Preclinical pharmacokinetics of MHAA4549A, a human monoclonal antibody to influenza A virus, and the prediction of its efficacious clinical dose for the treatment of patients with influenza A

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ABSTRACT
MHAA4549A is a human immunoglobulin G1 (IgG1) monoclonal antibody that binds to a highly conserved epitope on the stalk of influenza A hemagglutinin and blocks the hemagglutinin-mediated membrane fusion in the endosome, neutralizing all known human influenza A strains. Pharmacokinetics (PK) of MHAA4549A and its related antibodies were determined in DBA/2J and Balb-c mice at 5 mg/kg and in cynomolgus monkeys at 5 and 100 mg/kg as a single intravenous dose. Serum samples were analyzed for antibody concentrations using an ELISA and the PK was evaluated using WinNonlin software. Human PK profiles were projected based on the PK in monkeys using species-invariant time method. The human efficacious dose projection was based on in vivo nonclinical pharmacological active doses, exposure in mouse infection models and expected human PK. The PK profiles of MHAA4549A and its related antibody showed a linear bi-exponential disposition in mice and cynomolgus monkeys. In mice, clearance and half-life ranged from 5.77 to 9.98 mL/day/kg and 10.2 to 5.76 days, respectively. In cynomolgus monkeys, clearance and half-life ranged from 4.33 to 4.34 mL/day/kg and 11.3 to 11.9 days, respectively. The predicted clearance in humans was ~2.60 mL/day/kg. A single intravenous dose ranging from 15 to 45 mg/kg was predicted to achieve efficacious exposure in humans. In conclusion, the PK of MHAA4549A was as expected for a human IgG1 monoclonal antibody that lacks known endogenous host targets. The predicted clearance and projected efficacious doses in humans for MHAA4549A have been verified in a Phase 1 study and Phase 2a study, respectively.

Introduction
Influenza viruses are associated with significant human disease and cause annual epidemics during autumn and winter. Influenza is a respiratory illness with a broad clinical spectrum that can result in mild symptoms such as fever and cough from which most people recover without requiring medical attention. The standard-of-care therapy for patients with acute uncomplicated influenza consists of administration of neuraminidase inhibitors (NAI) that include but are not limited to oseltamivir, zanamivir, and peramivir.1-5

Influenza can, however, also cause serious complications requiring hospitalization, such as pneumonia, leading to shortness of breath and acute respiratory failure, secondary bacterial respiratory infections, and death, with the majority of infections caused by the influenza A virus. Seasonal influenza results in ~3–5 million cases of severe illness and up to 500,000 deaths worldwide per year.6 Influenza caused an estimated yearly average of 19,100 deaths between 1997 and 2009 in the United States.7 A comparable mortality and morbidity has been reported for European countries.8 Thus, a significant unmet medical need still exists for those at high-risk of developing influenza-complications, including children, the elderly, pregnant women, as well as individuals with underlying chronic medical conditions or weakened immune systems.

To address this unmet medical need, a highly-specific anti-influenza A antibody therapy (MHAA4549A) is being developed for the treatment of hospitalized patients with severe influenza A. MHAA4549A is a human immunoglobulin G1 (IgG1) monoclonal antibody (mAb) against the influenza A virus, which was cloned from a single human plasmablast cell isolated from an influenza vaccinated donor.9 Influenza A is a membrane-enveloped RNA virus that expresses 2 immunodominant surface proteins, hemagglutinin, and neuraminidase.10 Hemagglutinin promotes viral attachment and entry into the host cell,10,11 and is the target for the majority of neutralizing antibodies generated during a normal immune response.10,11 However, significant sequence diversity among hemagglutinin genes limits the ability of these antibodies to broadly protect against influenza A infection. MHAA4549A is capable of neutralizing all tested human influenza A strains by binding to a highly conserved epitope on the stalk of hemagglutinin with
high affinity, blocking fusion of the viral envelope with the host target cell endosomal membrane and preventing viral replication.

As part of our approach for the clinical development of MHAA4549A, we characterized the preclinical pharmacokinetics (PK) of MHAA4549A and its related antibodies (39.29 QVQ and 39.29 NYC, which have the same complementarity-determining region (CDR) as MHAA4549A with only a few amino acids changes on the IgG backbone) in mouse and monkey, and predicted its human PK. In addition, nonclinical efficacy data from mouse infection models of influenza A were combined with human PK estimates to project the efficacious dose for MHAA4549A in the clinic.

Results

Pharmacokinetics of MHAA4549A in DBA/2J and Balb-c mice

The PK profiles of MHAA4549A following a single intravenous (IV) bolus dose in DBA/2J and Balb-c mice at a dose of 5 mg/kg are shown in Fig. 1. Following a single IV bolus dose of 5 mg/kg in female DBA/2J or Balb-c mice, MHAA4549A showed a bi-exponential serum concentration-time profile with a short distribution phase followed by a long elimination phase. In female DBA/2J mice, clearance (CL) was 5.77 mL/day/kg, terminal half-life (t1/2, z) was 10.2 days, volume of distribution at steady state (Vss) was 86.2 mL/kg, and observed maximum concentration (Cmax) was 117 μg/mL. In female Balb-c mice, CL was 9.98 mL/day/kg, t1/2, z was 5.76 days, Vss was 65.8 mL/kg, and Cmax was 115 μg/mL.

Pharmacokinetics of 39.29 QVQ in cynomolgus monkeys

The PK profiles of 39.29 QVQ following a single IV bolus dose in cynomolgus monkeys at doses of 5 and 100 mg/kg are shown in Fig. 2. After a single IV bolus dose of 5 or 100 mg/kg in cynomolgus monkeys, 39.29 QVQ showed a bi-exponential serum concentration-time profile with a short distribution phase followed by a long elimination phase. Mean area under the serum concentration-time curve (AUC) and mean Cmax increased dose proportionally from 5 to 100 mg/kg, suggesting that 39.29 QVQ PK were dose proportional in a dose range of 5–100 mg/kg. Following a single IV bolus dose of 39.29 QVQ at 5 mg/kg, mean CL was 4.34 mL/day/kg, mean β-phase half-life (t1/2, β) was 11.9 days, mean Vss was 70.8 mL/kg, mean volume of the central compartment (V1) was 33.9 mL/kg and mean Cmax was 148 μg/mL. Following a single IV bolus dose of 39.29 QVQ at 100 mg/kg, mean CL was 4.33 mL/day/kg, mean t1/2, β was 11.3 days, mean Vss was 66.7 mL/kg, mean V1 was 30.5 mL/kg, and mean Cmax was 3340 μg/mL.

In vivo efficacy of 39.29 NYC to determine driver of efficacy in mouse Influenza models

An efficacy study with fractional doses was conducted to determine the driver of efficacy for anti-influenza A mAbs in mouse influenza infection models. 39.29 NYC was evaluated for efficacy against the A/PR/8/34 H1N1 influenza A strain. One group received a single IV bolus dose of ~5 mg/kg (100 μg/mouse) for 3 days, while the second group received a single IV bolus dose of ~15 mg/kg (300 μg/mouse) followed by 2 intraperitoneal (i.p) doses of water for 2 d. These two dosing regimens provide comparable AUC with different Cmax and time over a threshold concentration. The single dose of 15 mg/kg 39.29 NYC provided significant protection compared to multiple doses of 5 mg/kg over 3 d (p < 0.01, log-rank test), with animals exhibiting 60% mortality at the end of study in the 15 mg/kg group while 100% mortality was observed at day 7 in the 5 mg/kg group (Fig. 3). At 72 hours post infection before antibody intervention, mice infected with A/PR/8/34 H1N1 did not show any body weight loss (Fig. 4). Starting at Day 4 post infection, both groups of mice started losing body weight progressively until all the mice in the 5 mg/kg group succumbed to
infection at Day 7; the 15 mg/kg 39.29 NYC-treated mice that survived the infection exhibited a transient body weight loss, and regained their body weight to 100% of their pre-infection body weight at the end of the study. In contrast, 15 mg/kg 39.29 NYC-treated mice that succumbed to the infection continued to lose their body weight progressively until death, similar to the 5 mg/kg group (Fig. 4). These data demonstrate that the initial systemic concentrations of 39.29 NYC either C_{max} or time over threshold concentration likely drive its efficacy in the mouse models of influenza A infection.

**Prediction of human pharmacokinetics of MHAA4549A from 39.29 QVQ pharmacokinetics in cynomolgus monkeys**

39.29 QVQ PK data from the single IV dose PK study in cynomolgus monkeys were used to estimate human PK of MHAA4549A using a species-invariant time method.\(^{12}\) The estimated human serum concentration-time profiles obtained by this method were described well by a linear, 2-compartment model (Fig. 5). The following human PK parameter estimates of MHAA4549A were projected: CL of 2.60 mL/day/kg, V_{1} of 32.2 mL/kg, and \(t_{1/2, \beta}\) of 19.3 days, which are in the range of a typical human IgG1 antibody.\(^{13,14}\) As expected, the non-cross reactive mAb has a longer half-life in humans compared to that in animals.

**Projection of clinical target dose range for MHAA4549A**

The C_{max} in the mouse models was chosen as the most conservative target concentration to estimate the efficacious dose in humans. At the efficacious dose range of 15 mg/kg to 45 mg/kg in mouse infection models, the C_{max} was estimated to range from \(~345 \mu g/mL\) to \(1050 \mu g/mL\). A single IV dose ranging from \(~15 mg/kg\) to \(~45 mg/kg\) is likely to achieve efficacious exposures in \(\geq90\%\) patients based on the projected human PK parameters in conjunction with the target C_{max} range (Fig. 6A and 6B).

**Discussion**

MHAA4549A is a human IgG1 mAb that binds specifically and with high affinity to Group 1 and Group 2 influenza A hemagglutinins. MHAA4549A neutralized a panel of influenza A human strains that represent the wide genetic diversity of this virus, both geographic and temporal, indicating that MHAA4549A binds to a highly conserved hemagglutinin epitope, possibly shared by all human influenza A strains.\(^{9}\) With the high incidences of hospitalizations and associated morbidity and mortality as a result of severe Influenza A infections,\(^{8,15,16}\) MHAA4549A is being developed as a potential therapy for the treatment of patients hospitalized with severe influenza A infection. To gain a better understanding of the therapeutic potential of MHAA4549A, we assessed its or its related antibodies (39.29 QVQ and 39.29 NYC) PK in mouse, and monkey, as well as nonclinical efficacy in mouse infection models of influenza A. These data were then used to predict MHAA4549A human PK and efficacious dose range in the clinic. All three antibodies were expected to have similar efficacy and PK properties owing to their similar CDRs and backbone with only a few amino acid changes, which were confirmed in our PK and efficacy studies.

Following a single IV bolus dose of 5 mg/kg in female DBA/2J or Balb-c mice, and 5 or 100 mg/kg in cynomolgus monkeys, MHAA4549A and 39.29 QVQ showed a bi-exponential serum concentration-time profile with a short distribution phase followed by a long elimination phase. The clearance of MHAA4549A in Balb-c mice appeared to be \(~1.7\) times faster than MHAA4549A clearance in DBA/2J mice. Of note, the Balb-c strain of mice have more inter-individual variability especially after Day 10, only one mouse seems to be driving the concentrations up on Day 21 while all 3 mice have greatly reduced concentrations on Day 28 (Fig. 1). It is perhaps due to
different PK behavior among animals and/or the presence of anti-therapeutic antibodies (ATA) in Balb/c mice, although ATAs were not measured in the study. Overall, the PK profile of MHAA4549A and 39.29 QVQ in mice and monkeys (Figs. 1–2) was consistent with that of a human IgG1 mAb that lacks known endogenous host targets.14

The PK of 39.29 QVQ in cynomolgus monkey was used to predict human PK of MHAA4549A. The cynomolgus monkey was selected to scale PK to humans as it is generally considered the most relevant species for predicting the human PK of mAbs.13 It has been shown that the clearance and volume distribution for a mAb that lacks known endogenous host targets in humans can be reasonably predicted based on monkey data, since disposition and elimination pathways are so similar in the 2 species.13,14,17,18 In monkeys, the estimated CL was 4.34 mL/day/kg, which is in the expected range of clearance of a typical human IgG1. The predicted CL in humans of 2.60 mL/day/kg, V1 of 32.2 mL/kg, and t1/2, β of 19.3 days, are also as expected for a typical human IgG1. The Phase 1 PK data in healthy volunteers further confirmed the human PK projections where the PK of MHAA4549A was observed to be linear in a dose range of 1.5 mg/kg to 45 mg/kg, with average mean t1/2 of ~23 d and apparent CL ranging from 2.33 to 2.90 mL/day/kg.19

In vivo efficacy of MHAA4549A as a single agent in mouse influenza models was reported previously by Nakamura et al.9 Briefly, MHAA4549A was evaluated for efficacy as a single dose IV at 3 dose levels of ~5 mg/kg, ~15 mg/kg, and ~45 mg/kg against 4 different influenza A strains that included both Group 1 (H1N1) and Group 2 (H3N2) subtypes in DBA/2J mice: A/PR/8/34 H1N1, A/Hong Kong/1/68 H3N2, A/Port Chalmers/1/73 H3N2, or A/Aichi/2/68 H3N2. The efficacy studies demonstrated the strong antiviral activity of MHAA4549A as a single agent in Group 1 and Group 2 influenza A mouse infection models. A dose less than 15 mg/kg cannot provide 100% protection of survival. An efficacious dose range of ~15 mg/kg to 45 mg/kg was determined to achieve 100% protection of survival in these models.9

The clinical target dose range for MHAA4549A was based on the nonclinical efficacy data from the in vivo mouse influenza A infection models8 and the predicted human PK. The nonclinical efficacy studies were done in 4 different influenza A mouse model that include both Group 1 (H1N1) and Group 2 (H3N2) subtypes, and the dose-response relationship is different for each model.4 Therefore, a simple generalized conservative method was used for human efficacious dose projection. In these efficacy studies, mice in the control group started showing mortality as early as 3 d post-dose, which was 100% prevented by treatment with ~15 mg/kg to 45 mg/kg of MHAA4549A. Additional efficacy studies with fractional dose showed that the initial systemic concentration of MHAA4549A (i.e., Cmax) or time over a threshold appears to drive its efficacy as observed previously with other anti-viral drugs where Cmax or time above threshold is the driver of efficacy.20,21 A66 is another broadly neutralizing anti-influenza A mAb discovered by Sui et al.22 We have observed that the initial systemic exposure of A66 was comparable between infected mice and uninfected mice following a single IV dose of 5 mg/kg (Fig. S1), which is expected for MHAA4549A and its related antibodies too, since influenza disease is restricted mostly to the respiratory tract and there is generally no viremia associated with the disease.23 Assuming that the viral kinetics in the mouse infection models mimic those in human influenza A infection, the Cmax in the mouse models was chosen as the most conservative target concentration to estimate the efficacious dose. Using the projected human PK parameters in conjunction with the target Cmax range, a single IV dose ranging from ~15 mg/kg to 45 mg/kg was predicted to achieve efficacious exposures in ≥~90% patients. The projected efficacious dose in humans is similar with that in mice, due to matching Cmax between mice and humans for the efficacy. Cmax is determined by V1, which is comparable between mice and humans for MHAA4549A based on current data and assumptions.

Efficacy data from a Phase 2a study with dose ranging from ~5 mg/kg to 45 mg/kg confirmed the proof of antiviral activity at the 45 mg/kg dose level (data not shown).24 The 45 mg/kg efficacious dose seems to be high for a mAb compared to marketed biologics; however, intravenous
immunoglobulin (IVIG) preparations have been used for over 30 y in the clinic with doses up to 2 g/kg. Treatment of severe influenza A (H1N1) infection with 400 mg/kg of hyperimmune immunoglobulin was associated with clinical benefits. A 100 mg/kg dose of anti-LINGO-1 mAb has been tested in 2 Phase 1 studies with favorable safety profile. In addition, high doses further reduce the risk for development of resistance. Finally, MHAA4549A has a large safety margin based on predicted human PK and nonclinical safety study, where MHAA4549A was well tolerated when administered once weekly for 4 weeks (five total doses) up to 150 mg/kg in naive rats (data not shown).

In summary, the PK of MHAA4549A and its related antibody in mice and monkeys was consistent with that of other human IgG1 mAbs that lack known endogenous host targets. The predicted CL in humans estimated from the cynomolgus monkey PK data is similar to typical human IgG1 antibodies and was confirmed in a Phase 1 study. The projected human efficacious dose of 45 mg/kg (i.e., 3600 mg for an 80 kg subject) was confirmed in patients in a Phase 2a study.

Materials and methods
MHAA4549A, 39.29 QVQ and 39.29 NYC anti-influenza A antibodies were generated at Genentech, Inc. (South San Francisco, CA), and formulated in 10 mM sodium succinate, 240 mM sucrose and 0.02% (w/v) polysorbate 20, 200 mM arginine and 137 mM succinic acid, and phosphate-buffered saline (PBS) respectively, for the in vivo PK and efficacy studies. Antibody 39.29 NYC was the first sequence cloned and did not match the germline in areas of high conservation. It is different from antibody 39.29 QVQ only in the framework region by 3 amino acids, which amount to one residue in the light chain (proline in 39.29 NYC versus serine in 39.29 QVQ) and 2 residues in the heavy chain (proline in 39.29 NYC vs. leucine in 39.29 QVQ on 2 positions). Antibodies MHAA4549A and 39.29 QVQ varied by 1 amino acid residue (glutamine in 39.29 NYC versus glutamic acid in 39.29 QVQ), which enhanced antibody stability.

MHAA4549A pharmacokinetic study in DBA/2J and Balb-c mice
The PK study in DBA/2J and Balb-c mice was approved by the Institutional Animal Care and Use Committee at Genentech, Inc. Naïve female DBA/2J and Balb-c mice that weighed between 14.3 and 19.7 g each, received a single IV dose of 5 mg/kg of MHAA4549A via the tail vein (n = 18/group). Blood samples were collected via retro-orbital bleeds and the terminal blood sample was collected via cardiac stick from each animal in each dosing group at the following time points: 15 and 30 min, 1, 8, and 24 h; and 3, 7, 10, 14, 17, 21, and 28 d, and processed to collect serum. Two blood samples were taken from each mouse (one retro-orbital bleed and one terminal bleed), and there were 3 mice per time point. Sera collected were stored at approximately −70°C until analyzed by enzyme-linked immunosorbent assay (ELISA) for antibody concentrations.

39.29 QVQ pharmacokinetic study in cynomolgus monkeys
The PK study in cynomolgus monkeys was approved by the Institutional Animal Care and Use Committee and conducted at Charles River Laboratories (Reno, NV). Two male and 2 female cynomolgus monkeys that weighed between 2.0 to 2.5 kg were given a single IV bolus dose of 5 or 100 mg/kg of 39.29 QVQ via the saphenous vein. Blood samples (~1 mL) for PK analysis were collected from each animal via the femoral vein or peripheral vein and processed to collect serum. Serum samples (n = 4 per group/timepoint) were collected at following timepoints: 15 min, 4 and 8 h; and 1, 3, 5, 7, 10, 14, 21, 28 and 42 d post-dose, and analyzed for antibody concentrations by ELISA methods.

Bioanalysis of serum samples from pharmacokinetic studies
Serum samples from DBA/2J mice, Balb-c mice and cynomolgus monkeys were analyzed for MHAA4549A and 39.29 QVQ antibody concentrations respectively, using a quantitative ELISA. For mouse samples, the assay utilized a polyclonal donkey anti-human Fc (Jackson ImmunoResearch Laboratories Inc.) as the capture reagent and polyclonal goat anti-human IgG Fc (Jackson ImmunoResearch Laboratories Inc.) conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories Inc.) as the detection reagent. The minimum quantifiable concentration (MQC) in this assay was 15.6 ng/mL.

For cynomolgus monkey samples, the assay utilized a lectin coat as the capture reagent to immobilize soluble hemagglutinin protein present in a lysate of influenza A-infected cells (prepared at Genentech, Inc.). MHAA4549A present in serum samples was then captured by immobilized hemagglutinin, followed by detection using polyclonal goat anti-human IgG (Fc specific) antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories Inc.). The MQC in this assay was 156 ng/mL.

Pharmacokinetic data analysis
Group mean serum concentration–time profiles were used to estimate the following PK parameters in mouse, using non-compartmental analysis with a naive-pooled approach (i.e. pooled data were treated as from a single animal) to provide one estimate per group (Phoenix WinNonlin, version 6.3; Pharsight Corporation, Mountain View, CA): total drug exposure defined as area under the serum concentration–time curve extrapolated to infinity (AUC0–∞, data not shown), CL, t1/2, Vss, and Cmax. The nominal dose administered for each group was used for modeling. Data points at Day 28 in Balb-c mice were excluded from analysis per WinNonlin best-fit criteria for terminal phase slope determination.

For cynomolgus monkeys, individual serum concentration profiles were used to estimate the following PK parameters using a 2-compartment analysis (WinNonlin, version 5.2.1, Model 7; IV bolus input using 1/y^2 weighting and linear trapezoidal [linear interpolation] rule): AUC (data not shown), Cmax, CL, t1/2, β, Vss, and volume of distribution of the central
In vivo efficacy of 39.29 NYC to determine driver of efficacy in mouse influenza models

In vivo efficacy of 39.29 NYC was tested in Balb-c mice challenged with a minimum lethal intranasal dose of $5 \times 10^6$ pfu of A/PR/8/34 H1N1. The infection was allowed to progress for 72 h before IV administration of a single dose of 15 mg/kg of 39.29 NYC followed by i.p administration of water for the next 2 d in one group, while the other group received 5 mg/kg 39.39 NYC for 3 d. Mice were monitored for body conditioning and survival until 21 d after infection. Results were presented as percent survival versus time (days). The virus inoculation amount, dose level and treatment window were selected based on a pilot study as well efficacy studies reported by Nakamura et al.\textsuperscript{9}

**Prediction of human pharmacokinetics of MHAA4549A from 39.29 QVQ pharmacokinetics in cynomolgus monkeys**

Human PK of MHAA4549A was projected based on 39.29 QVQ PK data in cynomolgus monkeys. The monkey serum concentration–time profiles were transformed to human concentration–time profiles using a species-invariant time method as described previously using the following equations.\textsuperscript{12-14}

$$Time_{human} = Time_{cyno} \times \left( \frac{BW_{human}}{BW_{cyno}} \right)^{Exponent_{volume} - Exponent_{clearance}}$$

(1)

$$Concentration_{human} = Concentration_{cyno} \times \frac{Dose_{human}}{Dose_{cyno}}$$

(2)

Where $Time_{human}$ is pharmacokinetic time in human; $Time_{cyno}$ is pharmacokinetic time in cynomolgus monkey; $Concentration_{human}$ is mAb serum concentration in human; $Concentration_{cyno}$ is mAb serum concentration in cynomolgus monkey; $Dose_{human}$ is dose in human PK study (mg/kg); $Dose_{cyno}$ is dose in cynomolgus monkey PK study (mg/kg). A scaling exponent of 0.85 was used to estimate human CL, and a scaling exponent of 1 was used to estimate human $V_1$\textsuperscript{13,28}

**Projection of clinical target dose range for MHAA4549A**

The projected human serum concentration–time data obtained from the species-invariant time method were used to predict population PK parameter estimates in humans using a 2 compartmental PK model with linear 1st order elimination. The estimated MHAA4549A human PK parameters were then used to estimate the clinical dose to achieve the target efficacious exposure identified in the mouse efficacy studies, in $\geq 90\%$ patients using population simulations by NONMEM (version 7.1.2, ICON Development Solutions, Ellicott City, MD). Targeting $\geq 90\%$ patients achieving projected efficacious exposure is a conservative in terms of efficacy. For the simulations, the inter-individual variability on CL and $V_1$ was assumed to be 30% and the correlation between CL and $V_1$ was assumed as 0.5 based on what are generally observed in humans for monoclonal antibodies.\textsuperscript{13,14,28}

**Disclosure of potential conflicts of interest**

All authors are employees of Genentech, Inc., a member of the Roche Group, and stockholders in Roche Holdings AG during their involvement in this study.

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