated in several types of human cancer [1, 2]. In 15–20% of multiple myeloma patients, as a result of t(4;14) chromosomal translocation, FGFR3 is ectopically overexpressed and associated with poor prognosis [3, 4]. FGFR3 has been established as an important oncogenic driver and hence a potential therapeutic target in multiple myeloma [5–7]. FGFR3 is expressed as two major isoforms, FGFR3-IIIb

and FGFR3-IIIc, which differ by approximately 50 amino acids in the second half of Ig-like domain 3 (D3). These isoforms have distinct ligand specificity and tissue distribution; FGFR3-IIIb is primarily expressed in epithelial cells, and FGFR3-IIIc is generally expressed in mesenchymal cells [1, 8]. To block FGFR3 function in cancer, it is optimal to antagonize both isoforms and cancer-associated mutant variants of FGFR3.

MFGR1877A is a phage-derived human immunoglobulin (Ig)G1 monoclonal antibody (mAb) that binds to FGFR3 with high affinity. It inhibits ligand binding to FGFR3, prevents receptor–receptor association, and blocks signaling from both wild-type (WT) and common mutant variants of FGFR3 linked with cancer [9]. MFGR1877A suppresses FGFR3-mediated cell proliferation and exerts strong anti-tumor activity in mouse xenograft models of t(4;14)-positive multiple myeloma through direct inhibition of FGFR3 signaling and engagement of effector function activities such as antibody-dependent cell-mediated cytotoxicity (ADCC) [9, data not shown]. MFGR1877A is being investigated as a potential therapy for relapsed/refractory FGFR3+ multiple myeloma.

MFGR1877A binds to the Ig-like domain 2 and domain 3 (D2–D3) of FGFR3 from multiple species including mice, rats, cynomolgus monkeys, and humans. The binding affinity of MGFR1877A to FGFR3-IIIc in mice, rats, cynomolgus monkeys, and humans, as well as to human FGFR3-IIIb is similar. Although the binding affinity of MFGR1877A to rat and cynomolgus monkey FGFR3-IIIb was not evaluated, FGFR3-IIIb appears highly conserved across species based on the approximately 90% homology between mouse and human FGFR3 D3 sequence [8, 10]. In this report, we have characterized the pharmacokinetics (PK) of MFGR 1877A in mouse, rat, and monkey and predicted its human PK. In addition, efficacy data from a multiple myeloma xenograft model were combined with human PK estimates to project the efficacious dose in the clinic.

Materials and methods

Antibodies

MFGR1877A was generated at Genentech Inc. for the in vivo PK and efficacy studies.

Pharmacokinetic study in athymic nude mice

The PK study in athymic nude mice was approved by the Institutional Animal Care and Use Committee at Genentech, Inc. Female athymic nude mice received a single intravenous (IV) dose of 1, 10, or 50 mg/kg of MFGR1877A via the tail vein (n = 15/group). Blood samples were collected via retro-orbital bleeds performed on alternating eyes, and the terminal blood sample was collected via cardiac stick from each animal in each dosing group at the following time points: 10 min, 1, 3, 5, 8, and 24 h; and 2, 3, 7, 10, 14, 17, 21, and 28 days, and processed to collect serum. Three blood samples were taken from each mouse (two retro-orbital bleeds from alternate eyes and one terminal bleed), and there were three mice per time point. Composite serum concentration–time profiles were constructed for pharmacokinetic analysis.

Pharmacokinetic study in Sprague–Dawley rats

The PK study in Sprague–Dawley rats was approved by the Institutional Animal Care and Use Committee at Genentech, Inc. Three female Sprague–Dawley rats were assigned to each of three treatment groups and given a single IV bolus dose of 1, 10, or 50 mg/kg of MFGR1877A via the femoral vein. Blood samples for PK and anti-therapeutic antibodies (ATA) were collected via jugular catheter in the first 12 h after dosing, and then through the tail vein on days 1–21. At the final time point (day 28), blood was collected via cardiac puncture. PK samples were collected at the following time points: 15 min, 2, 4, 8, and 12 h; and 1, 2, 3, 4, 7, 10, 14, 21, and 28 days, while ATA samples were collected at pre-dose, 14 and 28 days, and processed to collect serum.

Pharmacokinetic study in cynomolgus monkeys

The PK study in cynomolgus monkeys was approved by the Institutional Animal Care and Use Committee and conducted at Covance Laboratories, Inc. (Madison, WI). Two male and 2 female cynomolgus monkeys were assigned to each of three treatment groups and given a single IV bolus dose of 1, 10, or 30 mg/kg of MFGR1877A via the saphenous vein. Blood samples (~1 mL) for PK and ATA analysis were collected from each animal via the femoral vein and processed to collect serum. PK samples were collected on day 0 (pre-dose, 10 and 45 min, and 2, 4, 7, and 12 h post-dose), and 1, 2, 4, 6, 9, 13, 20, 27, 34, and 41 days post-dose, while ATA samples were collected pre-dose and 13, 20, 27, 34, and 41 days post-dose.

Anti-tumor efficacy study in mice bearing KMS11 human multiple myeloma xenografts

The efficacy studies in mice were approved by the Institutional Animal Care and Use Committee at Genentech, Inc. Female C.B.-17 SCID mice (8 weeks old) were injected with 20 million human multiple myeloma KMS11 cells, suspended in 0.2 mL of 50% HBSS and 50% Matrigel, subcutaneously in the right flank. When tumors reached a

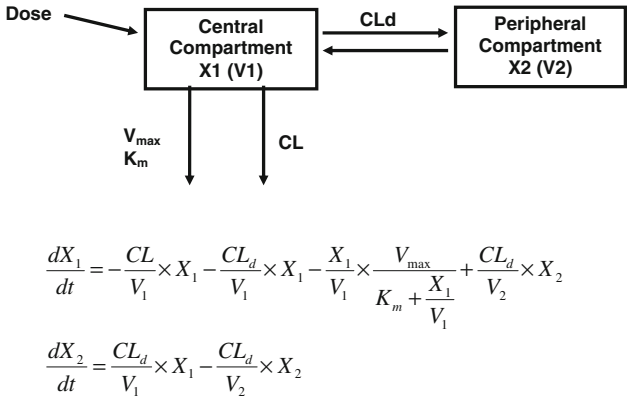
volume range of 119–288 mm^3, mice were randomized into seven groups ($n = 12$/per group) and received a single IV dose of vehicle (control group) or MFGR1877A (treatment groups) at doses of 0.1, 0.3, 1, 3, 10, and 30 mg/kg. For each group, blood samples were collected from the retro-orbital sinuses of 3 mice per time point at 15 min, 4 h, and 1, 7, 14, and 22 days post-dose and processed for serum. Composite serum concentration–time profiles were constructed for pharmacokinetic analysis. Tumors were measured twice each week for the duration of the study using UltraCal-IV calipers, and tumor volume was calculated using the following formula: tumor volume (mm^3) = (length × width^2) × 0.5. The results were shown as mean tumor volume ± standard error of the mean (SEM) for the time that at least half the animals in the group remained on study. Time to tumor-doubling (TTD) was calculated and is defined as the time in days for a tumor to double in volume from the day of randomization.

Bioanalysis of serum samples from pharmacokinetic and efficacy studies

Serum samples from athymic nude mice, SCID mice, Sprague–Dawley rats, and cynomolgus monkeys were analyzed for MFGR1877A concentrations using a quantitative enzyme-linked immunosorbent assay (ELISA). For the athymic mouse and SCID mouse serum samples, the ELISA utilized a wild-type human FGFR3-IIIC ECD protein with a histidine tag as the capture reagent and goat anti-hIgG1-Fc-horseradish peroxidase (HRP) as the detection reagent. The minimum quantifiable concentration (MQC) in this assay was 7.8 ng/mL. For Sprague–Dawley rat and cynomolgus monkey serum samples, the ELISA utilized a FGFR3-IIIC ECD protein with a histidine tag as the capture reagent and biotinylated 10C4 antibody (10C4.1-mouse anti-Genentech rhuMAb framework) and Avidin-HRP as the detection agents. The MQCs were 200 and 70 ng/mL for the rat and monkey serum assays, respectively.

Anti-therapeutic antibody (ATA) assay

A digoxigenin (DIG) format bridging ELISA was validated to detect antibodies to MFGR1877A in cynomolgus monkey serum and Spraque–Dawley rat serum in the PK studies. The assay used biotin-conjugated MFGR1877A capturing the antibodies into streptavidin-coated plates and DIG-conjugated MFGR1877A with mouse monoclonal anti-DIG–HRP conjugate as detection agents. The assay cutpoint was determined from a panel of therapeutic-naïve individual serum samples.



$$\frac{dX_1}{dt} = -\frac{CL}{V_1} \times X_1 - \frac{CL_d}{V_1} \times X_1 - \frac{X_1}{V_1} \times \frac{V_{max}}{K_m + \frac{X_1}{V_1}} + \frac{CL_d}{V_2} \times X_2$$

$$\frac{dX_2}{dt} = \frac{CL_d}{V_1} \times X_1 - \frac{CL_d}{V_2} \times X_2$$

Fig. 1 Two-compartment non-linear PK model

Pharmacokinetic data analysis

Serum concentration–time profiles were used to estimate the following PK parameters in mouse, rat, and monkey, using non-compartmental analysis (WinNonlin, version 5.2.1; Pharsight Corporation, Mountain View, CA): total drug exposure defined as area under the serum concentration–time curve extrapolated to infinity (AUCinf), total clearance (CLtot), volume of distribution at steady state (Vss), and observed maximum serum concentration (Cmax). For the mouse efficacy study, a limited number of time points were taken as compared to the PK study, and only AUCinf was reported. A naïve pooled approach was used in mouse to provide one estimate for each dose group, while in rat and monkey, each animal was analyzed separately and results for each dose group were summarized as mean ± standard deviation (SD).

In order to further characterize the non-linear pharmacokinetics in cynomolgus monkey, the monkey serum concentration–time data were fit to a non-linear, two-compartment model comprising specific (target-mediated) and non-specific CL pathways [11] as shown in Fig. 1, using population PK analysis (NONMEM, version VI, ICON Development Solutions, Ellicott City, MD) to obtain the following PK parameters: clearance from the central compartment (CL) that is the non-specific clearance pathway, distributional CL (CLd), volumes of distribution of the central compartment (V1) and the peripheral compartment (V2), and parameters of the specific clearance pathway that are maximum target-mediated elimination rate under conditions of target saturation (Vmax), and the concentration for reaching 50% Vmax (Km). The results were summarized as one estimate for each parameter and the % standard error of the estimate (% SEE).

Prediction of human PK and clinical target dose range

The monkey serum concentration–time profiles were transformed to human concentration–time profiles using a species-invariant time method as described previously [12, 13]. A scaling exponent of either 0.75 or 0.90 was used to estimate human CL, and a scaling exponent of 1 was used to estimate the volume of the central compartment (V1) [12, 14]. The projected human serum concentration–time data obtained from this method were used to predict population PK parameter estimates for humans using the PK model shown in Fig. 1. The estimated human PK parameters were then used to estimate the clinical dose and regimen to achieve the target efficacious exposure using population simulations by NONMEM. For the simulations, the inter-individual variability on Vmax, CL, and V1 was assumed to be 30% based on what is generally observed in humans for monoclonal antibodies [12, 15].

Two approaches were used to estimate clinical target efficacious dose and regimen for multiple myeloma based on the non-clinical data. The first approach was to achieve the efficacious exposure observed in the anti-tumor efficacy study in mice implanted with KMS11 human multiple myeloma xenografts in greater than 90% of patients. The second approach was to target a steady state trough concentration (Ctrough) that was tenfold of the in vitro 90% inhibitory concentration (IC90) in cell proliferation assays. The IC90 of MFGR1877A was determined in mouse pro-B Ba/F3 cells stably expressing FGFR3 including wild-type (WT) FGFR3 and five of the most common cancer-associated mutants of FGFR3 as shown previously by Qing et al. [9].

Results

Pharmacokinetics in athymic nude mice

The PK profiles of MFGR1877A following a single IV bolus dose in athymic nude mice at doses of 1, 10, and 50 mg/kg are shown in Fig. 2, and PK parameters are summarized in Table 1. As the dose increased from 1 to 50 mg/kg, AUCinf increased in a greater-than-dose-proportional manner. Total clearance (CLtot) decreased with the increased doses, going from 69.6 mL/day/kg at the 1 mg/kg dose to 11.7 mL/day/kg at the 50 mg/kg dose, and the Vss ranged from 40 to 84 mL/kg at the doses tested. These data suggest that the pharmacokinetics of MFGR1877A were non-linear in a dose range of 1–50 mg/kg in female athymic nude mice.

Pharmacokinetics in Sprague–Dawley rats

The PK profiles of MFGR1877A following a single IV bolus dose in Sprague–Dawley rats at doses of 1, 10, and 50 mg/kg are shown in Fig. 3, and PK parameters are summarized in Table 2. As the dose increased from 1 to 50 mg/kg, AUCinf increased more than dose proportionally from 1 to 10 mg/kg, and dose proportionally from 10 to 50 mg/kg. CLtot decreased as dose increased from 1 to 10 mg/kg and remained constant as dose increased from 10 to 50 mg/kg. The mean CLtot values in groups given 1, 10, or 50 mg/kg were 39.4, 14.5, and 14.8 mL/day/kg, respectively. Vss ranged from 53.4 to 63.7 mL/kg at the doses tested. ATAs were detected in 5 of 9 animals (56%) given MFGR1877A, though their presence appeared to have minimal impact on the PK parameter estimates. These data suggest that the pharmacokinetics of MFGR1877A in female Sprague–Dawley rats were non-linear in a dose range of 1–10 mg/kg and linear in a dose range of 10–50 mg/kg.

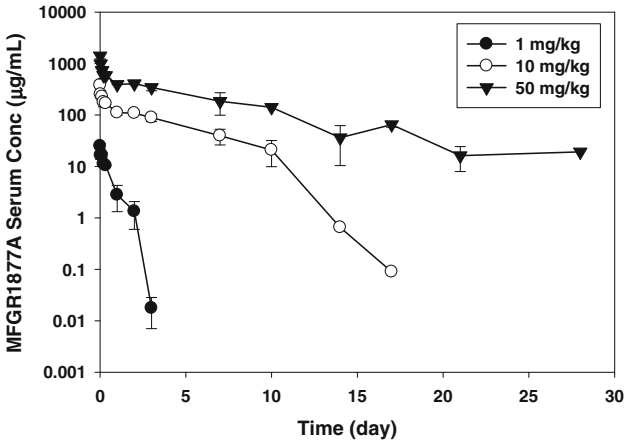


Fig. 2 MFGR1877A average serum concentration–time profiles in athymic nude mice after IV administration

Table 1 MFGR1877A pharmacokinetic parameters in athymic nude mice by non-compartmental analysis

Parameter	1 mg/kg	10 mg/kg	50 mg/kg
Measured dose (mg/kg)	0.832	15.4	44.6
Cmax (µg/mL)	25.0	379	1,410
AUCinf (day µg/mL)	12.0	765	3,830
CLtot (mL/day/kg)	69.6	20.1	11.7
Vss (mL/kg)	40.2	72.1	83.7

Pharmacokinetics in cynomolgus monkeys

The PK profiles of MFGR1877A following a single IV bolus dose in cynomolgus monkeys at doses of 1, 10, and 30 mg/kg are shown in Fig. 4, and PK parameters after non-compartmental analysis are summarized in Table 2. After a single IV bolus dose of 1, 10, or 30 mg/kg in cynomolgus monkeys, AUCinf increased more than dose proportionally from 1 to 10 mg/kg, and dose proportionally from 10 to

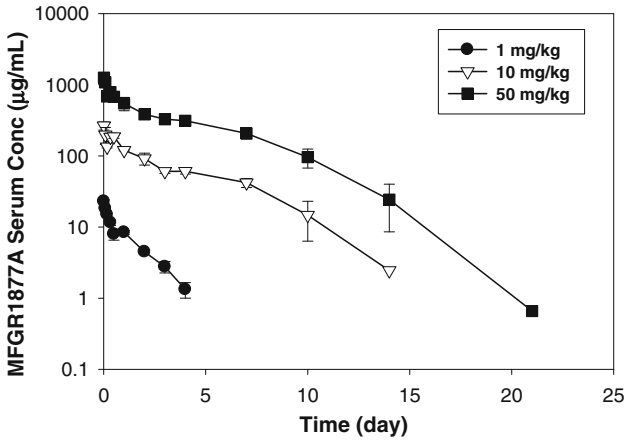


Fig. 3 MFGR1877A average serum concentration–time profiles in Sprague–Dawley rats after IV administration

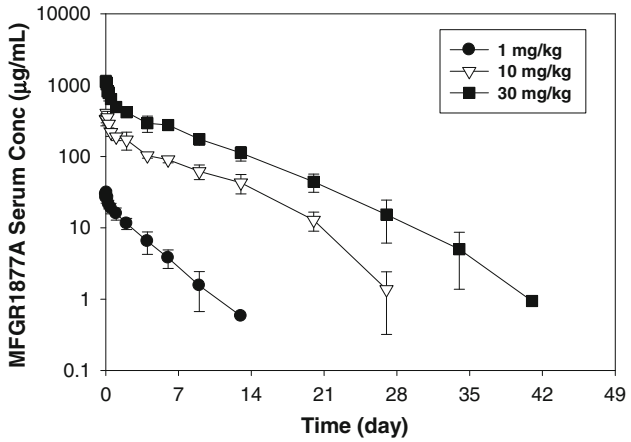


Fig. 4 MFGR1877A average serum concentration–time profiles in cynomolgus monkeys after IV administration

30 mg/kg. The mean CLtot values in groups dosed at 1, 10, and 30 mg/kg were 13.2, 6.37, and 6.69 mL/day/kg, respectively. The Vss ranged from 40.4 to 46.8 mL/kg at the doses tested. ATAs were detected in 1 of 12 (8%) animals given MFGR1877A, and their presence had minimal impact on the PK parameter estimates. These data indicate that MFGR1877A pharmacokinetics in cynomolgus monkeys were non-linear in a dose range of 1–10 mg/kg, and linear in a dose range of 10–30 mg/kg.

The monkey concentration–time profiles were described well by a non-linear, two-compartment model comprising specific (target-mediated) and non-specific CL pathways, and the estimated PK parameters are shown in Table 3. Using this model, the estimated non-specific CL was 6.02 mL/day/kg, which is in the expected range of clearance of a typical human IgG1 in monkeys [12]. Estimated values for the Vmax and Km for the specific CL pathway were 71 µg/day/kg and 2.55 µg/mL, respectively.

Table 3 MFGR1877A pharmacokinetic parameters in cynomolgus monkeys using a non-linear two-compartment model and population PK analysis

Parameter	Estimate	% SEE[a]
CL (mL/day/kg)	6.02	7.77
V1 (mL/kg)	28.6	4.97
Vmax (µg/day/kg)	71	27.9
Km (µg/mL)	2.55	56.9
CLd (mL/day/kg)	28	15.5
V2 (mL/kg)	25	8.4

[a] % Standard error of estimate

Anti-tumor efficacy in mice bearing KMS11 human multiple myeloma xenografts

MFGR1877A was evaluated for anti-tumor activity in the KMS11 human multiple myeloma xenograft model at 6 dose levels ranging from 0.1 to 30 mg/kg. This model was selected due to its previously characterized sensitivity to MFGR1877A [9]. As shown in Fig. 5, after a single IV dose, MFGR1877A showed substantial anti-tumor activity as determined by the tumor-doubling time (Table 4). Animals treated with vehicle or MFGR1877A at 0.1 or 0.3 mg/kg had tumors that doubled in size in 10.2 days. The tumor-doubling time increased to 12.7 days at 1 mg/kg of MFGR1877A and was more than twice that (26.7 days) at 3 mg/kg of MFGR1877A. At both higher doses (10 and 30 mg/kg), tumors from both groups had not doubled by the end of the study on day 33. Estimated AUCinf values at the various doses are shown in Table 4 and represent the total exposure to MFGR1877A after a single IV dose. The

Table 2 MFGR1877A pharmacokinetic parameters in Sprague–Dawley rats and cynomolgus monkeys by non-compartmental analysis

Parameter	Sprague–Dawley rats			Cynomolgus monkeys		
Dose (mg/kg)	1	10	50	1	10	30
Cmax (µg/mL)	23.0 ± 1.32	265 ± 34.6	1,260 ± 66.6	31.7 ± 2.55	423 ± 47.1	1,230 ± 140
AUCinf (day µg/mL)	25.5 ± 1.85	689 ± 44.8	3,420 ± 433	77.5 ± 12.8	1,602 ± 254	4,540 ± 558
CLtot (mL/day/kg)	39.4 ± 2.78	14.5 ± 1.02	14.8 ± 1.97	13.2 ± 2.19	6.37 ± 1.06	6.69 ± 0.80
Vss (mL/kg)	63.7 ± 5.00	53.4 ± 2.98	62.7 ± 6.90	46.2 ± 9.72	40.4 ± 3.25	46.8 ± 2.42

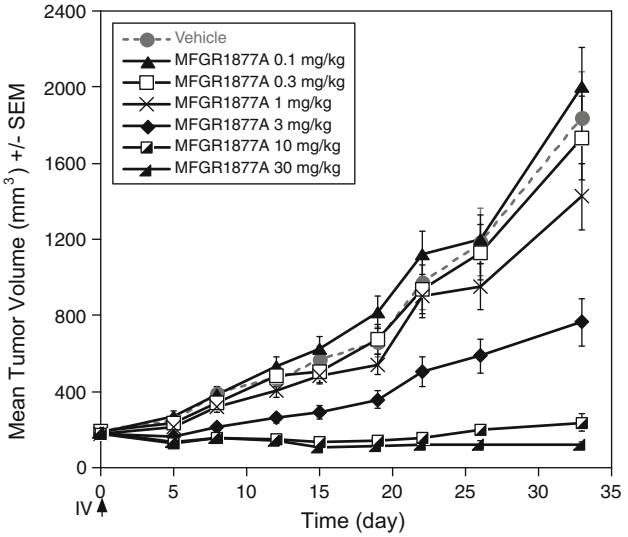


Fig. 5 Anti-tumor efficacy of MFGR1877A in KMS11 human multiple myeloma xenograft model after a single IV dose

Table 4 MFGR1877A exposure and anti-tumor activity in a KMS11 human multiple myeloma xenograft mouse model

Treatment group	AUCinf (day μg/mL)	Time to tumor-doubling (day)
Vehicle	–	10.2
MFGR1877A—0.1 mg/kg	NA[a]	10.2
MFGR1877A—0.3 mg/kg	2.54	10.2
MFGR1877A—1 mg/kg	19.8	12.7
MFGR1877A—3 mg/kg	121	26.7
MFGR1877A—10 mg/kg	945	>33
MFGR1877A—30 mg/kg	5,730	>33

[a] *NA* not applicable as concentrations were MQC after 4 h

AUCinf in the 0.1 mg/kg group could not be estimated as concentrations were below MQC after 4 h. As the dose increased from 0.1 to 30 mg/kg, AUCinf increased more than dose proportionally, indicating that the PK of MFGR1877A is non-linear in tumor bearing SCID mice.

Prediction of human pharmacokinetics

The cynomolgus monkey was selected to scale pharmacokinetics to humans because MFGR1877A binds to monkey FGFR3 with similar affinity as to human FGFR3, and cynomolgus monkey is generally considered the most relevant species for predicting the human PK of antibodies [12]. Pharmacokinetics from the single IV dose PK study in cynomolgus monkeys were used to estimate human pharmacokinetics using a species-invariant time method [13]. The estimated human serum concentration–time profiles

Table 5 MFGR1877A predicted human PK parameters using a non-linear 2-compartment model and population PK analysis

Parameter	Slow clearance (CL exponent = 0.75)		Fast clearance (CL exponent = 0.9)	
	Estimate	% SEE[a]	Estimate	% SEE[a]
CL (mL/day/kg)	2.63	8.63	4.39	7.84
V1 (mL/kg)	28.5	4.81	28.6	4.93
Vmax (μg/day/kg)	37.7	34.7	52	27.7
Km (μg/mL)	3.92	64.5	2.6	55.4
CLd (mL/day/kg)	13.3	14.5	20.5	15.7
V2 (mL/kg)	24.6	8.58	24.9	8.47

[a] % Standard error of estimate

obtained by this method were described well by a non-linear, two-compartment model comprising target-mediated and non-specific CL pathways, and the estimated population PK parameters are shown in Table 5. The estimated non-specific CL range was 2.63–4.39 mL/day/kg, which is in the range of a typical human IgG1 [12]. Estimated values for the Vmax and Km for the specific CL pathway were 37.7–52 μg/day/kg and 2.6–3.92 μg/mL, respectively.

Projection of clinical target dose range

Efficacy data obtained from the KMS11 xenograft model were used to estimate MFGR1877A exposure required to achieve tumor growth inhibition. The anti-tumor activity of MFGR1877A was evaluated in the KMS11 human multiple myeloma xenograft model in a dose range of 0.1–30 mg/kg given as a single IV dose. Minimal or no anti-tumor activity was detectable at the 0.1–1 mg/kg dose levels. At the 10 and 30 mg/kg dose levels, time to tumor-doubling was >33 days. Anti-tumor activity at the 10 mg/kg dose was maintained for at least 3 weeks after a single IV dose. Based on these data, 10 mg/kg was chosen as the target efficacious dose, and exposure at the 10 mg/kg dose over 3 weeks was used to estimate the target dose in humans (weekly AUC of 315 day μg/mL). The target steady state exposure for clinical dosing intervals of every week (Q1 W), every 2 weeks (Q2 W), every 3 weeks (Q3 W), and every 4 weeks (Q4 W), is 315, 630, 945, and 1,260 day μg/mL, respectively.

The second approach was to target a steady state Ctrough that is tenfold the in vitro IC90 in a cell proliferation assay. Using Ba/F3 cells stably expressing wild-type or mutated FGFR3 to establish in vitro cell-based assays, MFGR1877A was shown to dose-dependently inhibit the wild-type FGFR3 and all FGFR3 mutants evaluated [9], including variants that are ligand dependent (G372C, Y375C), constitutively active (R248C, S249C), or both (K652E), and IC90 values are shown in Table 6. The

Table 6 MFGR1877A IC90 values in Ba/F3 cells stably expressing WT and mutant FGFR3

Form of FGFR3 expressed in Ba/F3 cells	IC90 (μg/mL)
FGFR3-IIIb WT	0.21
FGFR3-IIIc WT	0.14
FGFR3-IIIb-R248C	0.06
FGFR3-IIIb-S249C	0.05
FGFR3-IIIc-G372C	0.25
FGFR3-IIIb-Y375C	0.11
FGFR3-IIIb-K652E	0.04

Table 7 Estimated MFGR1877A dose range to achieve target exposure in ≥90% of patients at steady state

Dose frequency	Q1 W	Q2 W	Q3 W	Q4 W
Dose range (mg/kg)	2–3	3–5	5–8	6–10
Percentage of patients with AUC ≥ target (315 day μg/mL weekly)	>98	>91	>94	>90
Percentage of patients with Ctrough ≥2.5 μg/mL (tenfold IC90)[a]	>99	>99	>99	>99

[a] In vitro 90% inhibitory concentration in cell proliferation assays

MFGR1877A IC90 for inhibition of cell proliferation in vitro was approximately ≤0.25 μg/mL; therefore, achieving steady state Ctrough ≥2.5 μg/mL (tenfold the IC90) was a second clinical PK target. The estimated human PK parameters were then used to estimate the clinical dose and dose regimen to achieve the target efficacious exposure and steady state Ctrough in ≥90% of the patients. Doses ranging from 2 to 3 mg/kg IV Q1 W and 6–10 mg/kg IV Q4 W are predicted to achieve a target exposure of 315 day μg/mL per week and maintain a Ctrough at steady state of ≥2.5 μg/mL and are summarized in Table 7.

Discussion

MFGR1877A is a unique antibody that inhibits not only WT FGFR3 but also common mutant variants of FGFR3 linked with cancer [9]. It was shown to have potent anti-tumor activity against t(4;14)-positive human multiple myeloma tumor models in mice by direct inhibition of FGFR3 signaling and engagement of antibody-dependent cell-mediated cytotoxicity [9, data not shown]. We are very interested in developing MFGR1877A as a therapy for relapsed/refractory FGFR3+ multiple myeloma. To gain a better understanding of the therapeutic potential of MFGR1877A, we assessed its pharmacokinetics in mouse, rat, and monkey and efficacy in xenograft models, and predicted its human PK and efficacious dose range in the clinic.

Following single IV dose administration, the pharmacokinetics of MFGR1877A in athymic nude mice appeared to be non-linear in the dose range of 1–50 mg/kg, while in Sprague–Dawley rats and cynomolgus monkeys, the pharmacokinetics of MFGR1877A appeared to be non-linear in the dose range of 1–10 mg/kg and linear at doses ≥10 mg/kg. The non-linear PK data suggest that the total clearance of MFGR1877A comprises a specific (target-mediated) CL component and a non-specific CL component, with the specific CL component having a greater contribution at the lower doses. The total clearance decreased with increased dose likely due to saturation of the target. PK in cynomolgus monkey was used to predict human PK since MFGR1877A binds to both monkey and human FGFR3 with similar affinity, and also because it has been shown that mAb clearance and volume distribution in humans can be reasonably predicted based on monkey data since its disposition and elimination pathways are so similar in the two species [12, 16, 17]. In monkeys, the estimated non-specific CL was 6.02 mL/day/kg, which is in the expected range of clearance of a typical human IgG1 [12]. Since monkey PK was used for human PK predictions, the predicted non-specific CL in humans of 2.63–4.39 mL/day/kg was also as expected for a typical human IgG1. In a clinical study in non-Hodgkin's lymphoma (NHL) patients with rituximab, a chimeric monoclonal antibody against CD-20 expressed on B cells, the clearance of rituximab was significantly reduced after a few treatment cycles, which was due to depletion of the target B cells upon rituximab treatment [18, 19]. Since our target antigen is expressed on multiple myeloma cells, it is possible that the FGFR3 density may decrease upon treatment with MFGR1877A due to depletion of the target cells, leading to time-dependent changes in clearance of MFGR1877A. Due to the uncertainty in the PK projection given the non-linear nature of the CL and the possibility of time-dependent changes in clearance, it is important to verify these predictions in Phase I. The observed human PK information from Phase I will be used to support the dose selection for subsequent clinical trials.

MFGR1877A showed strong anti-tumor activity in human multiple myeloma xenograft models [9]. KMS11 was chosen as a representative model to assess the efficacious dose range in humans due to its previously characterized sensitivity to MFGR1877A [9]. The KMS11 cells contain the t(4;14) translocation and express a mutant FGFR3 ($FGFR3^{Y373C}$). The anti-tumor activity of MFGR 1877A in this model appears to be due to blocking FGFR3 signaling as well as activating ADCC by efficient recruitment of FCγR-bearing immune effector cells [9]. Combining the efficacious exposure from the KMS11 xenograft model and the in vitro cell proliferation assays, and the

predicted human PK estimates, clinical doses for various regimens in patients were predicted to achieve a target exposure of 315 day μg/mL per week at steady state and maintain a Ctrough at steady state of ≥2.5 μg/mL.

In summary, the PK of MFGR1877A was non-linear in mouse, rat, and monkey. The predicted non-specific CL in humans is similar to typical human IgG1 antibodies and will be verified in a Phase 1 study. The projected human efficacious dose and dose regimen appear to be achievable in patients.

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